M.A. Hayat
Editor

Tumors of the Central Nervous System

Volume 10
Pineal, Pituitary, and Spinal Tumors

Springer

Tumors of the Central Nervous System
Volume 10

For further volumes:
http://www.springer.com/series/8812

Tumors of the Central Nervous System
Volume 10

Tumors of the Central Nervous System

Pineal, Pituitary, and Spinal Tumors

Edited by

M.A. Hayat
Distinguished Professor
Department of Biological Sciences
Kean University, Union, NJ, USA

Springer

Editor
M.A. Hayat
Department of Biological Sciences
Kean University
Room 213, Library building
Morris Avenue 1000
Union, NJ 07083, USA

ISBN 978-94-007-5680-9 ISBN 978-94-007-5681-6 (eBook)
DOI 10.1007/978-94-007-5681-6
Springer Dordrecht Heidelberg New York London

Library of Congress Control Number: 2012955032

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

"Although touched by technology, surgical pathology always has been, and remains, an art. Surgical pathologists, like all artists, depict in their artwork (surgical pathology reports) their interactions with nature: emotions, observations, and knowledge are all integrated. The resulting artwork is a poor record of complex phenomena."

Richard J. Reed, MD

One Point of View

All small tumors do not always keep growing, especially small breast tumors, testicular tumors, and prostate tumors. Some small tumors may even disappear without a treatment. Indeed, because prostate tumor grows slowly, it is not unusual that a patient may die at an advanced age of some other causes, but prostate tumor is discovered in an autopsy study. In some cases of prostate tumors, the patient should be offered the option of active surveillance followed by PSA test or biopsies. Similarly, every small kidney tumor may not change or may even regress. Another example of cancer or precancer reversal is cervical cancer. Precancerous cervical cells found with Pap test may revert to normal cells. Tumor shrinkage, regression, dormancy, senescence, reversal, or stabilization is not impossible. Can prosenescence therapy be an efficient alternative strategy to standard therapies for cancer prevention and treatment?

Another known example of cancer regression is found in pediatric neuroblastoma patients. Neuroblastoma shows one of the highest rates of spontaneous regression among malignant tumors. In addition to the well-known spontaneous regression in stage 4S disease, the high incidence of neuroblastoma remnants found during autopsy of newborns suggest that localized lesions may undergo a similar regression (Guin et al. 1969). Later studies also indicate that spontaneous regression is regularly seen in infants with localized neuroblastoma and is not limited to the first year of life (Hero et al. 2008). These and other studies justify the "wait and see" strategy, avoiding chemotherapy and radiotherapy in infants with localized neuroblastoma, unless *MYCN* gene is amplified. Infants with nonamplified *MYCN* and hyperdiploidy can be effectively treated with less intensive therapy. Infants with disseminated disease without *MYCN* have excellent survival with minimal or no treatment. Another example of spontaneous shrinkage and loss of tumors without any treatment is an intradural lipoma (Endoh et al. 1998).

Although cancers grow progressively, various lesions such as cysts and thyroid adenomas show self-limiting growth. Probably, cellular senescence occurs in many organ types following initial mutations. Cellular senescence, the growth arrest seen in normal mammalian cells after a limited number of divisions, is controlled by tumor suppressors, including p53 and p16, and so this phenomenon is believed to be a crucial barrier to tumor development. It is well-established that cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence.

Metastasis is the main cause of death from cancer. Fortunately, metastasis is an inefficient process. Only a few of the many cancer cells detached from the primary tumor succeed in forming secondary tumors. Metastatic inefficiency varies depending on the location within an organ, but the malignancy may continue to grow preferentially in a specific tissue environment. Some of the cancer cells shed from the primary tumor are lost in the circulation due to hemodynamic forces or the immune system, macrophages, and natural killer cells.

Periodic rejection of a drug by FDA, which was previously approved by the FDA, is not uncommon. Most recently, the FDA ruled that Avastin should not be used to treat advanced breast cancer, although it remains on the market to treat other cancers, including colon and lung malignancies. Side-effects of Avastin include high blood pressure, massive bleeding, heart attack, and damage to the stomach and intestines.

Unwanted side-effects of some drug excipients (e.g., propylene glycol, menthol) may also pose safety concerns in some patients. Excipients are defined as the constituents of the pharmaceutical formulation used to guarantee stability, and physicochemical, organoleptic, and biopharmaceutical properties. Excipients frequently make up the majority of the volume of oral and parenteral drugs. Not all excipients are inert from the biological point of view. Although adverse drug reactions caused by the excipients are a minority of all adverse effects of medicinal products, the lack of awareness of the possible risk from excipients should be a concern for regulatory agencies, physicians, and patients (Ursino et al. 2011). Knowledge of the potential side-effects of excipients is important in clinical practice.

It is known that chemotherapy can cause very serious side-effects. One most recent example of such side-effects was reported by Rubsam et al. (2011). Advanced hepatocellular carcinoma (HCC) induced by hepatitis C virus was treated with Sorafenib. It is an oral multikinase inhibitor that interferes with the serine/threonine kinases RAF-1 and B-Raf and the receptor tyrosine kinases of the vascular endothelial growth factor receptors and the platelet-derived growth factor receptor-beta. Although Sorafenib is effective in regressing HCC, it shows serious side-effects including increasingly pruritic and painful skin changes (cutaneous eruption).

An example of unnecessary surgery is the removal of all the armpit lymph nodes after a biopsy when a sentinel node shows early stage breast cancer; removal of only the sentinel node may be needed. Limiting the surgery to the sentinel node avoids painful surgery of the armpit lymph nodes, which can have complications such as swelling and infection (such limited surgery is already being practiced at the Memorial Sloan-Kettering Cancer Research Center). Radiation-induced second cerebral tumors constitute a significant risk for persons undergoing radiotherapy for the management of cerebral neoplasms. High-grade gliomas are the most common radiation-induced tumors in children (Pettorini et al. 2008). The actual incidence of this complication is not known, although it is thought to be generally low.

Medical Radiation

Chromosome aberrations induced by ionizing radiation are well-known. Medical radiation-induced tumors are well-documented. For example, several types of tumors (sarcomas, meningiomas) can develop in the CNS after irradiation of the head and neck region (Parent 1990). Tumorigenic mechanisms underlying the radiation therapy of the CNS are discussed by Amirjamshidi and Abbassioun (2000) (See below).

Radiation therapy is commonly used to treat, for example, patients with primary and secondary brain tumors. Unfortunately, ionizing radiation has limited tissue specificity, and tends to damage both neoplastic and normal brain tissues. Radiation-induced brain injury, in fact, is a potential, insidious later cerebral side-effect of radiotherapy. Most commonly it consists of damage in small arteries and capillaries, resulting in secondary processes of ischemia.

After radiation therapy, imaging techniques (CT, MRI, SPECT) can be used to assess treatment response and detect radiation-induced lesions and recurrent tumors. Optical spectroscopy has also been used for detecting radiation damage (Lin et al. 2005). The F_{500} nm spectral peak allows accurate selection of tissues for biopsy in evaluating patients with new, contrast enhancing lesions in the setting of previous irradiation. This peak is highly correlated with a histological pattern of radiation injury. Deep lesions require a stereotactic biopsy to be conclusive. Also, much of the radiation effect is mediated by acute and chronic inflammatory cellular reactions. Biopsy samples supplement pathological differentiation of radiation effect from tumor progression. It should be noted that most of the biopsies show radionecrosis as well as scattered tumor cells.

Women treated with therapeutic chest radiation may develop cancer. This possibility becomes exceedingly serious considering that 50,000–55,000 women in the United States have been treated with moderate to high-dose chest radiation (~20 Gy). This possibility is much more serious for pediatric or young adult cancer patients, because these women are at a significantly increased risk of breast cancer and breast cancer mortality following cure of their primary malignancy (Martens et al. 2008). A recent study also indicates that such young women develop breast cancer at a young age, which does not appear to plateau (Henderson et al. 2010). In this high-risk population, ironically there is a benefit associated with early detection. In other words, young women with early stage breast cancer following chest radiation have a high likelihood for favorable outcome, although life-long surveillance is needed.

Presently, although approximately 80% of the children with cancer are cured, the curative therapy could damage a child's developing organ system; for example, cognitive deficits following cranial radiotherapy are well known. Childhood survivors of malignant diseases are also at an increased risk of primary thyroid cancer (Sigurdson et al. 2005). The risk of this cancer increases with radiation doses up to 20–29 Gy. In fact, exposure to radiation therapy is the most important risk factor for the development of a new CNS tumor in survivors of childhood cancer, including leukemia and brain tumors.

The higher risk of subsequent glioma in children subjected to medical radiation at a very young age reflects greater susceptibility of the developing brain to radiation. The details of the dose–response relationships, the expression of excess risk over time, and the modifying effects of other host and treatment factors have not been well defined (Neglia et al. 2006).

A recent study indicates that childhood brain tumor survivors are at an increased risk of late endocrine effects, particularly the patients treated with cranial radiation and diagnosed at a younger age (Shalitin et al. 2011). Among children with cancer, the application of radiotherapy, therefore, should not be taken lightly, and it should be administered only when absolutely necessary to successfully treat the primary tumor. When radiotherapy is administered, use of the minimum effective dose tends to minimize the risk of second CNS neoplasms (late effect). Prolonged follow-up of childhood cancer survivors (particularly those treated with radiation) is necessary because of the long period between treatment and the development of malignancy. This practice should be a part of the effective therapy of the primary disease.

It is well established that radiation doses are related to risk for subsequent malignant neoplasms in children with Hodgkin's disease. It has been reported that increasing radiation dose was associated with increasing standardized incidence ratio ($p=0.0085$) in survivors of childhood Hodgkin's disease (Constine et al. 2008). Approximately, 75% of subsequent malignancies occurred within the radiation field. Although subsequent malignancies occur, for example, in breast cancer survivors in the absence of radiotherapy, the rise increases with radiation dose.

The pertinent question is: Is it always necessary to practice tumor surgery, radiotherapy, chemotherapy, or hormonal therapy, or a combination of these therapies? Although the conventional belief is that cancer represents an "arrow that advances unidirectionally", it is becoming clear that for cancer to progress, it requires cooperative microenvironment (niche), including immune system and hormone levels. However, it is emphasized that advanced (malignant) cancers do not show regression, and require therapy. In the light of the inadequacy of standard treatments of malignancy, clinical applications of the stem cell technology need to be expedited.

Prostate Cancer

There were an estimated 217,730 new cases of prostate cancer in the United States in 2010 with 32,050 deaths, making it the second leading cause of cancer deaths in men. Currently, there are more than 2,000,000 men in the United States who have had radical or partial prostate surgery performed. Considering this huge number of prostate surgeries and the absence of a cumulative outcome data, it seems appropriate to carefully examine the benefits of radical surgery, especially in younger men.

Clinical prostate cancer is very rare in men of the ages younger than 40 years. In this age group the frequency of prostate malignancy is 1 in 10,000 individuals. Unfortunately, the incidence of malignancy increases over the ensuing decades, that is, the chance of prostate malignancy may reach to 1 in

7 in men between the ages of 60 and 79 years. Reactive or aging-related alterations in the tumor microenvironment provide sufficient influence, promoting tumor cell invasion and metastasis. It has been shown that nontumorigenic prostate epithelial cells can become tumorigenic when cocultured with fibroblasts obtained from regions near tumors (Olumi et al. 1999).

Prostate cancer treatment is one of the worst examples of overtreatment. Serum prostate specific antigen (PSA) testing for the early detection of prostate cancer is in wide use. However, the benefit of this testing has become controversial. The normal cut-off for serum levels of PSA is 4 ng/ml, so a man presenting with a PSA above this level is likely to require a rectal biopsy, but only in 25% of men with serum levels of PSA between 4 ng and 10 ng/ml have cancer (Masters 2007). The PSA threshold currently being used for biopsy ranges between 2.5 and 3.4 ng/ml. Up to 50% of men presenting with prostate cancer have PSA levels within the normal range. It is apparent that screening of prostate cancer using PSA has a low specificity, resulting in many unnecessary biopsies, particularly for gray zone values (4 ng–10 ng/ml). According to one point of view, the risks of prostate cancer overdetection are substantial. In this context, overdetection means treating a cancer that otherwise would not progress to clinically significant disease during the lifetime of the individual. Overdetection results in overtreatment. The advantages and limitations of PSA test in diagnosing prostate cancer were reviewed by Hayat (2005, 2008).

Androgen deprivation therapy (ADT) is an important treatment for patients with advanced stage prostate cancer. This therapy is carried out by blocking androgen receptor or medical or surgical castration. Although ADT is initially very effective, treated tumors inevitably progress to androgen-independent prostate cancer (AIPC); which is incurable. One possible mechanism responsible for the development of AIPC is modulation of the tissue microenvironment by neuroendocrine-like cancer cells, which emerge after ADT (Nelson et al. 2007).

Recently, Pernicova et al. (2011) have further clarified the role of androgen deprivation in promoting the clonal expansion of androgen-independent prostate cancer. They reported a novel linkage between the inhibition of the androgen receptor activity, down-regulation of S-phase kinase-associated protein 2, and the formation of secretory, senescent cells in prostate tumor cells. It is known that several components of the SASP secretome, such as IL-6, IL-8, KGH, and epidermal growth factor, are capable of transactivating androgen receptor under androgen-depleted conditions (Seaton et al. 2008). It needs to be pointed out that androgen deprivation therapy, used in high-risk patients with prostate cancer, may cause reduced libido, erectile dysfunction, fatigue, and muscle loss; osteoporosis is also a late complication. Therefore, periodic bone density scanning needs to be considered.

Recently, the FDA cleared the use of NADiA (nucleic acid detection immunoassay) ProsVue prognostic cancer test. This proprietary nucleic acid detection immunoassay technology identifies extremely low concentrations of proteins that have not been routinely used as a diagnostic or prognostic aid. It is an *in vitro* diagnostic assay for determining the rate of change of serum total PSA over a period of time. The assay can quantitate PSA at levels < 1 ng/ml.

This technique can be used as a prognostic marker, in conjunction with clinical evaluation, to help identify patients at reduced risk for recurrence of prostate cancer for years following prostatectomy. It targets the early detection of proteins associated with cancer and infectious diseases. This technique combines immunoassay and real-time PCR methodologies with the potential to detect proteins with femtogram/ml sensitivity (10–15 g/ml). Additional clinical information is needed regarding its usefulness in predicting the recurrence.

A significant decrease in the risk of prostate cancer-specific mortality is observed in men with few or no comorbidities. Indeed, active surveillance in lieu of immediate treatment (surgery or radiation, or both) is gaining acceptance. Most men with prostate cancer, even those with high-risk disease, ultimately die as a result of other causes (Lu-Yao et al. 2009). Debate on this controversy is welcome, but narrow opinions and facile guidelines will not lead to facts and new information; men worldwide deserve it (Carroll et al. 2011). Automatic linking of positive diagnosis with treatment, unfortunately, is a common clinical practice. Unfortunately, even men who are excellent candidates for active surveillance in the United States often undergo some treatment. Deferment of treatment is advised in men with low-risk disease, especially of a younger age.

Active surveillance is proposed for patients with low-risk prostate cancer in order to reduce the undesirable effects of overdiagnosis. Prostate specific antigen serum level lower than 10 ng/L and Gleason score lower than 7 are the main criteria to select patients for active surveillance. The correct use of these two criteria is essential to differentiate between aggressive and nonaggressive prostate cancer. Autopsy studies indicate that approximately one out of three men older than 50 years show histological evidence of prostate cancer (Klotz 2008). Thus, a large proportion of prostate cancers are latent, never destined to progress, or affect the life of the patient. It is estimated that the percentage of low-risk prostate cancer is between 50 and 60% of newly diagnosed cases. A large number of patients die having prostate cancer, but not because of this cancer (Filella et al. 2011).

First whole genome sequences of prostate tumors were recently published online in *Nature* journal (vol. 470: 214–220 2011). This study revealed that rather than single spelling errors, the tumor has long "paragraphs" of DNA that seem to have broken off and moved to another part of the genome (rearrangement of genes), where they are most active. These portions of DNA contain genes that help drive cancer progression. The mutated genes involved include *PTEN*, *CADM2*, *MAG12*, *SPOP,* and *SPTA1*. This information may lead to the development of more efficient, less invasive ways to diagnose and treat this cancer. Such information, in addition, should lead to personalized therapeutics according to sequencing results of different gene mutations or chromosomal rearrangements. The urgent need of such studies becomes apparent considering the huge number of new cases of prostate problems reported every year.

In contrast to prostate cancer, cardiovascular disorders take the heavier toll of life. In other words, the risk of death for men in the United States between the ages of 55 and 74 years due to cardiovascular disease surpasses that of prostate cancer. Cardiovascular disease is the most common of the chronic

non-communicable diseases that impact global mortality. Approximately, 30% of all deaths worldwide and 10% of all healthy life lost to disease are accounted for by cardiovascular disease alone.

In conclusion, initial treatment with standard surgery, irradiation, chemotherapy, or hormonal therapy, or combination of these protocols can result in both local and systemic sequelae. Therefore, surveillance for late recurrence and secondary primary malignancies is recommended for most cancer patients. Patients with breast, lung, prostate, colorectal, and head and neck cancers constitute the largest groups requiring long-term monitoring and follow-up care.

Eric Hayat

References

Amirjamshidi A, Abbassioun K (2000) Radiation-induced tumors of the central nervous system occurring in childhood and adolescence. Childs Nerv Syst 16:390–397

Carroll PR, Whitson JH, Cooperberg MR (2011) Serum prostate-specific antigen for the early detection of prostate cancer; always, never, or only sometimes? J Clin Oncol 29:345–346

Constine LS, Tarbell N, Hudson MM et al (2008) Subsequent malignancies in children treated for Hodgkin's disease; associations with gender and radiation dose. Int J Radiat Oncol Biol Phys 72:24–33.

Endoh M, Iwasaki Y, Koyanagi I, Hida K, Abe H (1998) Spontaneous shrinkage of lumbosacral lipoma in conjunction with a general decrease in body fat: case report. Neurosurgery 43(1):150–151; discussion 151–152

Filella X, Alcover J, Molina R (2011) Active surveillance in prostate cancer: the need to standardize. Tumor Biol 32:839–843

Guin P, Gilbert E, Jones B (1969) Incidental neuroblastoma in infants. Am J Clin Pathol 51:126–136

Hayat MA (2005) Prostate carcinoma: an introduction. In: Immunohistochemistry and in situ hybridization of human carcinomas, vol 2. Elsevier, San Francisco, pp 279–297

Hayat MA (2008) Prostate carcinoma. In: Methods of cancer diagnosis, therapy, and prognosis, vol 2. Springer, New York, pp 391–396.

Henderson TO, Amsterdam A et al (2010) Surveillance for breast cancer in women treated with chest radiation for a childhood, adolescent or young adult cancer: a report from Children's Oncology Group. Ann Intern Med 152:1–22

Hero S, Simon T, Spitz R, Ernestus K, Gnekow A, Scheel-Walter H, Schwabe D, Schilling F, Benz-Bohm G, Berthold F (2008) Localized infant neuroblastomas often show spontaneuous regression: results of the prospective trials NB95-S and NB 97. J Clin Oncol 26:1504–1510

Klotz L (2008) Low-risk prostate cancer can and should often be managed with active surveillance and selective delayed intervention. Nat Clin Pract Urol 5:2–3

Lin W-C, Mahadevan-Jansen A, Johnson MD, Weil R, Toms SA (2005) *In vivo* optical spectroscopy detects radiation damage in brain tissue. Neurosurgery 57:518–525

Lu-Yao GL, Albertsen PC, Moore DF et al (2009) Outcomes of localized prostate cancer following conservative management. JAMA 302:1202–1209

Masters JR (2007) Clinical applications of expression profiling and proteomics in prostate cancer. Anticancer Res 27:1273–1276

Mertens AC, Liu Q, Neglia JP et al (2008) Cause-specific late mortality among 5-year survivors of childhood cancer: the childhood cancer survivor study. J Natl Cancer Inst 100:1368–1379

Neglia JP, Robison LL, Stovall M, Liu Y, Packer R/J et al (2006) New primary neoplasms of the central nervous system in survivors of childhood cancer: a report from the childhood cancer survivor study. J Natl Cancer Inst 98:1528–1537

Nelson EC, Cambio AJ, Ok JH, Lara PN Jr, Evans CP (2007) Clinical implications of nueroendocrine differentiation in prostate cancer. Prostate Cancer Prostatic Dis 10:6–14
Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR (1999) Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res 59:5002–5011
Parent AD (1990) Late complications of radiation-induced neoplasms. Neurosurgery 26:1090–1091
Pernicova Z, Slabakova E, Kharaishvill G, Bouchal J, Kral M, Kunicka Z, Machalam M, Kozubik A, Soucek K (2011) Androgen depletion induces senescence in prostate cancer cells through down-regulation of SKp2. Neoplasia 13:526–536
Pettorini BL, Park Y-S, Caldarelli M, Massimi L, Tamburrini G, DiRocco C (2008) Radiation induced brain tumors after central nervous system irradiation in childhood: a review. Childs Nerv Syst 24:793–805
Rubsam K, Flaig MJ, Ruzicka T, Prinz JC (2011) Erythema marginatum hemorrhagicum: a unique cutaneous side effect of sorafenib. J Am Acad Dermatol 64:1194–1196
Seaton A, Scullin P, Maxwell PJ, Wilson C, Pettigrew J, Gallagher R, O'Sullivan JM, Johnston PG, Waugh DJ (2008) Interleukin-8 signaling promotes androgen-independent proliferation of prostate cancer cells via induction of androgen receptor expression and activation. Carcinogenesis 6:1148–1156
Shalitin S, Gal M, Goshen Y, Cohen I, Yaniv I, Philip M (2011) Endocrine outcome in long-term survivors of childhood brain tumors. Horm Res Paediatr 76:113–122
Sigurdson AJ, Ronckers CM, Mertens AC et al (2005) Primary thyroid cancer after a first tumor in childhood (the childhood cancer survivor study): a nested case–control study. Lancet 365:2014–2023
Ursino MG, Poluzzi E, Caramella C, DePonti F (2011) Excipients in medicinal products used in gastroenterology as a possible cause of side effects. Regul Toxicol 60:93–105

Preface

It is recognized that scientific journals and books not only provide current information but also facilitate exchange of information, resulting in rapid progress in the medical field. In this endeavor, the main role of scientific books is to present current information in more details after careful additional evaluation of the investigational results, especially those of new or relatively new therapeutic methods and their potential toxic side-effects.

Although subjects of diagnosis, drug development, therapy and its assessment, and prognosis of tumors of the central nervous system, cancer recurrence, and resistance to chemotherapy are scattered in a vast number of journals and books, there is need of combining these subjects in single volumes. An attempt will be made to accomplish this goal in the projected ten-volume series of handbooks.

In the era of cost-effectiveness, my opinion may be minority perspective, but it needs to be recognized that the potential for false-positive or false-negative interpretation on the basis of a single laboratory test in clinical pathology does exist. Interobserver or intraobserver variability in the interpretation of results in pathology is not uncommon. Interpretative differences often are related to the relative importance of the criteria being used.

Generally, no test always performs perfectly. Although there is no perfect remedy to this problem, standardized classifications with written definitions and guidelines will help. Standardization of methods to achieve objectivity is imperative in this effort. The validity of a test should be based on the careful, objective interpretation of the tomographic images, photo-micrographs, and other tests. The interpretation of the results should be explicit rather than implicit. To achieve accurate diagnosis and correct prognosis, the use of molecular criteria and targeted medicine is important. Equally important are the translation of molecular genetics into clinical practice and evidence-based therapy. Translation of medicine from the laboratory to clinical application needs to be carefully expedited. Indeed, molecular medicine has arrived.

This is the tenth volume in the series, *Tumors of the Central Nervous System*. As in the case of the nine previously published volumes, this volume mainly contains information on the diagnosis, therapy, and prognosis of brain tumors. Various aspects of three types of tumors (Pineal, Pituitary, and Spinal Tumors) are discussed.

Introduction to new technologies and their applications to tumor diagnosis, treatment, and therapy assessment are explained. Molecular profiling of brain tumors to select therapy in clinical trials of brain tumors is included.

Several surgical treatments, including resection and radiosurgery, are discussed. The remaining volumes in this series will provide additional recent information on this and other aspects of CNS malignancies.

By bringing together a large number of experts (oncologists, neurosurgeons, physicians, research scientists, and pathologists) in various aspects of this medical field, it is my hope that substantial progress will be made against this terrible disease. It would be difficult for a single author to discuss effectively the complexity of diagnosis, therapy, and prognosis of any type of tumor in one volume. Another advantage of involving more than one author is to present different points of view on a specific controversial aspect of the CNS cancer. I hope these goals will be fulfilled in this and other volumes of this series. This volume was written by 93 contributors representing 20 countries. I am grateful to them for their promptness in accepting my suggestions. Their practical experience highlights their writings, which should build and further the endeavors of the reader in this important area of disease. I respect and appreciate the hard work and exceptional insight into the nature of cancer provided by these contributors. The contents of the volume are divided into three subheadings: Pineal Tumors, Pituitary Tumors, and Spinal Tumors for the convenience of the reader.

It is my hope that this volume will join the preceding volumes of the series for assisting in the more complete understanding of globally relevant cancer syndromes. There exists a tremendous, urgent demand by the public and the scientific community to address to cancer diagnosis, treatment, cure, and hopefully prevention. In the light of existing cancer calamity, financial funding by governments must give priority to eradicating this deadly malignancy over military superiority.

I am thankful to Dr. Kristie Reilly for recognizing the importance of medical research and publishing through an institution of higher education. I am also thankful to Jennifer Russo for her contribution to the preparation of this volume.

M.A. Hayat

Contents

Contents of Volume 1

Contents of Volume 2

Contents of Volume 3

Contents of Volume 4

Contents of Volume 5

Contents of Volume 6

Contents of Volume 7

Contents of Volume 8

Contents of Volume 9

Contributors

Karen K. Anderson Department of Neurosurgery, University of Kansas Medical Center, Kansas City, KS, USA

Ihsan Anik Department of Neurosurgery, School of Medicine, Kocaeli University, Izmit, Kocaeli, Turkey

Eduardo Aran-Echabe Department of Neurosurgery, University of Santiago de Compostela, Santiago de Compostela, Spain

Belachew Arasho Department of Neurology, University of Addis Ababa, Addis Ababa, Ethiopia

Paul M. Arnold Department of Neurosurgery, University of Kansas Medical Center, Kansas City, KS, USA

Damla Arslantunali Department of Biotechnology, BIOMAT, Middle East Technical University, Ankara, Turkey

Albert Beckers Department of Endocrinology, Centre Hospitalier Universitaire de Liege, Domaine Universitaire du Sart-Tilman, Liege, Belgium

Vladimír Beneš III Department of Neurosurgery, Regional Hospital Liberec, Liberec, Czech Republic

David G. Brachman Division of Neurological Surgery, Barrow Neurological Institute, Saint Joseph's Hospital and Medical Center, Phoenix, AZ, USA

Michael Buchfelder Department of Neurosurgery, University of Erlangen, Erlangen, Germany

Pavel Buchvald Department of Neurosurgery, Regional Hospital Liberec, Liberec, Czech Republic

Henriett Butz Faculty of Medicine, Second Department of Medicine, Semmelweis University, Budapest, Hungary

Susana Boluda Casas Neuropathology Department, Hospital Universitario de Bellvitge, Barcelona, Spain

Alfonso Cerase Unit of Neuroimaging and Neurointervention, Azienda Ospedaliera Universitaria Senese, Santa Maria alle Scotte General Hospital, Siena, Italy

Savas Ceylan Department of Neurosurgery, School of Medicine, Kocaeli University, Izmit, Kocaeli, Turkey

Alejandro Fernández Coello Neurosurgery Department, Hospital Universitario de Bellvitge, Barcelona, Spain

William Couldwell Department of Neurosurgery, University of Utah School of Medicine, Salt Lake City, UT, USA

Cristina Micheletto Dallago Neuroendocrinology Center, Complexo Hospitalar Santa Casa, Universidade Federal de Ciências da Saúde de Porto Alerge, Porto Alerge, RS, Brazil

Adrian Daly Department of Endocrinology, Centre Hospitalier Universitaire de Liege, Domaine Universitaire du Sart-Tilman, Liege, Belgium

Alberto Torres Díaz Neurosurgery Department, Hospital Universitario de Bellvitge, Barcelona, Spain

Daniel C. Dim Department of Pathology, University of Missouri at Kansas City School of Medicine, Kansas City, MO, USA

Kemal Dizdarevic Department of Neurosurgery, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Marianne S. Elston Department of Endocrinology, Waikato Hospital, Hamilton, Waikato, New Zealand

Monica Fedele Instituto di Endocrinologia Sperimentale (IEOS) del CNR, Naples c/o Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università degli Studi di Napoli "Federico II", Naples, Italy

Andreas Filis Department of Neurosurgery, University of Erlangen, Erlangen, Germany

Tania W. Furlanetto Internal Medicine Division, Hospital de Clínicas de Porto Alerge, Porto Alerge, RS, Brazil

Alfredo Fusco Naples c/o Dipartimento di Biologia e Patologia Cellulare e Molecolare, Instituto di Endocrinologia Sperimentale (IEOS) del CNR, Università degli Studi di Napoli "Federico II", Naples, Italy

Guilherme Geib Internal Medicine Division, Hospital de Clínicas de Porto Alerge, Porto Alerge, RS, Brazil

Miguel Gelabert-González Department of Neurosurgery, University of Santiago de Compostela, Santiago de Compostela, Spain

Murat Gokden Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Wiesława Grajkowska Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Science, Warsaw, Poland

Amgad Hanna Department of Neurological Surgery, University of Wisconsin, Madison, WI, USA

James Harrop Department of Neurological Surgery, University of Wisconsin, Madison, WI, USA

Vasif Hasirci Departments of Biotechnology and Biological Sciences, BIOMAT, Middle East Technical University, Ankara, Turkey

Lee Hwang Department of Neurosurgery, Division of Pediatric Neurosurgery, Johns Hopkins University, Baltimore, MD, USA

Seung-Jae Hyun Department of Neurosurgery, Seoul National University College of Medicine, Seoul, South Korea

Kyoko Ishimoto Department of Oral and Maxillofacial Prosthodontics, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Toru Iwama Department of Neurosurgery, Gifu University Graduate School of Medicine, Gifu City, Japan

Takeo Iwata Department of Medical Pharmacology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Yusuf Izci Department of Neurosurgery, Gulhane Military Medical Academy, Etlik, Ankara, Turkey

Marie-Lise Jaffrain-Rea Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy

George Jallo Department of Neurosurgery, Division of Pediatric Neurosurgery, Johns Hopkins University, Baltimore, MD, USA

M. Yashar S. Kalani Division of Neurological Surgery, Barrow Neurological Institute, Saint Joseph's Hospital and Medical Center, Phoenix, AZ, USA

Ricky R. Kalra Department of Neurosurgery, University of Utah School of Medicine, Salt Lake City, UT, USA

Irem Ayse Kanneci Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Istanbul, Turkey

Hyun-Jib Kim Department of Neurosurgery, Seoul National University College of Medicine, Seoul, South Korea

Ki-Jeong Kim Department of Neurosurgery, Seoul National University College of Medicine, Seoul, South Korea

Grzegorz Kiwic Department of Neurosurgery, Provincial Specialistic Hospital, Jastrzebie-Zdroj, Poland

Gamze Torun Kose Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Istanbul, Turkey

Katarzyna Kotulska Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Science, Warsaw, Poland

Magdalena Larysz-Brysz Department of Physiology, Center of Excellence for Research and Teaching of Matrix Biology and Nanotechnology, Network of CoE BioMedTech Silesia, Medical University of Silesia, Katowice, Poland

Sara Leonini Unit of Neuroimaging and Neurointervention, Azienda Ospedaliera Universitaria Senese, Santa Maria alle Scotte General Hospital, Siena, Italy

Joanna Lewin-Kowalik Department of Physiology, Center of Excellence for Research and Teaching of Matrix Biology and Nanotechnology, Network of CoE BioMedTech Silesia, Medical University of Silesia, Katowice, Poland

Andrew S. Little Division of Neurological Surgery, Barrow Neurological Institute, Saint Joseph's Hospital and Medical Center, Phoenix, AZ, USA

Izabela Malinowska Translational Medicine Division, Brigham and Women's Hospital, Boston, MA, USA

Marek Mandera Division of Neurosurgery, Department of Pediatric Surgery, Medical University of Silesia, Katowice, Poland

Wiesław Marcol Department of Physiology, Center of Excellence for Research and Teaching of Matrix Biology and Nanotechnology, Network of CoE BioMedTech Silesia, Medical University of Silesia, Katowice, Poland

Juan José Acebes Martín Neurosurgery Department, Hospital Universitario de Bellvitge, Barcelona, Spain

Noriko Mizusawa Department of Medical Pharmacology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Phi Nguyen Department of Neurological Surgery, University of Wisconsin, Madison, WI, USA

Toba Niazi Department of Neurosurgery, University of Utah School of Medicine, Salt Lake City, UT, USA

Hiroshi Nishioka Department of Hypothalamic and Pituitary Surgery, Toranomon Hospital, Okinaka Memorial Institute for Medical Research, Minatoku, Tokyo, Japan

Christoph Nöthen Department of Neurosurgery, University of Erlangen, Erlangen, Germany

Miriam da Costa Oliveira Neuroendocrinology Center, Complexo Hospitalar Santa Casa, Universidade Federal de Ciências da Saúde de Porto Alerge, Porto Alerge, RS, Brazil

Maura F. O'Neil Department of Neurosurgery, University of Kansas Medical Center, Kansas City, KS, USA

Shinji Ono Department of Medical Pharmacology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Attila Patocs Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary

Juan Pablo Petiti Centro de Microscopía Electrónica, Facultad de Ciencias Médicas, Universidad de Córdoba, Córdoba, Argentina

Hemanshu Prabhakar Department of Neuroanaesthesiology, All India Institute of Medical Sciences, New Delhi, India

Zhi Rong Qian Department of Human Pathology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Wilson Z. Ray Department of Neurosurgery, University of Utah, Salt Lake City, UT, USA

Pooyan Sadr-Eshkevari Farzan Clinical Research Institute, Tehran, Iran

Nora Sandu Department of Neurosurgery, University of Lausanne, Lausanne, Switzerland

Bernhard Schaller Department of Neurosurgery, University of Oradea, Oradea, Romania

Meic H. Schmidt Department of Neurosurgery, University of Utah, Salt Lake City, UT, USA

Ramón Serramito-Garcia Department of Neurosurgery, University of Santiago de Compostela, Santiago de Compostela, Spain

Makoto Shibuya Department of Diagnostic Pathology, Ibaraki Medical Center, Tokyo Medical University, Tokyo, Japan

Shahidan Wan Nazatul Shima Department of Medical Pharmacology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Harminder Singh Department of Neurological Surgery, University of Wisconsin, Madison, WI, USA

Petr Suchomel Department of Neurosurgery, Regional Hospital Liberec, Liberec, Czech Republic

Philipp Taussky Department of Neurosurgery, University of Utah School of Medicine, Salt Lake City, UT, USA

Sina Tok Department of Neurosurgery, Division of Pediatric Neurosurgery, Johns Hopkins University, Baltimore, MD, USA

Alicia Inés Torres Centro de Microscopía Electrónica, Facultad de Ciencias Médicas, Universidad de Córdoba, Córdoba, Argentina

Ali Varan Department of Pediatric Oncology, Institute of Oncology, Hacettepe University, Ankara, Turkey

Vladimir Vasilev Department of Endocrinology, Centre Hospitalier Universitaire de Liege, Domaine Universitaire du Sart-Tilman, Liege, Belgium

William L. White Division of Neurological Surgery, Barrow Neurological Institute, Saint Joseph's Hospital and Medical Center, Phoenix, AZ, USA

Hirohito Yano Department of Neurosurgery, Gifu University Graduate School of Medicine, Gifu City, Japan

Chester K. Yarbrough Department of Neurosurgery, University of Utah, Salt Lake City, UT, USA

Nalan Yazici Faculty of Medicine, Department of Pediatric Oncology, Başkent University, Ankara, Turkey

Sang Hoon Yoon Department of Neurosurgery, Seoul National University College of Medicine, Seoul, South Korea

Katsuhiko Yoshimoto Department of Medical Pharmacology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Deniz Yucel Department of Histology and Embryology, School of Medicine, Acibadem University, Istanbul, Turkey

Cuiqi Zhou Department of Medicine, Cedars-Sinai Medical Center, David Geffen School of Medicine, University of California, Los Angeles, CA, USA

Part I

Pineal Tumors

Papillary Tumor of the Pineal Region

1

Alejandro Fernández Coello, Alberto Torres Díaz,
Susana Boluda Casas, and Juan José Acebes Martín

Contents

Abstract

Tumors of the central nervous system (CNS) demonstrating papillary features, and particularly in the pineal region, are rare. Papillary tumor of the pineal region (PTPR) is a recently described rare neuroepithelial tumour included in the fourth edition of the World Health Organization (WHO) classification of tumours of the CNS, published in 2007. This new histopathological entity manifests in children and adults, with magnetic resonance (MR) imaging showing variable degree of T1 hyperintensity signal as well as contrast enhancement. In addition to the initial report of six cases by Jouvet et al. (Am J Surg Pathol 27:505–512, 2003) several case reports have been published in the literature to date. PTPR is characterized by presenting an epithelial-like cytology and a papillary architecture with immunoreactivity for broad-spectrum cytokeratins. Ultrastructurally observed features suggest ependymal differentiation and a possible origin from specialized ependymal cells of the subcommissural organ (SCO) has been postulated. The differential diagnosis of PTPR include pineal parenchymal tumors, choroid plexus tumors, papillary ependymoma, papillary meningioma, and metastasis. As a result of the rarity of these tumors, the biological behaviour of PTPR is not yet clear. It may correspond to WHO grades II or III. The high risk of local recurrence makes accurate surgery followed by radiotherapy necessary to achieve optimal treatment.

A.F. Coello (✉) • A.T. Díaz • J.J.A. Martín • S.B. Casas
Neurosurgery Department, Hospital Universitario
de Bellvitge, Feixa Llarga, s/n, 08907 L'Hospitalet
de Llobregat, Barcelona, Spain
e-mail: jandrocoello@gmail.com

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_1,

Introduction

Pineal region tumors constitute <1% of all intracranial tumors. The anatomical relations of this location include the quadrigeminal plate cistern, the posterior part of the thalamus, vein of Galen or the posterior third ventricle. Despite sharing a common brain area and often displaying similar imaging characteristics, pineal region tumors are very heterogeneous. Because of the variety of tumor types that occur in this region, usually histologic diagnosis is required to give an appropriate therapy. This tissue diagnosis can be made via stereotactic or endoscopic biopsy or an open surgical approach.

Historically, surgical management of pineal region tumors has been strongly influenced by unfavorable results after direct surgical intervention being considered as unresectable lesions. Now we know that ~30% of pineal tumors are benign and can be cured with gross total resection. The prognosis for malignant lesions has already improved as a result of combined therapy including surgery, radiation, radiosurgery, and chemotherapy.

PTPR was first described in 2003 when Jouvet et al. reported a series of six cases of papillary tumors of the pineal region with distinct features. Recently, it has been included as a new clinicopathological entity in the latest edition of the WHO Classification of Tumours of the CNS (Jouvet et al. 2007).

Clinical Presentation

According to the series reported by Fèvre-Montange et al. (2006), patients' ages ranged from 5 to 66 years with a mean age of 29.9 years. A slight female preponderance was noted with a female to male ratio of 17:14. The majority of patients with pineal region tumors present with symptoms of obstructive hydrocephalus, such as headache, vomiting, lethargy or impaired upgaze (Parinaud phenomena). PTPR is not an exception. Furthermore, it is characterized by progressive growth, frequent local recurrence, and rare spinal dissemination.

Recently, a case of a young patient with early cerebrospinal fluid dissemination and a possible multicentric origin was reported by Sato et al. (2009). Different series in the literature describe an initial clinical presentation consisting of headache without other neurological signs. Until now, it remains difficult to predict the biological behavior and natural course. The different growth and recurrence rates reported are probably due to the intrinsic characteristics of these tumors demonstrating a large spectrum of variability.

Radiology

Neuroradiological data are still limited, making PTPR radiological diagnosis a true challenge. Brain MRI is the gold standard to distinguish it from other tumors that may occur in the same location. A pineal mass with variable degree of intrinsic T1 hyperintensity and contrast enhancement suggests the diagnosis of PTPR (Chang et al. 2008). The common features of PTPR on imaging are its well-circumscribed and cystic component (Fig. 1.1). It is known that intrinsic tumor cells with well-differentiated secretory functions produce proteins, glycoproteins and other T1-shortening substances. This heterogeneous content in the cystic spaces of the tumor should explain this variable enhancement. When fat, hemorrhage, melanin or calcification in a mass of the posterior commissure or pineal region are ruled out with CT and MRI, the diagnosis of PTPR may be suggested so that specific studies should be performed for a definitive diagnosis.

Histology

PTPR is thought to arise from the specialized ependymal cells in the subcommissural organ (SCO) of the pineal region (Jouvet et al. 2003). This hypothesis is supported by the similarities in morphologic and ultrastructural features between the ependymal cells and the tumor cells in PTPR. The SCO is an ependymal gland located at the entrance of the third ventricle that secretes glycoproteins into the ventricular cerebrospinal fluid.

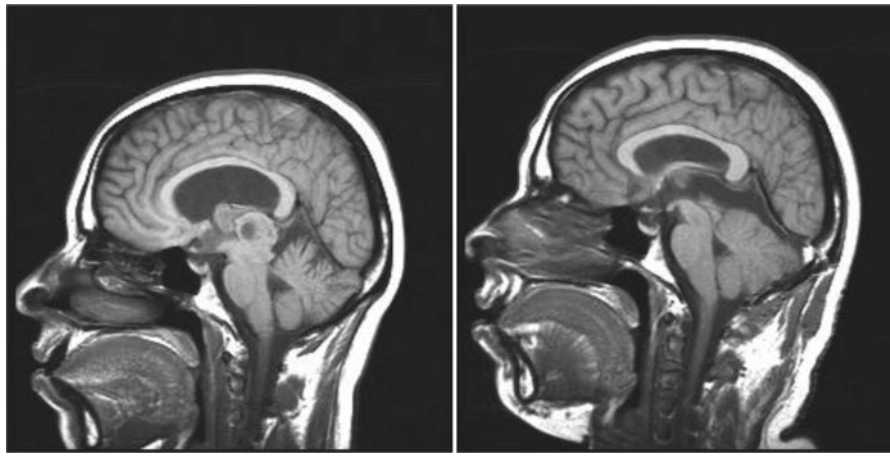

Fig. 1.1 *Left*: MRI showing a well-circumscribed and cystic pineal region mass with intrinsic hyperintensity causing supratentorial obstructive hydrocephalus. *Right*: MRI performed after surgical resection

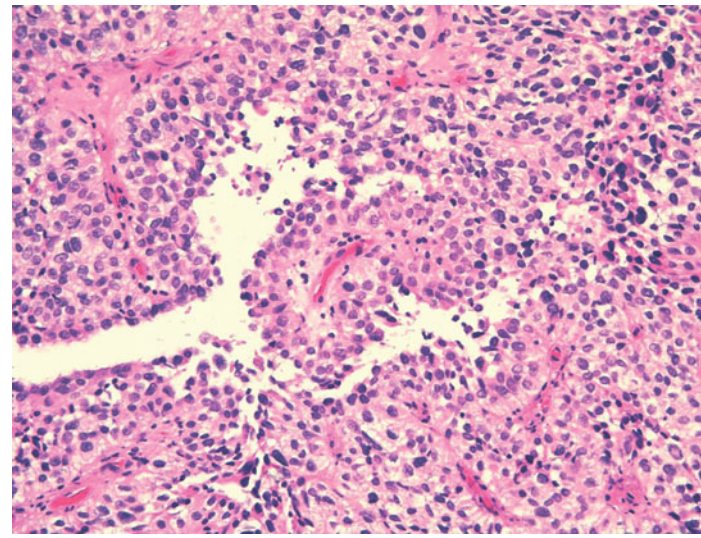

Fig. 1.2 Pathologic examination showing an epithelial-appearing tumor with papillary features

The SCO regresses after birth; therefore, only remnants of the specialized SCO cells invading the pineal gland can be found in adults.

PTPR is an epithelial-like cellular tumor with papillary features as its main histological hallmark (Fig. 1.2). Areas of solid cellular sheets and the presence of perivascular pseudorosettes, true ependymal rosettes and "microlumens", which appear to be rosettes (Shibahara et al. 2004; Boco et al. 2008) are also seen. Large cuboidal or columnar epithelial-like cells with clear, eosinophilic or vacuolated cytoplasm are identified in most reported cases (Buffenoir et al. 2008). The presence of necrosis has been reported in most of the cases while microvascular proliferation is consistently absent in the tumor. Mitotic activity is usually moderate ranging from 0 to 10 mitoses/10HPF.

Immunohistochemical profiles of PTPR are quite diverse as the SCO expresses different proteins throughout the development process (Hasselblatt et al. 2006). Cells of the SCO transiently express CK, this fact can be related to the constant expression of cytokeratins including

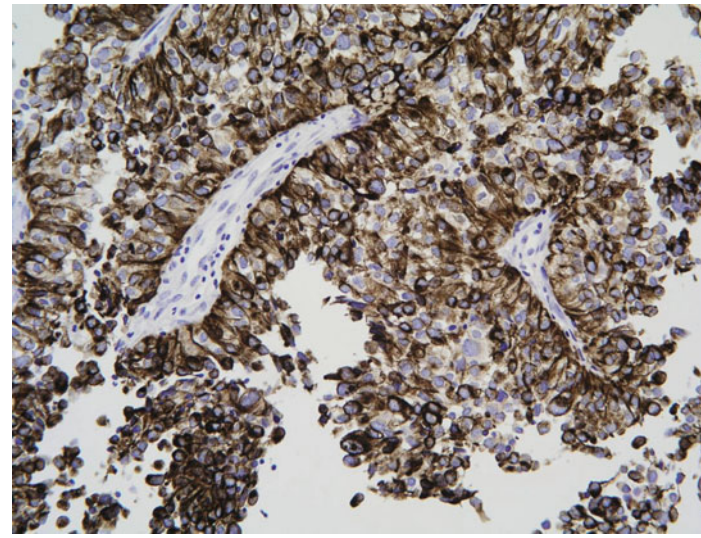

Fig. 1.3 Tumor cells are diffusely and intensely immunoreactive for CAM 5.2

AE1/AE3, CAM5.2, and cytokeratin 18, more evident in the papillary component (Fig. 1.3). PTPR also shows EMA immunoreactivity, usually restricted to the cell surface or as small ring or dot-like intracytoplasmic reactivity. Vimentin, S100 protein, and neuron-specific enolase immunolabeling is very frequently positive. Transthyretin, synaptophysin, and chromogranin immunolabeling can be seen but is usually weak and focal. Furthermore, weak GFAP labeling has been reported but is limited to the perivascular zones and reactive astrocytes. The Ki-67 proliferation index ranges between 5 and 10% being higher than 10% in some cases.

Ultrastructural features such as junctional complexes formed by tight junctions and zonulae adherentes are indicative of ependymal differentiation. The nuclei are round-to-oval shaped, sometimes indented, and situated in the basal pole of the tumor cells. On the other hand, microvillous cytoplasmic surface at the apical pole is observed, but cilia are rare. The cytoplasm contains many organelles as phagolysosomes, endocytosis vesicles or microtubules. Furthermore, most of the cells have a fair number of mitochondria and rough endoplasmic reticulum (Jouvet et al. 2003).

Owing to the papillary architecture of PTPR, other pineal neoplasms showing papillary features must be considered among the differential diagnoses such as pineal parenchymal tumor, choroid plexus tumors, papillary ependymoma, meningioma and, in adults, metastasis. However, these entities may be distinguished from PTPR based on specific morphology, immunohistochemistry and ultrastructural findings.

Pineal parenchymal tumors show strong immunoreaction for neuronal markers such as synaptophysin, unlike PTPR in which none or focal reactivity can be seen. Ependymomas can present with papillary features but in contrast to PTPR they frequently express GFAP. In addition, cytokeratin immunoreactivity is seen in all reported PTPR cases; however, it is only focally identified in ependymomas. Despite an immunohistochemical reactivity profile similar to choroid plexus tumors, PTPR is less papillary than choroid plexus papilloma and is more differentiated than choroid plexus carcinoma. Moreover, EMA labeling is much weaker in PTPR than in choroid plexus tumors. Papillary meningiomas, in contrast to PTPR, usually have meningothelial-like regions (whorls and psammoma bodies) in addition to the papillary features, lack true rosettes, have widespread EMA immunoreactivity, and are GFAP negative (Shibahara et al. 2004; Hasselblatt et al. 2006; Boco et al. 2008). PTPR can resemble papillary metastatic carcinomas; however, the latter appear more epithelial and are immunoreactive to cytokeratins and EMA

antibodies, while S100, GFAP, and synaptophysin are nonreactive. Germ cell tumors and other third ventricle or other CNS papillary tumors pose no real diagnostic problem and can be ruled out based on morphologic features, ultrastructural findings, and immunohistochemistry.

Natural History, Management and Outcome

Presently, given the rarity of these tumors, it remains difficult to predict the biological behavior, natural course, and appropriate management of PTPR (Cerase et al. 2009). It has been reported that the different growth and recurrence rates are probably due to intrinsic characteristics of these tumors demonstrating a large spectrum of variability. Most cases showed aggressive behavior, 5-year overall survival and progression-free survival have been estimated as 73 and 27%, respectively, on Kaplan–Meier survival curves (Fèvre-Montange et al. 2006).

The currently recommended treatment is surgical resection, which must be as complete as possible, because it is the only prognostic factor that has a tendency to correlate with overall survival and recurrence currently identified in the literature. Occipital craniotomy and supracerebellar infratentorial approach is the most used procedure to perform the resection of this deep localized tumor (Kern et al. 2006; Dagnew et al. 2007).

Local recurrence rates makes complementary radiotherapy necessary. Fractionated radiotherapy at a dose of 50 Gy currently appears to be the standard regimen, but the value of radiotherapy in terms of disease progression needs to be further investigated, as reported by Santarius et al. (2008). Chemotherapy has been proposed in specific cases: rapid spinal dissemination, a patient treated by first-line radiotherapy, and a patient who developed local recurrence. However, the benefit of chemotherapy is yet to be determined.

In conclusion, papillary tumor of the pineal region is a newly described and rare neuroepithelial tumour included in the last edition of the World Health Organization (WHO) classification of tumours of the CNS. Histologic diagnosis is difficult as many primary or secondary tumors of the pineal region can present papillary architecture, being papillary ependymoma and choroid plexus tumors, the two main differential diagnoses. The clinicopathologic profile of PTPR remains incomplete, appearing to run a diverse spectrum of clinical courses. However, most cases tend to show an aggressive behavior, with high rates of local recurrence. Therefore, it is important to plan a tumoral resection as radical as possible, followed by radiotherapy to achieve the best results in this type of tumor.

References

Boco T, Aalaei S, Musacchio M, Byrne R, Cochran E (2008) Papillary tumor of the pineal region. Neuropathology 28:87–92

Buffenoir K, Rigoard P, Wagner M, Ferrand S, Coulon A, Blanc JL, Bataille B, Listrat A (2008) Papillary tumor of the pineal region in a child: case report and review of the literature. Childs Nerv Syst 24:379–384

Cerase A, Vallone IM, Di Pietro G, Oliveri G, Miracco C, Venturi C (2009) Neuroradiological follow-up of the growth of papillary tumor of the pineal region: a case report. J Neurooncol 95:433–435

Chang AF, Fuller GN, Debnam JM, Karis JP, Coons SW, Ross JS, Dean BL (2008) MR imaging of papillary tumor of the pineal region. Am J Neuroradiol 29:187–189

Dagnew E, Langford L, Lang F, DeMonte F (2007) Papillary tumors of the pineal region: case report. Neurosurgery 60:953–955

Fèvre-Montange M, Hasselblantt M, Figarella-Branger D, Chauveinc L, Champier J, Saint-Pierre G, Taillandier L, Coulon A, Paulus W, Fauchon F, Jouvet A (2006) Prognosis and histopathologic features in papillary tumors of the pineal region: a retrospect of 31 cases. J Neuropathol Exp Neurol 65:1004–1011

Hasselblatt M, Blümcke I, Jeibmann A, Rickert CH, Jouvet A, van de Nes JAP, Kuchelmeister K, Brunn A, Fèvre-Montange M, Paulus W (2006) Immunohistochemical profile and chromosomal imbalances in papillary tumours of the pineal region. Neuropathol Appl Neurobiol 32:278–283

Jouvet A, Fauchon F, Liberski P, Saint-Pierre G, Didier-Bazes M, Heitzmann A, Delisle MB, Biassette HA, Vincent S, Mikol J, Streichenberger N, Ahboucha S, Brisson C, Belin MF, Fèvre-Montange M (2003) Papillary tumor of the pineal region. Am J Surg Pathol 27:505–512

Jouvet A, Nakazato Y, Scheitahauer BW, Paulus W (2007) Papillary tumour of the pineal region. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) World Health Organization classification of

tumours of the central nervous system. IARC, Lyon, pp 118–129

Kern M, Robbins P, Lee G, Watson P (2006) Papillary tumor of the pineal region. A new patological entity. Clin Neuropathol 25:185–192

Santarius T, Joseph JA, Tsang KT, O'Donovan DG, Kirollos RW (2008) Papillary tumour of the pinal region. Br J Neurosurg 22:116–220

Sato TS, Kirby PA, Buatti JM, Moritani T (2009) Papillary tumor of the pineal region: report of a rapidly progressive tumor with possible multicentric origin. Pediatr Radiol 39:188–190

Shibahara J, Todo T, Morita A, Mori H, Aoki S, Fukayama M (2004) Papillary neuroepithelial tumor of the pineal region. A case report. Acta Neuropathol 108:337–340

Pineal Region Tumors: Clinical Aspects

2

Nalan Yazici and Ali Varan

Contents

Abstract

Pineal region tumors are rare in children. They are predominantlly seen in males and median age is usually reported less than 10 years according to different studies. They show diversity in histopathology and the treatment of these tumors depends majorly on this feature. Germ cell tumors compose nearly 60% of all cases and pineal parenchymal tumors were the second most common tumor type in this region. Unlike the other parts of the brain, gliomas and other tumors constitute less than 5% of the tumors of this region. Lesions of benign histology should also be considered in differential diagnosis. Endocrinological signs and symptoms may also prominently accompany the symtoms and signs of increased intracranial pressure and hydrocephalus because of the regulatory function of the pineal gland on the whole endocrine system. Besides tumor histopathology, the prognosis is also associated with treatment modalities such as surgery which is the mainstay of the treatment and appopriate adjuvant and/or neoadjuvant treatment modalities which would be discussed later in the subsequent chapter.

N. Yazici (✉)
Faculty of Medicine, Department of Pediatric Oncology, Başkent University, Ankara, Turkey
e-mail: nalanyaz@yahoo.com

A. Varan
Department of Pediatric Oncology, Institute of Oncology, Hacettepe University, Ankara, Turkey

Introduction

Pineal region is the part of the central nervous system where tumors with extremely different histopathology, either benign or malignant, can be seen. Since fundamental of management depends

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_2,

on histopathology, differential diagnosis of these lesions is noteworthy. Pineal region is a deep seated part of the brain and management is sometimes difficult. Diagnostic procedures other than surgery are sometimes mandatory as previously used. Herein, the epidemiology, pathology, clinical features and issues of laboratory diagnosis involving the pineal tumors are discussed from the view of pediatric oncology.

Epidemiology

Tumors arising in the pineal region are rare. They usually account less than 1% of all brain tumors in adults but they have been reported between 0.4 and 2% of all primary nervous system tumors in children (Blaney et al. 2006). The incidence reported from different parts of the world varied from 2.2 to 12.5%, which was markedly higher in the Northeast Asia (The comittee of Brain Tumor Registry of Japan 1992; Graziano et al. 1987; Koide et al. 1980; Rubinstein 1981; Cho et al. 1998; Yazici et al. 2009; Fuller et al. 1994; Packer et al. 1984) Recent data from 'The Surveillence Epidemiology and End Results' (SEER) revealed that the pineal tumors account for 0.8% of all 77,264 central nervous system tumors between 1973 and 2005 with an age range of 0–83 years (Al-Hussaini et al. 2009). According to this cohort, 56% of the cases were under 18 years-old (n = 355).

Male predominance was reported in majority of the reports. Konavalov and Pitskhelauri (2003) and Packer et al. (1984) reported the lowest male to female ratio which was 1.3 each. The other experiences revealed male to female ratio ranging from 2 to 3.36 (Cho et al. 1998; Kang et al. 1998; Tamaki and Yin 2000; Kumar et al. 2006) The SEER data recently revealed this ratio as 3.1 in which was a huge experience with 477 pineal tumors in males vs. 156 in females (Al-Hussaini et al. 2009). In 2 pediatric experiences except Packer et al. (1984), male to female ratio was reported as 2 and 2.6 (Yazici et al. 2009; Shin et al. 1998)

Median age was between 13 and 20 years in series with both pediatric and adult patient populations (Al-Hussaini et al. 2009; Konovalov and Pitskhelauri 2003; Tamaki and Yin 2000; Cho et al. 1998) and it was between 6 and 9.5 years in cases only with pediatric series (Packer et al. 1984; Pendl and Vorkapic 1985; Shin et al. 1998; Yazici et al. 2009). The difference could be resulting from the upper range limit of the studies which was 15 in one series and 20 in the other (Shin et al. 1998; Knierim and Yamada 2003).

The recent report from SEER with 633 pineal tumors of adults and children revealed that germ cell tumors composed 59% of all cases and pineal parenchymal tumors were the second most common tumor type of this region with around 30%. Gliomas constituted 5%, other tumors were about 5% and atypical teratoid rhabdoid tumors were less than 1% (Al-Hussaini et al. 2009).

Anatomy, Histology, and Physiology of the Pineal Gland

The pineal gland occurs as a diverticulum in second month of gestation in the roof of third ventricle. The gland is a midline structure of the epithalamus and it is attached to the diencephalon by the pineal stalk. It projects backward so that it lies posterior to the midbrain. Third ventricle is laid in front of the gland. The base of the pineal stalk possesses a recess that is continuous with the cavity of the third ventricle. The gland is posteriorly surrounded by a capsule, composed of connective tissue which continues with the leptomeninges. The arterial blood is transported via the posterior cerebral artery and internal cerebral veins and the vein of Galen are responsible from the venous return. The gland was innervated with noradrenerjic symphatic nerves (Snell 1992). The gland is usually 8–10 mm in length and its weight is about 0.15–0.20 g. Pineal gland parenchyma was composed of lobules with different size (200–1,000 μm) and shape. The connective tissue from the capsule enters into the gland which forms septae around these lobules

and contains a loose connective tissue of vessels (de Girolami et al. 2008).

Pineal gland is histologically composed of three major groups of cells. These are pineal parenchymal cells (pineocytes or pinealocytes), glial cells (astrocytes, ependymal cells), and connective tissue cells (blood vessels and nerves) (de Girolami et al. 2008). The pineal parenchymal cells are located in the lobules. The processes of the cells are located at the outer edge of the lobule, around the small vessels and subependymal regions in order to secrete the products of the cell to the circulation and cerebrospinal fluid. The pineal parenchymal cell has a round or oval nucleus which is about 5–12 μm in size with a pale and scattered chromatin usually with not prominent nucleoli and spherical inclusions. The pineal parenchymal cells and their processes can be demonstrated immunohistochemically by synaptophysin, neurofilament protein antibodies, S-100; antibodies to the S-antigen, chromogranin and in some of the cells by rhodopsin stain. Besides the pineal parenchymal cells, rarely GFAP positive astrocytes, fenestrated and unfenestrated capillaries of circulation, autonomic neurons are also detected in the structure of the gland.

When first detected, the pineal gland was historically thought to be the source of multiple functions of the body and the location of the *'soul'* (Guyton 1991). It is now recognised as an endocrine organ. Anatomical studies showed that this gland was the remnant of the third eye in the lower vertebrates. It has been reported that pineal gland in fish, amphibians and reptiles have clear photoreceptor and neuroendocrine differentiation but in birds and mamals the gland shows regressive changes (de Girolami et al. 2008). When the gland is removed or a loss of innervation of the gland is established in the lower vertebrates, seasonal reproduction periods are lost in these animals. In human pineal gland, light and dark cells according to electron density and cytoplasm content have been displayed. Structural findings showed dense core granules, granular vesicles in different sizes and synaptic contacts regarding the neuroendocrine function. Cytoplasmic melanin granules are also detected in the gland. In human pinealocytes, the structural features of these dense vesicles and numerous, small, clear, synaptic-like vesicles are believed to have neurosecretory and neurosensory function. Pineal gland by age, progressively undergoes cystic degeneration and calcification which is called 'brain sand' or acervuli.

Pineal gland is regulated by the amount of daylight through the eyes. It has been shown that the gland is stimulated in a hamster after a 13 h stay in darkness whereas little portion of darkness has no effect on stimulation of the pineal gland (Guyton 1991). The light stimulus arrives to the pineal gland through the hypothalamic suprachiasmatic nucleus and stimulates the secretion of the gland. Pineal gland is considered as an endocrine organ with indolamine secretions such as melatonin and serotonin. These neurohormones are involved in the regulation of circadian rhythms. It has been shown that the pineal gland has an essential role in sleep physiology in relation to light and dark cycles and hybernation in some animals. The melatonin is produced from tryptophan by the enzymes tryptophan hydroxylase, N-acetyl transferase and hydroxyl indole-O-methyl transferase (HIOMT). Melatonin and other polypeptides are circulated via the bloodstream or cerebrospinal fluid of the third venticle to the anterior pituitary which regulates the secretion of the gonadotrophins. In some of the animals, by the help of the pineal secretion, the gonadotrophins are reduced especially in winter leading to the regression of the gonads. Later, gonadotrophins reduces the effect of the pineal gland inhibitory function, leading to the activation of the gonads and reproduction activities in the spring. In humans, some of the neoplasms of pineal body relieves the inhibititory function of the gland on the production of the gonadotrophic hormones and endocrine syndromes such as precocious pubert may be detected.

Pathology

The tumors of the pineal gland are extraaxial brain tumors and they may originate from the oncogenic transformation of pineal parenchymal

cells, glial elements, the cells of the supporting structures, meninges, autonomic nerves and the progenitor germ cells. It has been reported that embryologic remnants of germ cells could have left the primordium and might have led to neuroaxial seedings in the midline which explains the similarity of the germ cell tumors of the central nervous system and the gonadal and mediastinal tumors of the same origin in terms of histopathology (de Girolami et al. 2008).

Previously, the term 'pinealoma' was both used for suprasellar atypical teratomas and pineal parenchymal tumors but this term has no longer been used. Although several classifications were made for the tumors in this region, the latest one is the classification of the pineal region tumors which had been published in 2007 by World Health Organization (Louis et al. 2007a). However, this classification was according to the cell origin so, only the tumors of neuroepithelial tissue in this region were revealed as pineal region tumors in four subgroups. The germ cell tumors and the other tumors are also defined by the grouping of de Girolami et al. (2008) which summarizes all of the tumors in this region.

Pineal Parenchymal Tumors

According to SEER, pineal parenchymal tumors are the second most common tumors of the pineal region (Al-Hussaini et al. 2009). Single center experiences revealed the rate of pineal parenchymal tumors as low as 5.6% (Tamaki and Yin 2000) and as high as 41.6% (Yazici et al. 2009). Several reports from different institutions revealed highy variable incidence rates between 10 and 38.8% (Pendl and Vorkapic 1985; Kang et al. 1998; Cho et al. 1998; Knierim and Yamada 2003; Shin et al. 1998; Konavalov and Pitskhelauri 2003; Packer et al. 1984; Kumar et al. 2006).

The SEER data revealed grade 1 pineal parenchymal tumors as 9%, grade 2 and 3 were 4 and 6% respectively and grade 4 tumors were 81% (Al-Hussaini et al. 2009). Another pathological study from India previously had shown ten pineoblastomas, seven pineal parenchymal tumors with intermediate differentiation and four cases with pineocytoma (Kumar et al. 2006).

These types of tumors have structural, immunohistochemical and biochemical similarites with normal pineal gland. In some instances, there may be a real difficulty in recognising the normal pineal gland from a very differentiated tumor of pineal parenchymal origin. Neural markers such as synaptophysin, neurofilaments, neuron-specific enolase, chromogranin show positive staining as well as some reactivity with retinal S-antigen, rhodopsin and melatonin pathway enzymes. Ultrastrucural features of better differantiated pineal parenchymal tumors include both mamalian and submamalian normal pineal gland features such as light and dark cells, secretory granules, synaptic structures, filamentous bundles, mitochondria and membranous whorls. In undifferantiated neoplasms and pineoblastomas, the structures of normal pineal gland mostly are not detected (Jouvet et al. 2000). Molecular genetic features of pineal parenchymal tumors also reveal difference according to the differentiation of the tumor.

Pineocytoma

This tumor is rarely seen in children. Only few cases were reported in single center experiences in children which usually accounts less than 10% of all the pineal region tumors (Knierim and Yamada 2003; Packer et al. 1984; Pendl and Vorkapic 1985; Shin et al. 1998). The majority of these tumors are seen in young adults and middle-aged. It is a WHO grade 1 tumor. de Girolami et al. (2008) reported that it was grossly well-circumscribed, non necrotic in nature, lacked mitotic activity, had round to oval nuclei of the cells which were embedded in fibrillary material, forming occasionally Homer-Wright rosettes with non infiltrative compressions to the normal pineal gland and abrupt margins of the tumor. In immunohistochemistry, synaptophysin is positive, s-100 and GFAP are negative. Chromogranin, NSE, neurofilament proteins, S-antigen, rhodopsin, serotonin, tryptophane hydroxylase are other used immunohistochemical markers in diagnosis.

Pineoblastoma

It is a WHO grade 4 tumor which is highly agressive like medulloblastoma and is one of the primitive neuroectodermal tumors of the central nervous system. It is majorly a tumor of children. The five of the six cases were under 7 years old in the experience of Knierim and Yamada (2003). Similarly, among six cases, only two of the patients were between 10 and 15 years old and there were no cases over the age of 16 in the report by Cho et al. (1998). Only one patient was 49 years old in the review of Kumar et al. (2006) among ten pineoblastomas.

It has been mentioned by de Girolami et al. (2008) that the tumor is infiltrative to the adjacent structures like ventricles, subarachnoid space and is not well circumscribed and it has a high potential to form seeding metastases. Besides, high cellularity with mitoses, high Mib-1 index, endothelial proliferation, cystic degeneration, necrosis, and hemorrage are common reported features by the authors (de Girolami et al. 2008). The nuclei of the tumor cells are rounded and pleomorphic and they have scanty cytoplasm. Some cells form rosettes like Homer Wright or Flexner-Wintersteiner rosettes as seen in other primitive neuroectodermal tumors. Immunohistochemical markers for neuronal differentiation (NSE, synaptophysin, NF) are focally positive, particularly in neuropil-like regions (de Girolami et al. 2008) In view of molecular genetic findings, monosomy of 20 and 22, trisomy of 14, chromosomal gains and losses were defined in pineoblastoma. Besides, APC/APCL genes which are important in Wnt signaling were found as downregulated in this tumor.

Among 62 patients with supratentorial primitive neuroectodermal tumors, 23% of the cases had tumors in the pineal region (Taylor et al. 2009). Unlike the other sPNETs, Gilheeney et al. (2008) recently revealed a better prognosis in pineal region PNETs with gross total resection, multiagent chemotherapy and radiotherapy. Pineal primary site for this group of tumor was also found to be a good prognostic factor by Taylor et al. (2009). In children, this tumor may be associated with bilateral retinoblastoma which was previously called as trilateral retinoblastoma (de Girolami et al. 2008; Varan et al. 1998).

Pineal Parenchymal Tumor with Intermediate Differentiation

According to WHO 2007 classification of CNS tumors, this tumor was grouped between WHO grade 2 and 3 tumors (Louis et al. 2007a). The incidence was reported as 33% in 21 pineal parenchymal tumors in the study of Kumar et al. (2006). This tumor has been reported both in children and adult cases frequently in the second and third decades of life (de Girolami et al. 2008), and the age range differed from 11 to 46 years in the report by Kumar et al. (2006). According to de Girolami et al. (2008), the difference of this tumor from pineocytoma is more sheet like architecture and cytologic atypia compared with pineocytoma, but less celularity and malignant appearrence than pineoblastoma. Besides, the authors mention that immunohistochemistry also reveals features that are between the pineocytoma and pineoblastoma, with variable eosinophilic neurophil background, much more mitosis compared to pineocytoma and variably elevated Mib index (de Girolami et al. 2008). All tumors showed positive staining with synaptophysin and negative staining for GFAP in the study of Kumar et al. (2006). Molecular genetic studies suggested that PRAME, CD24, POU4F2 and HOXD13 might have been used for grading pineal parenchymal tumors with intermediate differentiation (Févre-Montange et al. 2008).

In conclusion, it has been suggested that pineal parenchymal tumors should be classified in four subgroups by several authors (de Girolami et al. 2008; Jouvet et al. 2000; Berns and Pearl 2006):

1. Grade 1 pineocytoma
2. Grade 2 pineal parenchymal tumor intermedia: distinctive neuronal immuno-localization and less than six mitoses per high power field
3. Grade 3 pineal parenchymal tumor intermedia: Faint neuronal immunolocalization and less than six mitoses per high powerfield or greater than six mitoses per high powerfield
4. Grade 4 pineoblastoma

Gliomas

According to recent SEER data, gliomas constituted 5% of the pineal region tumors (Al-Hussaini et al. 2009). In other single center experiences, glial tumors were reported between 8.4 and 40% by several authors (Yazici et al. 2009; Shin et al. 1998; Cho et al. 1998; Konavolov and Pitskhelauri 2003; Knierim and Yamada 2003; Packer et al. 1984; Kumar et al. 2006). Astrocytoma, oligodendroglioma, glioblastome multiforme, ependymoma and papillary tumor of subcomissural origin, choroid plexus papilloma and carcinoma are all seen in the pineal region of central nervous sytem. It is mentioned that it seemed difficult to estimate that these tumors were really originating from the pineal region whether they were the tumors of the posterior part of the third ventricle that had invaded this area and a suggestion to clarify this subject is a careful revision of the signs and symptoms of the patients as well as the neuroradiological findings (de Girolami et al. 2008). An example of this subject was overviewed by Pendl and Vorkapic (1985) who had reported eight patients with glial tumors in the pineal region. Among 20 cases, only 1 case of 8 glial tumors were truely reported as originated from pineal gland whereas the other 7 tumors were thought as midbrain lesions.

The most common glial tumors in the pineal region were reported as pilocytic astrocytomas (n = 8) and fibrillary astrocytomas (n = 3) in a pathological study of 54 pineal tumors (Kumar et al. 2006). The pilocytic astrocytomas in the pineal region displayed the classical histopathological features of GFAP positivity, glial fibrillar background, Rosenthal fibers, microcystic changes and low proliferation index like other pilocytic astrocytomas located in different parts of the central nervous system (de Girolami et al. 2008).

Ependymoma is another tumor of glial origin which is also rare. In the study of Kumar et al. (2006) 3 cases were detected among 54 histopathological material. It usually occurs at the posterior third ventricle and invade the pineal gland. The papillary variant of pineal tumor is reported to possibly derive from the columnar or cuboidal epithelial tissue on the fibrovascular stalk of the gland and shows less reactivity to chromogranin and synaptophysin but more reactivity to S-100; and has positive epithelial markers such as CK, but weak expression of GFAP and EMA (de Girolami et al. 2008).

Glioblastoma, anaplastic astrocytoma, subependymal giant cell astrocytoma, oligodendroglima were all rarely diagnosed in this region (Kumar et al. 2006; de Girolami et al. 2008). Glioblastoma, anaplastic astrocytoma, oligodendroglioma and ependymoma were detected in adult patients but subependymal giant cell astrocytoma and pilocytic astrocytomas occured under 16 years in the study of Kumar et al. (2006). Fibrillary astrocytoma was detected both in children and adults (Kumar et al. 2006).

Germ Cell Tumors

Germ cell tumors are common in children and young adults with male predominance. They originate from the gonadal embryonic rests during the migration of these cells through the dorsal caudal axis where they possibly lead to tumors in the midline structures such as the gonads, retroperitoneum, sacrococcygeal region, mediastinum and suprachiasmatic and pineal regions. Germ cell tumors according to embryonic differetiation and migration properties were outlined in Fig. 2.1. Experience with 389 previously published primary intracranial germ cell tumors revealed that 65% were germinomas, 18% were teratomas, 5% embryonal carcinomas, 7% endodermal sinus tumors and 5% were choriocarcinomas (Jennings et al. 1985). The majority of germinomas (57%) in this series had arisen in the suprasellar region while most of the nongerminomatous germ cell tumors (68%) preferentially involve the pineal gland.

Germinomas

As mentioned above, the majority of the tumors in this region are germinomas. The 285 patients were pure germinomas in 373 pineal gland germ cell tumors reviewed in SEER data in all the patients registered (Al-Hussaini et al. 2009). In a recent report from SEER database involving the

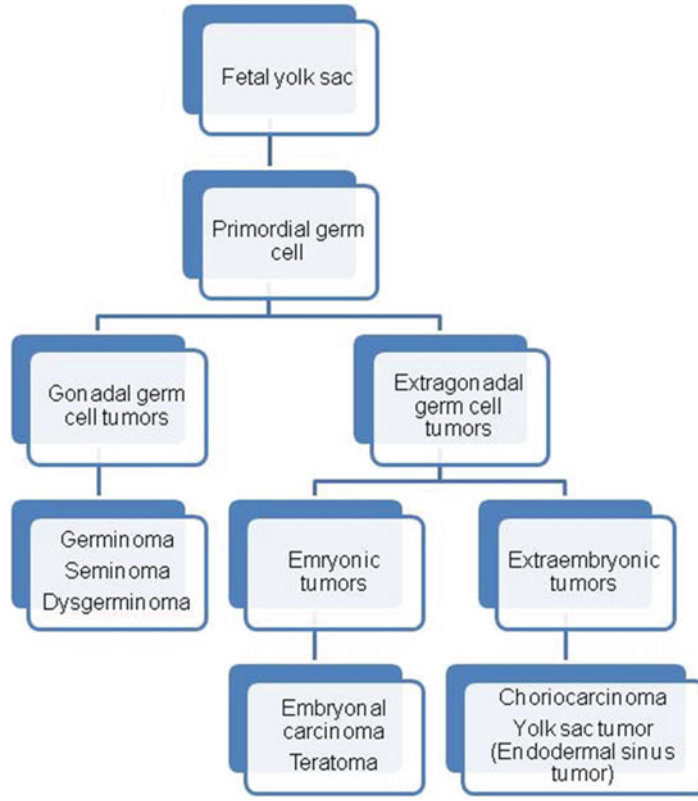

Fig. 2.1 Germ cell tumors according to embryonic differentiation and migration

patients under 30 years-old revealed 218 germinomas located in the pineal region among 322 pineal germ cell tumors (Goodwin et al. 2009). Several reports from all over the world had reported germinomas between 11.6 and 66.6%, however these were the mixed series of adult and pediatric patients (Konovalov and Pitskhelauri 2003; Kang et al. 1998; Tamaki and Yin 2000) Series with only pediatric cases or separating the pediatric datain these studies revealed the incidence of germinomas as 4–30.5% (Packer et al. 1984; Pendl and Vorkapic 1985; Yazici et al. 2009; Knierim and Yamada 2003; Shin et al. 1998; Cho et al. 1998; Edwards et al. 1988). The differences in these series could be associated with the patient profile of the institutions or the source of the reports such as pediatric oncology or neurosurgery departments. It has been shown by Goodwin et al. (2009) that the males significantly had more germinomas in this region than females. A better prognosis was reported compared with other non germinomatous germ cell tumors (Hoffman et al. 1991; Choi et al. 1998).

It has been reported that germinomas are generally circumscribed tumors that invade all of the gland and grow with expansion with the potential of extending to the subarachnoid space and cause seeding metastasis by cerebrospinal fluid circulation which may lead to aquaductal obstruction and hydrocephalus (de Girolami et al. 2008). The tumor is reported to have lobules of uniform cells with fibrous septae around (Kumar et al. 2006). Two cell populations are defined: round cells with vesicular nuclei containing prominent nucleoli and clear cytoplasm with marked cytoplasmic borders; and small T-lymphocytes, which aggregate in clusters and form germinal-center-like nodules (de Girolami et al. 2008). Immunohistochemical findings were defined as positive staining of tumor cells with cytoplasmic PAS, and generally positive staining with PLAP, α-fetoprotein, CD 117 whereas β-HCG may be positive in the

tumor when there is syncytio-trophoblast like cells are present (de Girolami et al. 2008). Proliferation index of these tumors are high and Mib-1 index is reported as high as 80% by de Girolami et al.(2008). Admixtures of non-germinamatous germ cell components and cystic teratomatous foci, and usually after a dermoid/epidermoid cyst rupture into the tumor, a considerable giant cell reaction can be seen (de Girolami et al. 2008).

Non-germinomatous Germ Cell Tumors

Yolk sac tumor (endodermal sinus tumor), embryonal carcinoma, choriocarcinoma and teratomas are the tumor types in this group. New evidence showed that non-germinomatous germ cell tumors included 6 embryonal carcinomas (2%), 5 yolk sac tumors (1%), 25 immature teratomas (7%), 2 choriocarcinomas (0.5%), 50 mixed germ cell tumors (13.5%) (Al-Hussaini et al. 2009). Nongerminomatous germ cell tumors were more common around birth and in the age range of 10–20 years and may extend to the age of 25 years (Goodwin et al. 2009). *Yolk sac tumor* is a rare tumor and its histopathologic appearrence resembles to the tumors that are outside the central nervous system. It is described as a a papillary primitive epithelial tumor with myxoid matrix in organoid structure containing a blood vessel surrounded by an epithelial-line (Schiller duval body) and tumor cells have PAS + diastase resistant eosinophylic intracytoplasmic globules. Besides, α-fetoprotein is an important immunohistochemical marker in this tumor and the tumor has usually a high mitotic ratio (de Girolami et al. 2008).

Embryonal carcinoma is a rare tumor seen particularly in children. Packer et al. (1984) defined 5 cases in 25 pediatric pineal tumors. Kang et al. (1998) reported 3 in 38 germ cell tumors and Tamaki and Yin (2000) reported only 1 case among 30 germ cell tumors in the pineal region. It should be also considered that this tumor may be a component of a mixed germ cell tumor as previously detected (Knierim and Yamada 2003; Shin et al. 1998). It is described by de Girolami et al. (2008) that it is a high grade tumor with elevated mitotic ratio and tumor is composed of primitive epithelial carcinoma cells growing in sheets or forming gland like structures. It has multinucleated giant cells which produce β-HCG. Immunohistochemically the cells stain positive with PLAP and cytokeratin.

Choricarcinoma is an extremely rare and agressive non germinamatous germ cell tumor which secretes β-HCG. In a series of 30 germ cell tumors, only 1 case was reported (Tamaki and Yin 2000). It is defned as an hemorrhagic tumor with cytotrophoblasts and syncytiotrophoblasts, giant cell elements in malignant primitive epithelial sheets of cells lack of a spesific architecture. Cytotrophoblasts are closely located uniform and medium sized cells with clear cytoplasm and distinct cell margins and vesicular nuclei. In immunohistochemistry, syncytiotrophoblasts are positively stained by β-HCG. (de Girolami et al. 2008; Cushing et al. 2006).

Teratomas are the most common childhood germ cell tumors independent from the site of occurrence. These are tumors which are composed of elements of three germ layers, endoderm, ectoderm, and mesoderm. Any type of tissue may exist in the structure and may show physiologic activity such as enzyme or hormone secretion. Components of the teratoma may vary according to the site of this germ cell tumor. There are three types of teratomas, namely mature, immature and malignant teratoma. Mature solid teratomas contain skeletal muscle, brain, skin, hair, teeth, cartilage etc. In immature teratoma, three germ layers are present with fetal or immature tissue of usually neuroepithelium as well as ectodermal, mesodermal and endodermal components. Although it is used for ovarian immature teratomas, grading systems are used for classification (Cushing et al. 2006):

Grade 0: Mature tissue only,

Grade 1: Immature tissue less than 1 low-power field per slide;

Grade 2: Immature tissue, 1–3 low-power fields per slide

Grade 3: Abundant immature tissue

In malignant teratoma, there is malignant transformation in the sarcomatous or carcinomatous tissue in the solid part of the teratoma (de Girolami et al. 2008). In histologic classification of pediatric gonadal and extragonadal germ cell tumors, malignant teratomas of the gonads (ovary) was classified as follows (Cushing et al. 2006):

Teratoma with associated malignant germ cell tumor component

Teratoma with associated malignant somatic component (squamous carcinoma, glioblastoma, peripheral neuroectodermal tumor, etc.)

The classification of extragonadal teratomas of the sacral, mediastinal retroperitoneal, pineal and the other sites was classified as (Cushing et al. 2006):

Teratoma+/– Endodermal sinus tumor

Teratoma+/– Emryonal carcinoma

As specific to this region, and although they are considered sometimes as non neoplastic lesions, dermoid cyst and epidermoid cyst is also frequently seen. Dermoid cyst is composed of a cyst wall with keratinizing squamous epithelium with adnexial structures of skin and epidermoid cysts is the name of the same lesion without adnexial structures of the skin.

Other Tumors

Various types of tumors, such as chemodectoma, sarcoma, ganglion cell and melanocytic tumors, lipoma, meningioma, hemangioma are malignant and benign neoplasms which had been previosly defined in this region (de Girolami et al. 2008). Adamantinomatous type craniopharyngioma, choroid plexus papilloma and carcinoma were also detected (Kumar et al. 2006; Knierim and Yamada 2003). Interestingly, ganglioneuroblastoma in a pediatric series was also reported (Packer et al. 1984). Metastasis to the pineal region was rarely reported in previous series of pineal tumors (Tamaki and Yin 2000) and they are usually discovered incidentally in post mortem studies from the common adult tumors such as lung and breast cancer as well as others (de Girolami et al. 2008).

Non-neoplastic Lesions

In differential diagnosis, calcific deposits of the pineal gland (acervuli, brain sand), pineal cysts, pineal apoplexia (hemorrhagic necrosis), autoimmune inlammatory cell infiltration which is called idiopathic pinealitis, arachnoid cysts, demyelinating disorders, encephalitis, sarcoidosis, cerebrovascular accidents, arteriovenous malformation and tuberculous abcess should be considered as well as tumors in this region (Knierim and Yamada 2003; de Girolami et al. 2008; Kumar et al. 2006).

Signs and Symptoms

The majority of the patients present with headache and elevated intracranial pressure. Additional presenting signs and symptoms were outlined in Table 2.1. In infants, macrocephaly, developemental delay and irritability may also ocur. Pineal region masses that extend rostrally or anteriorly obstruct third ventricular outflow and result in hydrocephalus and symptoms of raised intracranial pressure (Packer et al. 1984). The lesions in the posterior part of the midbrain cause paralysis of vertical gaze, a response in the pupils in which they react poorly to light but better to accomodation, conversion nystagmus and lid retraction which is totally called Parinaud's syndrome. This clinical feature may accompany the symptoms of increased intracranial pressure (Packer et al. 1984). Lesions that compress or infiltrate the overlying thalamus cause hemiparesis, incoordination, visual difficulty or movement disorders. Lethargy, seizures and multifocal neurological deficits without raised intracranial pressure were also reported in two children (Packer et al. 1984). Signs and symptoms of raised intracranial pressure was the most common reported clinical feature which accounts for more than half of all features at presentation of the patients (48.8–91%) (Konovalov and Pitskhelauri 2003; Kang et al. 1998; Cho et al. 1998; Shin et al. 1998; Yazici et al. 2009). Visual disturbances and eye movement disorders were also frequent features

Table 2.1 Presenting signs and symptoms

Signs
Increase in intracranial pressure (including papilledema, abducens palsy, 3rd nevre palsy)
Hemiparesis
Parinaud's
Seizure
Diabetes insipidus
Diencephalic syndrome
Ataxia and other cerebellar signs
Precocious puberty
Sudden tumor bleeding
Hearing impairment
Extrapiramidal signs
Hypopituitarism
Symptoms
Headache-nausea-vomiting
Visual disturbances (including diplopia, blurred vision)
Polyuria –polydipsia
Cachexia
Gait disturbances
Lethargy

of the tumors in this region reported as high as 76% (Konovalov and Pitskhelauri 2003). Since the gland has regulatory effects on the endocrine system of the body, diabetes insipidus, precocious puberty, hypopituitarism were also prominent in these patients. Diabetes insipidus was reported 2.8, 4.8, 6, 6.9 and 11.6, and 18% of cases respectively (Knierim and Yamada 2003; Yazici et al. 2009; Konavalov and Pitskhelauri 2003; Shin et al. 1998; Kang et al. 1998; Cho et al. 1998). Precocious puberty was around 1.4–2.3% (Knierim and Yamada 2003; Konavolov and Pitskhelauri 2003; Shin et al. 1998). Hypopituitarism was 5% (Konavalov and Pitskhelauri 2003). Diencephalic syndrome was also rarely reported as 2% (Yazici et al. 2009).

Diagnostic and Laboratory Interventions

Radiology

Pior to the extensive use of magnetic resonance imaging, all patients underwent computed tomography (CT) with and without contrast injection. Germ cell tumors are usually irregular lesions of mixed density with or without calcifications which usually enhance heterogeneously, rarely homogeneously. Pineal parenchymal tumors are also seen as irregular masses with isodense or mixed density with or without calcification. They usually enhance diffusely but heterogeneous enhancement was also reported in some cases (Packer et al. 1984). Glial tumors were hypointense or isointense on computed tomography with variable contour properties and enhancement changes. They have been reported to have a tendency to involve the mesencephalon and diencephalon (Knierim and Yamada 2003). Pineoblastomas may be discriminated from other tumors by diffuse subarachnoid enhancement. In those selected, bilateral internal carotid and vertebral angiography were also performed additional to the computed tomography previously (Packer et al. 1984). Arteriography was useful for the demonstration of the venous and arterial supply of the tumor. Computed tomography was used for diagnositic purposes of germinomas before histopathological evaluation of the pineal tumor by the evidence of rapid response to radiation therapy after V/P shunting which was named as 'biological biopsy' (Balmaceda et al. 1996; Knierim and Yamada 2003).

Magnetic resonance imaging (MRI) markedly improved the anatomic localization of tumors by imaging in three orthogonal planes without the need to move the patient, no need for X-irradiation and creating a better image quality with improved soft tissue contrast. Although especially in the emergency setting, CT without contrast is still extensively used in pediatric patients, however it should have been remembered that gadolinum enhanced MRI is superior to CT using iodine-based contrast mediums for evaluating the extension of the tumor. Besides routine examination with T1 weighted imaging before and after gadolinum and T2- and proton density weighted imaging, MRI has other advantages such as fluid-attenuated inversion recovery (FLAIR) sequences, fast echo planar imaging, volumetric measurement, angiography, spectroscopy, gradient echo, diffusion-weighted, perfusion and activation functional imaging techniques. Pineal region tumors

like extraaxial sellar tumors and meningiomas usually lack a blood brain barrier and enhance quickly after contrast administration. Juvenile pilocytic astrocytoma, glioblastome multiforme and metastatic tumors usually enhance homogenously but in some instances glioblastome multiforme displays heterogeneous and irregular enhancement. Anaplastic astrocytoma, oligodendroglioma and metastatic lesions in this region may also show heteregenous and irregular enhanncement patterns. Oligodendroglioma and ganglioglioma were known as focal noduler enhancing lesions in this region. Low grade fibrillary astrocytoma usually lack of contrast enhancement. In meningioma, ependymoma, pineoblastoma and metastatic tumors display of low to isointense imaging property to gray matter in T-2 signal sequences whereas glial tumors show hyperintensity to gray matter. Cystic appearrence are prominent with juvenile pilocytic astrocytoma, ganglioglioma, oligodendroglioma; hemorrage may be apparent with glioblastoma, anaplastic astrocytoma, ependymoma and oligodendroglioma; Calcification may be a radiological feature of oligodendroglioma and ependymoma and necrosis can be seen with glioblastoma and metatstatic tumors. Both CT and MRI have still being used extensively in different indications and clinical situations, MRI was found particularly valuable in showing the varied tissue character in patients with mixed germ cell tumors (Kang et al. 1998). Retrospective evaluation of MRI and CT images of 32 patients with histologically proven pure germinomas without elevation of tumor markers, showed a pathognomonic CT and MRI sign of 'butterfly' in 43% of the cases. This sign was caused by a symmetric, predominantly subependimal germinomatous infiltration of both thalamus with enlarged pineal calcification along the midline (Konavalov and Pitskhelauri 2003).

Nuclear Medicine

Thallium 201 single-photon emission CT (SPECT) is rarely used in pediatric brain tumors. T1 Spect was found less specific and sensitive than gadolinum enhanced MRI and in a series the researchers did not find useful clinical indication for the technique except distinguishing the postradiation necrosis from tumopr recurrence in brainstem gliomas (Rollins et al. 1995; Blaney et al. 2006). In-111 Pentetreotide showed intense focal uptake in a patient with recurrent pineocytoma (Serrano et al. 2009).

Positron emission tomography (PET) with 18 F-fluorodeoxyglucose (18 F-FDG) indicates metabolically active tissue. It is extensively used in clinical practice, especially in oncology but clinical data is growing in neurooncology (Plowman et al. 1997; Chen 2007). 18 F-FDG/PET follows the pathway of glucose metabolism and indicates glycolysis; where it is greater than the background uptake and shows hypermetabolism which may suggest a high grade tumor. 18 F-FDG/PET positivity has been shown and supported the diagnosis of pineal/suprasellar germ cell tumor with negative markers however there were also limitations of the technique in terms of lack of hypermetabolism in either an isolated lesion or characteristics of multiple lesions as well as the high rate of the physiologic glucose metabolism in normal brain tissue (Janmohamed et al. 2002; Kelly et al. 2009). It should be concluded that 18 F-FDG/PET positivity should be a useful diagnositic tool but has to be used cautiously in follow–up especially in recurrence.

Tumor Markers

The levels of oncofetoproteins, α-fetoprotein and β-HCG in cerebrospinal fluid should be obtained during surgery in pineal region tumors for diagnostic purposes and from lumbar puncture for the follow up in case of response to chemotherapy and radiotherapy as well as in the suspicion of recurrence. Marker positive tumors of the pineal region are tumors of germ cell origin. Beta human chorionic gonadotrophin is secreted from syncytiotrophoblasts and found frequently in germinomas, embryonal carcinoma and choriocarcinoma. These markers were suggested to be useful for the discrimination of postradiation necrosis from postradiation recurrence as well (Knierim and Yamada 2003). Germinomas have a special

appearrence of multicentricity in radiology. Although not suggested, tumor markers and the special radiological appearrence led to treatment in some cases with inadequate histopathological material or morbidity of surgery in case of suspected germinomas by several experiences (Yazici et al. 2009; Cho et al. 1998; Kang et al. 1998; Tamaki and Yin 2000).

In a report of Konovalov and Pitskhelauri (2003), the tumor types which were marker positive account for 26% of analysed cases (n=68) but this study had reported 87 germ cell tumors. These cases were choriocarcinoma in 5, germinoma in 4, mixed germ cell tumors in 3 embryonal carcinoma in 3, immature teratoma in 2 and endodermal sinus tumor in 1 patient. Among 12 patients with germ cell tumors (9 with histologically proven germ cell tumors, 3 diagnosed with radiology and elevated tumor markers), positive tumor markers could be obtained in 7 patients (58%) in another experience (Yazici et al. 2009). Tamaki and Yin (2000) revealed 18 cases of 23 germ cell origin with assayed tumor markers. In all three studies, lack of data was present in some cases with germ cell tumors whether these cases had non secreting or there was a difficulty of obtaining a sample for a tumor marker. However, in the study of Kang et al. (1998), levels of either β-HCG and α-fetoprotein in serum and CSF were positive in 18 cases of assayed 38 tumors of germ cell origin (47.4%).

Surgery

Surgery remains the mainstay of diagnostic procedures and as well as treatment in brain tumors. Stereotactic biopsy and third ventricle endoscopic procedures are exteensively used in diagnosis. Although in case of pure germinoma with typical butterfly sign in radiology, surgery and stereotactic biopsy may be omitted and radiotherapy should be applied. In also non pure germinomas chemoradiotherapy should be started without histopathological diagnosis. In most cases pineal region tumors and lesions and unresponsive tumors to adjuvant treatment modalities of radiotherapy and chemoterapy, histological diagnosis is relevant (Konavalov and Pitskhelauri 2003) so surgery becomes the principal diagnostic and treatment modality.

In conclusion, pineal gland tumors are rare and account less than 1% of all primary brain tumors but the incidence is higher in pediatric age group. Epidemiology, pathology, clinical features and essential laboratory investigations are outlined in this chapter. The prognostic factors other than histopathology which are surgery and appopriate adjuvant and/or neoadjuvant treatment modalities would be discussed later in the subsequent chapter.

References

Al-Hussaini M, Sultan I, Abuirmileh N, Jaradat I, Qaddoumi I (2009) Pineal gland tumors: experience from SEER database. J Neurooncol 94:351–358

Balmaceda C, Heller G, Rosenblum M, Diez B, Villablanca JG, Kellie S, Maher P, Vlamis V, Walker RW, Leibel S, Finlay JL (1996) Chemotherapy without irradiation—a novel approach for newly diagnosed CNS germ cell tumors: results of an international cooperative trial. The First International Central Nervous System Germ Cell Tumor Study. J Clin Oncol 14:2908–2915

Berns S, Pearl G (2006) Review of pineal anlage tumor with divergent histology. Arch Pathol Lab Med 130:1233–1235

Blaney SM, Kun LE, Hunter J, Rorke-Adams LB, Lau C, Strother D, Pollack IF (2006) Tumors of the central nervous system. In: Pizzo PA, Poplack DG (eds) Principles and practice of pediatric oncology, 5th edn. Lippincott, Williams and Wilkins, Philadelphia, pp 786–864

Chen W (2007) Clinical applications of PET in brain tumors. J Nucl Med 48:1468–1481

Cho BK, Wang KC, Nam DH, Kim DG, Jung HW, Kim HJ, Han DH, Choi KS (1998) Pineal tumors: experience with 48 cases over 10 years. Childs Nerv Syst 14:53–58

Choi JU, Kim DS, Chung SS, Kim TS (1998) Treatment of germ cell tumors in the pineal region. Childs Nerv Syst 14:41–48

Cushing B, Perlman EJ, Marina NM, Castleberry RP (2006) Germ cell tumors. In: Pizzo PA, Poplack DG (eds) Principles and practice of pediatric oncology. Lippincott, Williams and Wilkins, Philadelphia, p 1116

de Girolami U, Fêvre-Montange M, Seilhean D, Jouvet A (2008) Pathology of tumors of the pineal region. Rev Neurol 164:882–895

Edwards MS, Hudgins RJ, Wilson CB, Levin WA, Wara WM (1988) Pineal region tumors in children. J Neurosurg 68:689–697

Fèvre-Montange M, Szathmari A, Champier J, Mokhtari K, Chrétien F, Coulon A, Figarella-Branger D, Polivka M, Varlet P, Uro-Coste E, Fauchon F, Jouvet A (2008) Pineocytoma and pineal parenchymal tumors of intermediate differentiation presenting cytologic pleomorphism: a multicenter study. Brain Pathol 18:354–359

Fuller BG, Kapp DS, Cox R (1994) Radiation therapy of pineal region tumors: 25 new cases and a review of 208 previously reported cases. Int Radiat Oncol Biol Phys 28:229–245

Gilheeney SW, Saad A, Chi S, Turner C, Ullrich NJ, Goumnerova L, Scott RM, Marcus K, Lehman L, de Girolami U, Kieran MW (2008) Outcome of pineoblastoma after surgery, radiation therapy and chemotherapy. J Neurooncol 89:89–95

Goodwin TL, Sainani K, Fisher PG (2009) Incidence patterns of central nervous system germ cell tumors: a SEER study. J Pediatr Hematol Oncol 31:541–544

Graziano SL, Paolozzi FP, Rudolph AR, Stewart WA, Elbadawi A, Comis RL (1987) Mixed germ-cell tumor of the pineal region. J Neurosurg 66:300–304

Guyton AC (1991) Endocrinology and reproduction: epiphysis (pineal gland). In: Textbook of physiology, Saunders, 8th edn. Philadelphia, p 1399

Hoffman HJ, Otsubo H, Hendrick EB, Humphreys RP, Drake JM, Becker LE, Greenberg M, Jenkin D (1991) Intracranial germ cell tumors in children. J Neurosurg 74:545–551

Janmohamed S, Grossman AB, Metcalfe K, Lowe DG, Wood DF, Chew SL, Monson JP, Besser GM, Plowman PN (2002) Suprasellar germ cell tumours: specific problems and the evolution of optimal management with a combined chemoradiotherapy regimen. Clin Endocrinol (Oxf) 57:487–500

Jennings MT, Gelma R, Hochberg F (1985) Intracranial germ-cell tumors: natural history and pathogenesis. J Neurosurg 63:155–167

Jouvet A, Saint-Pierre G, Fauchon F, Privat K, Bouffet E, Ruchoux MM, Chauveinc L, Fèvre-Montange M (2000) Pineal parenchymal tumors: a correlation of histological features with prognosis in 66 cases. Brain Pathol 10:49–60

Kang JK, Jeun SS, Hong YK, Park CK, Son BC, Lee IW, Kim MC (1998) Experience with pineal region tumors. Childs Nerv Syst 14:63–68

Kelly PA, Metcalfe K, Evanson J, Sabin I, Plowman PN, Monson JP (2009) Positron emission tomography in the diagnosis and management of intracranial germ cell tumours. Horm Res 72:190–196

Knierim DS, Yamada S (2003) Pineal tumors and associated lesions: the effect of ethnicity on tumor type and treatment. Pediatr Neurosurg 38:307–323

Koide O, Watanabe Y, Sato K (1980) A pathological survey of intracranial germinoma and pinealoma in Japan. Cancer 45:2119–2130

Konovalov AN, Pitskhelauri DI (2003) Principles of treatment of the pineal region tumors. Surg Neurol 59:250–268

Kumar P, Tatke M, Sharma A, Singh D (2006) Histological analysis of lesions of the pineal region: a retrospective study of 12 years. Pathol Res Pract 202:85–92

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2007a) WHO classification of tumors of the central nervous system, 4th edn. International Agency of Research on Cancer, Lyon

Louis DN et al (2007b) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114:97–109

Packer RJ, Sutton LN, Rosenstock JG, Rorke LB, Bilaniuk LT, Zimmerman RA, Littman PA, Bruce DA, Schut L (1984) Pineal region tumors of childhood. Pediatrics 74:97–102

Pendl G, Vorkapic P (1985) Surgery of pineal region lesions in childhood. Acta Neurochirurgica Suppl 35:114–118

Plowman PN, Saunders CA, Maisey M (1997) On the usefulness of brain PET scanning to the pediatric neuro-oncologist. Br J Neurosurg 11:525–532

Rollins NK, Lowry PA, Shapiro KN (1995) Comparisoon of gadolinum enhanced MRI and thallium −201 single photon emission computed tomography in pediatric brain tumors. Pediatr Neurosurg 22:8–14

Rubinstein LJ (1981) Cytogenesis and differentiation of pineal neoplasms. Hum Pathol 12:4481–4484

Serrano J, Infante JR, Rayo JI, Dominguez L, Garcia-Bernardo L, Duràn C, Sànchez R (2009) In-111 pentetreotide in recurrent pineocytoma. Clin Nucl Med 34:117

Shin HJ, Cho BK, Jung HW, Wang KC (1998) Pediatric pineal tumors: need for a direct surgical approach and complications of the occipital transtentorial approach. Childs Nerv Syst 14:174–178

Snell RS (1992) The cerebrum: epithalamus. In: Clinical neuoanatomy for medical students, 3rd edn. Little Brown and Company, Boston/Toronto/London, pp 252–254

Tamaki N, Yin D (2000) Therapeutic strategies and surgical results for pineal region tumours. J Clin Neurosci 7:125–128

Taylor RE, Donachie PH, Weston CL, Robinson KJ, Lucraft H, Saran F, Ellison DW, Ironside J, Walker DA, Pizer BL (2009) Children's Cancer and Leukaemia Group CNS Tumour Division. Impact of radiotherapy parameters on outcome for patients with supratentorial primitive neuro-ectodermal tumours entered into the SIOP/UKCCSG PNET 3 study. Radiother Oncol 92:83–88

The comittee of Brain Tumor Registry of Japan (1992) Brain tumor registry of Japan (Tokyo). Neurol Med Chir 32:381–547

Varan A, Akyüz C, Büyükpamukçu M (1998) A rare presentation of trilateral retinoblastoma. Tumori 84:515–516

Yazici N, Varan A, Soylemezoglu F, Zorlu F, Kutluk T, Akyüz C, Büyükpamukçu M (2009) Pineal region tumors in children: a single center experience. Neuropediatrics 40:15–21

Papillary Tumor of the Pineal Region: Diagnosis

3

Hirohito Yano and Toru Iwama

Contents

Abstract

Papillary tumor of the pineal region (PTPR) has been recently included in the 2007 World Health Organization (WHO) "Classification of tumors of the central nervous system." PTPRs are considered to originate from the subcommissular organ (SCO), which consists of secretory ependymocytes. The origin of the tumor is reflected in the neuroradiological, microscopic, immunohistochemical, and ultrastructural findings. In non-contrast T1-weighted magnetic resonance imaging (MRI) scans, the lesion is represented as a heterogeneous hyperintense area due to the presence of secretory glycoprotein inclusions. PTPRs are most frequently misdiagnosed as ependymomas or choroid plexus (CP) tumors owing to the morphological similarity among these tumors. PTPRs are histologically characterized by loose papillary and densely cellular diffuse, patternless areas showing pseudorosettes with fibrovascular cores covered by several layers of columnar or cuboidal cells. PTPRs are composed of a greater number of epithelial cells than that in ependymomas and lesser number of papillary cells than choroid plexus (CP) tumors. Characteristic immunohistochemical findings of PTPRs include characteristic small ring- and dot-like staining patterns indicative of cytokeratin 18 immunoreactivity, which are similar to those obtained for CP tumors. Furthermore, both PTPRs and CP tumors showed focal transthyretin (prealbumin; TTR) immunoreactivity. On the other

H. Yano (✉) • T. Iwama
Department of Neurosurgery, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu City 501-1194, Japan
e-mail: hirohito@gifu-u.ac.jp

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_3,

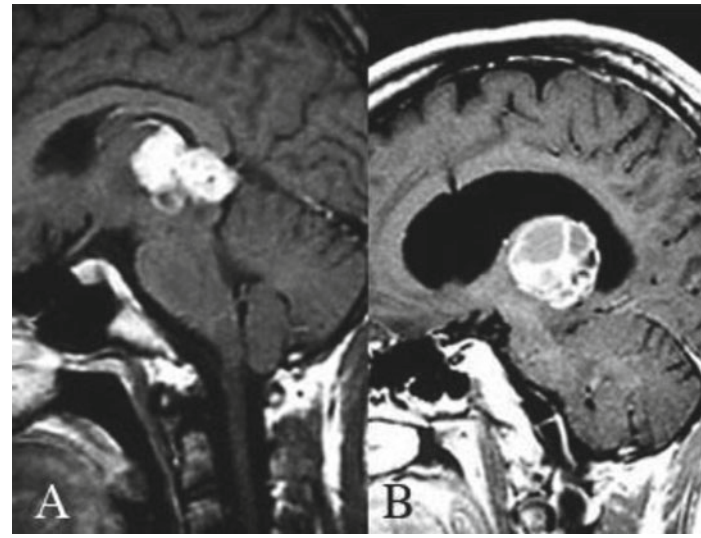

Fig. 3.1 (**a**) Magnetic resonance imaging (MRI) with gadolinium (Gd) enhancement shows a gourd-shape lesion in the pineal region. (**b**) MRI with Gd enhancement 9 years after the initial examination (Fig. 3.1a) shows re-growth of the lesion with multiple cystic components

hand, PTPRs and ependymomas occasionally showed immunoreactivities to epithelial membranous antigen (EMA), glial fibrillary acidic protein (GFAP), and neural cell adhesion molecule (NCAM). In addition to the abovementioned makers, strong diagnostic markers for PTPRs and factors that distinguish PTPRs from ependymomas or CP tumors are expression of neuronal markers, including microtubule-associated protein 2 (MAP2), neuron-specific enolase (NSE), and neuronal nuclei (NeuN). These findings can be attributed to the extensive involvement of SCO in neuronal differentiation. Ultrastructural examination of PTPRs showed the presence of microvilli and desmosomes—characteristics of ependymoma cells—and abundant rough endoplasmic reticulum, lipid droplets, etc.—characteristics of CP tumor cells.

Introduction

Papillary tumor of the pineal region (PTPR), first described as a distinct entity by Jouvet et al. (2003), was recently included as a rare pineal tumor in the 2007 World Health Organization (WHO) "Classification of tumors of the central nervous system" (Louis et al. 2007). PTPRs are frequently misdiagnosed as papillary pineocytomas (Nakamura et al. 2009), papillary ependymomas (Yano et al. 2003), CP tumors, and metastatic pineal tumors. In this study, we describe the neuroradiological, microscopic, immunohistochemical, and ultrastructural characteristics of PTPRs that distinguish it from the abovementioned pineal tumors.

Symptoms

In almost all reported cases of PTPRs, the patients usually presented with headaches due to hydrocephalus, which develops as the result of the compression of the mesencephalic aqueduct (Roncaroli and Scheithauer 2007). Some patients present with recurrent attacks of generalized epilepsy and markedly reduced cognitive function without focal neurological deficits (Kuchelmeister et al. 2006).

Magnetic Resonance Imaging Findings

Magnetic resonance imaging (MRI) of PTPRs typically shows a well-circumscribed mass lesion in the pineal region (diameter, 2–3 cm) (Fig. 3.1a). The lesion consists of a well-enhanced solid component and a cystic component in many cases

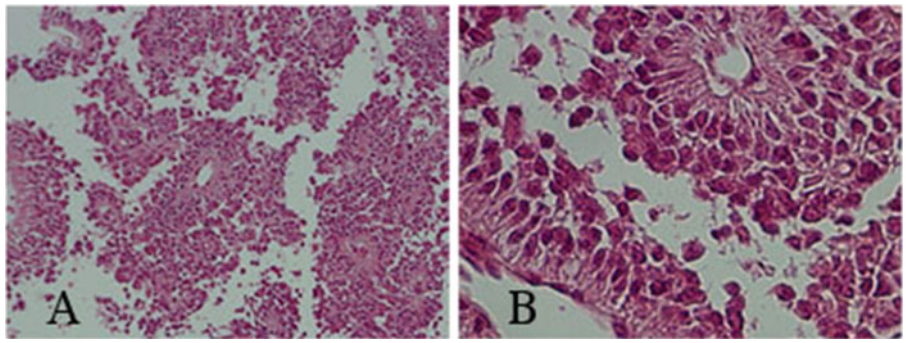

Fig. 3.2 (**a**) Hematoxylin-eosin (H&E) staining showing papillary structures with growth pattern similar to the epithelial growth pattern. (**b**) Stratified epithelial cells surrounding the vessels

(Fig. 3.1b) (Shibahara et al. 2004). In sagittal T1-weighted images, a PTPR is frequently visualized as a macrolobulated lesion compressing the tectum and the aqueduct in the posterior part of the third ventricle (Fig. 3.1a) (Kuchelmeister et al. 2006). Accordingly, triventricular occlusive hydrocephalus is observed in many cases. The lesion is usually represented by a heterogeneously hyperintense area on non-contrast T1-weighted images. These findings can be attributed to the presence of secretory glycoprotein inclusions in the tumor cells (Chang et al. 2008). On the contrary, a few cases have been reported in which lesions with cystic components showed isointense or low-intensity areas on non-contrast T1-weighted images (Kawahara et al. 2007).

Histology

The histological hallmark of a PTPR is loose papillary and dense cellular diffuse, patternless areas showing pseudorosettes with fibrovascular cores, true rosettes, or tubes and necrosis and, occasionally, vacuolated tumor cells (Fèvre-Montange et al. 2006). The presence of numerous extensive epithelial papillary structures around vascular structures in these tumors gives the appearance of perivascular pseudorosettes, because of which PTPR is wrongly diagnosed as papillary ependymoma (Fig. 3.2a). A large proportion of pseudorosettes consist of stratified columnar or cuboidal cells (Fig. 3.2b) (Hasselblatt et al. 2006; Kern et al. 2006), which frequently occur in multiple layers. Occasionally, a single layer of columnar epithelial cells surrounding a central vessel is observed in the papillae. The interstitium may deeply stain with eosin, and occasionally, the cells may show moderate mitotic activity. Focal necrosis is frequently observed. Generally, the conspicuous, pathognomonic vacuolated tumor cells appear signet-ring-like in shape (Fig. 3.2b) (Hirato et al. 1997). This finding is also observed in a smear preparation (Dagnew et al. 2007). A few papillary areas contained granular cytoplasmic material that showed a periodic acid-Schiff positive reaction. A few papillary areas contained granular cytoplasmic material that showed a periodic acid-Schiff positive reaction; this material was considered to be glycoprotein (Roncaroli and Scheithauer 2007; Fèvre-Montange et al. 2006; Kern et al. 2006). Obvious vascular proliferation is not observed; however, the vessels often showed slight endothelial hyperplasia or hyalinization (Jouvet et al. 2003; Fèvre-Montange et al. 2006). If a relapse of PTPR occurs, the papillary structures are often more prominent, thinner, and fewer than those observed in the initial specimens (Roncaroli and Scheithauer 2007; Yano et al. 2009).

Immunohistochemistry

Immunohistochemical analysis enabled the differential diagnosis of PTPRs, papillary pineocytomas, other pineal parenchymal tumors, papillary ependymoma, CP tumors, and metastatic tumors.

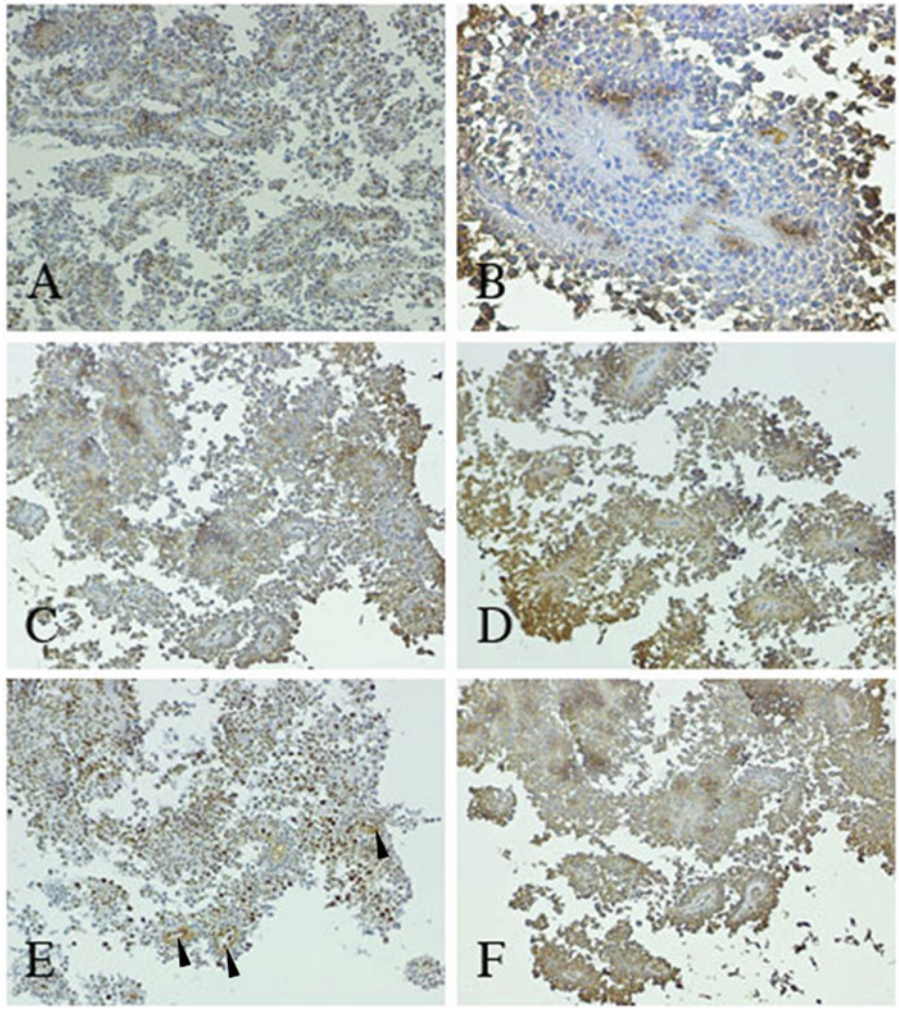

Fig. 3.3 (**a**) Strong cytokeratin 18 immunoreactivity in the epithelia is seen in the form of a small dot-like staining pattern. (**b**) EMA immunoreactivity is restricted to the cell surface. (**c**) Strong and extensively positive immunoreactivity of microtubule-associated protein 2. (**d**) Diffuse strong anti-neuron-specific enolase immunostaining. (**e**) The nucleus and some of the perivascular structures are strongly and partly positive for anti-neuronal nuclei immunoreactivity (*arrowhead*). (**f**) Diffuse strong anti-synaptophysin immunostaining

Positive results of staining for epithelial and neuronal markers can facilitate the diagnosis of PTPRs. Immunohistochemical studies show that PTPRs are positive for cytokeratin 18 (CK 18) (Fig. 3.3a), epithelial membranous antigen (EMA) (Fig. 3.3b), microtubule-associated protein (MAP2) (Fig. 3.3c), neuron-specific enolase (NSE) (Fig. 3.3d), neuronal nuclei (NeuN) (Fig. 3.3e), synaptophysin (Syn) (Fig. 3.3f), and neural cell adhesion molecule (NCAM) (Kern et al. 2006; Yano et al. 2009). The number of CK-positive neoplastic cells differs among cases. Strong CK 18 immunoreactivity, elicited as a small ring- and dot-like staining pattern, was observed in the epithelia (Fig. 3.3a) (Roncaroli and Scheithauer 2007; Jouvet et al. 2003; Kuchelmeister et al. 2006; Yano et al. 2009). In contrast, EMA immunoreactivity is weak and restricted to the cell surface; in many cases, EMA immunoreactivity is observed in the form of a dot-like staining pattern, particularly, on the pole facing the vessels (Roncaroli and Scheithauer

2007; Fèvre-Montange et al. 2006). The staining pattern for both CK-18 and EMA antibodies can be useful for the diagnosis of PTPRs. Diffuse strong immunostaining for anti-NSE and Syn is observed in the cytoplasm in many cases of PTPR. Those staining pattern is similar. In contrast, strongly positive anti NeuN immunoreactivity was observed in the nucleus of the tumor cells and around the vessel core in the rosettes (Fig. 3.3e) (Yano et al. 2009). Most of the PTPR tumor cells are strongly positive for MAP2 in a majority of the cases, showing a dot-like pattern occasionally (Fig. 3.3c) (Fèvre-Montange et al. 2006). Widespread NCAM immunoreactivity is seen in the cell membrane (Kern et al. 2006). Although choromogranin A-positive neoplastic cells are found in a few cases, staining for neurofilament protein is reported to be negative in the majority of cases (Jouvet et al. 2003). Neuron-specific beta III-tubulin (TuJ1) protein, considered to be a marker for immature neurons (Lee et al. 1990), has been reported to be negative in a reported case (Yano et al. 2009). GFAP expression is reported to be weak and focal in perivascular areas at the periphery of the tumor or in scattered reactive astrocytes (Jouvet et al. 2003). Focal transthyretin (prealbumin; TTR) immunoreactivity is moderately observed on the cell surface of the epithelia in half of the reported cases (Shibahara et al. 2004; Fèvre-Montange et al. 2006). Nuclear and cytoplasmic staining for S-100 protein in papillary structures may be stronger than that in the diffuse areas (Jouvet et al. 2003). Ki67 proliferation indices are lower than 10% in two third and higher than 10% in one third of the cases (Fèvre-Montange et al. 2006).

Electron Microscopy

Discussion

Origin of the Tumor

To discuss the neuroradiological and histological findings of PTPR, it is necessary to know its origin. PTPR is thought to originate from the subcommisular organ (SCO), which consists of specialized ependymocytes (Jouvet et al. 2003). Chordoid glioma in the third ventricle has also been reported to originate from the specialized ependyma of the SCO-related tumor (Cenacchi and Glangaspero 2004). The SCO is considered to be a part of the circumventricular organs (CVOs). The CVOs are unique midline glandular structures in the brain that lie outside the blood–brain barrier and line the third and fourth ventricles (Kern et al. 2006). The papillary structures of the PTPRs are similar to those of the SCO cells (Boco et al. 2008). Ependymal cells originating from the SCO express cytokeratin, which is a major marker for PTPR. SCO has also been reported to express TTR, which is a possible marker for PTPR (Shibahara et al. 2004; Rodrigeus et al. 2001). The expression of CK 18 and TTR in PTPR are in agreement with the hypothesis that PTPR originates from the SCO. EMA immunoreactivity in PTPR is frequently observed on the cell surface, particularly on the poles facing the vessels in some papillae. The ring- and dot-like staining pattern is considered as a highly characteristic finding of PTPRs, reflecting their ependymal origin (Kuchelmeister et al. 2006). EMA-immunoreactivity in PTPR was reported to be weaker than that in choroid plexus tumor (Cruz-Sanchez et al. 1989).

SCO-spondin has been reported to play a role in the development of Reissner's fiber (RF), which is extensively involved in neuronal differentiation (Meiniel 2001). This may provide evidence to support the fact that PTPR shows immunoreactivity for some kinds of neuronal markers.

Differential Diagnosis

The differential diagnosis of PTPR includes papillary pineocytomas, pineal parenchymal tumors, papillary ependymoma, CP tumors, and metastatic tumors. PTPRs have been most frequently misdiagnosed as ependymomas or CP tumor because of the morphological similarities among these tumors (Yano et al. 2003; Hasselblatt et al. 2006). PTPR is characterized by an epithelial-like growth pattern in which the

thick fibrovascular core in the papillae is covered by several layers of columnar or cuboidal cells (Kern et al. 2006). The misdiagnosis of PTPR as papillary ependymoma can be attributed to these histological findings. Papillary ependymomas lack distinct thick fibrovascular cores and fibrillary stromal backgrounds. PTPRs are composed of a greater number of epithelial cells than that in ependymomas and lesser number of papillary cells than choroid plexus (CP) tumors (Jouvet et al. 2003). Immunohistochemical analysis revealed that a papillary ependymoma is usually positive for EMA, GFAP (Jouvet et al. 2003), and NCAM (Chang et al. 2008), but negative for CK 18 (Kern et al. 2006), E-cadherin (Fèvre-Montange et al. 2006), and MAP2 (Hasselblatt et al. 2006).

Cytokeratin is one of the diagnostic markers for PTPR; however, ependymoma is generally negative for cytokeratin, except for AE1 and AE3, and shows only weak focal staining (Kern et al. 2006). EMA immunoreactivity in ependymomas is usually seen in the form of dot- and ring-like staining patterns; therefore, the immunoreactivity in PTPR reflects its ependymal nature (Fèvre-Montange et al. 2006; Kuchelmeister et al. 2006). Ependymomas are distinctly positive for GFAP; however, it has been reported that only focal immunostaining for GFAP is seen at the perivascular areas in the periphery of some PTPR cells (Jouvet et al. 2003; Fèvre-Montange et al. 2006). In addition, ependymomas do not express E-cadherin but strongly express NCAM; since this pattern is also seen in PTPR, it reflects the ependymal nature of the tumor (Figarella-Branger et al. 1995). The immunoreactivities for MAP2 (Blümcke et al. 2004), NSE, and NeuN (Preusser et al. 2006) were reported to be negative in the majority of the cases of classic ependymoma; in contrast, PTPRs demonstrate distinct patterns in staining for MAP2 (Hasselblatt et al. 2006), NSE (Jouvet et al. 2003), and NeuN (Yano et al. 2009). Ependymomas are generally negative for Syn, and immunoreactivity for Syn has been reported in a few cases. Therefore, critical diagnostic findings for PTPR and the distinguishing point between PTPR and ependymoma are CK 18 expression and neuronal marker, including MAP2, NSE, and NeuN.

The thick fibrovascular cores covered by several layers of columnar to cuboidal cells are not observed in choroid plexus papilloma in which papillae with broad fibrovascular cores are generally covered with a monolayer of cuboidal or columnar epithelial cells (Kern et al. 2006). Immunohistochemical analysis revealed that both CP tumors and PTPRs express cytokeratin and TTR; however, CP tumors can be distinguished from PTPRs by negative staining for neuronal markers, including MAP2, NSE, Syn, and NeuN (Fèvre-Montange et al. 2006). Immunohistochemical analyses revealed mature neuronal differentiation in PTPR, which may help to distinguish the tumor from CP tumors (Hasselblatt et al. 2006; Yano et al. 2009). CP tumors usually show stronger EMA-positivity, without dot- and ring-like patterns, than that in PTPRs (Kuchelmeister et al. 2006). The differential expression of NCAM and E-cadherin in PTPRs and CP tumors can also serve as additional diagnostic criteria (Fèvre-Montange et al. 2006). The intense immunoreactivity to NCAM is a likely characteristic of PTPRs, but not of CP tumors (Figarella-Branger et al. 1995). In contrast, E-cadherin immunoreactivity is a likely characteristic of CP tumors, but not of PTPRs. The majority of PTPRs is characterized by lack of membrane staining for Kir7.1 and lack of cytoplasmic staining of stanniocalcin-1, both of which are positive for CP tumors (Hasselblatt et al. 2006). On the other hand, CP tumors are generally considered to be negative for neuronal markers, including MAP2 and NeuN. Therefore, the distinguishing characteristic between PTPRs and CP tumors is immunoreactivity for NCAM and neuronal markers, including MAP2 and NeuN.

Generally, other papillary tumors in the pineal region, including papillary pineocytoma, pineoblastoma, astroblastoma, medulloepithelioma, yolk sac tumor, and metastatic papillary carcinoma, can be easily distinguished from PTPR on the basis of their serological tumor markers, clinical neuroradiological findings, and histopathological features.

References

Blümcke I, Müller S, Buslei R, Riederer BM, Wiestler OD (2004) Microtubule-associated protein-2 immunoreactivity: a useful tool in the differential diagnosis of low-grade neuroepithelial tumors. Acta Neuropathol 108:89–96

Boco T, Aalaei S, Musacchio M, Byrne R, Cochran E (2008) Papillary tumor of the pineal region. Neuropathology 28:87–92

Cenacchi G, Glangaspero F (2004) Emerging tumor entities and variants of CNS neoplasms. J Neuropathol Exp Neurol 63:185–192

Chang AH, Fuller GN, Debnam JM, Karis JP, Coons SW, Ross JS, Dean BL (2008) MR imaging of papillary tumor of the pineal region. AJNR Am J Neuroradiol 29:187–189

Cruz-Sanchez FF, Rossi ML, Hughes JT, Coakham HB, Figols J, Eynaud PM (1989) Choroid plexus papillomas: an immunohistological study of 16 cases. Histopathology 15:61–69

Dagnew E, Langford LA, Lang FF, DeMonte F (2007) Papillary tumor of the pineal region: case report. Neurosurgery 60:953–955

Fevre-Montange M, Champier J, Szathmari A, Brisson C, Reboul A, Mottolese C, Fauchon F, Claustrat B, Jouvet A (2006) Prognosis and histopathologic features in papillary tumors of the pineal region: a retrospective multicenter study of 31 cases. J Neuropathol Exp Neurol 65:1004–1011

Figarella-Branger D, Lepidi H, Poncet C, Gambarelli D, Bianco N, Rougon G, Pellissier JF (1995) Differential expression of cell adhesion molecules (CAM), neural CAM, and epithelial cadherin in ependymomas and choroid plexus tumors. Acta Neuropathol 89:148–157

Hasselblatt M, Blümcke I, Jeibmann A, Rickert CH, Jouvet A, van de Nes JA, Kuchelmeister K, Brunn A, Fevre-Montange M, Paulus W (2006) Immunohistochemical profile and chromosomal imbalances in papillary tumors of the pineal region. Neuropathol Appl Neurobiol 32:278–283

Hirato J, Nakazato Y, Iijima M, Yokoo H, Sasaki A, Yokota M, Ono N, Hirato M, Inoue H (1997) An unusual variation of ependymoma with extensive tumor cell vacuolization. Acta Neuropathol 93:310–316

Jouvet A, Fauchon F, Liberski P, Saint-Pierre G, Didier-Bazes M, Heitzmann A, Delisle MB, Biassette HA, Vincent S, Mikol J, Streichenberger N, Ahboucha S, Brisson C, Belin MF, Fèvre-Montange M (2003) Papillary tumor of the pineal region. Am J Surg Pathol 27:505–512

Kawahara I, Tokunaga Y, Yagi N, Iseki M, Abe K, Hayashi T (2007) Papillary tumor of the pineal region-case report. Neuro Med Chir (Tokyo) 47:568–571

Kern M, Robbins P, Lee G, Watson P (2006) Papillary tumor of the pineal region—a new pathological entity. Clin Neuropathol 25:185–192

Kuchelmeister K, Hügens-Penzel M, Jödicke A, Schachenmayr W (2006) Papillary tumor of the pineal region: histodiagnostic considerations. Neuropathol Appl Neurobiol 32:203–208

Lee MK, Rebhun LI, Frankfurter A (1990) Posttranslational modification of class III β-tubulin. Proc Natl Acad Sci USA 87:7195–7199

Louis DN, Ohgaki H, Wiestler OD, Cavence WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumors of the central nervous system. Acta Neuropathol 114:97–109

Meiniel A (2001) SCO-spondin, a glycoprotein of the subcommissural organ/Reissner's fiber complex: evidence of a potent activity on neuronal development in primary cell cultures. Microsc Res Tech 52:484–495

Nakamura H, Makino K, Kochi M, Nakazato Y, Kuratsu J (2009) Successful treatment of neoadjuvant therapy for papillary tumor of the pineal region. Brain Tumor Pathol 26:73–77

Preusser M, Laggner U, Haberler C, Heinzl H, Budka H, Hainfellner JA (2006) Comparative analysis of NeuN immunoreactivity in primary brain tumours: conclusions for rational use in diagnostic histopathology. Histopathology 48:438–444

Rodrigeus EM, Oksche A, Montecinos H (2001) Human subcommissural organ, with particular emphasis on its secretory activity during the fetal life. Microsc Res Tech 52:573–590

Roncaroli F, Scheithauer BW (2007) Papillary tumor of the pineal region and spindle cell oncocytoma of the pituitary: new tumor entities in the 2007 WHO classification. Brain Pathol 17:314–318

Shibahara J, Todo T, Morita A, Mori H, Aoki S, Fukayama M (2004) Papillary neuroepithelial tumor of the pineal region. A case report. Acta Neuropathol 108:337–340

Yano H, Oka N, Okumura A, Shinoda J, Sakai N (2003) Clinicopathological study of recurrent ependymoma in childhood (in Japanese). Nerv Syst Child 28:274–281

Yano H, Ohe N, Nakayama N, Shinoda J, Iwama T (2009) Clinicopathological features from long-term observation of a papillary tumor of the pineal region (PTPR): a case report. Brain Tumor Pathol 26:83–88

Pineal Parenchymal Tumors: Immunohistochemistry

4

Wiesław Marcol, Izabela Malinowska, Joanna Lewin-Kowalik, Katarzyna Kotulska, Wiesława Grajkowska, Magdalena Larysz-Brysz, and Marek Mandera

Contents

Abstract

Pineal parenchymal tumors (PPTs) are rare intracranial neoplasmatic growths in pineal region, affecting both children and adults. The current WHO classification of PPTs include: pineocytoma, pineoblastoma, pineal parenchymal tumor of intermediate differentiation (PPTID), and papillary tumor of pineal region (PTPR). However, the biology and prognosis of these tumors remains to be better understood. Immunohistochemistry is used to look at tissue-specific antigens, like neuronal and glial markers, as well as proliferation or apoptosis markers, specific pineal markers and others. This technique may greatly contribute to recognize PPTs biology and prognosis in individual cases. Identification of reliable diagnostic markers and prognostic factors for pineal region tumors is the key challenge for investigations in this field. In this section, the review of immunohistochemical studies on those tumors is presented.

Introduction

The World Health Organization (WHO) classification of brain tumors includes 126 types of neoplasms (Ikota et al. 2006). The variety and low incidence of some of them limits systemic studies. Pineal parenchymal tumors (PPTs) present less than 1% of primary central nervous system (CNS) tumors according to Sato and

W. Marcol (✉) • J. Lewin-Kowalik • M. Larysz-Brysz
Department of Physiology, Medical University of Silesia, Katowice, Poland
e-mail: wmarcol@o2.pl

I. Malinowska
Translational Medicine Division, Brigham and Women's Hospital, Boston, MA, USA

K. Kotulska
Department of Neurology and Epileptology, The Children's Memorial Health Institute, Warsaw, Poland

W. Grajkowska
Department of pathology, The Children's Memorial Health Institute, Warsaw, Poland

M. Mandera
Division of Neurosurgery, Department of Pediatric Surgery, Medical University of Silesia, Katowice, Poland

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_4,

Kubota (2009), thus they are extremely difficult to characterize with accurate statistical evaluation. The WHO 2007 classification of PPTs includes: pineocytoma (PC Grade I), PPT with intermediate differentiation (PPT-ID Grade II/III), pineoblastoma (PB Grade IV), (Nakazato 2008; Arivazhagan et al. 2008), and papillary tumor of pineal region (PTPR Grade II/III) (Hasselblatt et al. 2006). Pineal parenchymal neoplasms represent a broad spectrum of histologic differentiation, from well-differentiating lesions to rapidly growing, disseminating tumors. All those tumors may be composed of cells showing features of neuronal, glial or/and photoreceptor or retinal differentiation and papillary tumor of pineal region (Hasselblatt et al. 2006). All these tumors may be composed of cells presenting features of neuronal, glial or/and photoreceptor or retinal differentiation. Pineoblastomas are found with the highest prevalence in children (first two decades of life). PPT-IDs affect mainly young adults whereas pineocytomas are found in adults, although very rarely both types of tumors may also be found in children (Sato and Kubota 2009).

The diagnosis of pineal region tumor may be very difficult if it is based on the examination of a small amount of tissue obtained during pineal mass biopsy. Moreover, histological classification is sometimes not sufficient to predict postoperative survival in particular patients. Immunohistochemistry (IHC) adds new advantages to diagnostic procedures in pineal gland neoplasms. It may also present some prognostic value by identification of specific markers positively or negatively correlating with patients survival.

Multiple and extensive studies have been already carried on neuronal markers in PPTs with use of different antibodies against: synaptophysin, tubulin, neurofilament proteins, neural specific enolase (NSE). Glial markers were explored in less extent, because they are considered to be not informative in pineal gland tumors. Proliferative markers, like Ki-67, and indicators of apoptosis, gained more interest recently as they were found to correlate well with clinical picture of many tumors (Sato and Kubota 2009).

Immunohistochemistry: Methodology

Currently, immunohistochemistry is a widely used technique in pathology. It is based on binding of antibodies to specific antigens in examined tissues. It is performed on fresh frozen sections or paraffin embedded organs/tumors sections. Frozen tissues need to be fixed in acetone:methanol (1:1), methanol or 5% paraformaldehyde and before staining they must undergo rehydration in phosphate buffer solution (PBS). Paraffin sections are first deparaffinized in xylenes and rehydrated in set of ethanol solutions graded from 100 to 70% and then in PBS. Processing tissues in paraffin may result in change of antigens conformation. Thus, variable antigen retrieval methods are used, including high temperature treatment-boiling/microwaving, and chemical methods using reactions with citrate buffer pH 6.0 or EDTA buffer pH 8.0, or enzymes with protease activity (e.g. proteinase K, trypsin).

Final color antigen-positive reaction is usually generated by use of substrate 3,3-diaminobenzidine (DAB- black, blue, brown) or aminoethylcarbazole (AEC- red) for enzymatic reaction catalyzed by horse-radish peroxidase (HRP). To avoid non-specific background staining, peroxidase blocking solution (e.g. 0.3% hydrogen peroxide in methanol) is used before specific antibody application. The antibody against the antigen of interest is the primary antibody (I) that can be directly conjugated to HRP, but more often is detected by use of secondary (II) anti-species antibody-HRP conjugated by manufacturer or by additional step in protocol. Enzyme is attached to secondary antibody by biotin-avidin interaction. Though, all the preparation should be modified depending on what antibodies are used, and serum blocking (host serum for IInd antibody) and/or biotin/avidin blocking must be applied in these cases to avoid unspecific reaction. All the incubations of the slides covered with antibodies are led in wet chamber for 1 h in room temperature or overnight in 4 °C. After each incubation, washing step is necessary to remove excess of antibodies that did not bind to antigens. Working concentration of antibodies is usually established by set of

dilutions checked on positive control tissue. Traditionally hematoxylin is used as counterstain, to identify if antigen of interest is localized in nucleus or/and cytoplasm of the cells observed in bright field light microscope (Cuello 1993).

Immunofluorescence (IF)

Immunofluorescent technique is similar to immunohistochemistry, with that main difference the primary or secondary antibodies are conjugated with fluorochrome emitting light of the specific wavelength. Fluorescent microscope is needed to detect signal from antigen-positive tissues. This method is more quantitative, but also more sensitive to formalin fixation of the tissue. Using secondary antibodies with two/three different fluorochromes enables also the co-staining and co-localization of different antigens in the same tumor section (Javois 1995).

Scoring

Most pathologists and scientists use different scales or scores to describe color reaction or fluorescence, especially for statistical analysis of specific antigen expression in particular tissue.

In fluorescence methods, semiquantitative method for co-localized markers, and direct light intensity measurement can be used for this purpose. For some markers there are standard counting schemes, e.g. Ki-67 labeling index reflects the percentage (n per hundred) of Ki-67-positive tumor nuclei divided by the total number of tumor cells examined.

Pediatric Pineal Parenchymal Tumors Markers in Immunohistochemistry

Immunohistochemistry is a valuable method in pineal parenchymal tumors diagnosis. PPTs were always regarded as difficult to describe histologically unless they had typical pineocytomatous rosettes in pineocytomas or fleurettes and Homer-Wright or Flexner-Wintersteiner rosettes in pineoblastomas, and sometimes necrosis and ganglioid cells in pleomorphic type (Sato and Kubota 2009). Pineal parenchymal tumors are divided in four groups: pineoblastoma (Figs. 4.1a–c and 4.2), pineocytoma (Fig. 4.1d–f), PPT of intermediate differentiation (PPTID), and papillary tumors of pineal region (PTPR) (Fig. 4.1g–i). The biological behaviour and clinical course cannot be predicted only on the basis of histological features. Identification of specific differentiation and proliferation antigens by IHC is a valuable tool to distinguish tumor grades. Expression of specific markers in PPT can give more accurate prognosis than sole classic histologic evaluation of hematoxylin and eosin (H&E) stained samples.

Neuronal Markers

Normal pineal gland is build with pineocytes and connective tissue stroma (Fig. 4.1j–l). Pineocytes are neuroepithelial cells immunopositive for synaptophysin and neurofilaments, chromogranin A, retinal S-antigen, serotonin, and melatonin (Fig. 4.1l) (Sato and Kubota 2009). Pineocytomas correspond histologically to WHO grade I lesions and they are composed of small, mature-appearing pineocytes often forming pineocytomatous rosettes. These neoplastic cells imitate differentiation of normal pineocytes, therefore they show immunoreactivity for synaptophysin and neurofilaments, chromogranin A, serotonin, and melatonin (Hirato and Nakazato 2001) (Fig. 4.1d–f). Synaptophysin is mainly found in the cytoplasm and the cytoplasmic processes. Neurofilament protein (NFP) 68 kDa is also expressed in cytoplasmatic processes, whereas the cytoplasm of pineocytoma cells is positive for neuron specific enolase (NSE), and sometimes for NFP 68 kDa, chromogranin A, β-tubulin III, and αB crystalline (Jouvet et al. 1994) (Fig. 4.1f). Photosensory differentiation is associated with immunoreactivity for retinal S-antigen and rhodopsin (Fukuda et al. 2010).

Pineoblastomas are highly malignant embryonal tumors of the pineal gland, mainly affecting children, frequently associated with CSF dissemination.

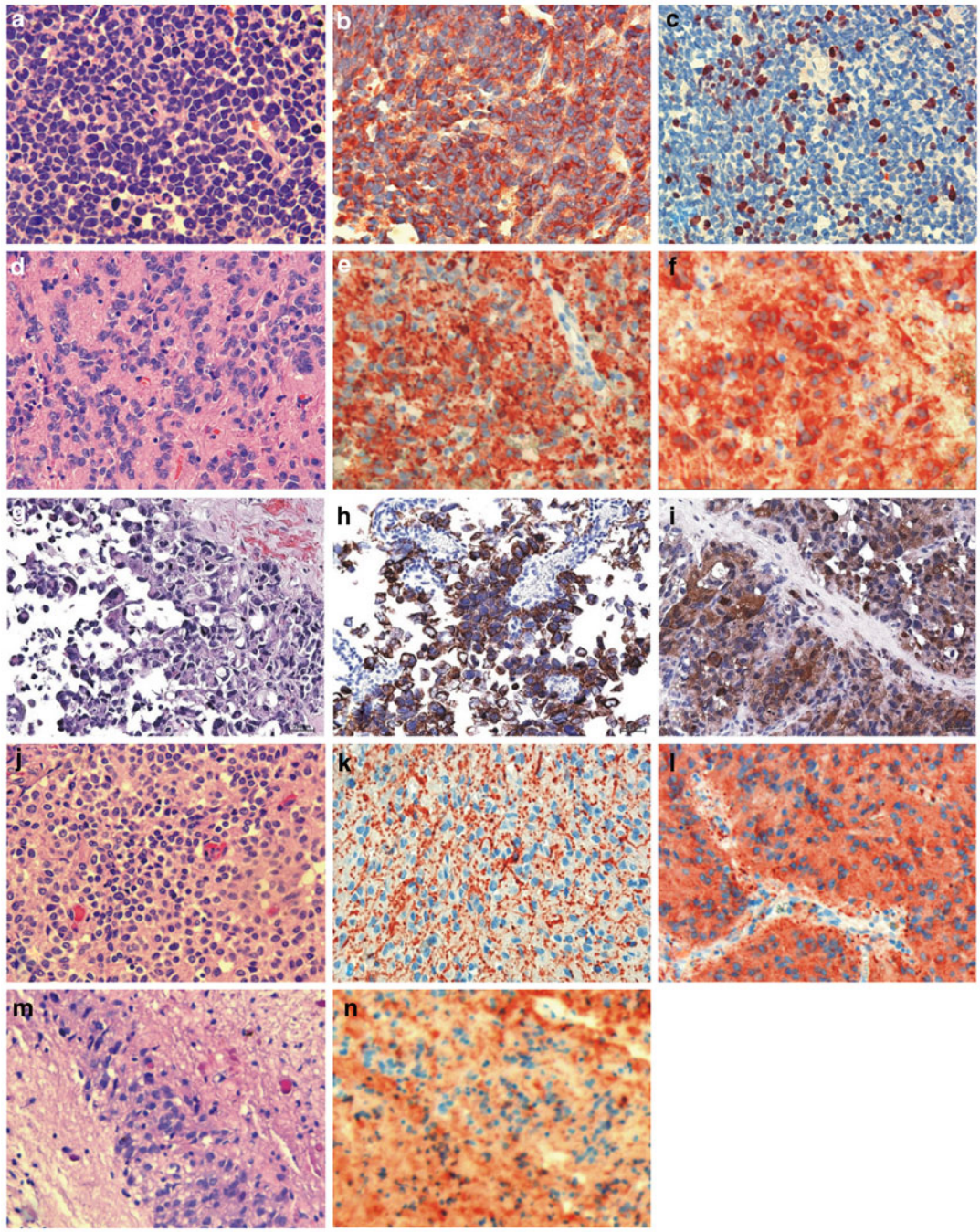

Fig. 4.1 (**a**) Pineoblastoma: diffuse sheets of small hyperchromatic cells. HE staining; (**b**) Pineoblastoma: strong expression of synaptophysin; (**c**) Pineoblastoma: high Ki-67 labeling index; (**d**) Pineocytoma: irregulary placed tumor cells around islands of neuropil. HE staining; (**e**) Pineocytoma: strong expression of neurofilament; (**f**) Pineocytoma: strong expression of NSE; (**g**) Papillary tumor of the pineal region: papillary areas. HE staining; (**h**) Papillary tumor of the pineal region: cytokeratin positivity; (**i**) Papillary tumor of the pineal region: NSE expression; (**j**) Pineal gland: uniform lobularity. HE staining; (**k**) Pineal gland: GFAP- positive astrocytes with long processes characteristic for normal pineal gland; (**l**) Pineal gland: synaptophysin- positive pineal tissue; (**m**) Pineal glial cyst: the sharp interface between glial layer and normal pineal gland. HE staining; (**n**) Pineal glial cyst: strong GFAP expression. Light microscopy. Original magnification – 40×

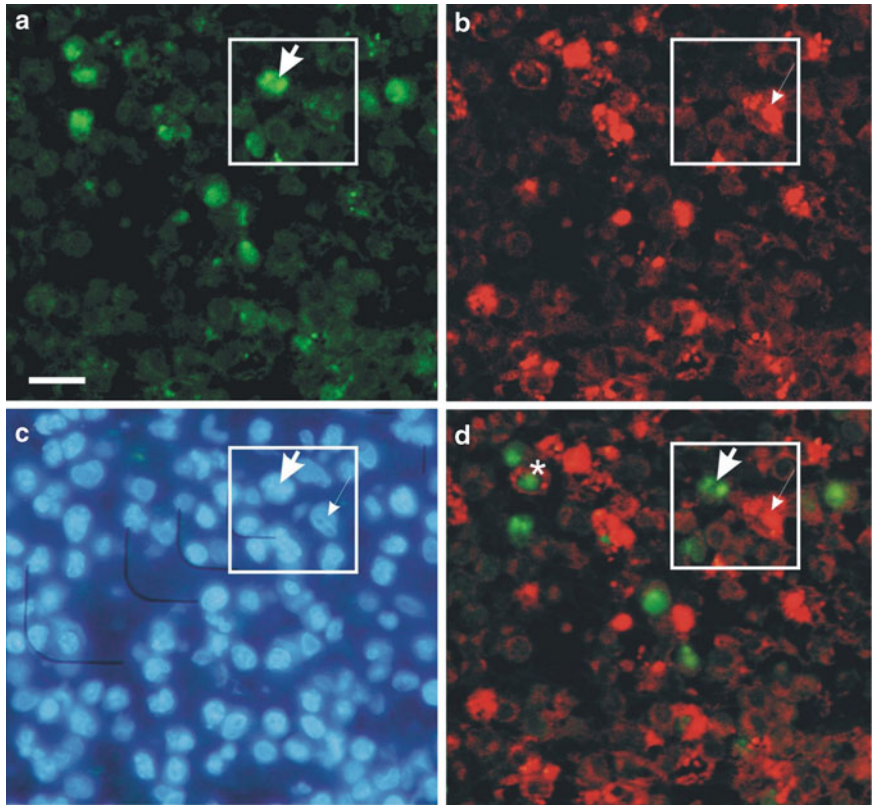

Fig. 4.2 Microphotograph of pineoblastoma specimen analyzed with the immunohistochemical double labeling demonstrating clearly nuclear positive reaction for Ki-67 (*green*, **a**), positive reaction for Bcl-2 antigen (*red*, **b**; this protein is present only in the cells' cytoplasm), DAPI stained cells found in this specimen (*blue*, **c**), and co-localisation of Ki-67/Bcl-2 (*green/red color*) only in single cells – *asterisk* (**d**). Note lack of co-localisation of these two antigens in majority of cells. *Arrows* in the *box*, examples of single labeled cells: *big arrow* presents Ki-67 positive cell, *small arrow* – Bcl-2 labeled neuron. Confocal microscopy. Original magnification – 200× (from: Marcol et al. (2006) J Mol Histol 37:5–7)

These tumors are composed of sheets of small, undifferentiated cells with round nuclei and scant cytoplasm. Pineoblastomas correspond histologically to WHO grade IV. The immunophenotype of these tumors is similar to that of pineocytomas and quite often they show expression of neural antigens with variable expression intensity (Fig. 4.1a–c). They may also show morphological features and markers of photoreceptors expressing retinal S-antigen. Synaptophysin staining is usually positive in all types of PPTs. Kumar et al. (2006) showed synaptophysin cytoplasmic reactivity in pineoblastomas, and in cytoplasm as well as fibrillary core of rosettes in pineocytomas.

Neurofilament protein 68 kDa was found to be of high diagnostic utility (Jouvet et al. 1994; Yamane et al. 2002), leading to additional PPTs reclassification by Jouvet et al. (1994) in grades according to number of mitosis and NFP positivity. Moreover, Arivazhagan et al. (2008) described neurofilament immunoreactivity as indicator of better prognosis, which correlated with patients survival, irrespective of the histological subgroup.

Marcol et al. (2009) presented recently a study on neuronal specific enolase expression in pineal region tumors. The NSE expression was weak, but present in pineoblastomas, and evident in all pineocytomas. Statistically positive correlation between patient's survival and NSE expression was found. Tubulin III was found by the same group more often and with stronger signal in pineocytomas than in pineoblastomas, and positively correlated with patients' survival.

Glial Markers

Stroma of normal pineal gland may consist of some astrocytes and they are expressing typically glial fibrillary acidic protein (GFAP) and S-100 protein (Sato and Kubota 2009) (Fig. 4.1k). Glial differentiation in pineal parenchymal tumors is very rare phenomenon, and most GFAP-positive cells are reactive astroglia entrapped in the tumor (Yamane et al. 2002) (Fig. 4.1m, n). Historically GFAP-negative (together with synaptophysin-positive) pattern was considered as a good marker distinguishing pineocytoma from glioma of astrocytic or ependymal type, which present opposite reaction (Schild et al. 1993; Kumar et al. 2006). Marcol et al. (2009) found GFAP-positive cells in 10 out of 27 pineal gland tumors: in 2 out of 11 pineoblastomas, and in 8 out of 16 pineocytomas. The difference between groups was not statistically significant, and expression of GFAP did not correlate with patients survival.

Nestin

Nestin is early neuroectodermal marker typical for immature cells that can differentiate either in neurons or in glia. In healthy adult human nestin-positive cells can be found only in dentate gyrus of the hippocampus and in olfactory bulb. Nestin intermediate filament was found by Sugawara et al. (2002) in proliferating endothelium in malignant gliomas. It was also found in many other malignant tumors in central nervous system. This marker was included in Marcol et al. (2009) work as a putative indicator of not differentiated proliferating cells in pineal parenchymal tumors. Nestin was found only in 3 of 11 pineoblastomas and intermediately differentiated PPTs, and in none of pineocytomas. Nestin showed evident negative correlation with patients' survival in this study.

Pineal Markers

Yoichi Nakazato's laboratory developed seven antibodies against pinealocytes: PP1-PP7, and three antibodies reacting with pineal interstitial cells: PI1, PI2, PX1 (Yamane et al. 2002). These antibodies show different patterns of immunoreactivity in normal pineal glands. PP1, PP4 and PP6 show granular stain in cytoplasm, whereas PP2 and PP5 are diffused in cytoplasm. PP3 is a membranous antigen. PP7 labels apical parts of pinealocytes and cell processes (Ikota et al. 2006; Yamane et al. 2002). Only PP5 was found to be useful in discrimination astrocytic versus oligodendroglial tumors of central nervous system (Ikota et al. 2006). The other two: PP1 and PP6 show significant differences of reactivity between pineocytoma, intermediate differentiated PPT, and pineoblastoma. Expression of PP1 and PP6 in pineocytoma was strong, in pineoblastoma it was very weak, and was average in intermediate differentiated PPT (Yamane et al. 2002). Tumors do not stain positively for interstitial cells markers PI1, PI2, PX1. Only GFAP-immunoreactive cells seem to have also PI1, PI2 antigens present, but usually they are not part of tumor, but normal or reactive astrocytes.

Hydroxyindole-*O*-methyltransferase(HIOMT) catalyzes the final reaction in melatonin synthesis. In normal pineal gland, HIOMT is expressed in pineal parenchymal cells. It is also expressed in pineal parenchymal tumors, including pineocytoma, pineal parenchymal tumor of intermediate differentiation, and pineoblastoma (Fukuda et al. 2010). There was an association between tumor biology and the percentage of HIOMT-positive cells reported: the lower the differentiation of the tumor, the lower the percentage of HIOMT-immunoreactive cells. As shown by Fèvre-Montagne et al. (2008a, b), PTPR does not express HIOMT. Tryptophan hydroxylase (TPH), another enzyme involved in melatonin biosynthesis, was found to be expressed in PPT cells (Fèvre-Montagne et al. 2008a, b), but not in PTPR.

Proliferative Markers

Proliferative index is made on basis of counting Ki-67 (MIB-1)-positive nuclei per hundred tumor cells (Figs. 4.1c and 4.2). It is higher in pineoblastomas (>8%), and pineocytomas with anaplasia (<7%) than in PPTID (3–10%), and pineocytomas

(0.27%) so correlates with proliferative potential of tumor (Sato and Kubota 2009). The MIB-1 labelling index of normal parenchymal cells of pineal gland is typically zero. Though, Tsumanuma et al. (1999) and Arivazhagan et al. (2008) found it rather not predictive for tumor recurrence. Interestingly, they noticed MIB-1 index to be lower in neurofilament protein-positive cases, suggesting that NF-protein may be associated with good prognosis.

Apoptotic Markers

Apoptosis is a programmed cell death. It is supposed to be the natural way of elimination of abnormal cells in healthy organism. This process is disregulated in tumors and leads to neoplastic cells expansion and accumulation of DNA aberrations. In normal pineal body, Marcol et al. (2006) described apoptotic index at almost invisible level with Bcl-2 expression in 0.7% of cells. There were no Bax-immunopositive pinealocytes. Bcl-2-positive cells were mature neurons, neither immature ones nor glia. The same authors studied the apoptotic markers in pineal parenchymal tumors (Marcol et al. 2009). Bcl-2 expression profile was higher in pineoblastomas when compared to pineocytomas, and strongly correlated with patients' shorter survival after surgery (Fig. 4.2). Overexpression of Bcl-2 was established as independent prognostic factor, even if found in tubulin- and NSE-positive cells. No significant differences in Bax expression were found. Bax was present in GFAP- positive cells only.

p53 Protein

Protein p53 is tumor suppressor protein, playing an important role in cell cycle regulation. Mutations in p53-encoding gene may lead to abnormal localization of the protein and cell cycle dysregulation. In pineal parenchymal tumor its role is controversial. Tsumanuma et al. (1995) in immunohistochemical analysis revealed no positive staining for p53 protein in pineal region tumors. Molecular genetic testing revealed that p53 gene mutation is rare in pineal gland tumors. Marcol et al. 2009 found positive staining for p53 in 7 out of 27 cases. In most of them, the reaction was weak, still it correlated negatively with survival time in those cases.

In conclusion, Jouvet et al. (1994) was first to state that standard histological examination is insufficient to give the detailed diagnosis and prognosis in pineal parenchymal tumors in subgroups, and immunohistochemistry helps in accurate evaluation. Immunohistochemistry is a valuable technique, but it should be kept in mind that PPTs are very rare tumors, so well-designed multicenter studies are required for identification of reliable diagnostic and prognostic markers. Retrospective studies, however, indicate that some neuronal, glial, pineal, proliferative, apoptotic, and other markers might have not only diagnostic but also prognostic value. Neurofilament protein, neuron specific enolase and tubulin are neuronal markers which correlate positively with patients' survival (Yamane et al. 2002; Marcol et al. 2009). Better prognosis is independent of histological types of pineal parenchymal tumors, as far as they are immunoreactive for those markers (Arivazhagan et al. 2008). Presence of neuronal markers does not mean these are really mature neurons, and can be misleading. Co-expression of Bcl-2 anti-apoptotic factor and NSE- or beta III tubulin is associated with markedly worse prognosis (Marcol et al. 2009). Tumors positive for nestin and p53 protein were also shown to be characterized by shorter survival of patients. Proliferative index based on Ki-67 (MIB-1)-positive cells count correlates negatively with patients's survival. Nevertheless, when index is low (up to 7%), even pleomorphic pineocytomas and PPTID may have benign clinical outcome (Fèvre-Montange et al. 2008a, b). Glial markers, like GFAP, are not distinctive in pineal parenchymal tumors, but can help in differential diagnosis (Schild et al. 1993). Pineal markers also have limited value. Only PP1 and PP6 antibodies distinguish histological types of PPTs by different intensity of signal (Yamane et al. 2002), but do not give favorable or unfavorable prediction.

PTPR is a rare neuroepithelial tumor of the pineal region mainly in adults, characterized by papillary architecture and epithelial feature. This tumor may corresponds to grades II or III (Hirato and Nakazato 2001). The most characteristic immunohistochemical feature of PTPR is the reactivity neoplastic cells for keratin (AE1/AE3, CAM5.2, CK18). Focal GFAP immunoexpression may be seen. PTPRs reveal expression of vimentin, S-100 protein, NSE, MAP2, N-CAM. NFP immunoreactivity is never seen, while the neuroendocrine markers such as synaptophysin and chromogranin may be focally expressed. The Ki-67 labelling index is moderate, highest in tumors of young patients (Hirato and Nakazato 2001).

References

Arivazhagan A, Anandh B, Santosh V, Chandramouli BA (2008) Pineal parenchymal tumors – utility of immunohistochemical markers in prognostication. Clin Neuropathol 27:325–333

Cuello AC (1993) Immunohistochemistry II, vol 14, IBRO handbook series: methods in the neurosciences. Wiley, Chichester, 474 p

Fèvre-Montange M, Szathmari A, Champier J, Mokhtari K, Chrétien F, Coulon A, Figarella-Branger D, Polivka M, Varlet P, Uro-Coste E, Fouchon F, Jouvet A (2008a) Pineocytoma and pineal parenchymal tumors of intermediate differentiation presenting cytologic pleomorphism: a multicenter study. Brain Pathol 18:354–359

Fèvre-Montange M, Champier J, Szathmari A, Brisson C, Reboul A, Mottolese C, Fouchon F, Claustrat B, Jouvet A (2008b) Histological features and expression of enzymes implicated in melatonin synthesis in pineal parenchymal tumours and in cultured tumoural pineal cells. Neuropathol Appl Neurobiol 34:296–305

Fukuda T, Akiyama N, Ikegami M, Takahashi H, Sasaki A, Oka H, Komori T, Tanaka Y, Nakazato Y, Akimoto J, Tanaka M, Okada Y, Saito S (2010) Expression of hydroxyindole-O-methyltransferase enzyme in the human central nervous system and in pineal parenchymal cell tumors. J Neuropathol Exp Neurol 69:498–510

Hasselblatt M, Blümcke I, Jeibmann A, Rickert CH, Jouvet A, van de Nes JA, Kuchelmeister K, Brunn A, Fèvre-Montange M, Paulus W (2006) Immunohistochemical profile and chromosomal imbalances in papillary tumours of the pineal region. Neuropathol Appl Neurobiol 32:278–283

Hirato J, Nakazato Y (2001) Pathology of pineal region tumors. J Neurooncol 54:239–249

Ikota H, Kinjo S, Yokoo H, Nakazato Y (2006) Systematic immunohistochemical profiling of 378 brain tumors with 37 antibodies using tissue microarray technology. Acta Neuropathol 111:475–482

Javois LC (1995) Immunocytochemical methods and protocols, vol 34, Methods in molecular biology. Humana Press, Totowa, 456 p

Jouvet A, Fèvre-Montange M, Besançon R, Derrington E, Saint-Pierre G, Belin MF, Pialat J, Lapras C (1994) Structural and ultrastructural characteristics of human pineal gland, and pineal parenchymal tumors. Acta Neuropathol 88:334–348

Kumar P, Tatke M, Sharma A, Singh D (2006) Histological analysis of lesions of the pineal region: a retrospective study of 12 years. Pathol Res Pract 202:85–92

Marcol W, Kotulska K, Larysz-Brysz M, Malinowska-Kołodziej I, Mandera M, Lewin-Kowalik J (2006) Expression of Bcl-2 and Bax protein in normal pineal gland in children and young adult. J Mol Histol 37:5–7

Marcol W, Mandera M, Grajkowska W, Malinowska-Kołodziej I, Olakowska E, Właszczuk A, Lewin-Kowalik J (2009) Apoptotic proteins and markers of differentiation in childhood pineal parenchymal tumors: relationships and prognostic value. Cen Eur Neurosurg 70:115–124

Nakazato Y (2008) Revised WHO classification of brain tumours. Brain Nerve 60:59–77

Sato K, Kubota T (2009) Pathology of pineal parenchymal tumors. Prog Neurol Surg 23:12–25

Schild SE, Scheithauer BW, Schomberg PJ, Hook CC, Kelly PJ, Frick L, Robinow JS, Buskirk SJ (1993) Pineal parenchymal tumors. Clinical, pathologic, and therapeutic aspects. Cancer 72:870–880

Sugawara K, Kurihara H, Negishi M, Saito N, Nakazato Y, Sasaki T, Takeuchi T (2002) Nestin as a marker for proliferative endothelium in gliomas. Lab Invest 82:345–351

Tsumanuma I, Sato M, Okazaki H, Tanaka R, Washiyama K, Kawasaki T, Kumanishi T (1995) The analysis of p53 tumor suppressor gene in pineal parenchymal tumors. Noshuyo Byori 12:39–43

Tsumanuma I, Tanaka R, Washiyama K (1999) Clinicopathological study of pineal parenchymal tumors: correlation between histopathological features, proliferative potential, and prognosis. Brain Tumor Pathol 16:61–68

Yamane Y, Mena H, Nakazato Y (2002) Immunohistochemical characterization of pineal parenchymal tumors using novel monoclonal antibodies to the pineal body. Neuropathology 22:66–76

Pineal Parenchymal Tumors: Diagnostics and Prognosis

5

Wiesław Marcol, Marek Mandera, Joanna Lewin-Kowalik, Izabela Malinowska, and Grzegorz Kiwic

Contents

Abstract

Pineal parenchymal tumors (PPTs) are heterogeneous population of rare tumors with hard-to-predict clinical outcome. Their diagnosis is also problematic, as there are no specific and characteristic markers. Radiological imaging is mostly useful; however it does not give enough data about tumor type. There is a strong need to work out methods enabling as well earlier recognition and better prognosis of outcome of the disease as assessment of therapeutic effectiveness. In this chapter we summarize present, however still very limited, information about PPTs.

Introduction

Pineal region tumors are comparatively rare as they represent 0.4–1.0% of all intracranial tumors irrespective of age (Russel and Rubinstein 1977), for north-eastern Asian countries they account most frequently for 2.0–8% (Sano et al. 1983). They are relatively more common in children and young people accounting for 3–11% of all pediatric intracranial tumors (Cho et al. 1998; Hoffman et al. 1994). Among them, the most common are germ cell tumors. Pineal parenchymal tumors (PPTs) are rare and determine 14–30% of all tumors of the pineal region (Jouvet et al. 1994; Hirato and Nakazato 2001).

Pineal parenchymal tumors develop from pineocytes or their embryonic precursors, and they are ranging from tumors composed of

W. Marcol (✉) • J. Lewin-Kowalik
Department of Physiology, Medical University of Silesia, Katowice, Poland
e-mail: wmarcol@o2.pl

M. Mandera
Division of Neurosurgery, Department of Pediatric Surgery, Medical University of Silesia, Katowice, Poland

I. Malinowska
Translational Medicine Division, Brigham and Women's Hospital, Boston, MA, USA

G. Kiwic
Department of Neurosurgery, Provincial Specialistic Hospital, Jastrzebie-Zdroj, Poland

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_5,

mature elements to primitive immature cells. They can be divided into three histological types: pineocytoma, pineoblastoma and pineal parenchymal tumor of intermediate differentiation. Pineocytomas are well differentiated, slow-growing neoplasms composed of small mature pineocyte-resembling cells corresponding histologically to WHO grade II (Sato and Kubota 2009). However, pineocytomas are not as homogenous group as it is widely believed, particularly in younger patients, they can behave aggressively and have a high rate of recurrence (Deshmukh et al. 2004). Pineoblastomas are poorly differentiated, malignant, embryonic neoplasms corresponding histologically to WHO grade IV with a highly malignant biological behaviour (Karasek et al. 2007). Pineal parenchymal tumors of intermediate differentiation show an intermediate histological grade of malignancy with an unpredictable growth rate and clinical behaviour (Karasek et al. 2007).

Pineocytomas and pineoblastomas constitute respectively 45% of PPTs whereas pineal parenchymal tumors of intermediate differentiation make ca.10% of the entire group (Fauchon et al. 2000; Mena et al. 2000). Pineocytomas do not show any preference for sex while pineoblastoma shows a slight male predominance. They happen in patients of various age groups. Pineoblastomas tend to occur in the first two decades of life whereas pineocytoma in adults, especially in the third and fourth decade of life (Cho et al. 1998; Mena et al. 2000).

Clinical Symptomatology

The interval between initial symptoms and treatment may be less then month for pineoblastoma. Two distinct groups of symptoms may be distinguished in patients with pineal tumors, regardless of their histological forms:

- symptoms of increased intracranial pressure caused by obstructive hydrocephalus as a result of aqueduct occlusion by a tumor mass
- focal neurological deficits due to the compression or invasion of the tumor on neighboring neural structures; brain stem, thalamus or cerebellum.

The signs of increased intracranial pressure are the most common and usually first manifestations of pineal region tumors. Headache, nausea and vomiting constitute typical presentation of the increased intracranial pressure syndrome. Blurred or double vision, papilledema and drowsiness in the later stage are also commonly found (Kang et al. 1998; Mandera and Bazowski 2004).

The pathognomonic symptom for pineal and tectal region tumors is Parinaud's syndrome developing from a compression on the quadrigeminal plate. In Parinaud's syndrome, the paralysis of conjugate upward gaze with frequently impaired pupillary light reaction in the presence of intact accommodative response are observed. Other symptoms resulting from the pressure of adjacent brain structures, such as cerebellar ataxia, hemiparesis, changes in mental status, hypothalamic-based endocrine abnormalities rarely appear (Kang et al. 1998; Mandera and Bazowski 2004).

Diagnosis

Radiology

On CT examination, pineocytoma is usually a regular, spherical tumor less than 3 cm in diameter with frequently found calcifications or occasional cystic changes. Pineoblastoma often attains larger size and is less regular in shape. Although computed tomography (CT) allows identification of the pineal tumor, the magnetic resonance imaging (MRI) provides more detailed information and is superior in visualizing the tumor. Satoh et al. (1995) reported that PPTs are usually sharply demarcated and smoothly marginated from the normal brain tissue on MRI. They exhibit either iso- or hypointensity on T1-weighted images. On T2-weigthted images, pineocytoma shows usually high intensity while pineoblastoma appears as low-intensity lesion. The gadolinum enhancement of these lesions is usually heterogeneous (Satoh et al. 1995) (Figs. 5.1 and 5.2). Tumor relations to the quadrigeminal plate and deep veins are essential in the aspect of a planned surgical approach. The deep location, narrow

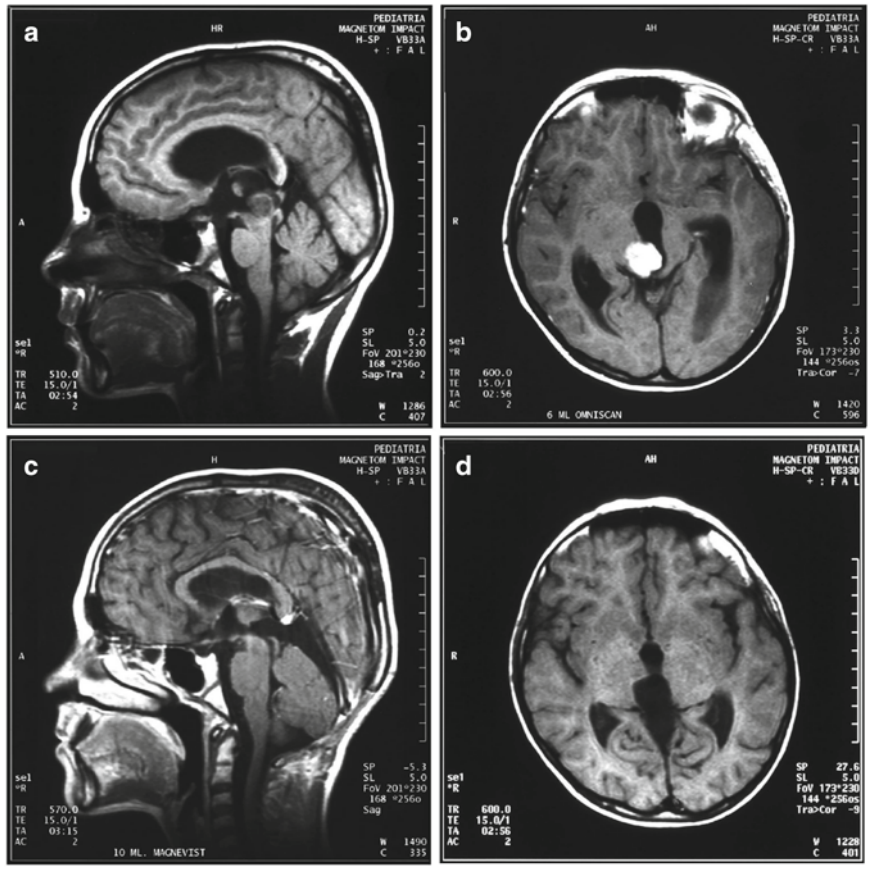

Fig. 5.1 Magnetic resonance imaging of pineocytoma. (**a**) and (**c**) Sagittal T1-weighted MRI without gadolinium; (**b**) and (**d**) axial T1-weighted MRI with gadolinium. *Upper panel* – before surgery; *lower panel* – after total surgical removal of the tumor

confines and density of vascular structures require detailed analysis of parenchymal, vascular, cisternal and ventricular architecture. Therefore, MRI is a first choice examination. Unfortunately, there are no characteristic features on CT and MRI that allow to differentiate the histological forms of neoplasms (Satoh et al. 1995; Tien et al. 1990).

Differentiation of pineocytoma from simple pineal cyst is very important. Pineal cysts are diagnosed when the lesion is 8 mm long or greater in single plane, although some authors classify lesions of 5 mm in diameter as cysts, especially in cases when the smooth and thin rim (<2 mm) of enhancement is visible after application of contrast material (Sawamura et al. 1995). They are well-circumscribed, homogenous, round formations that are isointensive or only slightly hyperintense relative to CSF on MR T1-weighted images. On T2-weighted and proton density-weighted images they are hyperintense relative to CSF (Engel et al. 2000; Mandera et al. 2003).

Biochemical Markers

Some biochemical markers like alpha-fetoprotein and beta-human chorionic gonadotropin in the serum and the cerebrospinal fluid have a well documented role in the diagnostics of the pineal gland pathology. They allow diagnosis of some forms of malignant germ cell tumors, mainly endodermal sinus tumor, chorioncarcinoma or embryonal carcinoma (Washiyama et al. 1987). Unfortunately, there

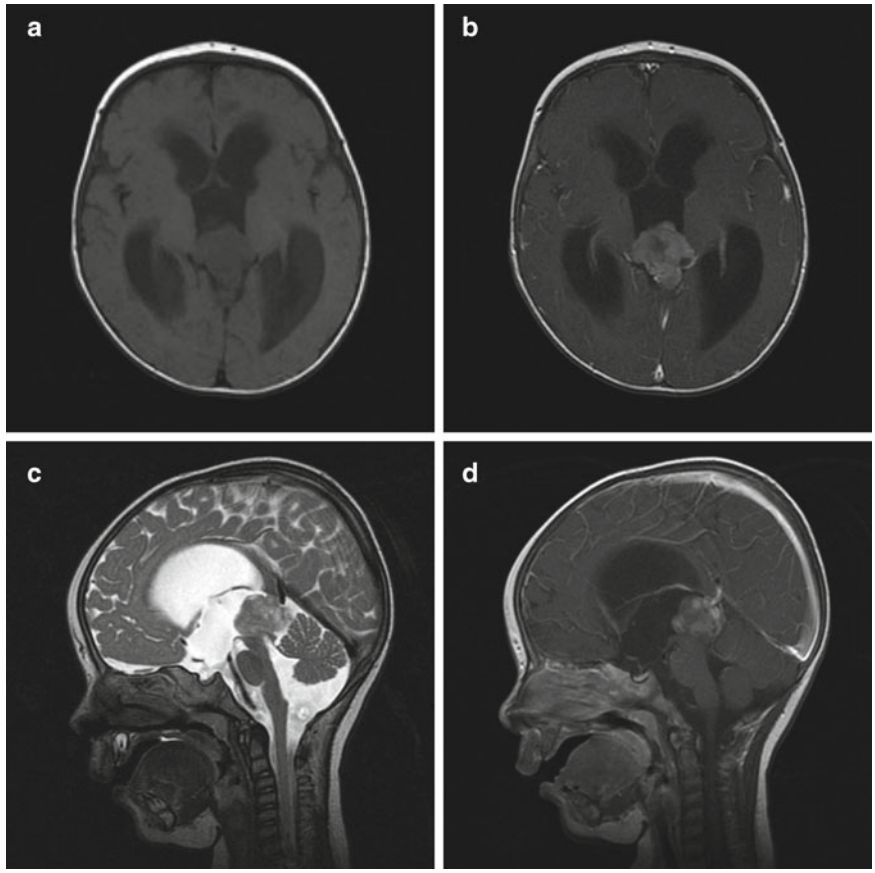

Fig. 5.2 Magnetic resonance imaging of pineoblastoma. *Upper panel*: Axial T1-weighted MRI images before (**a**) and after (**b**) gadolinium administration. *Lower panel*: Sagittal T2-weighted MRI image (**c**) and T1-weighted MRI with gadolinium (**d**)

are no biochemical markers specific both for PPTs as a whole group and for different types of PPTs.

There are also some reports on melatonin as a possible marker of PPTs (Mandera et al. 1999; Vorkapic et al. 1987). Webb and Puig-Domingo (1995) speculated that excessive secretion of melatonin could be present in tumors developing from pineal parenchyma, especially pineocytoma, while in lesions causing destruction or compression of pineal gland, e.g. teratoma or hypothalamic hamartoma, low values of melatonin should be found. Moreover, hydroxyindole-*O*-methyltransferase (HIOMT – an enzyme catalyzing the final step in melatonin synthesis) activity as well as expression of mRNA coding for HIOMT and serotonin *N*-acetyltransferase (the key enzyme in melatonin synthesis) are detectable in CSF in both pineocytoma and pineoblastoma patients (Kleihues and Cavenee 2000; Fukuda et al. 2010; Tsumanuma et al. 2000). Unfortunately, the results presented so far in the literature are insufficient and further studies on more numerous population are necessary to confirm melatonin usefulness as the PPTs biochemical marker. Some pineal cysts occasionally contain fibrillary astrocytes or Rosenthal fibers and demonstrate reactivity to glial fibrillary acid protein and the S100 protein stains (Al-Holou et al. 2010).

Cerebrospinal Fluid (CSF) Cytology

Apart from neuroimaging study and the detection of biochemical markers, a CSF cytology also provides an essential information in the presurgical

diagnostics. The presence of malignant cells in CSF indicates dissemination of the tumor along pathways of CSF flow. Neoplastic cells found in CSF are particularly common in cases of pineoblastoma and malignant germ cell tumors, reaching 45–100% of the cases depending upon the stage of the disease (Fauchon et al. 2000; Shibamoto et al. 1994) and 10–15% for low and intermediate grade PPTs (Luther et al. 2010).

Stereotactic and Endoscopic Biopsy

As histological verification is necessary for choosing an optimal therapeutic strategy, stereotactic and endoscopic or open biopsy is of some importance in diagnostics of pineal region tumors, however there are many controversies of their diagnostic value and safety.

Endoscopic biopsy allows not only to obtain tumor specimens for histopathological examination but also to treat hydrocephalus by performing ventriculocysternostomy (VCS) during one procedure. Therefore, it is recommended in cases with coexisting hydrocephalus. Despite of no significant increase of leptomeningeal dissemination followed by VCS, some authors recommend non-symptomatic patients a follow-up with spine MR imaging or CSF cytology (Ahn and Goumnerova 2010). Stereotactic biopsy may be considered in cases with normal size of the ventricles. Both methods are relatively low invasive, however a significant risk of hemorrhagic complications should be taken into consideration. The other disadvantage is that a small size of the tumor samples obtained with both types of biopsy may be a reason of false histological diagnosis (Oi et al. 2000; Regis et al. 1996; Ferrer et al. 1997). As technology has continued to improve, surgical goals have progressed from biopsy to save and complete tumor removal. PPTs have either been approached surgically (gross-total resection for benign tumors) with or without conventional external-bean radiation therapy. The radiosurgery can be successfully used in conjunction with a reduced-dose of craniospinal radiation planned as a boast modality (Lekovic et al. 2007). The most effective procedure for PPTs treatment relies on multidisciplinary approach combining surgery, radiation and/or radiosurgery and chemiotherapy if applicable.

Prognosis

Prognosis of patients with pineal region tumors correlates strongly with histology of the neoplasm. Pineocytoma has a good prognosis, although in some cases recurrence of the tumor is possible, especially if it is not totally removed. Five-year survival rate is estimated at 67–86% of patients with pineocytoma (Fauchon et al. 2000; Schild et al. 1993). The only exception is an extremely rare form of PPT – papillary pineocytoma – which has a poor outcome and needs aggressive treatment (Kawahara et al. 2007; Marcol et al. 2007).

Prognosis in pineoblastoma case is very poor. According to Fauchon et al. (2000) and Mena et al. (1995), average time of survival ranges from 16 to 25 months and 5-year survival rate was reported in 15% of pineoblastoma (Lapras et al. 1999). Pineal parenchymal tumors of intermediate differentiation are very rare and only limited data are available regarding their biologic and clinical characteristics. Thus, the prognosis in this entity is in many aspects unpredictable. Some authors, like Fèvre-Montange et al. (2008), report benign clinical course. However, there are also reports of possible malignant behaviour or even transformation of this neoplasm to pineoblastoma with less favourable outcome (Kim et al. 2009).

In conclusion, pineal parenchymal tumors are rare and constitute only 14–30% of all pineal region tumors. They can be divided into three histological types: pineocytoma, pineoblastoma and pineal parenchymal tumor of intermediate differentiation. Recently a new and very rare form of pineal region neoplasm has been described as a papillary tumor of pineal region. Initial diagnosis of pineal region tumors is based on MR study. Unfortunately, there are no characteristic features on MRI that allow to differentiate the histological forms of neoplasms. Some biochemical markers can help in differential diagnosis, however no specific marker for PPTs exists

so far. Thus, final diagnosis of PPT is always based on histological examination of the tumor tissue obtained by open surgery and removal of the tumor or at least endoscopic or stereotactic biopsy. Treatment strategy and prognosis depend on the histology of the tumor. Pineocytoma has a good prognosis, while pineoblastoma very poor. In cases of PPTs of intermediate differentiation prognosis is unpredictable.

References

Ahn ES, Goumnerova L (2010) Endoscopic biopsy of brain tumors in children: diagnostic success and utility in guiding treatment strategies. J Neurosurg Pediatr 5:255–262

Al-Holou wN, Maher CO, Muraszko KM, Garton JL (2010) The natural history of pineal cysts in children and young adults. J Neurosurg Pediatr 5:162–166

Boco T, Aalaei S, Musacchio M, Byrne R, Cochran E (2008) Papillary tumor of the pineal region. Neuropathology 28:87–92

Cho BK, Wang KC, Nam DH, Kim DG, Jung HW, Kim HJ, Han DH, Choi KS (1998) Pineal tumors: experience with 48 cases over 10 years. Childs Nerv Syst 14:53–58

Engel U, Gottschalk S, Niehaus L, Lehmann R, May C, Vogel S, Janisch W (2000) Cystic lesions of the pineal region – MRI and pathology. Neuroradiology 42:399–402

Deshmukh VR, Smith KA, Rekate HL, Coons S, Spetzler RF (2004) Diagnosis and management of pineocytomas. Neurosurgery. 55:349–55; discussion 355–357

Fauchon F, Jouvet A, Paquis P, Saint-Pierre G, Mottolese C, Hassel MB, Chauveinc L, Sichez J, Philippon J, Schlienger M, Bouffet E (2000) Parenchymal pineal tumors: a clinicopathological study of 76 cases. Int J Radiat Oncol Biol Phys 46:959–968

Ferrer E, Santamarta D, Garcia-Fructuoso G, Caral L, Rumia J (1997) Neuroendoscopic management of pineal region tumors. Acta Neurochir (Wien) 139:12–21

Fèvre-Montange M, Szathmari A, Champier J, Mokhtari K, Chrétien F, Coulon A, Figarella-Branger D, Polivka M, Varlet P, Uro-Coste E, Fauchon F, Jouvet A (2008) Pineocytoma and pineal parenchymal tumors of intermediate differentiation presenting cytologic pleomorphism: a multicenter study. Brain Pathol 18:354–359

Fukuda T, Akiyama N, Ikegami M, Takahashi H, Sasaki A, Oka H, Komori T, Tanaka Y, Nakazato Y, Akimoto J, Tanaka M, Okada Y, Saito S (2010) Expression of hydroxyindole-O-methyltransferase enzyme in the human central nervous system and in pineal parenchymal cell tumors. J Neuropathol Exp Neurol 69:498–510

Hirato J, Nakazato Y (2001) Pathology of pineal region tumors. J Neuroncol 54:239–249

Hoffman HJ, Yoshida M, Becker LE, Hendrick EB, Humphreys RP (1994) Pineal region tumours in childhood. Pediatr Neurosurg 21:91–104

Jouvet A, Fevre-Montange M, Besancon R, Derrington E, Saint-Pierre G, Belin MF, Pialat J, Lapras C (1994) Structural and ultrastructural characteristics of human pineal gland, and pineal parenchymal tumors. Acta Neuropathol 88:334–348

Kang JK, Jeun SS, Hong YK, Park CK, Son BC, Lee IW, Kim MC (1998) Experience with pineal region tumors. Childs Nerv Syst 14:63–68

Karasek M, Mandera M, Papierz W (2007) Pineal region tumors. In: Pandi-Perumal SR, Cardinali DP (eds) Melatonin: from molecules to therapy. Nova Science Publishers, Inc, New York, pp 483–497

Kawahara I, Tokunaga Y, Yagi N, Iseki M, Abe K, Hayashi T (2007) Papillary tumor of the pineal region. Neurol Med Chir (Tokyo) 47:568–571

Kim BS, Kim DK, Park SH (2009) Pineal parenchymal tumor of intermediate differentiation showing malignant progression at relapse. Neuropathology 29:602–608

Kleihues P, Cavenee WK (2000) Pathology and genetics of tumors of the nervous system. IARC Press, Lyon, pp 115–121

Lapras C, Mottolesese C, Jouvet A (1999) Pineal region tumors. In: Choux M, Di Rocco C, Hockley A, Walker M (eds) Pediatric neurosurgery. Churchill Livingstone, London, pp 549–560

Lekovic GP, Gonzalez LF, Shetter AG, Porter RW, Smith KA, Brachman D, Spetzler RF (2007) Role of Gamma Knife in the management of pineal region tumors. Neurosurg Focus 23(6):E12

Luther N, Stetler WR Jr, Dunkel IR, Christos PJ, Wellons JC III, Souweidame MM (2010) Subarachnoid dissemination of intraventricular tumors following simultaneous endoscopic biopsy and third ventriculostomy. J Neurosurg Pediatr 5:61–67

Mandera M, Bazowski P (2004) Surgical management of pineal region tumors in children. J Pediatr Neonat 1:36–40

Mandera M, Bażowski P, Wencel T, Dec R (1999) Melatonin secretion in patients with pineal region tumors – preliminary report. Neuroendocrinol Lett 20:167–170

Mandera M, Marcol W, Bierzyńska-Macyszyn G, Kluczewska E (2003) Pineal cysts in childhood. Childs Nerv Syst 19:750–755

Marcol W, Kotulska K, Grajkowska W, Gołka D, Własczczuk P, Drogosiewicz M, Mandera M, Lewin-Kowalik J, Roszkowski M (2007) Papillary pineocytoma in child: case report. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 151:121–123

Mena H, Rushing EJ, Ribas JL, Delahunt B, McCarthy WF (1995) Tumors of pineal parenchymal cells: a correlation of hitological features, including nucleolar organizer regions, with survival in 35 cases. Hum Pathol 26:20–30

Mena H, Nakazato Y, Jouvet A, Scheithauer BW (2000) Pineal parenchymal tumours. In: Kleihues P, Cavenee WK (eds) Pathology and genetics of tumours of the nervous system. IARC Press, Lyon, pp 115–121

Oi S, Shibata M, Tominaga J, Honda Y, Shinoda M, Takei F, Tsugane R, Matsuzawa K, Sato O (2000) Efficacy of neuroendoscopic procedures in minimally invasive preferential management of pineal region tumors: a prospective study. J Neurosurg 93:245–253

Regis J, Bouillot P, Rouby-Volot F, Figarella-Branger D, Dufour H, Peragut JC (1996) Pineal region tumors and the role of stereotactic biopsy: review of mortality, morbidity and diagnostic rates un 370 cases. Neurosurgery 39:907–914

Russel DS, Rubinstein LJ (1977) Pathology of tumors of the nervous system. Williams and Wilkins, Baltimore, pp 284–295

Sano K (1983) Pineal region tumors: problems in pathology and treatment. Clin Neurosurg 30:59–91

Sato K, Kubota T (2009) Pathology of pineal parenchymal tumors. Prog Neurol Surg 23:12–25

Satoh H, Uozumi T, Kiya K, Kurisu K, Arita K, Sumida M, Ikawa F (1995) MRI of pineal region tumours: relationship between tumours and adjacent structures. Neuroradiology 37:624–630

Sawamura Y, Ikeda J, Ozawa M, Minoshima Y, Saito H, Abe H (1995) Magnetic resonance images reveal a high incidence of asymtomatic pineal cysts in young women. Neurosurgery 37:11–15

Schild SE, Scheithauer BW, Scomberg PJ, Hook CC, Kelly PJ, Frick L, Robinow JS, Biskirk SJ (1993) Pineal parenchymal tumors. Clinical, pathologic and therapeutic aspects. Cancer 72:870–880

Shibamoto Y, Oda Y, Yamashita J, Takahashi M, Kikuchi H, Abe M (1994) The role of cerebrospinal fluid cytology in radiotherapy planning for intracranial germinoma. Int J Radiat Oncol Biol Phys 29:1089–1094

Tien RD, Barkowich AJ, Edwards MS (1990) MR imaging of pineal tumors. Am J Neuroradiol 11:557–565

Trojanowski JQ, Tascos NA, Rorke LB (1982) Malignant pineocytoma with prominent papillary features. Cancer 50:1789–1793

Tsumanuma I, Tanaka R, Ichikawa T, Washiyama K, Kumanishi T (2000) Demonstration of hydroxyindole-O-methyltransferase (HIOMT) mRNA expression in pineal parenchyma tumors: histochemical in situ hybridization. J Pineal Res 28:203–209

Vorkapic P, Waldhauser F, Bruckner R, Biegelmayer C, Schmidbauer M, Pendl G (1987) Serum melatonin levels: a new neurodiagnostic tool in pineal region tumors? Neurosurgery 21:817–824

Washiyama K, Sekiguchi K, Tanaka R, Yamazaki K, Kumanishi T, Oyake Y (1987) Immunohitochemical study on AFP, HCG, and PLAP in primary intracranial germ cell tumors. Prog Exp Tumor Res 30:296–306

Webb SM, Puig-Domingo M (1995) Role of melatonin in health and disease. Clin Endocrinol 42:221–234

Papillary Tumor of the Pineal Region: Diagnosis and Treatment

6

Alfonso Cerase and Sara Leonini

Contents

Abstract

In 2003, papillary tumor of the pineal region has been described as a distinct entity on the basis of a series of six cases with identical histological features. It is a rare neuroepithelial tumor of the pineal region, characterized by a papillary architecture and epithelial cytology, with immunopositivity for cytokeratins, and ultrastructural features suggesting ependymal differentiation. It stains for vimentin, S100 protein, NSE, MAP2, N-CAM, and TTR, while GFAP labeling is focal or absent. Possible origin from specialized ependymal cells of the subcommissural organ has been suggested. In 2007, the World Health Organization (WHO) formally recognized it as a distinct entity in the classification of tumors of the nervous system. A specific WHO grade has not been given but it has been suggested that the tumor corresponds to grade II or III. However, histological grading criteria remain to be defined. Papillary tumor of the pineal region manifests in both children and adults. Its biological behavior is variable. Clinically, it is characterized by progressive growth, frequent local recurrence (which has been reported in up to 70% of the patients), and rare spinal dissemination (which has been reported in up to 7% of the patients). The 5-year estimates for overall survival and progression-free survival have been evaluated in 73 and 27%, respectively. At neuroimaging, the tumor appears as a well-circumscribed, contrast-enhancing pineal mass. At magnetic resonance imaging, a reliable finding seems to be the

A. Cerase (✉) • S. Leonini
Unit NINT Neuroimaging and Neurointervention, Department of Neurological and Sensorineural Sciences, Azienda Ospedaliera Universitaria Senese, "Santa Maria alle Scotte" General Hospital, Siena, Italy
e-mail: alfonsocerase@gmail.com

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_6, © Springer Science+Business Media Dordrecht 2013

high-intensity signal on unenhanced T1-weighted images. When a mass of the posterior commissure or pineal region shows high-intensity signal on T1-weighted images, in the absence of fat, hemorrhage, melanin, or calcification, the diagnosis of a papillary tumor of the pineal region may be suggested so that specific immunohistochemical studies can be performed for a definitive diagnosis. Treatment of choice is surgery followed by irradiation, though the value of irradiation on disease progression is controversial. Incomplete resection and marked mitotic activity tend to be associated with recurrence and decreased survival.

Introduction

Tumors of the pineal region are infrequent lesions that account for only 1% of all intracranial tumors. Primary tumors of the pineal region with papillary features include papillary pineal parenchymal tumor, i.e. pineocytoma and pineoblastoma, papillary ependymoma, choroid plexus tumors, papillary meningioma, and germ cell tumors. In adulthood, differential diagnosis must consider papillary metastatic tumors from various primary sites.

Papillary tumor of the pineal region has been described for the first time as a distinct entity by Jouvet et al. (2003), on the basis of a series of six cases with identical histological features. In 2007, the World Health Organization (WHO) formally recognized it as a distinct entity in the classification of tumors of the nervous system. The provisional code proposed for the fourth edition of International Classification of Diseases for Oncology is 9395/3 (Louis et al. 2007).

Hasselblatt et al. (2006) described a series of papillary tumors initially diagnosed as choroid plexus papilloma, papillary ependymoma, or papillary pineal parenchymal tumor and subsequently reclassified as a primary papillary tumor of the pineal region after re-examination and immunohistochemical staining. Therefore, it is likely that other previous reports of unusual posterior third ventricle choroid plexus tumors, pineal parenchymal tumors, "papillary pineocytomas", papillary ependymomas, or even papillary meningiomas of the pineal region may actually represent early examples of a papillary tumor of the pineal region.

Epidemiology and Natural History

Papillary tumor of the pineal region is a rare tumor, thus incidence data are not available. The natural history is not completely known. The biological behavior of the tumor is variable.

Fèvre-Montange et al. (2006a, b), Hasselblatt et al. (2006), Jouvet et al. (2003), Kern et al. (2006), Kuchelmeister et al. (2006), Shibahara et al. (2004), and Li et al. (2011) reported examples of this tumor in both children and adults, with age ranging from 15 months to 66 years (mean, 32 years). No sex predilection has been noted. The most frequent clinical symptoms described were headache, tinnitus, vertigo, gait imbalance, memory loss, impairment of gaze and visual disturbance. Papillary tumor of the pineal region typically presents with symptoms and signs of obstructive hydrocephalus, such as occurred in the patient observed in our experience (Cerase et al. 2009). Papillary tumor of the pineal region shows

Fig. 6.1 (continued) show a iodine contrast-enhanced isodense mass lesion (*white arrow*) of the pituitary region. Note also the initial triventricular hydrocephalus. At unenhanced T2- (**c**) and T1-weighted (**d**), and gadolinium-enhanced T1-weighted (**e**) coronal MR images, the lesion wraps up the cranial portion of the quadrigeminal plate, and fills the cranial portion of the sylvian acqueduct. The lesion shows inhomogeneous intensity on T2-weighted images, subtle high-intensity signal on T1-weighted images (*black arrow*), and homogeneous contrast-enhancement. The lesion exhibited restricted diffusion at diffusion-weighted imaging and apparent diffusion coefficient maps (*not shown*). Twenty-two (**f**) and twenty-nine months (**g**, **h**) later, unenhanced T2-weighted (**f**, **g**) and gadolinium-enhanced T1-weighted (**h**) coronal MR images show clearcut enlargement of the lesion with the development of a cystic-necrotic component and inhomogeneous contrast-enhancement. Then, the patient underwent surgical resection and irradiation. Four years later, serial nonconsecutive gadolinium-enhanced T1-weighted coronal MR images (**i**–**k**) show findings consistent with leptomeningeal relapse in the posterior fossa, already evident 1 year before. This occurred in the absence of signs of local relapse.

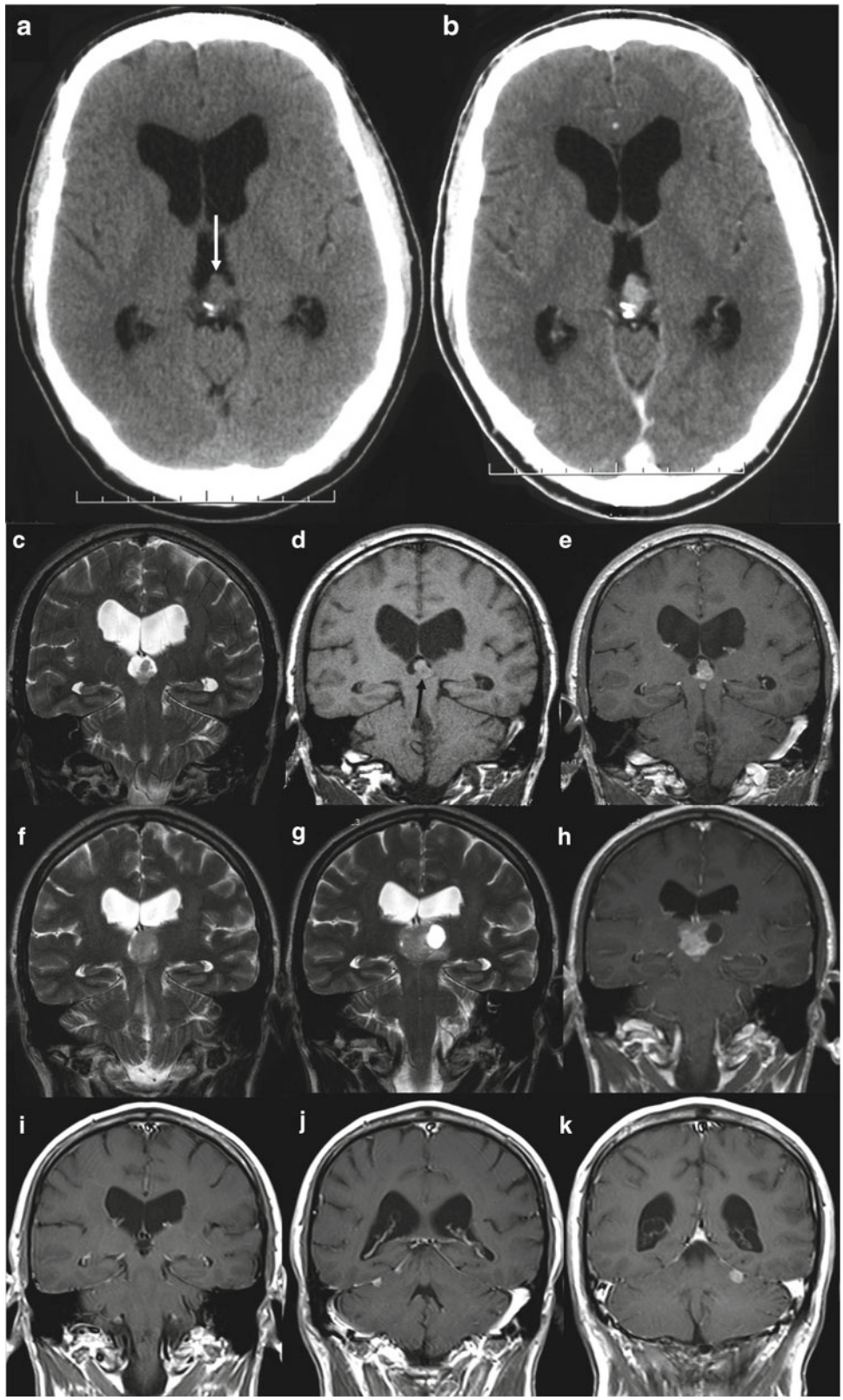

Fig. 6.1 Computed tomography (CT) and magnetic resonance (MR) imaging of the brain of the patient reported by Cerase et al. (2009). Unenhanced (**a**) and iodine contrast-enhanced (**b**) axial CT scans obtained at diagnosis

a progressive growth and frequent recurrence. Leptomeningeal (Fig. 6.1i–k) and spinal dissemination is possible, also at diagnosis. Sato et al. (2009) reported a young boy with early cerebrospinal fluid dissemination and a possible multicentric origin.

Neuroimaging

There are only limited neuroimaging reports of papillary tumor of the pineal region, which usually is described as a large, well circumscribed, heterogeneous pineal mass, occasionally featuring a cystic-necrotic component. At computed tomography (CT) (Fig. 6.1a), the lesion generally appears iso to hypodense when compared to the cortical gray matter. At magnetic resonance imaging (MRI), the lesion is generally heterogeneous on both T1- and T2-weighted images, possibly with cystic components, such as reported by Ballesteros et al. (1997), Chang et al. (2008), Dagnew et al. (2007), Smirniotopoulos et al. (1992). Low-intensity signal on T2-weighted images seems to be consistent with densely cellular areas. Chang et al. (2008) reported a characteristic high-intensity signal on T1-weighted images in four patients. They have hypothesized that protein, glycoprotein or other T1-shortening substances produced by tumor cells with well-differentiated secretory functions were the cause. When a pineal mass shows intrinsic high signal intensity on T1-weighted images, other lesions including teratoma, dermoid cyst, lipoma, hemorrhagic metastases such as from renal or thyroid cancer, or melanoma, should be excluded before suggesting the diagnosis of papillary tumor of the pineal region. High-intensity signal on T1-weighted images (Fig. 6.1d) has been confirmed as a reliable sign of papillary tumor of the pineal region also in our experience (Cerase et al. 2009). Notably, when CT and MRI rule out fat, hemorrhage, melanin or calcification in a mass of the posterior commissure or pineal region, the diagnosis of papillary tumor of the pineal region may be suggested so that specific immunohistochemical studies can be performed for a definitive diagnosis. However, the tumor might appear isointense on T1-weighted MR images. At both iodine contrast-enhanced CT (Fig. 6.1b) and gadolinium-enhanced T1-weighted MR imaging (Fig. 6.1e, h), the tumor shows variable contrast-enhancement. Patel et al. (2012) have presented a nonenhancing tumor at MR images. Regarding advanced MR techniques, Vaghela et al. (2010) have confirmed other reports showing that the tumor presents high level of perfusion, diffusion restriction, and presence of myo-inositol peak, and that these findings may correlate with high incidence of tumor recurrence. Inoue et al. (2008) and Sato et al. (2009) reported a significant radiotracer uptake with increased glucose metabolism suggesting a malignant tumor at both [^{18}F] fluorodeoxyglucose positron emission tomography and In-DTPA-pentetreotide scan, respectively.

Neuropathology and Genetics

Papillary tumor of the pineal region presents as a well-circumscribed mass, grossly indistinguishable from pineocytoma. It is an epithelial-appearing tumor with papillary architecture and more densely cellular areas, often exhibiting ependymal-like differentiation, i.e., true rosettes and tubes. Fèvre Montange et al. (2012) have reported the detailed description of the histopathologic features of a large series of tumors from 20 different centers and distinguished two subgroups with either a striking papillary growth pattern or a papillary and solid growth pattern. These two subgroups present similar clinical characteristics and immunophenotypes. A specific WHO grade has not been given but it has been suggested that papillary tumor of the pineal region corresponds to grade II or III, however histological grading criteria remain to be defined.

Jouvet et al. (2003) asserted that ultrastructural demonstration of ependymal, secretory and neuroendocrine features, and immunoistochemical findings suggest that papillary tumor of the pineal gland may have specialized ependymal derivation, probably arising from the subcommissural organ. Leonardt (1980), Meiniel (2007), Perez-Figares et al. (2001), and Rodriguez et al. (2001) have extensively described the subcommissural organ, i.e. a small ependymal brain gland located in the

third ventricle, adjacent to the pineal gland, at the entrance of the third ventricle, and secretes glycoproteins into the ventricular cerebrospinal fluid, such as reported by. It reaches full development during the embryonic period, and regresses after birth, such that only some remnants cells of the specialized subcommissural organ invading the pineal gland can be found in adults. The distinctive papillary appearance of these tumors results from the radial arrangement of large, cuboidal or columnar epithelioid cells around central, often hyalinized blood vessels. Fèvre Montange et al. (2012) have highlighted the findings that the tumor has unusual vessels with multiple lumina and frequently show detachment of the border of the tumoral cells from the vascular wall. Individual cells have abundant pale or eosinophilic cytoplasm. Nuclei are round to oval, with stippled chromatin; pleomorphic nuclei may be present. Fèvre-Montange et al. (2006b), Hasselblatt et al. (2006), Jouvet et al. (2003), and Scheithauer (1999) reported that mitotic index is moderate, ranging from 0 to 10 per 10 HPF. Kuchelmeister et al. (2006), and Shibahara et al. (2004) reported that the Ki67/MIB-1 labelling index is also moderate, with highest indices being seen in young patients. The prognostic significance of proliferation indices is not known. Necrotic foci may be seen. Microvascular proliferation is usually absent. There is a clear demarcation between tumor and the adjacent pineal gland.

Other tumors of the pineal region that may exhibit papillary features include choroid plexus tumors (papillomas and carcinomas), papillary ependymoma, and metastatic papillary carcinomas. Pineal parenchymal tumors, meningiomas, and germ cell tumors may rarely display papillary features. The histologic appearance of a papillary tumor of the pineal region is less papillary than choroid plexus papilloma and more epithelial than ependymoma, without the fibrillary background.

The recent neuropathology literature clearly defines the immunophenotypic profiles that distinguish papillary tumor of the pineal region from the other papillary-type masses of the pineal region, including a strong and consistent immunoreactiveness for cytokeratins (KL1, AE1/AE3, CAM 5.2, CK18), particularly in the papillary structures. Hasselblatt et al. (2006), Shibahara et al. (2004) observed immunoreactivity for vimentin, S100 protein, NSE, MAP2, N-CAM, and TTR. Kuchelmeister et al. (2006), Boco et al. (2008), Kawahara et al. (2007), Louis et al. (2007), and Yano et al. (2009) have shown that the neuroendocrine markers synaptophysin and chromogranin A are sometimes weakly and focally expressed, while NFP immunolabelling is never seen. Fèvre Montange et al. (2012) have confirmed and extended the results of previous ultrastructural studies on the presence of intercellular junctions at the apical part of tumoral cells. The expression of the tight junction proteins claudin-1, claudin-2, and claudin-3 was investigated by immunohistochemistry. Claudin-1 and claudin-3, but not claudin-2, were expressed in the tumor and in the fetal subcommissural organ. Claudin expression may help in the diagnosis of PTPRs and can be used in combination with other markers, such as CK18, NCAM, E-cadherin, MAP-2, and Kir 7.1.

Unlike classic ependymoma, GFAP may completely lack or be labelled only focally in papillary tumor of the pineal region, such as shown by Jouvet et al. (2003), and Kuchelmeister et al. (2006). Similarly, focal membrane or dot-like reactivity for EMA, which is typical of ependymoma, is rarely seen in papillary tumor of the pineal region. The weak and patchy synaptophysin reaction helps to rule out a papillary pineocytoma in which this marker is strongly positive, such as reported by Santarius et al. (2008). Hasselblatt et al. (2006) found that the majority of papillary tumor of the pineal region are characterized by the absence of staining for membranous Kir 7.1 or cytoplasmic stanniocalcin-1, both of which are frequently seen in choroid plexus tumors. Choroid plexus tumors have immunohistochemical similarities with papillary tumor of the pineal gland, but Figarella-Branger et al. (1995) reported that they do not show diffuse N-CAM immunoreactivity. Choroid plexus papilloma is generally much less well differentiated than papillary tumor of the pineal region, is immunonegative for NSE, and is rarely observed in the third ventricle in adults. Fèvre Montange et al. (2012) showed that all three claudins are expressed in choroid plexus papillomas.

At present, papillary tumor of the pineal region has not been associated with syndromic conditions, and there is no evidence of genetic

susceptibility. The most common chromosomal imbalances found were loss of chromosomes 10 and 22q as well as gain of chromosomes 4, 8, 9, 12, such as reported by Hasselblatt et al. (2006). Ang et al. (1990) reported a high expression of genes coding for proteins observed in the subcommisural organ, such as ZFH4, RFX3, TTR, and CGRP.

Treatment and Prognosis

Treatment guidelines for papillary tumor of the pineal region have not been established due to the small number of reported cases. The largest described patients' series is a multicenter retrospective study which has been reported by Fèvre-Montange et al. (2006a), and included 31 patients. The clinical course was evaluated in 29 patients over a mean follow-up period of 4.2 years. Gross total resection of the tumor was achieved in 21 patients. In the remaining patients, only incomplete resection of the tumor was feasible. Fifteen patients received heterogenous radiotherapy regimens after complete (n=9) or incomplete (n=6) resection of the primary tumor. During that time, 21 patients experienced tumor progression, including 19 local recurrences, one local and spinal, and one spinal recurrence, and eight patients died. Tumor progression occurred in 72% of cases, while the 5-year estimates for overall survival and progression-free survival have been evaluated in 73 and 27%, respectively. Their conclusions were that gross total resection was the only clinical factor that tended to be associated with overall survival and recurrence. In our experience (Cerase et al. 2009), a patient who had been treated with surgical gross total resection of the lesion (Figs. 6.1 and 6.2), and 45 Gy cranial conformational irradiation, showed signs of leptomeningeal disease relapse after 3 years. Most notably, aggressive local therapy with maximal surgical resection and adjuvant irradiation has been advocated, though the value of irradiation in terms of disease progression needs to be further investigated, such as stated by Boco et al. (2008), Nakamura et al. (2009), and Patel et al. (2012). El Majdoub et al. (2012) have presented the results of stereotactic (125)iodine brachytherapy performed in four patients, Cardenas et al. (2010) on a patient treated by gammaknife. Lorenzetti et al. (2011) treated a patient with adjunctive temozolomide.

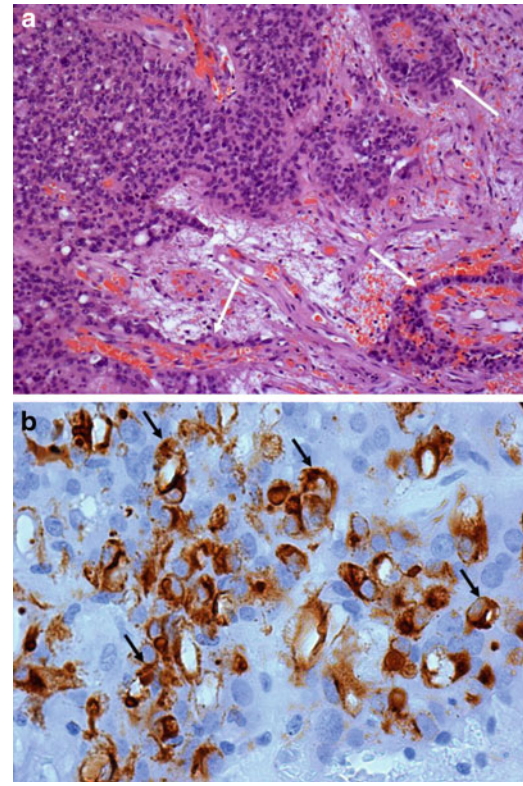

Fig. 6.2 Pathologic specimen obtained after resection of the pituitary lesion observed in Fig. 6.1. (*Courtesy of Prof. ssa Clelia Miracco, Department of Human Pathology, Section of Pathologic Anatomy, University of Siena, "Santa Maria alle Scotte" General Hospital, Siena, Italy*) (**a**) Hematoxylin and eosin (original magnification×100) show an epithelial appearing neoplasia organized in papillary structures (*white arrows*), and hyalinized vessels. (**b**) Immunohistochemical findings (streptavidin-biotin peroxidase complex method; chromogen: diaminobenzidine; original magnification×400) show that most tumor cells have a characteristic brown-stained cytoplasmic positivity (*black arrows*) to low-weighted cytokeratins. Additional pathological findings included microfoci of necrosis, mild atypia, focal positivity to S100 protein, and a MIB-1 proliferative index of 8% (*not shown*). No mitotic figures were evident

References

Ang LC, Taylor AR, Bergin D, Kaufmann JC (1990) An immunohistochemical study of papillary tumors in the central nervous system. Cancer 65:2712–2719

Ballesteros MD, Durán A, Arrazola J, Redondo MJ, Bordiú E, de Abajo S, Charro AL (1997) Primary intrasellar germinoma with synchronous pineal tumor. Neuroradiology 39:860–862

Boco T, Aalaei S, Musacchio M, Byrne R, Cochran E (2008) Papillary tumor of the pineal region. Neuropathology 28:87–92

Cardenas R, Javalkar V, Haydel J, Wadhwa R, Fowler M, Scheithauer B, Nanda A (2010) Papillary tumor of pineal region: prolonged control rate after gamma knife radiosurgery – a case report and review of literature. Neurol India 58:471–476

Cerase A, Vallone IM, Di Pietro G, Oliveri G, Miracco C, Venturi C (2009) Neuroradiological follow-up of the growth of papillary tumor of the pineal region: a case report. J Neurooncol 95:433–435

Chang AH, Fuller GN, Debnam JM, Karis JP, Coons SW, Ross JS, Dean BL (2008) MR imaging of papillary tumor of the pineal region. AJNR Am J Neuroradiol 29:187–189

Dagnew E, Langford LA, Lang FF, DeMonte F (2007) Papillary tumors of the pineal region: case report. Neurosurgery 60:E953–E955, discussion E953-5

El Majdoub F, Blau T, Hoevels M, Bührle C, Deckert M, Treuer H, Sturm V, Maarouf M (2012) Papillary tumors of the pineal region: a novel therapeutic option-stereotactic (125)iodine brachytherapy. J Neurooncol 109:99–104

Fèvre Montange M, Vasiljevic A, Bergemer Fouquet AM, Bernier M, Champier J, Chrétien F, Figarella-Branger D, Kemeny JL, Lechapt-Zalcman E, Michalak S, Miquel C, Mokthari K, Pommepuy I, Quintin Roué I, Rousseau A, Saint-Pierre G, Salon C, Uro-Coste E, Varlet P, Kratzer I, Ghersi-Egea JF, Jouvet A (2012) Histopathologic and ultrastructural features and claudin expression in papillary tumors of the pineal region: a multicenter analysis. Am J Surg Pathol 36:916–928

Fèvre-Montange M, Hasselblatt M, Figarella-Branger D, Chauveinc L, Champier J, Saint-Pierre G, Taillandier L, Coulon A, Paulus W, Fauchon F, Jouvet A (2006a) Prognosis and histopathologic features in papillary tumors of the pineal region: a retrospective multicenter study of 31 cases. J Neuropathol Exp Neurol 65:1004–1011

Fèvre-Montange M, Champier J, Szathmari A, Wierinckx A, Mottolese C, Guyotat J, Figarella-Branger D, Jouvet A, Lachuer J (2006b) Microarray analysis reveals differential gene expression patterns in tumors of the pineal region. J Neuropathol Exp Neurol 65:675–684

Figarella-Branger D, Lepidi H, Poncet C, Gambarelli D, Bianco N, Rougon G, Pellissier JF (1995) Differential expression of cell adhesion molecules (CAM), neural CAM and epithelial cadherin in ependymomas and choroid plexus tumors. Acta Neuropathol 89:248–257

Hasselblatt M, Blümcke I, Jeibmann A, Rickert CH, Jouvet A, van de Nes JA, Kuchelmeister K, Brunn A, Fèvre-Montange M, Paulus W (2006) Immunohistochemical profile and chromosomal imbalances in papillary tumors of the pineal region. Neuropathol Appl Neurobiol 32:278–283

Inoue T, Kumabe T, Kanamori M, Sonoda Y, Watanabe M, Tominaga T (2008) Papillary tumor of the pineal region: a case report. Brain Tumor Pathol 25:85–90

Jouvet A, Fauchon F, Liberski P, Saint-Pierre G, Didier-Bazes M, Heitzmann A, Delisle MB, Biassette HA, Vincent S, Mikol J, Streichenberger N, Ahboucha S, Brisson C, Belin MF, Fèvre-Montange M (2003) Papillary tumor of the pineal region. Am J Surg Pathol 27:505–512

Kawahara I, Tokunaga Y, Yagi N, Iseki M, Abe K, Hayashi T (2007) Papillary tumor of the pineal region. Neurol Med Chir (Tokyo) 47:568–571

Kern M, Robbins P, Lee G, Watson P (2006) Papillary tumor of the pineal region—a new pathological entity. Clin Neuropathol 25:185–192

Kuchelmeister K, Hügens-Penzel M, Jödicke A, Schachenmayr W (2006) Papillary tumour of the pineal region: histodiagnostic considerations. Neuropathol Appl Neurobiol 32:203–208

Leonardt H (1980) Ependym und circumventriculäre organe. In: Okschem A, Vollrath L (eds) Neuroglia I: handbuch der mikroskopischen anatomie des menschen. Springer, Berlin, pp 177–665

Li J, Recinos PF, Orr BA, Burger PC, Jallo GI, Recinos VR (2011) Papillary tumor of the pineal region in a 15-month-old boy. J Neurosurg Pediatr 7:534–538

Lorenzetti M, Motta F, Campanella R, Bauer D, Assi A, Arienta C, Gaini SM, Caroli M (2011) Adjuvant temozolomide chemotherapy for treatment of papillary tumor of the pineal region. World Neurosurg 76: 160–163

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumors of the central nervous system. Acta Neuropathol 114:97–109

Meiniel A (2007) The secretory ependymal cells of the subcommissural organ: which role in hydrocephalus? Int J Biochem Cell Biol 39:463–468

Nakamura H, Makino K, Kochi M, Nakazato Y, Kuratsu J (2009) Successful treatment of neoadjuvant therapy for papillary tumor of the pineal region. Brain Tumor Pathol 26:73–77

Patel SK, Tomei KL, Christiano LD, Baisre A, Liu JK (2012) Complete regression of papillary tumor of the pineal region after radiation therapy: case report and review of the literature. J Neurooncol 107:427–434

Perez-Figares JM, Jimenez AJ, Rodriguez EM (2001) Subcommissural organ, cerebrospinal fluid circulation, and hydrocephalus. Microsc Res Tech 52:591–607

Rodriguez EM, Oksche A, Montecinos H (2001) Human subcommissural organ, with particular emphasis on its secretory activity during the fetal life. Microsc Res Tech 52:573–590

Santarius T, Joseph JA, Tsang KT, O'Donovan DG, Kirollos RW (2008) Papillary tumor of the pineal region. Br J Neurosurg 22:116–120

Sato TS, Kirby PA, Buatti JM, Moritani T (2009) Papillary tumor of the pineal region: report of a rapidly progressive tumor with possible multicentric origin. Pediatr Radiol 39:188–190

Scheithauer BW (1999) Pathobiology of the pineal gland with emphasis on parenchymal tumors. Brain Tumor Pathol 16:1–9

Shibahara J, Todo T, Morita A, Mori H, Aoki S, Fukayama M (2004) Papillary neuroepithelial tumor of the pineal region. A case report. Acta Neuropathol (Berl) 108: 337–340

Smirniotopoulos JG, Rushing EJ, Mena H (1992) Pineal region masses: differential diagnosis. Radiographics 12:577–596

Vaghela V, Radhakrishnan N, Radhakrishnan VV, Menon G, Kesavadas C, Thomas B (2010) Advanced magnetic resonance imaging with histopathological correlation in papillary tumor of pineal region: report of a case and review of literature. Neurol India 58: 928–932

Yano H, Ohe N, Nakayama N, Shinoda J, Iwama T (2009) Clinicopathological features from long-term observation of a papillary tumor of the pineal region (PTPR): a case report. Brain Tumor Pathol 26:83–88

Pineal Region Tumors: Optimal Neurosurgical Treatment

7

Kemal Dizdarevic

Contents

Abstract

Pineal region tumors are rare intracranial tumors which are diverse in nature involving various different kinds of tissues. These tumors are generally more prevalent in males and children. Currently, surgery still plays a crucial role in the treatment of most pineal region tumors and the two main microsurgical approaches are infratentorial supracerebellar and occipital transtentorial. Although management of pineal region tumors must include involvement of a multidisciplinary team, the role of the surgeon remains important as successful resection of the tumors require a thorough understanding of the relevant anatomy and high technical skills in order to reduce mortality and ensure favourable outcomes.

Introduction

The pineal region is a midline area that includes the pineal gland, the posterior third ventricle, the surrounding cisterns of the quadrigeminal plate and velum interpositum and the adjacent solid tissues of the brainstem, the thalami, and the overlying splenium of the corpus callosum (Ringertz et al. 1954). The pineal gland develops during the second month of gestation from the most caudal portion of the roof of the third ventricle (Langman 1975). The principal cell of the gland is the pineal parenchymal cell. Pineal gland is richly innervated with sympathetic noradrenergic input from a pathway originating from the retina.

K. Dizdarevic (✉)
Department of Neurosurgery, Clinical Center University of Sarajevo, Bolnika 25, 71000 Sarajevo, Bosnia and Herzegovina
e-mail: kemaldiz@bih.net.ba

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_7,

Upon stimulation the gland produces melatonin, which has regulatory effects on certain hormones such as follicle stimulating hormone and luteinizing hormone. Additionally, the gland plays a central role in maintaining the human circadian rhythm by acting as a neuroendocrine transducer, synchronizing hormonal release with phases of daily light–dark cycle.

Pineal region tumors are derived from cells located in and around the pineal gland. Lesions occurring in this area may be neoplastic or non-neoplastic and are diverse in nature, involving various types of tissues. Most of the tumors are malignant with a propensity to seeding. Combined, these lesions represent less than 1% of all adult intracranial tumors in the United States but the occurrence of pineal masses is higher in Asia with an unexplained higher incidence in Japan (Sano 1998). Pineal region tumors are ten times more common in children than in adults (Abay et al. 1981) and can be found in 3–8% of all pediatric intracranial tumors. Most children are aged 10–20 years at presentation while adults typically present around the age of 30. Jennings et al. (1985) showed that males are 2.2 times more likely to get pineal region tumors than female. This review will discuss the diagnosis, types and optimal neurosurgical treatment of pineal region tumors.

Diagnosis of Pineal Region Tumors

Clinical Presentation

The clinical signs and symptoms of tumors in the pineal region are associated with normal pineal anatomy and specific tumor histology. Symptomatic hydrocephalus or occulomotor signs are generally the first clinical manifestations of pineal region tumors. Hydrocephalus is triventricular by compression of the aqueduct of Sylvius and can be acute or chronic. Acute obstructive hydrocephalus might be severe enough to result in a downward transtentorial herniation. In slow-growing tumors, chronic hydrocephalus may develop and cause dementia. Hydrocephalus is common at the time of diagnosis. Other symptoms and signs include headaches, visual problems and gait. Occulomotor signs can frequently occur through direct compression of the superior colliculi or the posterior commisure (Sawamura and de Tribolet 2002). Pineal region masses that compromise the superior colliculi may result in Parinaud's syndrome. This syndrome, described by Parinaud (1883) involves vertical gaze palsy (usually paralysis of the upward gaze), eyelid retraction and convergence/refractory nystagmus; it has become pathognomonic for lesions involving the quadrigeminal plate. Patients can also present with motor impairment such as dysmetria and ataxia due to the compression of cerebellar efferent fibres within the superior cerebellar peduncle. Children with pineal region tumors can also present with endocrine malfunction such as diabetes insipidus and precocious puberty. Pineal apoplexy can rarely occur as a presenting feature of pineal region tumors (Kobayashi et al. 2001). Hemorrhage into a vascular-rich pineal tumor can occur preoperatively and is a well-described postoperative complication.

Neuroimaging

The radiological investigation of choice is magnetic resonance imaging (MRI) which will reveal the tumor and its relations to adjacent anatomical structures. Particular attention has to be given to T1 with gadolinium sequences, high resolution T2 sequences for surrounding vessels (flow-void) and cranial nerves, phlebo-MRI sequences for assessing the 3D anatomy of the deep venous system and its relation with the tumor. A computed tomography (CT) scan is also useful to detect intra-tumoral calcifications or hemorrhage. Even if the different pineal tumor types may have a preferential appearance on imaging, no such characteristics are specific for one or another tumor type and this does not preclude obtaining tissue for histological examination. One exception is the benign pineal cysts, which have a homogenous cyst content with a thin enhancing rim and have no or only mild mass effect on surrounding structures. Except for pineal region meningiomas, angiography is usually not necessary (Sawamura and de Tribolet 2002). The role of magnetic resonance angiography (MRA) to study the patency of the venous system

and the arterial supply still remains inferior to digital subtraction angiography (DSA). DSA allows studying details of the vascular anatomy, which is highly complex in the pineal region. It should be mentioned that not visualizing on DSA a vein or a sinus encased in a tumor does not always mean that the structure is occluded and could be safely sacrificed without intraoperative examination.

Tumor Markers

Serum and cerebrospinal fluid (CSF) markers contribute to the diagnosis of pineal tumors and assessment of their malignancy. Embryonic proteins such as beta human chorionic gonadotrophin (βhCG) and alfa-fetoprotein (AFP) are found in germ cell tumors. βhCG is mainly positive in choriocarcinomas, embryonal carcinomas and mixed germ cell tumors. The expression of βHCG is usually low in germinomas which are often positive for placental alkaline phosphate (PLAP) on immunohistochemistry (Sawamura 1998). AFP is expressed by yolk sac tumors (high levels), embryonic carcinomas, immature teratomas and mixed germ cell tumors.

Biopsy

Histological diagnosis is obtained either by stereotactic or endoscopic transventricular biopsy or directly during open surgery. For large pineal tumors a stereotactic biopsy is a safe initial procedure to obtain diagnosis. For tumors extending into the posterior part of the third ventricle, endoscopic transventricular biopsy allows access to tumor tissue as well as third ventriculostomy to treat hydrocephalus.

Types of Pineal Region Tumors

Germ Cell Tumors

Pineal germ cell tumors derive from pluripotential germ cells and affect mainly children and adolescents (90% occurs in the first 25 years of life with a peak between 10 and 14 years). It is more common in males. An accurate histological diagnosis is critical for appropriate treatments and prognosis. It can be said that only germinoma and teratoma are found as pure tumor types.

Germinoma (WHO-2007 Grade 4)

Germinoma is the most common type of pineal region masses, accounting for approximately 65% of germ cell tumors and 40% of all pineal region tumors. About 80% of intracranial germinomas are in the pineal area. Male patients are affected up to 17 times more often than female patients. The peak age of presentation is in the second decade and only a few patients are older than 30 years old at initial presentation. A large number of young children have germinomas associated with precocious puberty (Simson et al. 1968).

Germinoma is a malignant tumor with a biphasic pattern. It is composed of a mixture of large multipotential primitive germ cells with prominently nucleolated large nuclei and fibrovascular septa infiltrated with T-lymphocytes and plasma cells. Because germinomas are unencapsulated, they may invade the adjacent structures of the brain, leading to the fact that at initial presentation the patient would normally already have disseminated disease. Measurement of serum and CSF tumor markers plays a vital role in the initial investigation of patients with germ cell tumors; the expressions of AFP and βhCG are suggestive of malignant germ cells (Allen et al. 1979). In CT scans obtained without contrast, germinomas appear as homogenous masses that either have attenuation equal or slightly higher to that of the gray matter. Similarly, on MRI scans, the signal intensity of germinomas tends to be equal to that of the gray matter on images obtained with both short and long pulse sequences. Therefore, the findings of a non-calcified, homogenous pineal region mass with such signal intensity are strongly suggestive of a germinoma. Although germinomas appear highly enhanced on both CT and MRI scans with contrast material, the enhancement is non-specific and gadolinium-enhanced MRI is recommended to help distinguish metastases from CSF seeding. Germinoma is not a surgical lesion and it is a highly radiosensitive tumor with 85% 10-year survival rate.

Teratoma (Mature: WHO-2007 Grade 1–2; Immature: WHO-2007 Grade 3–4)

Pineal teratoma is the second most common pineal region tumor, contributing to almost 15% of all pineal region masses. Teratomas have a male predilection that ranges from 2:1 to 8:1. They are well-circumscribed benign tumors of multipotential cells that undergo normal organogenesis thereby producing tissues consisting of a mixture of two or more of the embryologic layers of ectoderm, mesoderm and endoderm. Hosoi (1930) first used the term "teratoid" to describe less structured tumors in which derivatives of all three germinal layers could not be distinguished easily. These tumors may contain bone, cartilage and hair. Pineal region teratomas are usually partially or totally encapsulated; however they may also be unencapsulated and locally invasive (Jennings et al. 1985).

Although teratomas do not secrete AFP, the less-differentiated immature teratomas can produce detectable amounts. Immature teratomas contain incompletely differentiated components, resembling fetal tissues and can have primitive neuroectodermal elements like rosettes, arrays and melanotic neuroepithelium. In imaging studies, teratomas tend to be heterogenous, multilocular, ring or ring-enhanced lesions. They may have areas of mixed CSF, lipid and soft-tissue characteristics, as well as calcification. On TI-weighted MRI scans, teratomas may show evidence of fatty or lipid components as areas of signal hyperintensity (Zee et al. 1991). Teratomas may also demonstrate contrast enhancement on both CT and MRI scans. Pineal region teratoma may rupture spontaneously or at surgery, spilling their varied contents and causing chemical meningitis. The third type of teratoma is designated as teratoma with malignant transformation (WHO-2007 4 grade). It usually contains a cancer, mainly rhabdomyosarcoma or undifferentiated sarcoma and less commonly enteric-type adenocarcinoma.

Choriocarcinoma (WHO-2007 Grade 4)

Choriocarcinomas make up less than 5% of all pineal masses and also have a male predilection. They arise from the differentiation of the pluripotential germ cells into the extraembryonic placenta-like tissues. This tumor contains cytotrophoblast (large, mononucleate cells with vesicular nuclei and eosinophilic cytoplasm) and syncytiotrophoblast (giant, βHCG-positive cells with multiple densely hyperchromatic nuclei arranged in knot-like clusters). Typical findings for these tumors are ectatic vascular channels in stroma and extensive hemorrhage necrosis. Choriocarcinomas secrete large amounts of βhCG and are associated with elevated levels of both CSF and plasma βhCG. Their appearance at CT scans is non-specific and like germinomas they are often represented by areas of high attenuation and show prominent contrast enhancement. Other types of germ cell tumors are yolk sac (endodermal sinus) tumors and embryonal carcinomas. Both tumors are very malignant (WHO-2007 grade 4). Yolk sac tumor contains epithelial cells which form Schiller-Duval bodies (papillary perivascular cuboidal epithelium). Embyonal carcinoma contains large cells lining spaces replicating structure of the early embryo (embryoid bodies).

Pineal Parenchymal Tumors

Pineal parenchymal tumors are the second major group of pineal region tumors, accounting for up to 30% of all tumors in this location. These tumors can occur at any age and should be considered as a true neoplasm of pineal glandular tissue.

Pineocytoma (WHO-2007 Grade 1)

Pineocytoma is a slow-growing tumor usually composed of well-differentiated mature cells that are almost indistinguishable from the normal pineal parenchyma (Preslock 1984). The tumor cells are small and uniform, often forming large pineocytomatous rosettes. It is very rare that large ganglion cells accompanied by small cells with nuclear pleomorphism and hyperchromasia occur. Usually the only feature distinguishing a tumor from a normal tissue is the presence of a mass or an obviously enlarged pineal gland. Pineocytomas constitute approximately 45% of pineal parenchymal tumors. There is no sex predilection but pineocytomas have a tendency to affect young adults. Although pineocytoma is an unencapsulated

tumor, it remains locally confined and is unlikely to spread via the CSF system though it may extend into the third ventricle. Because it is a benign tumor, hemorrhage and necrosis are uncommon. Microscopically, pineocytoma is a moderately cellular tumor with large pseudorosettes and abundant cytoplasmic processes. The pseudorosettes (pineocytomatous rosettes), vary in size and shape. Pineal parenchymal cell tumor markers are less well-characterized than their germ cell counterparts. They include melatonin and the S antigen though neither of these proteins has been proven valuable in the diagnosis of pineal parenchymal tumors. On CT scans, pineocytomas are usually globular, demarcated masses measuring less than 3 cm in diameter. They appear hypodense and homogenous, but some show peripheral calcification or occasional cystic changes. Most tumors exhibit homogenous contrast enhancement. On MRI scans, the tumors tend to be low or isodense on T1- and hyperintense on T2-weighted images with strong, homogenous contrast enhancement. It is typical that 5-year survival rate of pineocytoma is very high (86–100%) and there is no relapses following total surgical resection. Tumor with divergent differentiation shares a similar prognosis.

Pineoblastoma (WHO-2007 Grade 4)

Pineoblastoma is a fast-growing, highly malignant primitive embryonic tumor of the pineal gland and composed of dense, patternless sheets of small cells with round to somewhat irregular nuclei and scant cytoplasm. It represents a typical primitive neuroectodermal tumor (PNET). Pineoblastomas comprise approximately 40% of all pineal parenchymal tumors. They originate from immature neoplastic cells of the parenchyma and while they may occur at any age, most present in the first two decades of life with a certain predilection for children. There is no gender preference. Median post-surgical survivals vary from 24 to 30 months (Abay et al. 1981); 5-year survival rate is more that 50%. Histologically, pineoblastoma is highly cellular, made up of small cells arranged either in sheets or rosettes. Homer-Wright and Flexner-Wintersteiner rosettes and fleurettes may occur. There are numerous mitoses and intratumoral necrosis. The cells tend to seed within the subarachnoid space and metastasize outside the cerebrum. In contrast to pineocytoma, the CT appearance of pineoblastoma is that of a large, lobulated or poorly-demarcated homogenous mass which is hyperdense after contrast enhancement. On TI-weighted MRI scans, pineoblastomas are hypo- or isodense though they show heterogenous contrast enhancement. Calcification is infrequent. Pineal parenchymal tumors may be inseparable at imaging but may be distinguished from the germ cell tumors because they displace preexisting pineal calcifications, producing an exploded appearance. With the exception of pineocytomas, all other pineal parenchymal tumors are potentially aggressive. Extent of disease at the time of diagnosis, as determined by CSF examination and MRI of the spine, directly affects the survival of patients with pineoblastoma. Not surprisingly, metastases of the pineal tumor within the CNS and vertebral column are the most common cause of death.

Pineal Parenchymal Tumor of Intermediate Differentiation; PPTID (WHO-2007 Grade 2–3)

This tumor type is a pineal parenchymal tumor of intermediate-grade malignancy, affecting all ages and composed of diffuse sheets of large lobules of uniform cells with mild to moderate nuclear atypia and low to moderate level mitotic activity. This tumor group accounts for at least 20% of all pineal parenchymal tumors. The gross appearance of PPTID shows intermediate degrees of development in the club-shaped expansions, dendritic processes, clear vesicles and dense core vesicles between pineocytomas and pineoblastomas (Jouvet et al. 2000). This tumor is diffuse neurocytoma-like tumor with tendency to form nucleus-free perivascular zones. The clinical behavior of this type of tumor is variable and 5-year survival rate is 39–74%. Since Schild et al. (1993) first introduced the term “PPTID”, other subtypes such as mixed pineocytoma/pineoblastoma, malignant pineocytomas and pineoblastomas with lobules have been included in the category.

Others

Other tumors found in the pineal region include metastases, gliomas (fibrillary astrocytoma, pilocytic astrocytoma, glioblastoma, oligodendroglioma, ependymoma and choroid plexus papilloma), melanomas, neurocytomas, hemangioblastomas, cavernous hemangiomas, gangliogliomas, symptomatic pineal cysts and meningomas.

Meningioma

Meningiomas in the vicinity of the pineal gland irrespective of their insertion on the falco-tentorial dura can be considered as meningiomas of the pineal region. According to origin, there are two main groups of pineal region meningiomas. The first group has the origin at the velum interpositum without dural attachment and occupies the pineal region and the posterior third ventricle (Konovalov et al. 1996). The second group arises from the falco-tentorial junction and has a dural attachment. These meningiomas also extend into the pineal region and the posterior third ventricle. Pineal region meningiomas are very rare and account for about 0.5–1% of all intracranial meningiomas (Raco et al. 2004; Okami et al. 2001). The first reports on falco-tentorial junction meningiomas were published by Balado (1927). A more detailed description of the falco-tentorial junction meningiomas was presented by Cushing and Eisenhardt in 1938; they described these tumors as a subtype of posterior falx meningiomas.

Pineal region meningiomas can be classified as follows: (a) meningiomas lying freely in the pineal space without dural attachment, (b) meningiomas attached to the tentorium and/or the falx without functional compromise of the venous system and (c) meningiomas with attachment and occlusion of the galenic system (Konovalov et al. 1996). Pineal meningiomas can be further divided according to their insertion and projection on sagittal MRI as anterior/posterior and superior/inferior, and on axial MRI as midline symmetrical and midline asymmetrical (Asari et al. 1994, 1995). Yasargil (1994) has given a very valuable contribution in microsurgical treatment and classification of tentorial meningiomas including pineal region meningiomas.

Neurosurgical Treatment

Microsurgical Anatomy

Pineal region tumors lie deep in the center of the cranium and are surrounded by critical nervous and vascular structures. Therefore, a precise knowledge of the pineal region anatomy is of paramount importance (Matsuno et al. 1988; Ono et al. 1984). The pineal gland is located on the midline and forms an appendix of the caudal end of the diencephalon embracing the pineal recess of the third ventricle. The pineal stem is continuous with the habenular commissure dorsally and the posterior commissure ventrally. The pineal body projects posteriorly in the quadrigeminal cistern where it is flanked by the splenium superiorly and lies on the tectal quadrigeminal plate between the left and right superior colliculi. The pineal gland is mainly vascularized by the medial and lateral posterior choroidal arteries. The tumor of this region is also vascularized by branches of the same arteries. The medial posterior choroidal arteries are branches of the posterior cerebral artery and in addition to the pineal body they supply the superior and inferior colliculi, and the choroidal plexus of the third ventricle. These arteries are displaced laterally by pineal tumors in the cistern and rostrally in the posterior part of the third ventricle together with internal cerebral veins. The lateral posterior choroidal artery supplies the pulvinar and is generally displaced laterally by pineal tumors. During surgical approaches to the pineal gland, the major anatomical obstacle is the Galenic venous system. The great vein of Galen originates a few millimeters behind the pineal gland and runs posterosuperiorly to drain into the straight sinus. There are several surgically significant vessels draining into the great vein. The midline-located superior vermian vein and the precentral cerebral vein run into the dorsal part of the great vein. The internal cerebral veins and the pineal veins join ventrally. Pineal tumors always rostrally elevate the posterior portion of the internal cerebral veins and the veins are occasionally separated from each other. On the lateral aspect of the great vein the medial

occipital veins, the third segment of the basal veins of Rosenthal and the posterior mesencephalic veins join. The pineal veins are the draining veins of pineal tumors and drain into either the internal cerebral veins or the vein of Galen. It means that pineal tumors are adherent to the internal cerebral vein and/or the vein of Galen. An injury to the basal veins or the internal cerebral veins is associated with major complications. Also, a transection of a major medial occipital vein may cause homonymous hemianopsia or visual seizures.

Indications for Surgery and Microsurgical Approaches

For benign pineal tumors total surgical resection is a primary goal as surgery alone can be curative (Bruce and Stein 1995). For malignant tumors surgery is only a part of the treatment which consists of adjuvant therapies and therefore radical surgical resection is not an objective (Sawamura and de Tribolet 2002). In all cases focus should be given to reduce post-treatment morbidity. If a newly diagnosed pineal mass is accessible by stereotactic or endoscopic biopsy and the cranial MRI is compatible with a germinoma, a biopsy should first be done in order to avoid an unnecessary craniotomy. If the radiological examination is compatible with an asymptomatic benign pineal cyst and the serum and CSF markers are negative, the patient can be followed up without treatment. The treatment of other pineal tumors requires surgery but the choice of total or subtotal resection will depend on the diagnosis of the presurgical biopsy or the intraoperative frozen section. Benign tumors such as mature teratomas, pineocytomas or meningiomas require total surgical resection when feasible without compromising surrounding neurovascular structures. More aggressive tumors, such as malignant teratomas, pinealoblastomas, embryonal carcinomas, choroicarcinomas and yolk sac tumors require a combination of surgery, radiation therapy and chemotherapy. In any case, the prime goal of surgery should be avoiding surgical morbidity even at the cost of a less radical surgical resection. The choice of approach is a matter of evaluating the anatomical relation of the tumor with the surrounding structures. A steep angle of the straight sinus makes the infratentorial supracerebellar approach (Krause 1926; Stein 1971) difficult as an extensive retraction of the cerebellum is required to visualize and reach the pineal area. Moreover, the lateral exposure of the surgical field is restricted and this renders the resection of larger tumors more complicated.

Evaluating the relationship of the tumor with the quadrigeminal plate is also important. For smaller midline tumors located in the posterior part of the third ventricle and displacing the quadrigeminal plate and the tegmentum of the midbrain caudally, the infratentorial supracerebellar approach is favored as it allows simple, direct and symmetrical exposure of the walls of the third ventricle and internal cerebral veins on both sides. In cases where the tumor lies more caudally and extends into the upper portion of the aqueduct of Sylvius, lying therefore cranially at the tectum, the infratentorial approach is inappropriate as the quadrigeminal plate obstructs the surgical exposure. Finally, the occipital transtentorial approach (Poppen 1966; Jamieson 1971) is preferred in huge tumors with lateral extension in the pulvinar thalami as it gives a better lateral exposure of the walls of the third ventricle (Sawamura and de Tribolet 2002). Giant tumors of the pineal region can be removed by the combined occipital, transtentorial, and supracerellar transsinus approach described by Sekhar and Tzortzidis (1999) (Figs. 7.1 and 7.2).

Neurosurgical Operative Modalities

Stereotaxy

Stereotactic procedures are basically diagnostic tools for some pineal tumors such as germinomas or those that are not amenable to resection. Stereotactic biopsy is nowadays easy to use and more advanced with minimal imminent risk of procedure. The vessels of pineal region are frequently displaced from their normal position by the tumor so the complication rate in this kind of biopsy is quite low (1% mortality, 7% morbidity). The diagnostic value of stereotaxy is very high and is more than 90%.

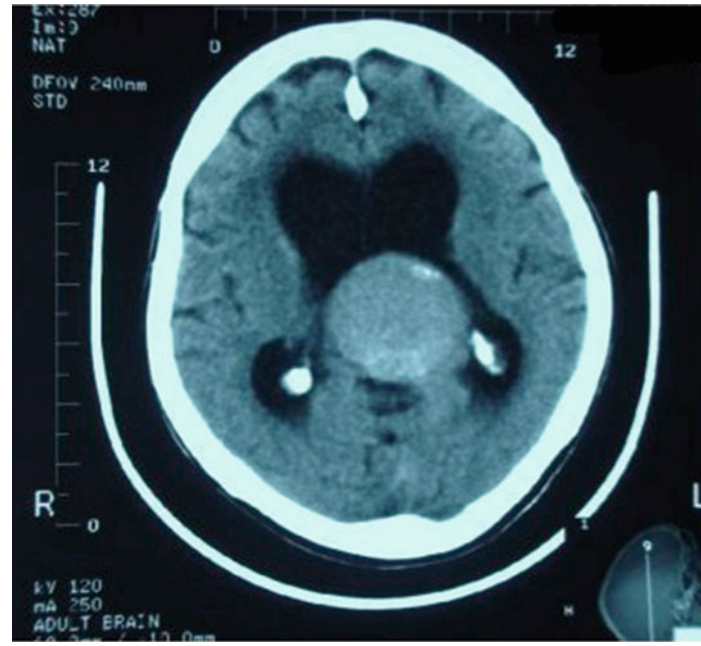

Fig. 7.1 Preoperative CT, large pineal region meningioma

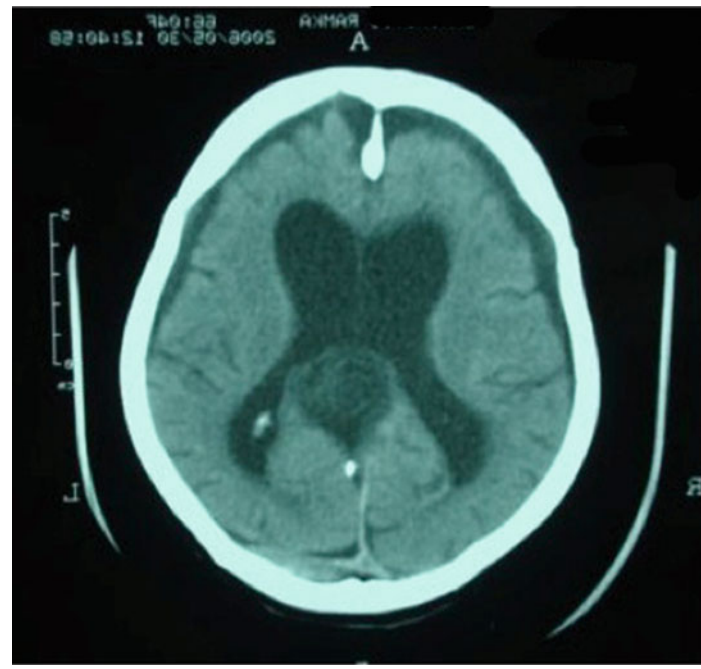

Fig. 7.2 Postoperative CT after radical meningioma resection through the combined Sekhar's approach

Endoscopy

Hydrocephalus may be controlled with either tumor resection or a CSF diversion procedure. Classical treatment of acute hydrocephalus caused by pineal mass lesion was CSF diversion by ventriculoperitoneal (VP) shunt or external ventricular drainage. Also, surgical resection with the main goal of opening the

aqueduct of Sylvius was one of the procedures for tumoral acute hydrocephalus. At present, the treatment of choice for hydrocephalus is the endoscopic third ventriculostomy. This procedure eliminates the necessity of a VP shunt and the risk of peritoneal dissemination of malignant tumor cells. Neuroendoscopy allows an inspection of posterior third ventricle and tumor biopsy at the same procedure with ventriculostomy. Potential risks of endoscopic ventriculostomy include hemorrhage from the basilar artery and posterior thalamoperforators and the tuber cinereum damage with consequent development of diabetes insipidus.

Microsurgery

Microsurgery is recommended in most adult cases and in selected pediatric cases. The five most common surgical approaches to the pineal gland have been described in the twentieth century. Dandy (1921) was the very first neurosurgeon who approached pineal region tumors. He used a parietal parasagittal transcallosal (posterior transcallosal) approach with interhemispheric fissure as an entry point. Following the retraction of parietal lobe and splitting of the splenium, the internal cerebral veins and vein of Galen can be seen. Ten years later, Van Wagenen (1931) describes the transventricular approach. This approach was limited to tumors in the nondominant hemisphere and patients with eccentric tumor with ventriculomegalia because the entry point was in the cortex of posterior part of superior temporal gyrus and the surgical angle was more lateral compared with other approaches. These two approaches resulted in high morbidity and mortality rates. Microsurgical technique gives some credit to these approaches but they are mainly abandoned nowadays.

Krause (1926) described and successfully used the infratentorial supracerebellar approach. During the 1970s, Stein (1971) developed and popularized this approach by using microsurgical techniques. The occipital transtentorial approach was pioneered by Foerster in 1928 and described by Poppen (1966). This method required extensive lifting of the occipital lobe after CSF drainage via a ventricular catheter. It was modified by Jamieson (1971) who preferred to mobilize the occipital lobe laterally rather than proceeding below it. Shekar described the combined occipital, transtentorial, supracerebellar, transsinus approach which combined the advantages of the supracerebellar infratentorial and occipital transtentorial approaches (Sekhar and Tzortzidis 1999). The occipital transtentorial approach, the infratentorial supracerebellar approach and combined Shekar's approach are nowadays accepted as the main standard accesses to the pineal region. Regardless of any chosen approach, an en-bloc tumor removal is rarely possible and as such piece-meal intracapsular decompression is an important step in preparing good cleavage planes especially the nearby quadrigeminal plate.

Occipital Transtentorial Approach

The occipital transtentorial approach can be performed with the patient placed in a prone, park-bench or sitting position. Currently, the park-bench position is preferred by majority of neurosurgeons. In this position the head is flexed and rotated, allowing the occipital lobe to fall aside with the help of gravity and the whole procedure can be done without placing any retractors on the brain. Approaching from the right side is more common although tumor extension and the torcular characteristic should always be taken into consideration before any decision is made. The typical skin incision is in an inverted U-shape manner starting 1 cm on the left of the occipital protuberance, extending upwards, turning right and finally downwards to reach the right mastoid. Two midline burr holes are performed, one over the torcular and another 6 cm apart and upward. A standard occipital craniotomy exposing 2 cm of the transverse sinus, the torcular and 6 cm of the superior sagittal sinus is completed. The inverted C-shape dural opening is made with the base on the superior sagittal sinus. After exposing the occipital lobe, the ipsilateral occipital horn is taped to release CSF and gain the space. The falx, tentorium and tumor can be visualized. The entry point is the unilateral interhemispheric space between the falx and the medial part of occipital lobe. The large occipital bridging veins are usually not present, but if veins are encountered,

they should be preserved. The tentorium is incised from posterior to anterior and begins 2 cm anterior to the torcular and just lateral to the straight sinus and proceeds parallel to the sinus until the falco-tentorial junction is reached.

In some pineal region meningiomas, the straight sinus and the falco-tentorial junction are encased by the tumor. The tentorial incision is then made laterally to the tumor and proceeds again posteroanteriorly to the free tentorial edge interrupting the vascular supply on the way. The venous channels of the tentorium sometimes cause significant bleeding which can be controlled by bipolar coagulation. The surgeon can see the superior cerebellar artery and the cranial nerve IV running around the brainstem after reflecting the incised tentorium laterally. If the vein of Galen and the internal cerebral veins are extensively invaded by tumor, the residual tumor tissue around these veins can be left in place and treated by radiosurgery.

For extrinsic tumors freely lying in the pineal region, the technique is essentially the same as for pineal gland intrinsic neoplasms. After the tentorium has been divided, the dura reflected, one stay suture can be placed on the dura to increase visibility. When the thick arachnoid cistern of the great vein of Galen becomes visible, a gentle retraction of the occipital lobe is made, avoiding an over-compression of the calcarine sulcus and an avulsion of veins, which could cause hemianopsia. The splenium of corpus callosum comes into sight. An extensive dissection of the arachnoidea helps to expose the ipsilateral medial occipital vein, the pericallosal veins, the precentral cerebellar vein and the tributary veins. The superior vermian vein and the precentral vein can be coagulated and sectioned. The dissection proceeds towards the right ambient cistern to identify the P3 segment of posterior cerebral artery, the cranial nerve IV emerging below the inferior colliculus, and the third segment of the basal vein.

The quadrigeminal plate is a crucial nervous structure, which must be intraoperatively located before deep microsurgical manipulation for tumor resection is started. This plate is situated inferiorly in the operative filed. If the tumor is posterior, the quadrigeminal plate will be covered by it, whereas if it is more anterior, the quadrigeminal plate will be pushed backwards and downwards making it identifiable just after opening of the cistern. The dissection can proceed on both sides laterally to separate the tumor from the pulvinar thalami. During this dissection one should be aware of Rosenthal's veins, which delineate the superior and lateral margins of operative view. Final dissection involves entering of the third ventricle and removing the superior part of the tumor adherent to the velum interpositum, the internal cerebral veins, and the anterior aspect of the vein of Galen. The main entry to the roof of the third ventricle is between the vein of Galen and the splenium. It can be done easily after cutting the posterior pericallosal veins which allows the splenium to be detached from the great vein. The bilateral internal cerebral veins will appear in the velum interpositum cistern. A dissection of the cistern will expose the anterior choroïdal artery in the third ventricle as well as the ventral part of tumor.

Another entry is below the vein of Galen and the internal cerebral vein. It is better to leave residual tumor behind rather than to damage the internal cerebral veins. If bleeding occurs, hemostasis should be achieved by packing surgicel without coagulation. After complete removal of the tumor, the surgeon will have a good view into the third ventricle, which can be extended to the foramina of Monroe and the lamina terminalis. Finally, care should be taken to "unplug" the aqueduct of Sylvius. When this is properly achieved, no postoperative drainage of CSF will be necessary.

Infratentorial Supracerebellar

The infratentorial supracerebellar approach is a gold standard in treating pineal gland tumors. Also, this approach is very useful for the resection of pineal region meningioma without dural attachment and which originates from the velum. The patient is always placed in a sitting position in which the cerebellum can fall inferiorly (Figs. 7.3 and 7.4). The preventative measures against air embolism including a central venous line, oesophageal Doppler and end-tidal CO_2 monitoring are mandatory. A vertical midline skin

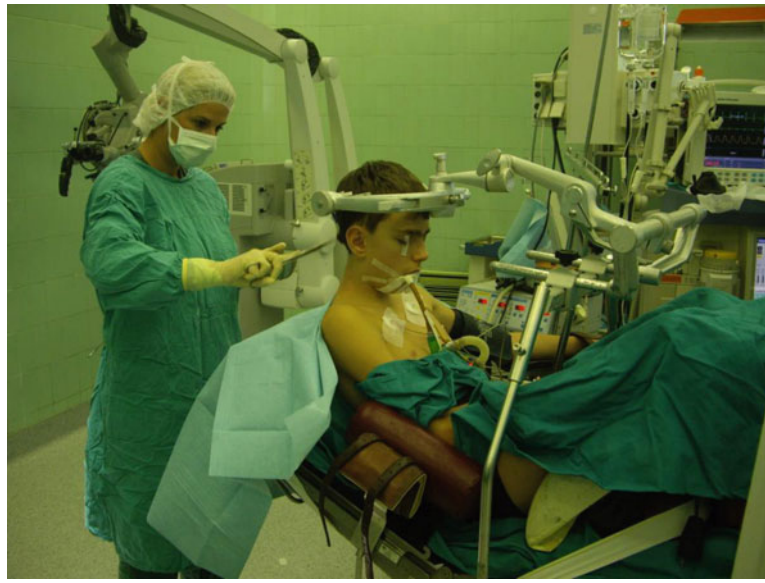

Fig. 7.3 The sitting position

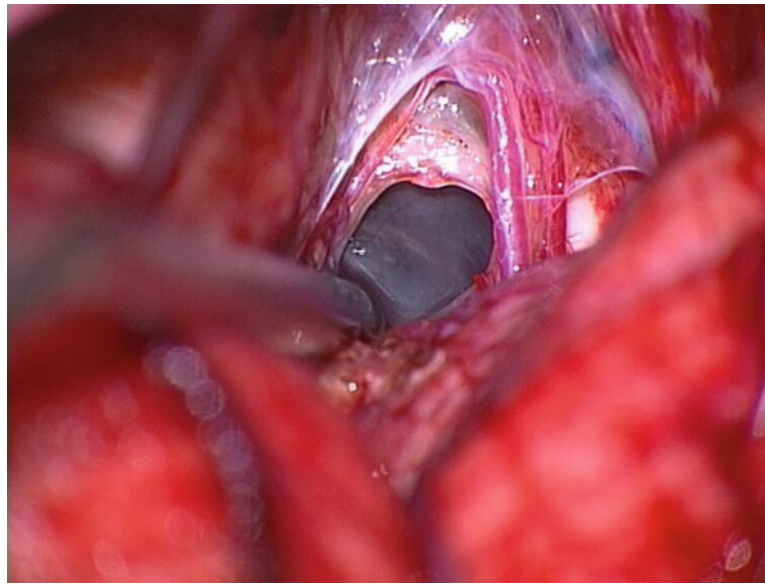

Fig. 7.4 Infratentorial supracerebellar approach

incision extends from 2 cm above the external occipital protuberance down to the level of the C2 spinal process, or lower when a patient has a thick neck. A high-speed drill (instead of a craniotome) is used to make two burr holes just beside the torcular on each side of the midline and to crosscut the sinuses. The craniotomy should not involve the foramen magnum. After craniotomy, any venous leaks must be sealed due to avoid air embolism. The dura is incised in a dull "U" shaped fashion, and the dural flap is suspended upwards. The posterior bridging veins between

the cerebellum and the tentorium are coagulated and transected. One retractor is then placed to pull up the tentorium. This creates a trajectory that leads along the straight sinus to the great vein of Galen. Pursuing the dissection of the arachnoid plane on both sides, a downward traction of the upper part of cerebellum exposes the deeply situated pineal area. A further retraction of the vermis can be executed after sectioning of the superior vermian and precentral veins.

This approach offers a view through the midline that can provide an easy orientation and a symmetrical exposure of both walls and the roof of the third ventricle. Under direct observation, tight tumor adhesion can be freed from the internal cerebral veins or the great veins. The opening of the third ventricle can be closed by fibrin sealant to prevent an excessive CSF leakage. A watertight dural closure should be done with a running suture only after compression of the jugular veins (by anesthesiologist) and sealing of all venous leaks are completed.

The Combined Occipital, Transtentorial, Supracerebellar, Transsinus Approach

Shekar has described a combined supratentorial-infratentorial approach for large pineal region tumor especially for giant falco-tentorial meningiomas. This approach provides a wide view of the pineal region and all venous complexes. The patient is placed in a semiprone position and a suboccipital-occipital craniotomy is performed in three pieces. The infratentorial and supratentorial dura, the superior sagittal and transverse sinuses as well as the torcular are exposed. The cisterna magna is opened to relax the cerebellum and gain the operative space. A needle (20-gauge) attached to a manometer is placed into the transverse sinus just lateral to the torcular. The sinus is occluded for 5 min lateral to the needle. If no brain swelling is observed and venous pressure does not rise more than 5 mmHg, the transverse sinus can be divided on the non-dominant side. The tentorium is divided parasagittally toward the tentorial notch. The occipital lobe and the cerebellum as well as the tentorium and the falx cerebri can then be easily retracted to reach the pineal region tumor. The transverse sinus can sometimes be resutured or even reconstructed by a short vein graft. However, in majority of cases it is safe to leave the sinus permanently occluded.

The advantages of this approach are the wide exposure of the tumor and the deep veins that can restrict the exposure through the occipital transtentorial approach, the decreased retraction of the occipital lobe and the cerebellum, and finally the better control of the posterior third ventricle and the medullary velum. However, there are some concerns about dividing the transverse sinus in a situation where the venous return can easily decompensate.

In conclusion, contemporary management of pineal region tumors requires a multidisciplinary cooperation where surgery represents only one aspect of the treatment plan. However, with the exception of germinoma where only a biopsy is needed, the role of the surgeons still remains prominent as resection of pineal tumors requires high technical skill and experience as well as precise clinical judgment. The infratentorial supracerebellar approach and the occipital transtentorial approach when used appropriately allow access to nearly every type of pineal region neoplasm.

References

Abay EO, Laws ER, Grado GL, Bruckman JE, Forbes GS, Gomez MR, Scott M (1981) Pineal tumors in children and adolescents. J Neurosurg 55(6):889–895

Allen JC, Nisselbaum J, Epstein F, Rosen G, Schwartz MK (1979) Alphafetoprotein and human chorionic gonadotropin determination in cerebrospinal fluid. An aid to the diagnosis and management of intracranial germ-cell tumors. J Neurosurg 51(3):368–374

Asari S, Yabuno N, Ohmoto T (1994) Magnetic resonance characteristics of meningiomas arising from the falcotentorial junction. Comput Med Imaging Graph 18(3):181–185

Asari S, Maeshiro T, Tomita S, Kawauchi M, Yabuno N, Kinugasa K, Ohmoto T (1995) Meningiomas arising from the falcotentorial junction. Clinical features, neuroimaging studies, and surgical treatment. J Neurosurg 82(5):726–738

Balado T (1927) Tumor de la hoz del cerebro pediculado a desarrollo subtentorial. Arch Argent de Neurol 1: 297–310

Bruce JN, Stein BM (1995) Surgical management of pineal region tumors. Acta Neurochir (Wien) 134: 130–135

Dandy WD (1921) An operation for the removal of pineal tumors. Surg Gynecol Obstet 33:113–119
Hosoi K (1930) Teratoma and teratoid tumors of the brain. Arch Pathol 9:1207–1219
Jamieson KG (1971) Excision of pineal tumors. J Neurosurg 35(5):550–553
Jennings MT, Gelman R, Hochberg F (1985) Intracranial germ-cell tumors: natural history and pathogenesis. J Neurosurg 63(2):155–167
Jouvet A, Saint-Pierre G, Fauchon F, Privat K, Bouffet E, Ruchoux MM, Chauveinc L, Fèvre-Montange M (2000) Pineal parenchymal tumors: a correlation of histological features with prognosis in 66 cases. Brain Pathol 10:49–60
Kobayashi S, Kamagata M, Nakamura M, Nakazato Y, Sasaki T (2001) Pineal apoplexy due to massive hemorrhage associated with cavernous angioma: case report. Surg Neurol 55(6):365–371
Konovalov AN, Spallone A, Pitzkhelauri DI (1996) Meningioma of the pineal region: a surgical series of 10 cases. J Neurosurg 85(4):586–590
Krause F (1926) Operative Freilegung der Vierhüge, nebst Beobachtungen bein Hirndruck un Dekompression. Zentralbl Chir 53:2812–2819
Langman J (1975) Medical embryology, 3rd edn. Williams and Wilkins, Baltimore, pp 175–178, 318–364
Matsuno H, Rhoton AL Jr, Peace D (1988) Microsurgical anatomy of the posterior fossa cisterns. Neurosurgery 23:58–80
Okami N, Kawamata T, Hori T, Takakura K (2001) Surgical treatment of falcotentorial meningioma. J Clin Neurosci 8(1):15–18
Ono M, Rhoton AL Jr, Peace D, Rodriguez RJ (1984) Microsurgical anatomy of the deep venous system of the brain. Neurosurgery 15:621–657
Parinaud H (1883) Paralysie des movements associe's des yeux. Arch de Neurol 5:145–172
Poppen JL (1966) The right occipital approach to a pinealoma. J Neurosurg 25(6):706–710
Preslock JP (1984) The pineal gland: basic implications and clinical correlations. Endocr Rev 5:292–308
Raco A, Agrillo A, Ruggeri A, Gagliardi FM, Cantore G (2004) Surgical options in the management of falcotentorial meningiomas: report of 13 cases. Surg Neurol 61(2):157–164
Ringertz N, Nordenstam H, Flyger G (1954) Tumors of the pineal region. J Neuropathol Exp Neurol 13(4):540–561
Sano K (1998) Pineal and posterior third ventricular tumors: a surgical overview. In: Apuzzo MLJ (ed) Surgery of the third ventricle. Williams and Wilkins, Baltimore, pp 801–819
Sawamura Y (1998) Overview for management. In: Sawamura YS, Shirato H, de Tribolet N (eds) Intracranial germ cell tumors. Springer, Wien/New York, pp 169–191
Sawamura Y, de Tribolet N (2002) Neurosurgical management of pineal tumours. Adv Tech Stand Neurosurg 27:217–244
Schild SE, Scheithauer BW, Schomberg PJ, Hook CC, Kelly PJ, Frick L, Robinow JS, Buskirk SJ (1993) Pineal parenchymal tumors: clinical, pathologic, and therapeutic aspects. Cancer 72:870–880
Sekhar LN, Tzortzidis F (1999) Approaches to the pineal region. In: Sekhar LN, de Oliveira E (eds) Cranial microsurgery: approaches and techniques. Thieme, New York
Simson LR, Lampe I, Abell MR (1968) Suprasellar germinomas. Cancer 22:533–544
Stein BM (1971) The infratentorial supracerebellar approach to pineal lesions. J Neurosurg 35:197–202
Van Wagenen WP (1931) A surgical approach for the removal of certain pineal tumors: report of a case. Surg Gynecol Obstet 53:216–220
Yasargil MG (1994) Microsurgery. In: Yasargil MG (ed) Microsurgery of CNS tumours IVB. Georg Thieme Verlag Stuttgart New York, pp 134–165
Zee CS, Segall H, Apuzzo M, Destian S, Colletti P, Ahmadi J, Clark C (1991) MR imaging of pineal region neoplasms. J Comput Assist Tomogr 15:56–63

Part II

Pituitary Tumors

Pituitary Tumors: Genetics and Heritable Predisposition

8

Ricky R. Kalra, Philipp Taussky, Toba Niazi, and William Couldwell

Contents

R.R. Kalra • P. Taussky • T. Niazi • W. Couldwell (✉)
Department of Neurosurgery, University of Utah, Clinical Neurosciences Center, 175 North Medical Drive East, Salt Lake City, UT 84132, USA
e-mail: neuropub@hsc.utah.edu

Abstract

Pituitary tumors are the most common intracranial tumors. Most pituitary tumors are thought to be sporadic, with estimates showing that genetic heritability in the form of traditional syndromes, including multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC), accounts for only 5% of all cases. The monoclonality of pituitary tumors is a widely established model in which a genetic mutation in one cell leads to the formation of an adenoma. On a larger scale, however, the pituitary gland may contain multiple hyperplastic cells, each with its own origin. The predominant cell type within the adenoma is dependent on a variety of oncogenes and tumor suppressor genes including *GSP*, *RAS*, Cyclin D1, *PTTG,* and *p53.* Multiple other studies show germline mutations in a variety of additional genes, including *AIP, BMP-4, CDKN1B, CDKN2A, CDKN2C, GADD45G, PDt-FGFR4, PKC, PRKAR1A, RB, WIF1,* and *ZAC*. More recently, studies of genetic mutations leading to pituitary adenomas and population studies of patients and families with pituitary adenomas have revealed a significant heritable predisposition for pituitary tumors outside of traditional syndromes. These studies, while confirming the heritability of pituitary tumors, unfortunately only provide a glimpse into the multifactorial cause of pituitary adenomas.

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_8,

Introduction: Background and Epidemiology

The most common pathological condition of the pituitary gland is intrinsic tumor growth. The most common tumors, benign adenomas of the pituitary, are a diverse group of tumors that have historically been classified according to size as micro- (less than 1 cm) or macroadenomas (greater than or equal to 1 cm). They are also classified as either functional or nonfunctional, depending on their hormonal activity in vivo.

Pituitary tumors are the most common intracranial tumors, estimated to be present in 16.7% of the population (Ezzat et al. 2004). They comprise up to 10–15% of intracranial tumors found at surgery, 6–25% of intracranial tumors observed at autopsy, and 20% of all primary brain and central nervous systems tumors. They are the second most common type overall by histology in patients between the ages of 20 and 35 years, according to the Central Brain Tumor Registry of the United States (www.cbtrus.org). The majority of these tumors are thought to be sporadic, with estimates showing that genetic heritability in the form of traditional syndromes, including multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC), accounts for only 5% of all cases (Tichomirowa et al. 2009). More recently, studies of genetic mutations leading to pituitary adenomas and population studies of patients and families with pituitary adenomas have revealed a significant heritable predisposition for pituitary tumors outside of traditional syndromes. These heritable tumors have been characterized as familial isolated pituitary adenomas (FIPAs) (Beckers and Daly 2007).

Pituitary Tumors

Nonfunctioning Adenomas

Nonfunctioning adenomas account for approximately 30% of pituitary tumors. The term nonfunctioning reflects the fact that these adenomas do not cause clinical hormone hypersecretion. These adenomas are generally heterogeneous and large and come to medical attention because of their compression effects on the chiasm and on the functioning pituitary, which can lead to hypopituitarism. Despite the lack of clinical hormone secretion, immunocytochemical staining reveals evidence of hormone expression in 80% of cases (Rengachary and Ellenbogen 2005). These are endocrinologically inactive peptides, with the dominant product being the α-subunits, which have no known systemic effects.

The enlargement of nonfunctioning adenomas into the suprasellar region results in optic chiasmal compression and visual field deficits. Additionally, the growing tumor causes progressive loss of native pituitary function over months to years. Gonadotropin function is generally lost first, followed by loss of growth hormone (GH) function, thyroid function, and, finally, adrenocorticotropic hormone (ACTH) function. Loss of vasopressin function is almost never a presenting or eventual symptom. Although progressive visual loss and clinical manifestations of hypopituitarism are generally the presenting symptoms, some patients present secondary to hemorrhage or infarction (apoplexy). With large hemorrhages into the gland, patients may present with sudden headache, a decreased level of consciousness, visual loss, and acute hormonal insufficiency.

Functioning Adenomas

Functioning pituitary adenomas are benign monoclonal tumors that arise from the cells comprising the anterior pituitary gland, which is responsible for secretion and regulation of peptide hormones and stimulating factors. Under physiologic conditions, the anterior pituitary gland depends on the hypothalamus, the portal circulation, and the pituitary stalk for normal hormone secretion. The anterior pituitary hormones ACTH, GH, prolactin, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) are controlled by the hypothalamic hormones corticotropin-releasing hormone (CRH), growth-hormone-releasing factor (GRF), dopamine, thyrotopin-releasing hormone (TRH), and

gonadotropin-releasing hormone (GnRH), respectively. This regulation is accomplished via the portal vascular system linking the hypothalamus and the pituitary gland.

Prolactin-Secreting Adenomas

Prolactin-secreting adenomas are the most common form of pituitary tumor, representing 40% of all pituitary adenomas. The normal regulation of prolactin is achieved by secretion of dopamine by the hypothalamus, which inhibits the anterior pituitary from producing prolactin. Aberrant growth of prolactin-secreting cells results in hyperprolactinemia and leads to amenorrhea, galactorrhea, and osteoporosis in women and diminished sexual drive and impotence in men.

GH-Secreting Adenomas

Acromegaly results from the hypersecretion of GH by the pituitary. Growth hormone is normally released in response to GRF from the hypothalamus, leading to increased levels and activity of insulin-like growth factor-1 (IGF-1), which, in turn, negatively feeds back to limit the production of GH. In addition, GH secretion is tightly regulated by somatostatin. The effects of uncontrolled hypersecretion of GH are gradual and result in gigantism in a child whose epiphyseal plates have not yet closed or in classic acromegalic features in an adult. There is typically an insidious coarsening of the facial features and an increase in the soft tissues. A significant number of patients present because of the local compressive effects of an expending pituitary mass, rather than somatic disturbances.

Glycoprotein-Secreting (TSH, FSH, LH) Adenomas

Glycoprotein-secreting tumors were traditionally thought to represent less than 1% of all pituitary tumors, but improved immunohistochemical techniques have shown that many "nonfunctioning" adenomas have evidence of glycoprotein production. Whereas TSH-secreting tumors cause hyperthyroidism, FSH- and LH-secreting tumors do not produce any specific clinical syndromes. Consequently, these tumors are usually only discovered when they cause symptoms relating to mass effect, and therefore they require surgical resection. Unfortunately, surgical cure is rarely achieved because of the suprasellar extension and involvement of the cavernous sinuses by the time they present to medical attention.

ACTH-Secreting Adenomas (Cushing Disease)

Hypercortisolemia causes multiple clinical problems; patients feel poorly and have diffuse muscle pain and weakness, emotional lability, and profound fatigue. They present with accelerated atherosclerosis, hypertension, diabetes mellitus, osteoporosis, obesity, susceptibility to infections, peptic ulcer disease, and thrombosis. Cushing disease is a serious medical condition that will shorten the life of afflicted individuals because of associated comorbidities. Most cases of Cushing disease in the adult population are caused by microadenomas of the anterior pituitary gland.

Genetic Syndromes Associated with Pituitary Tumors

Two autosomal dominant syndromes have been associated with a familial inheritance of pituitary adenomas: MEN1 and CNC. The familial syndrome MEN-1 affects the parathyroid gland, the endocrine pancreas, and, with less frequency, the pituitary gland. The syndrome is inherited in an autosomal-dominant fashion with reduced penetrance. Hyperparathyroidism develops in 90% of patients with MEN-1, and islet cell tumors of the pancreas develop in 60–70% of patients. Pituitary tumors occur in 24–45% of the patients, with symptoms consistent with hyperprolactinemia occurring in the majority of these patients (Lemos and Thakker 2008). The syndrome is caused by an inactivating mutation in the *MEN1* gene on chromosome 11q13, which encodes the nuclear protein menin. The clinical presentation of *MEN1* has been extensively characterized, and pituitary adenomas occur in 40% of patients with the syndrome (Lemos and Thakker 2008). All tumor phenotypes can occur, but prolactinomas predominate in this patient population. Over 350

mutations of the *MEN1* gene have been found, but more than 10% of patients with the clinical disease have no known gene mutations (Lemos and Thakker 2008). This suggests that epigenetic factors may also be involved in the development of the tumors (Lemos and Thakker 2008).

Carney complex is an autosomal-dominant familial neoplasia syndrome characterized by spotty skin pigmentation, cardiac myxomas, primary pigmented nodular adrenocortical disease, pituitary tumors, and schwannomas. Among the endocrine tumors, GH-producing pituitary adenomas are seen in approximately 10% of patients (Yin et al. 2008). Carney complex is a rare condition that is linked in more than 50% of cases to an inactivating mutation in the gene encoding protein kinase A at 17q24. The primary abnormality in CNC pituitary disease is multifocal cell hyperplasia. Thus, about 75% of patients with CNC exhibit subclinical increases in growth hormone, IGF-1, and prolactin levels, but only 10% of patients actually exhibit symptoms of acromegaly (Kirschner et al. 1998).

The recently defined syndrome FIPA has been identified with more frequency in the literature. Isolated familial somatotropinoma (IFS) was the first form of this syndrome described. It was defined as the occurrence of at least two cases of acromegaly in a single family in the absence of MEN1 and CNC. IFS was subcategorized within the broader FIPA syndrome category once data appeared to support the theory that pituitary adenomas of all types—not limited to IFS—can occur in a familial setting in the absence of MEN1 and CNC (Beckers and Daly 2007). The identification of FIPA as a "wastebasket" of heritable pituitary tumors indicates the vast amount of genetic mutations associated with pituitary tumors and the genetic predisposition linked to these mutations. Furthermore, heritable genetic mutations in families with incomplete penetrance indicate significant epigenetic factors associated with the development of pituitary adenomas.

Genetic studies have pinpointed one of the hereditary abnormalities associated with FIPA within a region on chromosome 11q13.3 in 15% of patients; however, multiple other chromosomes have been implicated in other forms of FIPA. The location of the 11q13.3 mutation is associated with the *aryl hydrocarbon receptor interacting protein (AIP)* gene (Vierimaa et al. 2006).

Pituitary Tumorigenesis: Genetic Basis

The monoclonality of pituitary tumors is a widely established phenomenon in which a genetic mutation in one cell leads to the formation of an adenoma. On a larger scale, however, the pituitary gland may contain multiple hyperplastic cells, each with its own origin. The predominant cell type within the adenoma is dependent on a variety of oncogenes and tumor suppressor genes. The most important oncogene implicated in sporadic tumors is *GSP*, which encodes a protein that regulates growth-hormone-releasing-hormone (GHRH) effects. The oncogene *RAS* has also been identified in tumorigenesis and is associated with aggressive pituitary tumors, including pituitary carcinomas. Cyclin D1, a cell cycle protein, is disrupted in a portion of pituitary adenomas and is overexpressed in approximately 70% of nonfunctioning tumors and 40% of somatotropinomas (GH-secreting pituitary adenomas) (Hibberts et al. 1999). The *pituitary tumor transforming gene (PTTG)* is usually down-regulated in healthy glands but shows up-regulation in functional adenomas (Zhang et al. 1999). Cell cycle regulator *p53* has also been shown to be mutated in a percentage of tumors (Tanizaki et al. 2007). Multiple other studies show germline mutations in a variety of genes, including *AIP, BMP-4, CDKN1B, CDKN2A, CDKN2C, GADD45G, PDt-FGFR4, PKC, PRKAR1A, RB, WIF1,* and *ZAC* (Tichomirowa et al. 2009). We will discuss some of the well-studied mutations further in this chapter.

Molecular Genetics and Epigenetic Alterations of Pituitary Tumors

Origin of Pituitary Adenomas

The clinical effects of pituitary tumors have been extensively studied, but until the 1990s researchers

lacked a good understanding of the initiating events of tumorigenesis. It was not known whether most pituitary adenomas arose from a single mutation or if they formed from multiple cells simultaneously stimulated by factors released from the hypothalamus. Using DNA restriction fragment length polymorphisms, Jacoby et al. (1990) studied X chromosome inactivation in DNA isolated from pituitary adenomas in women with three different hormonal subtypes. Analysis of the DNA fragments showed in each of the three samples that only one X chromosome was active in all cells within the adenoma, thus elucidating the monoclonal origin of pituitary adenomas. Multiple additional studies confirmed that pituitary adenoma tumorigenesis occurred in a single cell, leading to a clonal population of tumor cells (Jacoby et al. 1990).

Genetic Abnormalities of Carney Complex and Multiple Endocrine Neoplasia

The majority of genetic abnormalities in pituitary tumors result in dysregulation of hormone signaling, dysregulation of growth-factor signaling, dysregulation of signaling proteins, or cell cycle regulation (Spada et al. 2007).

CNC—PRKAR1A Gene

At the genetic level, CNC can be caused by null mutations in the *PRKAR1A* gene, encoding the type 1a regulatory subunit of protein kinase A. To further elucidate the role of this gene on pituitary tumorigenesis, researchers produced a tissue-specific knockout of this gene in a mouse model (Yin et al. 2008). The frequency of pituitary tumors was significantly increased in the knockout mice. At a hormonal level, GH levels in serum of knockout mice were markedly elevated compared with those of controls, regardless of whether a visible GH adenoma developed or not. Mice with heterozygous knockout did not have increased frequency of pituitary tumors. Thus, a null mutation at the *PRKAR1A* gene is necessary for development of pituitary tumors in the mouse CNC model.

Protein kinase A pathway abnormalities are well known to cause human pituitary adenomas. This is well illustrated by the activating mutations in G protein subunit Gs-α (encoded by the *gsp* oncogene). Mutations in the protein kinase A pathway lead to constitutive activation of the G protein, with subsequent stimulation of cAMP and protein kinase activity, leading to changes in GHRH effects and thus GH-secreting adenomas. *PRKAR1A* also causes changes the protein kinase A pathway, producing GH adenomas in patients with CNC.

Familial MEN-1 Syndrome

At the genetic level, the *MEN1* gene, which is responsible for the syndrome, encodes a 610-amino-acid protein, menin, on chromosome 11. The chromosome 11q13 germline mutation in *MEN-1* is revealed by a "second hit" on the remaining normal allele and is visualized as a loss of heterozygosity (LOH) using PCR technology to evaluate the gene. The "two-hit" requirement for phenotypic expression of the syndrome is further validated by the presence of truncated *MEN1* gene regions in up to 85% of families with the MEN-1 syndrome. LOH of the wild-type chromosome results in the development of tumors in patients with heterozygotic inheritance of the allele, including pancreatic (40% by 9 months after LOH), parathyroid (24% by 9 months after LOH), and pituitary (26% by 16 months after LOH) tumors (Williamson et al. 1995). The role of *MEN1* mutations in pituitary tumorigenesis in humans is not readily apparent yet. The predominance of prolactinomas in familial MEN-1 suggests that pituitary tumors might be caused by similar mutations. Since mutation of the wild-type allele in a heterozygous patient at the *MEN1* gene is observed in up to 30% of sporadic pituitary adenomas, the *MEN1* mutation may play a role in the progression but not the initiation of sporadic pituitary tumors. However, DNA sequence analysis shows that *MEN1* mutations occur in less than 2% of sporadic pituitary adenomas (Spada et al. 2007).

Genes Associated with Adenomas and Epigenetic Factors

Pituitary tumorigenesis, whether in the sporadic form or as part of a heritable syndrome, frequently involves mutations of oncogenes, tumor

Table 8.1 Germline and somatic gene abnormalities associated with pituitary adenomas

Gene	Product/Defect
Oncogenes	
Cyclin D1	Overexpression in nonsecreting adenomas and somatotropinomas
Gsp	Somatic activating mutations in up to 40% of somatotropinomas
PTTG	Increased expression in more aggressive pituitary tumors
RAS	Somatic activating mutations in pituitary carcinomas
c-myc	Transcription factor associated with PTTG gene mutations
Tumor suppressor genes	
MEN1	Inactivating mutations in all pituitary adenoma types
p53	Somatic inactivating mutations and overexpression in pituitary carcinomas
Retinoblastoma	Somatic mutations and promoter methylation in pituitary adenomas
Cell cycle regulators	
AIP	Germline mutations and loss of heterozygosity in 15% of FIPA cases
CDKN1B (p27)	Germline heterozygous nonsense mutations
PKC	Point mutation in invasive pituitary adenomas
PRKAR1A	Truncating mutations in Carney complex leading to hyperplasia and adenomas
ZAC	Promoter methylation in nonfunctioning adenomas
Growth factors and cytokines	
Pdt-FGFR4	Alternative transcription initiation in pituitary adenomas
BMP-4	Increased expression in prolactinoma

suppressor genes, cell cycle regulator genes, and epigenetic factors (Table 8.1). Recently published data indicate that pituitary tumors may possess a greater heritability than previously thought (Couldwell and Cannon-Albright 2010). Thus, an understanding of the genetic basis of these tumors is paramount in understanding the possible risk these tumors present to offspring of affected individuals (Tichomirowa et al. 2009).

Oncogenes

An oncogene is a gene that, when mutated or expressed at high levels, helps turn a normal cell into a tumor cell. Many abnormal cells normally undergo a programmed form of death, apoptosis. Activated oncogenes can cause those cells to survive and proliferate instead.

Several oncogenes have been implicated in the development of pituitary adenomas.

Cyclin D1

Cyclin D1, located on chromosome 11q13, regulates the cell cycle, specifically progression through the G_1 phase. Mutations in *cyclin D1* result in unregulated growth of pituitary cells. *Cyclin D1* amplification is more frequent in nonfunctioning adenomas and invasive tumors. The gene mutation is overexpressed in 70% of nonfunctioning tumors and 40% in somatotropinomas (Hibberts et al. 1999).

GSP

The most important oncogene implicated in sporadic tumors is *gsp*, which encodes a protein that regulates GHRH effects. Located on chromosome 20, mutation of the α subunit of the stimulatory guanine nucleotide-binding protein produces an unregulated active adenyl cyclase signaling system, which causes an inhibition of GTP hydrolysis and maintains Gsα in constitutively active state, thus increasing cAMP, leading to GH hypersecretion via upregulation of GHRH effects. Mutations of the *gsp* gene have been noted in approximately 40% of GH adenomas, 10% of nonfunctioning adenomas, and 6% of ACTH adenomas (Clayton 1999).

PTTG and C-myc

The PTT gene, also known as *securin*, is generally poorly expressed in normal pituitary glands but is up-regulated in most pituitary adenomas. *Securin*

appears to induce expression and secretion of basic fibroblast growth factor, a strong mitogenic and angiogenic factor (Zhang et al. 1999). Additionally, *securin* is involved in chromosome separation during mitosis, and mutations in the gene lead to improper cell cycle function, causing chromosomal instability and aneuploidy, and thus giving *PTTG* its oncogenetic potential (Zou et al. 1999). Basic fibroblast growth factor activation has also been shown to activate the *c-myc* oncogene. *C-myc* codes for proteins that bind to DNA and serves as a transcription factor. Overexpression of the *c-myc* oncogene, located on chromosome 8q24, has been reported in nearly one third of all pituitary adenomas (Pei 2001).

RAS

Expression of the *ras* oncogene, located on chromosome 5p13, has been reported in pituitary carcinomas and aggressive prolactinomas. The gene expression is absent in benign adenomas, which indicates that its activation may be a secondary event causing further de-differentiation to a more aggressive tumor type. By itself, *ras* does not likely play a significant role in initiating tumor development.

Tumor Suppressor Genes

A tumor suppressor gene, or anti-oncogene, is a gene that protects a cell from becoming a tumor cell. When this gene is mutated to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes. Unlike oncogenes, tumor suppressor genes generally follow the 'two-hit hypothesis,' which implies that both alleles that code for a particular gene must be affected before an effect is manifested. This is because if only one allele for the gene is damaged, the second can still produce the correct protein. Thus, mutant tumor suppressor alleles are usually recessive whereas mutant oncogene alleles are typically dominant. Several tumor suppressor genes have been implicated in the development of pituitary adenomas.

MEN1

Among the earliest recognized tumor suppressor genes associated with pituitary adenomas was the *MEN1* gene located on chromosome 11q13. Twenty-five to forty-five percent of patients with the autosomal-dominant inherited germ-line mutation in *MEN1* gene, who thus have *MEN1* syndrome, have pituitary tumors. *MEN1* mutations have been reported in sporadic tumors of all major subtypes, including ACTH and GH adenomas, prolactinomas, and nonfunctioning adenomas. The biological role of *MEN1* appears to be in part as a tumor suppressor gene with an immense series of interactions within the cell. Menin regulates or interacts with promoter regions of hundreds of genes and has a wide regulatory role in transcription (Agarwal et al. 2007).

p53

Cell cycle regulator and tumor suppressor gene *p53* has also been implicated in pituitary tumorigenesis. *p53* encodes a nuclear protein that regulates cyclin-dependent kinase (CDK) inhibitor p21. p21 induction has been shown to restrain cell cycle progression and thus pituitary tumor growth. Mutations in *p53* result in decreased p21 activation and uncontrolled replication (Chesnokova et al. 2008). The mutated *p53* gene, located on chromosome 17p13, has been reported in high percentage of patients with Cushing disease and in invasive nonfunctioning adenomas. Additionally, a recent study demonstrated *p53* mutations resulting in high expression of *p53* protein by pituitary tumor cells, both noninvasive and invasive ACTH adenomas (Tanizaki et al. 2007).

Retinoblastoma Gene (Rb)

The retinoblastoma protein Rb is a tumor suppressor protein that is dysfunctional in many cancers. A primary function of the versatile protein is to prevent excessive cell growth by inhibiting cell cycle progression until a cell is ready to divide. *Rb* also serves as recruiter of several chromatin remodeling enzymes such as methylases and acetylases. Differing results in regard to the role of the retinoblastoma gene (*Rb*) in tumorigenesis of pituitary adenomas have been reported. Somatic *Rb* gene loss via deletion in a locus on the long arm of chromosome 13 has been reported in invasive adenomas (Pei et al. 1995). Hypermethylation of the promoter region of the *Rb* gene has also

been implicated in tumor development. Overall, there are no definitive studies showing the specific mechanism by which *Rb* initiates or enhances pituitary tumor growth (Tichomirowa et al. 2009).

Cell Cycle Regulators

Cell cycle regulators function to initiate, control, and stop cell growth and development. Dysregulation of the cell cycle components may lead to aberrant cell cycle initiation and thus lead to hyperplasia and tumor development. The following cell cycle regulators have been implicated in the development of pituitary adenomas.

Aryl Hydrocarbon Receptor Interacting Protein (AIP)

Aryl hydrocarbon receptor interacting protein was discovered to cause adenomas in a comprehensive genetic study in families with multiple members diagnosed with pituitary tumors. Studies of patients in the FIPA cohort showed that *AIP* was associated with familial presentation of somatotropinomas and prolactinomas in 15% of cases (Vierimaa et al. 2006). Immunohistochemical methods showed that in normal cases, *AIP* was localized within GH- and prolactin-secreting cells; however, in sporadic and familial tumors, it was expressed in all tumor types (Tichomirowa et al. 2009).

The mechanism by which *AIP* mutations lead to adenomas in sporadic as well as FIPA cases is not well understood. To date, all germline *AIP* mutations reported have been heterozygous in nature. Some researchers have speculated that homozygous germline *AIP* mutations are not compatible with life. Thus, *AIP* mutations resulting in abnormal protein production are due to a 'second-hit' phenomenon. *AIP* mutations described to date result in either truncation or misfolding of the protein. The *AIP* gene product interacts with multiple cell cycle proteins including phosphodiesterases, heat shock proteins, cAMP, and aryl hydrocarbon receptors. Thus, abnormal cell cycle regulation is hypothesized to lead to aberrant growth (Daly et al. 2007).

CDKN1B (p27)

In pituitary adenomas, CDK inhibitor *p27*, located on chromosome 12, appears to have an important function, as evidenced by animal studies showing development of pituitary tumors in homozygous *p27* deletions. p27 protein quantitative reduction has also been shown to occur in adenomas but not in normal pituitary glands. The reduction in protein was most predominant in ACTH tumors (Lidhar et al. 1999).

Protein Kinase C

Protein kinase C (PKC) is an enzyme involved in the regulation of cellular growth, proliferation, and differentiation of the anterior pituitary gland. Increased PKC activity and expression have been reported in pituitary adenomas, especially in the invasive forms. In particular, the PKC α-isoform (α PKC) is overexpressed in these tumors. Furthermore, the α-isoform can undergo a point mutation, structurally altering the protein and resulting in a more aggressive tumor form (Couldwell et al. 1996).

PRKAR1A

Carney complex is associated with mutations in the *PRKAR1A* gene. This gene has been identified in 60% of patients with CNC (Veugelers et al. 2004). The *PRKAR1A* gene encodes the protein kinase A regulatory subunit Iα, and mutation of the gene results in mRNA instability leading to absent or decreased gene product. Decreased protein function within the cell increases cAMP effects and leads to tumor growth. *PRKAR1A* homozygous gene deletion is not compatible with life; *PRKAR1A* mutations resulting in deficient protein production are due to a 'second-hit' phenomenon (Kirschner et al. 2000).

ZAC

The *ZAC* gene encodes a zinc finger protein that also induces cell apoptosis and cell cycle arrest. The protein, located on chromosome 6, is normally expressed in high quantities in healthy pituitary glands. In nonfunctional adenomas, however, *ZAC* expression is virtually absent. Interestingly, loss of expression of the ZAC protein was not associated with a mutation of the *ZAC* gene; thus, an alternative epigenetic mechanism of gene inactivation must exist. One such mechanism implicated in the *ZAC* gene defect is promoter methylation leading to decreased protein product (Pagotto et al. 2000).

Table 8.2 Epigenetic factors associated with pituitary adenomas

Gene	Product/Defect
CDKN2A (p16)	Promoter methylation in pituitary adenomas
DAP	Promoter methylation in invasive/metastatic adenomas
GADD45G	Promoter methylation in nonsecreting adenomas, prolactinomas, and somatotropinomas
Gsp	Somatic activating mutations and relaxation of imprinting
MEG3a	Promoter methylation in nonsecreting adenomas and gonadotropinomas
Pdt-FGFR	Alternative transcription initiation in pituitary adenomas
Rb	Promoter methylation in pituitary adenomas
WIF 1	Promoter methylation in pituitary adenomas
ZAC	Promoter methylation in nonfunctioning adenomas

Growth Factors and Cytokines

Although pituitary adenomas arise from a clonal expansion of a single cell, the monoclonal growth may occur within a hyperplastic environment secondary to disturbances in paracrine regulation, including cytokines and growth factors. Abnormal responses to target organ feedback can also lead to paracrine abnormalities and play a role in creating this environment (Vallar et al. 1987).

Examples of such responses include:

1. Overexpression of FR-α. Pathological staining and microarray analysis has revealed that the folate receptor FR-α is significantly overexpressed in clinically nonfunctioning adenomas. It is a high-affinity folate transporter and thus may provide a growth advantage to dysplastic cells (Evans et al. 2008).
2. Potentiation of prolactin-producing cells by estrogen during pregnancy and lactation.
3. Loss of negative feedback resulting in tumor growth in the setting of adrenalectomy (Nelson syndrome) (Ando et al. 2001).
4. Mutations in fibroblast growth factor receptors (FGFRs). FGFRs assist in the growth and development of tissues. Mutations in such factors have been shown to lead to hyperplasia; specifically, a truncated pituitary tumor–associated form of FGFR4 has resulted in invasive pituitary tumorigenesis in animal models via alternative transcription initiation (Ezzat and Asa 2006).
5. Effects on bone morphogenic proteins (BMPs). These members of the tumor growth factor (TGF) β family interact with downstream regulators to control the cell cycle and cell proliferation. Specifically, BMP-4 has a stimulatory role on prolactin-secreting cells and supports the development of prolactinomas, but it has an inhibitory action on the corticotropic cells. Thus, cytokines in general, and the TGFβ family specifically, play a complicated role in tumorigenesis and tumor inhibition (Giacomini et al. 2007).

Epigenetic Factors

Epigenetics is the study of inherited changes in phenotype and gene expression caused by mechanisms other than changes in the underlying DNA sequence. Three main types of epigenetic inheritance have been studied: DNA methylation, genomic imprinting, and histone modification (Table 8.2). In many cancers, epigenetic changes have been implicated in development and growth of tumors, including pituitary adenomas (Tichomirowa et al. 2009).

DNA Methylation

Cell cycle regulators *p16* (CDKN2A) and *Rb* are absent in virtually all tumor types. The mechanisms implicated in their loss include gene deletion, point mutations, and promoter methylation. In pituitary tumors, DNA methylation of *p16* with gene silencing is present in approximately 70% of sporadic tumors and represents an early change in pituitary tumorigenesis. In regard to *Rb* absence, only a small portion is attributable to gene-silencing via methylation compared with deletion (Farrell 2005). The death-associated protein kinase (DAP kinase) gene product is an apoptotic mediator via the activation of *p19*/*p53*.

Several studies have implicated the methylation and deletion of DAP kinase gene with highly invasive and/or metastatic pituitary tumors (Simpson et al. 2002). Methylation of the growth arrest and DNA damage-inducible gene (GADD45G) also leads to gene silencing and is associated with the development of somatotropinomas, prolactinomas, and nonfunctioning adenomas (Zhang et al. 2002).

Imprinting

Genomic imprinting of the maternal and paternal DNA during gametogenesis establishes conditions whereby a specific allele is more abundantly or exclusively expressed in the offspring. Relaxation of imprinting by activation of the nonimprinted gene can lead to tumor development. Recent studies indicate that the oncogene *gsp* normally undergoes imprinting with the maternal allele exclusively being expressed (Weinstein et al. 2002). Tumor development due to gsp abnormalities is generally associated with mutation of the gene, but data suggest that some tumors express biallelic gene products, indicating a loss of imprinting. Relaxation of imprinting of gsp may represent a secondary feature in the progression of these tumors (Hayward et al. 2001).

Histone Modification

Histones are subject to a wide variety of post-translational modifications. These modifications occur primarily within the histone amino-terminal tails protruding from the surface of the nucleosome as well as on the globular core region (Cosgrove et al. 2004). Histone modifications are proposed to affect chromosome function through at least two distinct mechanisms. The first mechanism suggests modifications may alter the electrostatic charge of the histone resulting in a structural change in histones or their binding to DNA. The second mechanism proposes that these modifications are binding sites for protein recognition modules. Thus, post-translational modifications of histones create an epigenetic mechanism for the regulation of a variety of normal and disease-related processes, including pituitary tumor growth (Tateno et al. 2010). Recently published data suggest that MAGE-A3, a member of the MAGE-I family of cancer-related antigens, is abundantly transcribed in pituitary tumors and has been implicated in transcriptional silencing of *p53* through histone modification (Monte et al. 2006). As mentioned earlier, *p53* serves as a cell cycle regulator and tumor suppressor gene. *p53* encodes a nuclear protein that regulates CDK inhibitor p21. p21 induction has been shown to restrain cell cycle progression and restrain pituitary tumor growth. The silencing of the *p53* gene results in decreased *p21* activation and uncontrolled replication (Chesnokova et al. 2008).

Overview of Current Understanding of Heritability

Utah Population Data Base

Numerous researchers have undertaken the effort to understand genetic alterations that contribute to the development of different disease states associated with pituitary tumors. The ultimate goal of these studies is to develop processes that will enable us to screen and treat patients afflicted with these specific diseases and someday prevent their development altogether. In addition to the laboratory studies evaluating the genetic contribution to pituitary tumors, another resource has now been applied to the study of these tumors. The Utah Population Database (UPDB) was developed with the goal of investigating the genetic contribution to cancer. During the last 30 years, use of the UPDB has expanded to include many tumor types, and the genetic contribution to disease phenotypes other than cancer have also been evaluated. This resource links genealogical information representing Utah's pioneers and their descendants with the individual cancer records represented in the Utah Cancer Registry (UCR), which collects data on all patients with cancer diagnosed in the state, and with annual updates from the Utah Department of Health for births, deaths, marriages, divorces, as well as records from the Utah Driver's License Division. The UPDB now encompasses over seven million individuals and has a subset of over 2.5 million individuals with at

least three generations of genealogical data; and some pedigrees now extend to 11 generations linking back to the initial settlers in Utah.

Evaluation of Heritable Contribution

The genetic contribution to specific phenotypes can be evaluated using one of three different methods: the Genealogical Index of Familiality (GIF), which was developed specifically for use with the UPDB; the estimation of the relative risks (RR); and the identification of high-risk pedigrees with specific phenotypes observed in significant excess (Couldwell and Cannon-Albright 2010). The GIF examines the estimation of the average relatedness among affected individuals who share a specific phenotype (Couldwell and Cannon-Albright 2010). If individuals in the database with a selected phenotype have a significantly greater average relatedness than matched controls, there is evidence of excess familiality. Similarly, phenotypes with a genetic contribution should occur more often in relatives of those affected with that phenotype than in the control population (i.e., higher RR) (Couldwell and Cannon-Albright 2010). Finally, high-risk pedigrees found in the database may suggest the predisposition genes responsible for the observed phenotypes.

Use of the UPDB for Evaluation of Heritability of Pituitary Tumors

Using the data available in the UPDB and the UCR, Couldwell and Cannon-Albright (2010) analyzed the genetic relationships among individuals diagnosed with benign or malignant pituitary tumors to investigate whether there was evidence for a heritable contribution to the disease in non-syndromic cases. Twenty-one patients with a malignant pituitary tumor and 720 individuals with a benign pituitary tumor recorded in the UCR since 1966 also had Utah genealogical data. The analysis of the GIF testing the hypothesis of no excess relatedness for all pituitary tumor cases demonstrated that the pituitary cases had a higher degree of relatedness than expected ($p < 0.001$). The average relatedness of all pituitary tumor cases was also significantly higher than expected when all relationships closer than third-degree relatives were ignored. The RR assessment demonstrated a significantly elevated risk to first and third-degree relatives of affected individuals. Using these two methods, the authors found strong evidence for a genetic contribution to predisposition to symptomatic pituitary tumors.

Use of Heritability Information

This information is particularly valuable for counseling family members of patients treated for pituitary adenomas. Relative risks for first- and third-degree relatives were significantly elevated ($RR = 2.83$ and 1.63, respectively) (Couldwell and Cannon-Albright 2010). It also may raise a higher index of suspicion for the detection of symptomatic pituitary tumors in such individuals. At the present time, the cost effectiveness of screening close relatives for pituitary tumors using magnetic resonance imaging and endocrine studies has yet to be determined, but there is the potential in the future that this information may be useful for early detection. This information could be a stepping stone for gene mapping in these high-risk groups and for creation of a low-cost screening tool using the gene mapping information to help to lessen the burden of the disease.

In conclusion, extensive molecular research into pituitary tumors has revealed an exhaustive list of genes responsible for the growth of pituitary cells. Furthermore, epidemiological data has elucidated the genetic predisposition to clinically significant pituitary tumors in family members of patients. The data linking specific genes to relative risk to family members is still sparse. The discovery of familial pituitary tumor syndrome (FIPA) and the AIP gene has shown that no single gene is solely responsible for heritable pituitary adenomas. As such information linking specific genes with pituitary tumors becomes complete and available, it will be extremely valuable in counseling family members of patients

treated for pituitary adenomas and for raising a higher index of suspicion for the detection of a symptomatic pituitary tumor in such individuals.

References

Agarwal SK, Impey S, McWeeney S, Scacheri PC, Collins FS, Goodman RH, Spiegel AM, Marx SJ (2007) Distribution of menin-occupied regions in chromatin specifies a broad role of menin in transcriptional regulation. Neoplasia 9:101–107

Ando S, Sarlis NJ, Oldfield EH, Yen PM (2001) Somatic mutation of TRbeta can cause a defect in negative regulation of TSH in a TSH-secreting pituitary tumor. J Clin Endocrinol Metab 86:5572–5576

Beckers A, Daly AF (2007) The clinical, pathological, and genetic features of familial isolated pituitary adenomas. Eur J Endocrinol 157:371–382

Chesnokova V, Zonis S, Kovacs K, Ben-Shlomo A, Wawrowsky K, Bannykh S, Melmed S (2008) p21(Cip1) restrains pituitary tumor growth. Proc Natl Acad Sci USA 105:17498–17503

Clayton RN (1999) Sporadic pituitary tumours: from epidemiology to use of databases. Baillieres Best Pract Res Clin Endocrinol Metab 13:451–460

Cosgrove MS, Boeke JD, Wolberger C (2004) Regulated nucleosome mobility and the histone code. Nat Struct Mol Biol 11:1037–1043

Couldwell WT, Cannon-Albright L (2010) A heritable predisposition to pituitary tumors. Pituitary 13:130–137

Couldwell WT, Law RE, Hinton DR, Gopalakrishna R, Yong VW, Weiss MH (1996) Protein kinase C and growth regulation of pituitary adenomas. Acta Neurochir Suppl 65:22–26

Daly AF, Vanbellinghen JF, Khoo SK, Jaffrain-Rea ML, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo AP, Tamburrano G, Raverot G, Barlier A, De Herder W, Penfornis A, Ciccarelli E, Estour B, Lecomte P, Gatta B, Chabre O, Sabate MI, Bertagna X, Garcia Basavilbaso N, Stalldecker G, Colao A, Ferolla P, Wemeau JL, Caron P, Sadoul JL, Oneto A, Archambeaud F, Calender A, Sinilnikova O, Montanana CF, Cavagnini F, Hana V, Solano A, Delettieres D, Luccio-Camelo DC, Basso A, Rohmer V, Brue T, Bours V, Teh BT, Beckers A (2007) Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. J Clin Endocrinol Metab 92:1891–1896

Evans CO, Yao C, Laborde D, Oyesiku NM (2008) Folate receptor expression in pituitary adenomas cellular and molecular analysis. Vitam Horm 79:235–266

Ezzat S, Asa SL (2006) Mechanisms of disease: the pathogenesis of pituitary tumors. Nat Clin Pract Endocrinol Metab 2:220–230

Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, McCutcheon IE (2004) The prevalence of pituitary adenomas: a systematic review. Cancer 101:613–619

Farrell WE (2005) Epigenetic mechanisms of tumorigenesis. Horm Metab Res 37:361–368

Giacomini D, Acuna M, Gerez J, Nagashima AC, Silberstein S, Paez-Pereda M, Labeur M, Theodoropoulou M, Renner U, Stalla GK, Arzt E (2007) Pituitary action of cytokines: focus on BMP-4 and gp130 family. Neuroendocrinology 85:94–100

Hayward BE, Barlier A, Korbonits M, Grossman AB, Jacquet P, Enjalbert A, Bonthron DT (2001) Imprinting of the G(s)alpha gene GNAS1 in the pathogenesis of acromegaly. J Clin Invest 107:R31–R36

Hibberts NA, Simpson DJ, Bicknell JE, Broome JC, Hoban PR, Clayton RN, Farrell WE (1999) Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. Clin Cancer Res 5:2133–2139

Jacoby LB, Hedley-Whyte ET, Pulaski K, Seizinger BR, Martuza RL (1990) Clonal origin of pituitary adenomas. J Neurosurg 73:731–735

Kirschner LS, Taymans SE, Stratakis CA (1998) Characterization of the adrenal gland pathology of Carney complex, and molecular genetics of the disease. Endocr Res 24:863–864

Kirschner LS, Sandrini F, Monbo J, Lin JP, Carney JA, Stratakis CA (2000) Genetic heterogeneity and spectrum of mutations of the PRKAR1A gene in patients with the carney complex. Hum Mol Genet 9:3037–3046

Lemos MC, Thakker RV (2008) Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. Hum Mutat 29:22–32

Lidhar K, Korbonits M, Jordan S, Khalimova Z, Kaltsas G, Lu X, Clayton RN, Jenkins PJ, Monson JP, Besser GM, Lowe DG, Grossman AB (1999) Low expression of the cell cycle inhibitor p27Kip1 in normal corticotroph cells, corticotroph tumors, and malignant pituitary tumors. J Clin Endocrinol Metab 84:3823–3830

Monte M, Simonatto M, Peche LY, Bublik DR, Gobessi S, Pierotti MA, Rodolfo M, Schneider C (2006) MAGE-A tumor antigens target p53 transactivation function through histone deacetylase recruitment and confer resistance to chemotherapeutic agents. Proc Natl Acad Sci USA 103:11160–11165

Pagotto U, Arzberger T, Theodoropoulou M, Grubler Y, Pantaloni C, Saeger W, Losa M, Journot L, Stalla GK, Spengler D (2000) The expression of the antiproliferative gene ZAC is lost or highly reduced in nonfunctioning pituitary adenomas. Cancer Res 60:6794–6799

Pei L (2001) Identification of c-myc as a down-stream target for pituitary tumor-transforming gene. J Biol Chem 276:8484–8491

Pei L, Melmed S, Scheithauer B, Kovacs K, Benedict WF, Prager D (1995) Frequent loss of heterozygosity at the retinoblastoma susceptibility gene (RB) locus in aggressive pituitary tumors: evidence for a chromosome 13 tumor suppressor gene other than RB. Cancer Res 55:1613–1616

Rengachary SS, Ellenbogen RG (2005) Principles of neurosurgery. Elsevier Mosby, Edinburgh/New York

Simpson DJ, Clayton RN, Farrell WE (2002) Preferential loss of Death Associated Protein kinase expression in invasive pituitary tumours is associated with either CpG island methylation or homozygous deletion. Oncogene 21:1217–1224

Spada A, Lania A, Mantovani G (2007) Hormonal signaling and pituitary adenomas. Neuroendocrinology 85:101–109

Tanizaki Y, Jin L, Scheithauer BW, Kovacs K, Roncaroli F, Lloyd RV (2007) P53 gene mutations in pituitary carcinomas. Endocr Pathol 18:217–222

Tateno T, Zhu X, Asa SL, Ezzat S (2010) Chromatin remodeling and histone modifications in pituitary tumors. Mol Cell Endocrinol 326:66–70

Tichomirowa MA, Daly AF, Beckers A (2009) Familial pituitary adenomas. J Intern Med 266:5–18

Vallar L, Spada A, Giannattasio G (1987) Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. Nature 330:566–568

Veugelers M, Wilkes D, Burton K, McDermott DA, Song Y, Goldstein MM, La Perle K, Vaughan CJ, O'Hagan A, Bennett KR, Meyer BJ, Legius E, Karttunen M, Norio R, Kaariainen H, Lavyne M, Neau JP, Richter G, Kirali K, Farnsworth A, Stapleton K, Morelli P, Takanashi Y, Bamforth JS, Eitelberger F, Noszian I, Manfroi W, Powers J, Mochizuki Y, Imai T, Ko GT, Driscoll DA, Goldmuntz E, Edelberg JM, Collins A, Eccles D, Irvine AD, McKnight GS, Basson CT (2004) Comparative PRKAR1A genotype-phenotype analyses in humans with Carney complex and prkar1a haploinsufficient mice. Proc Natl Acad Sci USA 101:14222–14227

Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, Gundogdu S, De Menis E, Makinen MJ, Launonen V, Karhu A, Aaltonen LA (2006) Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science 312:1228–1230

Weinstein LS, Chen M, Liu J (2002) Gs(alpha) mutations and imprinting defects in human disease. Ann N Y Acad Sci 968:173–197

Williamson EA, Johnson SJ, Foster S, Kendall-Taylor P, Harris PE (1995) G protein gene mutations in patients with multiple endocrinopathies. J Clin Endocrinol Metab 80:1702–1705

Yin Z, Williams-Simons L, Parlow AF, Asa S, Kirschner LS (2008) Pituitary-specific knockout of the Carney complex gene Prkar1a leads to pituitary tumorigenesis. Mol Endocrinol 22:380–387

Zhang X, Horwitz GA, Heaney AP, Nakashima M, Prezant TR, Bronstein MD, Melmed S (1999) Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas. J Clin Endocrinol Metab 84:761–767

Zhang X, Sun H, Danila DC, Johnson SR, Zhou Y, Swearingen B, Klibanski A (2002) Loss of expression of GADD45 gamma, a growth inhibitory gene, in human pituitary adenomas: implications for tumorigenesis. J Clin Endocrinol Metab 87:1262–1267

Zou H, McGarry TJ, Bernal T, Kirschner MW (1999) Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. Science 285:418–422

Xanthogranulomas Associated with Pituitary Adenomas: Magnetic Resonance Imaging

9

Hiroshi Nishioka and Makoto Shibuya

Contents

Abstract

Xanthogranuloma (cholesterol granuloma) in the sellar region was traditionally regarded as a hallmark of craniopharyngioma, but it can be associated with other lesion including pituitary adenomas. In adenomas, xanthogranulomas may develop in large tumors, probably following hemorrhagic processes, and patients present with various degrees of pituitary insufficiencies. On MRI, xanthogranulomas typically exhibit mixed signal intensities with heterogeneous gadolinium enhancement, reflecting their complex histological features. The most characteristic finding is cholesterol clefts that show T1-high and T2-low signal intensities. Fine evaluation of MRI findings is required for the accurate diagnosis and the appropriate surgical intervention that may improve pituitary function.

Introduction

Evaluation by MRI is indispensable for the diagnosis and treatment of pituitary adenomas. Most adenomas can be reliably diagnosed by routine MRI examinations. Not only the precise localization including anatomical relation to the surrounding tissues, but also nature of the tumor can be speculated on MRI. In a few sellar lesions that exhibit atypical findings on MRI, however, diagnosis still remains troublesome. Differential diagnosis of adenomas may include various lesions such as craniopharyngiomas, Rathke's cleft cysts, germ

H. Nishioka (✉)
Department of Hypothalamic and Pituitary Surgery, Toranomon Hospital, Okinaka Memorial Institute for Medical Research, 2-2-2 Toranomon, Minatoku, Tokyo 105-8470, Japan
e-mail: nishioka@tokyo-med.ac.jp

M. Shibuya
Department of Diagnostic Pathology, Ibaraki Medical Center, Tokyo Medical University, Tokyo, Japan

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_9,

cell tumors, and hypophysitis. Most of the atypical features on MRI are due to histological changes within the tumor. Xanthogranuloma is a complex granulomatous lesion characterized by cholesterol clefts, foamy macrophages, chronic inflammation, and fibrosis. It usually exhibits complex findings on MRI. Herein, we demonstrate MRI features and the differential diagnosis of pituitary adenomas accompanying this complex histological change.

Histological Changes Associated with Pituitary Adenomas

Pituitary adenomas are benign, slowly growing neoplasm, but they are often associated with histological changes such as hemorrhage, necrosis, fibrosis, hyaline deterioration, calcification, cyst formation, amyloid deposit, et al. These changes are usually more common in large adenomas and in prolactinomas. Apart from secondary reaction to surgical, pharmacological, or radiation treatment, these changes are caused either by hemorrhagic, ischemic, inflammatory, or degenerative processes. They are generally unrelated to aggressiveness or malignancy of the tumor, but may result in endocrine dysfunction by affecting the surrounding pituitary. In addition, adenomas with these changes are often more difficult to achieve radical surgical resection than those without them.

On preoperative MRI of adenomas, most of the mixed signal intensities and heterogeneous gadolinium enhancements are related to these histological features, *e.g.,* hemorrhage and calcification is high and low signal intensity, respectively, on T1-weighted image. These changes are not specific to adenomas but may also develop in other sellar lesion. When these changes are remarkably complex and/or predominate in an adenoma, it is often difficult to differentiate from other diagnosis by MRI.

Xanthogranulomas in the Sellar Region

Xanthogranuloma (cholesterol granuloma, xanthogranulomatous reaction) is a granulomatous lesion consisting of cholesterol clefts, macrophages

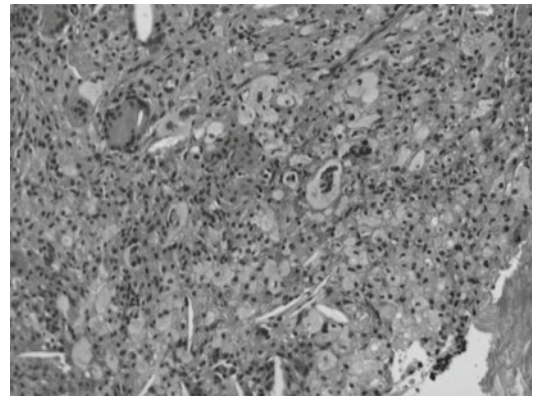

Fig. 9.1 Histological feature of xanthogranuloma associated with an adenoma showing chronic inflammatory infiltrates, xanthoma cells, cholesterol clefts, hemosiderin deposits, and fibrosis

(xanthoma cells), hemosiderin deposits, chronic inflammatory infiltrates, and fibrous proliferation (Fig. 9.1). Most xanthogranuloma in the sellar region is associated with epithelial lesion. Traditionally, xanthogranuloma had been regarded as a hallmark of adamantinomatous type craniopharyngioma (Janzer et al. 2000). However, various other lesions, including Rathke's cleft cyst, colloid cyst of the third ventricle, and epithelial cyst, may occasionally accompany xanthogranuloma (Tashiro et al. 2002). In addition, pituitary adenomas may, albeit rarely, undergo xanthogranulomatous change (Nishioka et al. 2010).

On the other hand, xanthogranuloma that lack epithelial component, *i.e.,* those with unknown origin, is termed "xanthogranuloma of the sellar region" (Paulus et al. 1999). Although it still remains unknown whether they represent a distinct entity, their characteristic clinicopathological features have been reported (Janzer et al. 2000; Paulus et al. 1999). Compared to craniopharyngiomas, they tend to show predominance in young age patients, intrasellar location, small tumor size, severe pituitary dysfunction, and benign prognosis. However, "xanthogranuloma of the sellar region" cannot radiologically be differentiated from other diagnosis (Jung et al. 2006).

Meanwhile, Erdheim-Chester disease, or non-X histiocytosis, is a rare xanthogranulomatous disease of multiple organ systems, primary involving the metaphysis and diaphysis of long bones. They occasionally show intracranial involvement affecting

the hypothalamic-pituitary axis. Although it has a progressive fatal prognosis and rarely manifests with intracranial involvement, neurosurgeons need to be aware of the possibility of this rare disease in patients with unusual neuroimaging findings and pituitary dysfunction (Abla et al. 2010).

Histogenesis of the Sellar Xanthogranulomas

The histogenesis of xanthogranuloma is controversial and is probably heterogeneous. It is generally considered to develop following hemorrhage, inflammation, or degeneration (Burt et al. 2003; Paulus et al. 1999; Yonezawa et al. 2003). Craniopharyngiomas and Rathke's cleft cysts are known to cause foreign-body inflammatory reaction in the cyst wall and the surrounding tissues (Nishioka et al. 2006; Tashiro et al. 2002). The inflammation occasionally extends to the pituitary resulting in secondary hypophysitis. The relatively higher incidence of pituitary insufficiency and diabetes insipidus in these lesions compared to adenomas are due to the chronic inflammation. Since hemorrhage is uncommon, it is suggested that xanthogranulomatous reaction is mainly induced by inflammatory process in these lesions. On the other hand, Paulus et al. (1999) who proposed the term "xanthogranuloma of the sellar region" found hemosiderin deposits in most cases. They suggested that xanthogranuloma is related to obstruction of a cavity, hemorrhage, or both.

In our previous study of xanthogranulomas associated with adenoma, we found hemosiderin deposits and xanthochromic-like fluids in most cases (Nishioka et al. 2010). Despite absence of evident episodes of pituitary apoplexy, we suggested that xanthogranulomatous reaction was related to hemorrhagic or necrotic processes in these adenomas.

MRI Features of the Sellar Xanthogranulomas

MRI findings, particularly signal intensity varies considerably in each case of sellar xanthogranulomatous lesion. They exhibit mixed signal intensities on both T1- and T2-weighted images with heterogeneous enhancement, reflecting their complex histologic components (Fig. 9.2). In general, cholesterol clefts show T1-high and T2-low signal intensities, hemosiderin deposits show T1-iso and T2-low signal intensities, calcification shows T1-low and T2-low signal intensities, cysts containing xanthochromic-like fluid show T1-high/iso and T2-high signal intensities, and thick fibrosis (granulation) show both T1- and T2-low signal intensities. Since the most characteristic histological features are cholesterol clefts, sellar mixed-intensity lesions showing T1-high and T2-low signal intensities suggest the possibility of xanthogranuloma (Sugata et al. 2009). Thus, T1-high signal intensity in the sellar region may indicate cholesterol clefts (xanthoma cells) in addition to hematomas, fat, and high-protein materials or fluids (Jung et al. 2006; Nishioka et al. 2010; Yokoyama et al. 2004).

In cases of xanthogranuloma associated with adenoma, component of an adenoma can be usually detected adjacent to the xanthogranuloma on fine MRI (Fig. 9.2). Adenomas with hemorrhage, especially those with old hematoma, may exhibit similar MRI features, since old hematoma and xanthogranuloma share some common histologic features. In addition, xanthogranulomatous reaction is probably triggered by hemorrhagic process. Characteristic signal intensities of cholesterol clefts can be a clue to a differential diagnosis.

Differential Diagnosis of the Sellar Xanthogranulomas

Differential diagnosis of sellar granulomatous and xanthgranulomaous lesion includes craniopharynioma, Rathke's cleft cyst, germ cell tumor, and systemic granulomatous disorders (sarcoidosis, Langerhans cell histiocytosis, Wegener granulomatosis, tuberculosis, syphilis).

When a lesion is exclusively or predominantly composed of xanthogranulomatous tissue, differential diagnosis will be difficult. In such cases, diagnosis of the primary lesion

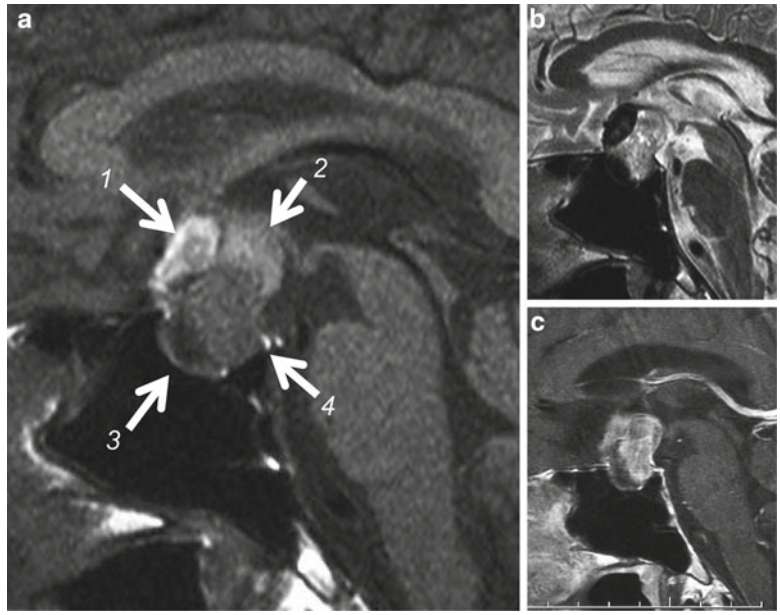

Fig. 9.2 Sagittal MR images of a case of adenoma accompanying xanthogranuloma showing complex signal intensities (**a**: T1-weighted, **b**: T2-weighted) and heterogeneous gadolinium-enhancement (**c**). On histology, each MRI components (**a**) corresponded mainly to xanthogranuloma with cholesterol clefts and xanthoma cells (*1*), old hematomas (*2*), thick fibrotic granulation tissue (*3*), and adenoma (*4*)

that accompanied xanthgranuloma is often difficult even with histological study. On macroscopic observation during surgery, these lesions usually demonstrate heterogeneous appearance including firm granulation tissue. The primary lesion may not be involved in small surgical specimens, whereas radical resection of the lesion can deteriorate the pituitary function and, thus, is usually unnecessary (Murakami et al. 2008). Furthermore, the primary lesion might have been destructed by the severe inflammation. It has been suggested that some, if not many, cases of idiopathic granulomatous and xanthogranulomatous hypophysistis have a relation to Rathke's cleft cyst (Murakami et al. 2008; Tashiro et al. 2002).

Since hypopituitarism is a common manifestation in patients with xanthogranulomas, appropriate surgical intervention may improve pituitary function. Although their MRI features are often complex reflecting the heterogeneous histologies, fine evaluation of unusual MRI findings is required for the accurate diagnosis.

References

Abla AA, Wilson DA, Eschbacher JM, Spetzler RF (2010) Neurosurgical biopsy as the initial diagnosis of xanthogranuloma of the Erdheim-Chester disease variety of the infundibulum and optic apparatus: letter to the editor. Acta Neurochir 152:925–927

Burt MG, Morey AL, Turner JJ, Pell M, Sheehy JP, Ho KKY (2003) Xanthomatous pituitary lesions: a report of two cases and review of the literature. Pituitary 6:161–168

Janzer RC, Burger PC, Giangaspero F, Paulus W (2000) In: Kleihues P, Cavenee WK (eds) Pathology & genetics. Tumours of the nervous system. IARC Press, Lyon, pp 244–246

Jung CS, Schanzer A, Hattingen E, Plate KH, Seifert V (2006) Xanthogranuloma of the sellar region. Acta Neurochir 148:473–477

Murakami M, Nishioka H, Izawa H, Ikeda Y, Haraoka J (2008) Granulomatous hypophysitis associated with Rathke's cleft cyst: a case report. Minim Inv Neurosurg 51:169–172

Nishioka H, Haraoka J, Izawa H, Ikeda Y (2006) Magnetic resonance imaging, clinical manifestations, and management of Rathke's cleft cyst. Clin Endocrinol 64:184–188

Nishioka H, Shibuya M, Ohtsuka K, Ikeda Y, Haraoka J (2010) Endocrinlogical and MRI features of pituitary adenomas with marked xanthogranulomatous reaction. Neuroradiology 52:997–1002

Paulus W, Honegger J, Keyvani K, Fahlbusch R (1999) Xanthogranuloma of the sellar region: a clinicopathological entity different from adamantinomatous craniopharyngioma. Acta Neuropathol 97:377–382

Sugata S, Hirano H, Yatsushiro K, Yunoue S, Nakamura K, Arita K (2009) Xanthogranuloma in the suprasellar region: case report. Neurol Med Chir (Tokyo) 49:124–127

Tashiro T, Sano T, Xu B, Wakatsuki S, Kagawa N, Nishioka H, Yamada S, Kovacs K (2002) Spectrum of different type of hypophysitis: a clinicopathologic study of hypophysitis in 31 cases. Endocr Pathol 18:183–195

Yokoyama S, Sano T, Tajitsu K, Kusumoto K (2004) Xanthogranulomatous hypophysitis mimicking a pituitary neoplasm. Endocr Pathol 15:351–358

Yonezawa K, Shirataki K, Sakagami Y, Kohmura E (2003) Panhypopituitarism induced by cholesterol granuloma in the sellar region: case report. Neurol Med Chir (Tokyo) 43:259–262

Pituitary Adenoma and Craniopharyngioma: An Overview

10

Murat Gokden

Contents

Abstract

This chapter discusses the pathologic features of pituitary adenoma and craniopharyngioma, the two most common and problematic neoplasms of the region of sella turcica. Pituitary adenoma, even when it is small, can present with very diverse clinical and pathological findings owing to its variety of cell types and its location. An increasing number of subtypes are being recognized based on histological and immunohistochemical features. Valuable data can be obtained from the pathologic evaluation in terms of biologic aggressiveness of the adenoma, and its further characterization as invasive and/or atypical. Craniopharyngioma is a low grade neoplasm that can be quite problematic due to its locally invasive nature and recurrences. Histologic subtypes, namely adamantinomatous and papillary, are reviewed; their pathologic features and prognoses are discussed. Finally, the interesting coexistence of these two neoplasms is discussed briefly.

Introduction

The region of sella turcica and the suprasellar region bring together a wide variety of tissues, such as neuroglial, adenohypophyseal, meningeal, vascular, soft tissue, bone and bone marrow, and upper respiratory epithelial, resulting in a large number of diverse lesions, both neoplastic and nonneoplastic. These various tissues are closely approximated and intermingled. Especially their

M. Gokden (✉)
Department of Pathology, University of Arkansas for Medical Sciences, 4301 West Markham Street, 517, Little Rock, AR 72205, USA
e-mail: mgokden@uams.edu

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_10,

neoplasms, even the most biologically benign ones, have the potential to compress and infiltrate into the neighboring tissues and structures to complicate matters more, causing problems by interfering with their crucial functions and presenting many diagnostic and therapeutic challenges. Due to the complex and unusual histology and its variations, additional challenges are faced by the neuropathologist in the diagnosis and classification of these lesions. In this chapter, the pathologic features of two of the most common neoplasms of this region, anterior pituitary neoplasms and craniopharyngioma, will be discussed with a diagnostic and prognostic perspective.

Pituitary Adenoma

Pituitary adenoma arises from the endocrine cells of the anterior pituitary gland. It is the most common neoplasm of the pituitary gland and constitutes 8% of all intracranial neoplasms (Surawicz et al. 1999), with an incidence of up to 20% in general population (Asa 1998). Its peak incidence is in the seventh to eighth decades of life (Surawicz et al. 1999). Clinical presentation of pituitary adenoma is usually due to the excess hormone it secretes or the mass effect it produces, or both. Some adenomas can compress and interfere with the function of the nonneoplastic pituitary tissue. While the great majority arise in the sella turcica and may grow out of the sella with or without invasion of various surrounding structures, rare adenomas are ectopic, with no abnormalities of the pituitary gland or the sella. Therefore, when a tissue sample is obtained, the diagnosis of the lesion as a pituitary neoplasm subsequent to its differential diagnosis from a variety of neoplastic and nonneoplastic lesions in the sellar-suprasellar region, the identification of its hormonal status with pathologic techniques and subclassification of the adenoma according to this information, the determination of its potential for invasion, recurrence and possibly metastasis, and finally, the integration of these data obtained from pathologic examination with the clinical, laboratory, radiologic and intraoperative findings, are crucial for the appropriate management of the patient. This section will focus on the pathologic diagnosis and classification of pituitary adenoma, as well as the prognostic data that can be obtained from pathologic examination.

General Pathologic Features

The anterior pituitary parenchyma is composed of a mixture of cell types referred to as acidophils, basophils and chromophobes based on their cytoplasmic tinctorial characteristics by routine histologic methods. They are arranged in a nesting pattern, surrounded by a delicate fibrovascular stroma (Fig. 10.1a). This architecture, characteristic for the typical histology of the anterior pituitary, is highlighted by a reticulin stain (Fig. 10.1b). When there is hyperplasia of this tissue, there is distortion of this architecture in the form of expansion and irregular branching (Fig. 10.1c). However, although distorted, the overall nesting pattern, as identified by reticulin stain, is still preserved (Fig. 10.1d). There may be a predominance of the hyperplastic cell type over the others. This may be highlighted further by immunohistochemical stains for pituitary hormones.

Adenomas are monomorphic lesions with sheets of similar cells and loss of nesting pattern (Fig. 10.1e). There is complete disruption of the reticulin network, which remains limited to perivascular areas (Fig. 10.1f). This histologic appearance constitutes the general pathologic hallmark of pituitary adenoma common to all types. The loss of reticulin network also explains the softer and more friable gross quality of the adenoma compared to the nonneoplastic gland.

These architectural differences between nonneoplastic and neoplastic pituitary tissue are also useful in intraoperative evaluations. Intraoperative cytology is very valuable in intraoperative neuropathology consultations. They reveal cellular preparations in adenomas due to the absence of reticulin, making it easier for adenoma cells to fall onto the slide from the loose sheets devoid of reticulin. Nonneoplastic tissue yields scantly cellular preparations, since not very many cells fall onto the slide due to rich reticulin network. Touch or smear preparations provide the best means for evaluation,

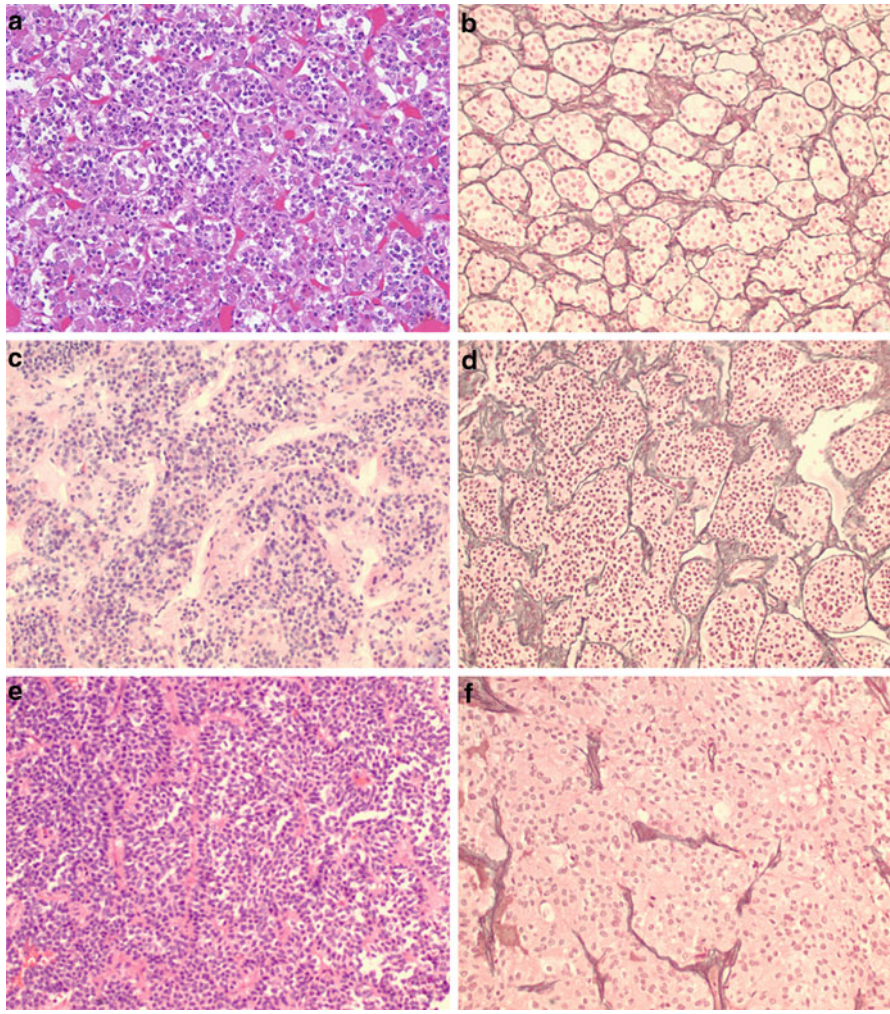

Fig. 10.1 (**a**) Normal histologic appearance of anterior pituitary with cellular groups composed of a mixture of cell types (H&E, 100×); (**b**) Nested pattern of reticulin network in normal anterior pituitary (reticulin stain; 100×); (**c**) Hyperplastic pituitary with irregular cell groups with relatively uniform cellularity (H&E; 100×); (**d**) Expanded and distorted reticulin pattern in hyperplastic pituitary (reticulin stain; 100×); (**e**) Pituitary adenoma with sheets of monotonous cells punctuated by focal areas of fibrovascular stroma (H&E; 100×); (**f**) Completely wiped-out reticulin network of pituitary adenoma (reticulin stain; 100×)

especially for the evaluation of chromatin pattern, revealing a finely-granular neuroendocrine chromatin pattern, usually referred to as "salt and pepper" chromatin. Nucleoli are very small or inconspicuous. Nuclei are usually round and uniform, although there may be considerable cytologic atypia and pleomorphism in some cases. Many cells tend to lose their friable cytoplasms, which may form a granular eosinophilic debris in the background. This is usually the case with the smear preparations and is likely the result of physical tension applied to the cells during smearing. Overall, cells tend to be distributed singly or in very small, loosely arranged groups. Cellularity in the cytologic preparations from the nonneoplastic pituitary tissue is composed of a mixture of cell types, while adenomatous tissue yields a monomorphic cell population. Actual frozen section preparations from the pituitary tissue are more difficult to assess due to the presence of technical artifacts. Cell cytoplasms may rupture during freezing and cutting of the tissue, resulting in a false impression of

monomorphism. However, frozen sections may still prove useful in evaluating the architecture, i.e., preservation or loss of nesting pattern. The preparation of frozen sections should be avoided in cases where the search is for a microscopic adenoma of only a few millimeters, as it may be lost to sectioning without a definitive diagnosis. Therefore, communication between the neurosurgeon and the neuropathologist, and familiarity with the radiologic findings are critical in terms of clarifying the expectations from the intraoperative consultation. Evaluation of surgical margins and capsular invasion by the adenoma by intraoperative pathology consultation should be discouraged, as it usually yields equivocal results, as well as risking loss of diagnostic features in small tissue samples.

Immunohistochemically, pituitary adenomas in general are largely positive for synaptophysin, chromogranin and keratin Cam 5.2, less so for pancytokeratin (AE1/AE3), and negative for cytokeratin 19 (O'Hara et al. 1998). Immunohistochemistry for specific hormones in nonneoplastic pituitary reveal positivity for a mixture of hormones, although one may predominate in hyperplasias. Adenomas, on the other hand and with the exception of null cell and plurihormonal adenomas, are usually positive for one type of hormone (see "specific types of pituitary adenomas" below). Electron microscopy shows general features of neuroendocrine cells, i.e., secretory granules of variable sizes and numbers, as well as cytoplasmic intermediate filaments (Ghadially 1982). These features may vary in different types of adenomas and some additional features diagnostic of certain types of adenomas can also be seen by electron microscopic examination; however, light microscopic evaluation, together with the current armamentarium of immunohistochemical markers and laboratory data, the diagnosis and classification of pituitary adenomas rarely require electron microscopic evaluation. Specific pathologic features will be mentioned in the discussion of adenoma subtypes below.

Adenomas are arbitrarily divided into micro- and macroadenomas. Microadenomas are smaller than 1.0 cm. in greatest dimension and are usually contained within the sella. Macroadenomas tend to distend the sella, compress the nonneoplastic pituitary gland and may invade the surrounding structures. Approximately 5% of adenomas are familial with more than 50% of this group being associated with Multiple Endocrine Neoplasia Type 1 (MEN-1) syndrome and Carney's Complex, while the remainder are familial isolated pituitary adenomas (FIPA) that are usually seen in a younger population and as larger adenomas (Daly et al. 2009). Those adenomas associated with MEN-1 and Carney's Complex are also more commonly macroadenomas of growth hormone and/or prolactin producing type (Scheithauer et al. 1987). Although areas of hemorrhage and necrosis are common findings in pituitary adenomas, some adenomas undergo a rapid and extensive hemorrhagic infarction. This is called pituitary apoplexy and constitutes a neurosurgical emergency.

Classification

While pituitary neoplasms are classified by the World Health Organization (WHO) based on biologic behavior into typical and atypical adenomas, and carcinoma, and are not officially graded or coded based on the hormone types (Lloyd et al. 2004), they are further examined according to their clinicopathologic and immunohistochemical features. Briefly, they are: Somatotroph adenoma (densely or sparsely granulated); mixed somatotroph-lactotroph adenoma; mammosomatotroph adenoma; acidophil stem cell adenoma; lactotroph adenoma (densely or sparsely granulated); thyrotroph adenoma; corticotroph adenoma (densely or sparsely granulated); gonadotroph adenoma; plurihormonal adenoma; null cell adenoma. Accurate classification of an adenoma requires work up with a panel of immunohistochemical stains, occasionally electron microscopic evaluation, and correlation with clinical and other laboratory features. Rarely, immunohistochemical markers are needed to identify the pituitary origin of the neoplasm (as discussed in "general pathologic features") in cases where pathologic features are obscure or other entities enter the differential diagnosis. Otherwise, immunohistochemistry is directed towards identification of the hormone(s)

secreted by the adenoma for its subclassification. Immunophenotype correlates with the histogenesis and functional status of the adenoma. Pituitary transcription factor-1 (Pit-1) expression indicates somatotroph, mammotroph and thyrotroph differentiation, along with the expression of growth hormone (GH), prolactin (PRL) and thyroid stimulating hormone (TSH), respectively. T-box transcription factor (Tpit) expression, together with adrenocorticotrophic hormone (ACTH), indicate corticotroph lineage. Steroidogenic factor-1 (SF-1), as well as follicle stimulating hormone (FSH) and luteinizing hormone (LH), expression indicates gonadotroph differentiation. Further details of immunohistochemical features will be discussed below under individual adenoma types.

Invasive Adenoma

Invasion by adenoma of the surrounding tissues such as dura mater, bone, into sphenoid sinus or nasopharynx mucosa, and cavernous sinus is considered a sign of aggressive growth (Fig. 10.2a). It is common to find microscopic invasion of dura mater in the great majority of cases (Selman et al. 1986), as long as representative specimen is available for evaluation. In spite of this seemingly aggressive growth pattern however, metastases are rarely seen in these adenomas, making the association of invasive growth with aggressive biologic behavior somewhat debatable. Nonetheless, invasive adenomas are prone to cause problems associated with incomplete resections and recurrences. In general, invasiveness appears to correlate with the size of the neoplasm (Sautner and Saeger 1991). Rarely, an adenoma may grow exophytically from the pituitary gland to invade the bone, without forming an intrasellar mass or distorting the pituitary gland (personal observation). In other instances such adenomas and others may present in an atypical manner imitating another lesion in the bone or upper respiratory tract. In still others, adenomas may arise from the ectopic pituitary tissue rests that remained along the development tract of the Rathke's pouch (Lloyd et al. 1986).

Atypical Adenoma

Typically, in pituitary adenoma, mitotic figures are difficult to find; Ki-67 is positive in approximately 1% or less of the adenoma cell nuclei and p53 is negative or is limited to scattered nuclei. Pituitary adenoma with an increased mitotic rate (Fig. 10.2b), greater than 3% proliferation index by Ki-67 immunohisitochemistry (Fig. 10.2c), and extensive nuclear positivity by p53 immunohistochemistry is classified as atypical adenoma (Lloyd et al. 2004). These "atypical" features attempt to identify those adenomas with a potentially more aggressive biologic behavior, including the potential to metastasize (Pernicone et al. 1997).

Pituitary Carcinoma

Based on current knowledge, since no other features appear to be reliable indicators of malignancy, only those adenomas that metastasize are considered pituitary carcinomas. Metastases are to distant areas of the subarachnoid space and brain parenchyma, i.e., excluding direct invasion, or to extracranial sites, mainly lymph nodes, lung, liver and bone (Asa 1998). Pituitary carcinoma is subject to the same immunohistochemical classification applied to adenoma. The pituitary origin of a metastatic neoplasm may not be readily apparent, which necessitates additional markers to confirm the pituitary origin. Pituitary carcinoma is rare. In the German Pituitary Tumor Registry, approximately 0.14% of adenomas evaluated in a 10-year period qualified as carcinomas (Saeger et al. 2007). Its incidence was 0.2% in Mayo Clinic series and showed a tendency to develop from a pituitary adenoma with atypical features (Pernicone et al. 1997).

Specific Types of Pituitary Adenomas

These are adenomas classified according to their hormone production status. In addition to the mass effect common to adenomas in general, there are usually clinical features associated with their hormonal status.

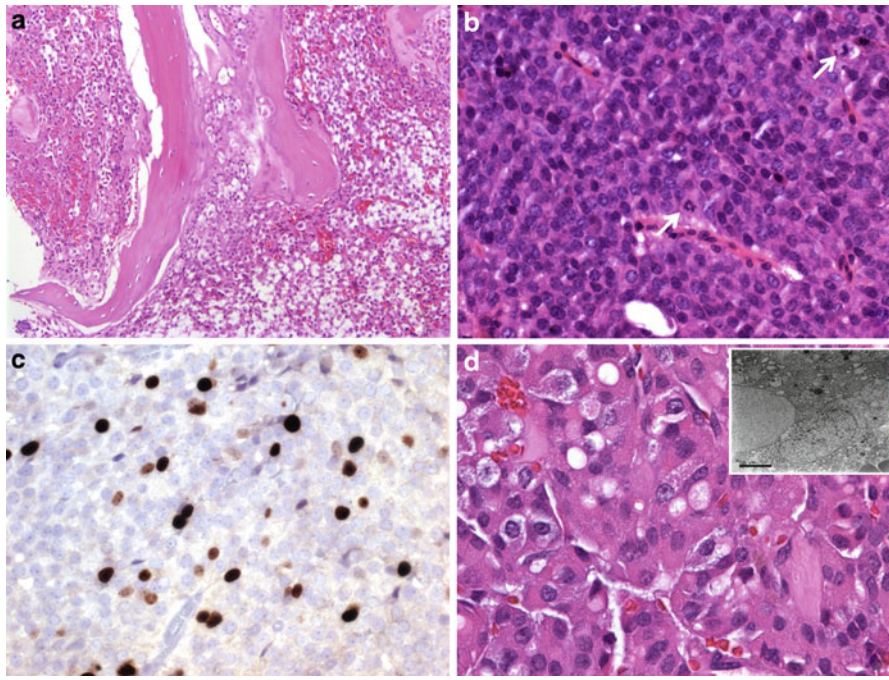

Fig. 10.2 (**a**) Sheets of adenoma cells infiltrating the bone marrow areas to *encircle* the bone trabeculae in invasive adenoma (H&E; 40×); (**b**) Increased mitotic activity (*arrows*) in atypical adenoma (H&E; 200×); (**c**) Increased proliferation index in atypical adenoma (Ki-67 immunohistochemistry; 200×); (**d**) Acidophil stem cell adenoma with eosinophilic cytoplasm and with cytoplasmic vacuoles (H&E; 200×); *inset* shows the electron microscopic appearance of giant mitochondria corresponding to the vacuoles (magnification bar 20 μm)

Growth hormone producing (somatotroph) adenoma constitutes 25–30% of all adenomas in surgical specimens (Kontogeorgos et al. 2004). Densely granulated somatotroph adenoma has cells with abundant acidophilic cytoplasm and is diffusely and strongly positive for growth hormone by immunohistochemistry. Approximately 50% of these adenomas are also positive for alpha-subunit. Sparsely granulated somatotroph adenoma cells are relatively chromophobic with irregular nuclei and have a cytoplasmic fibrous body that is positive for Cam 5.2. Staining for growth hormone is scant. Some somatotroph adenomas also have an admixture of lactotroph cells, i.e., mixed somatotroph-lactotroph adenomas, consisting of a mixture of acidophilic and chromophobic cells with immunoreactivity for growth hormone and prolactin in respective cell types. In contrast, mammosomatotroph adenoma is composed of uniformly acidophilic cells that are immunohistochemically positive for both growth hormone and prolactin in the same cell. Acidophil stem cell adenoma is a rare type that is positive for prolactin. Staining for growth hormone is weakly positive, or negative. It has fibrous bodies, as well. In addition, the cells appear to have cytoplasmic vacuoles (Fig. 10.2d) that correspond to swollen, giant mitochondria seen by electron microscopy (Fig. 10.2d, inset).

Prolactin producing adenoma constitutes up to 26% of pituitary adenomas with its incidence in surgical specimens decreasing after the introduction of medical treatment (Saeger et al. 2004). The majority is sparsely granulated and has chromophobic or faintly acidophilic cytoplasm with a paranuclear Golgi pattern of prolactin immunoreactivity, compared to the densely granular type with diffuse and strong positivity. Acidophil stem cell adenoma can also be considered together with prolactin producing adenomas due to its prolactin secretion. Medical treatment causes shrinkage of cells, resulting in smaller cells with hyperchromatic nuclei in a fibrotic stroma.

TSH producing adenoma is rare, has chromophobic cells that are variably positive for beta-TSH and alpha-subunit. It can be plurihormonal with additional growth hormone and/or prolactin secretion. (Osamura et al. 2004). ACTH producing adenoma has basophilic or chromophobic cells. The densely granulated adenomas have more diffuse and stronger positivity for ACTH than sparsely granulated adenomas. In addition, some adenomas can have cytoplasmic cytokeratin accumulation and are called Crooke cell adenomas. The rapid growth in an ACTH producing pituitary adenoma after bilateral adrenalectomy is Nelson's syndrome. Gonadotropin producing adenoma can have a peculiar arrangement of cells around blood vessels, trabecular or papillary architecture composed of acidophilic or chromophobic cells. It is variably but usually focally and weakly positive for beta-FSH and beta-LH, and frequently for alpha-subunit.

Plurihormonal adenoma is an adenoma with secretion of more than one type of hormone. The special situations of growth hormone, prolactin and TSH, as well as FSH and LH combinations that are described above are now excluded from this category (Horvath et al. 2004). Silent pituitary adenoma is an adenoma without clinical hormonal hyperfunction, but with immunohistochemical and ultrastructural evidence for hormonal activity. One particular type, silent subtype 3, is considered a silent plurihormonal adenoma with peculiar electron microscopic findings to confirm the diagnosis (Horvath et al. 2004). On the other hand, null cell adenoma is an adenoma with no immunohistochemical and electron microscopic evidence for hormone production and constitutes approximately 16% of adenomas (Kovacs et al. 1980). Based on this strict definition, its incidence is decreasing due to the advances in the identification of hormones. It can have chromophobic cells or variably-acidophilic cells, arranged in sheets or papillae with perivascular arrangements. Some null cell adenomas contain abundant mitochondria and have cells with eosinophilic granular cytoplasms, i.e., oncocytomas.

Prognostic Factors

Even when only the data from pathologic examination are considered, it becomes obvious that a definitive formula to predict biologic behavior is not available and the issue is complicated by multiple variables. Still, several characteristics that are pertinent to prognosis emerge based on the pathologic features outlined above. It is not possible for the neuropathologist to judge whether gross total resection has been achieved, or the size of the neoplasm, as the typical specimen is in the form of unoriented irregular fragments and no margin evaluation can be performed. Therefore, size and resection status can be better evaluated by the neurosurgeon and the neuroradiologist. Invasion of the surrounding structures can also be evaluated by imaging techniques, as well as intraoperatively; however, this can be confirmed by pathologic evaluation only if representative tissue is available. The definition and significance of invasion in pituitary adenoma are somewhat variable. It has been suggested that only the invasion that can be identified by the neurosurgeon or the neuroradiologist is clinically significant (Scheithauer et al. 1986), while others attribute increased recurrences and decreased survival to even the microscopic dural invasion identified by the neuropathologist (Meij et al. 2002). Invasion is seen more commonly in males with 56% of adenomas in male patients being invasive. In addition, invasion is more common in treated prolactin producing adenomas (56%), ACTH adenomas of Nelson's syndrome (56%), silent ACTH adenomas of subtype 2 (75%), and silent subtype 3 adenomas (92%) (Scheithauer et al. 2006). Interestingly, functional adenomas, in spite of their earlier detection due to hormone secretion and smaller size at the time of diagnosis, tend to be more invasive compared to nonfunctional adenomas (Scheithauer et al. 2006). Atypical adenomas, identified based on mitotic activity, increased proliferation rate and p53 positivity, have a poorer prognosis, as they are associated with rapid growth and more widespread invasion, and less of a chance of complete resection. They differ from pituitary carcinomas essentially by the absence of metastases (Lloyd et al. 2004).

The prognosis of pituitary carcinoma is dismal, with the rate of systemic metastases being highest in prolactin producing adenomas (71%) and ACTH producing adenomas (57%) (Pernicone et al. 1997). Although TSH producing adenomas are rare, they are frequently macroadenomas with invasive and aggressive growth pattern (Osamura et al. 2004). Acidophil stem cell adenoma implies a worse prognosis, due to rapid growth rate and incomplete resection as a result of invasive growth (Horvath et al. 1981). Apoplexy in a pituitary adenoma is an emergency and can be life-threatening if surgical intervention and hormone replacement are not carried out (Asa 1998).

Craniopharyngioma

Craniopharyngioma is a WHO Grade I neoplasm of the sellar/suprasellar region (Rushing et al. 2007) with an overall incidence of 0.13/100,000 persons/year (Bunin et al. 1998). It has a bimodal age distribution, being more common in 5–14 years and 50–74 years of age, with 96% of cases seen between 0 and 14 years of age (Bunin et al. 1998). There is also variation in age at presentation based on the histologic type; papillary craniopharyngioma is essentially exclusively seen in adults with a mean age of 44.7 years (Crotty et al. 1995; Weiner et al. 1994). Clinical presentation is commonly with visual disturbances, endocrine disorders and increased intracranial pressure due to its mass effect, and with psychiatric disturbances due to interference with hypothalamic function (Shin et al. 1999). This section will focus on the pathologic diagnosis and classification of craniopharyngioma, as well as the prognostic data that can be obtained from pathologic examination.

Pathologic Features

There are two histologic types of craniopharyngiomas: Adamantinomatous and papillary. The more common adamantinomatous craniopharyngioma is a cystic neoplasm containing a dark brown, thick fluid described as “machinery oil”. Cyst lining is irregular and friable. Pearly white flecks of keratinized material can be identified in the solid areas or floating within the cyst contents. The lesion is well-circumscribed and may give the false impression that it is surrounded by a capsule. It can grow into the sella turcica and/or superiorly, with pushing margins with the brain and other surrounding structures. Microscopic examination reveals a peculiar squamous epithelium in islands and in interconnecting trabeculae, admixed with variable amount of keratin. The epithelial islands are surrounded by basaloid cells with minimal amount of cytoplasm, arranged in a palisading pattern. The squamous cells inside the islands have more prominent cytoplasm and are stellate or even spindled. They form loosely-textured , discohesive areas referred to as “stellate reticulum”, where it is difficult to appreciate their epithelial nature. Small groups of squamous cells with eosinophilic cytoplasm can be identified in this background of stellate reticulum (Fig. 10.3a). This squamous epithelium lacks the granular layer and the flaky keratin. Admixed with the cellular areas or piled up on the epithelial surface are bright eosinophilic aggregates of anuclear ghost cells and keratin in a syncytial arrangement. They are called “wet-keratin” due to their thick, waxy quality (Fig. 10.3b), in contrast to the flaky keratin seen in other keratinizing squamous lesions. Calcification is common. Stroma is fibrotic and may show chronic inflammation. Cholesterol crystals are part of the cyst content and result in cholesterol clefts on tissue sections. They can be identified on direct, unfixed and unstained smears of the cyst fluid by polarized light examination. In spite of its well-circumscribed appearance, adamantinomatous craniopharyngioma tends to push into the brain parenchyma with broad finger-like extensions. It is common to find piloid gliosis in the surrounding brain tissue, usually with abundant Rosenthal fibers, creating a pitfall for the neuropathologist especially during intraoperative consultations, as similar changes can also be seen in pilocytic astrocytoma and as suprasellar-hypothalamic location

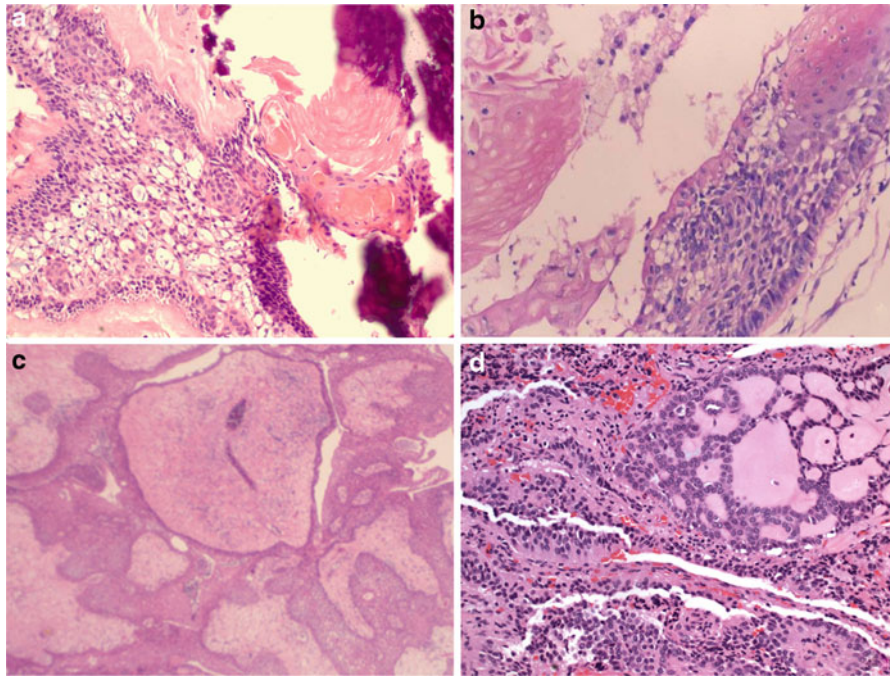

Fig. 10.3 (**a**) Adamantinomatous craniopharyngioma with cellular areas showing loosely-textured stellate reticulum, groups of squamous cells with eosinophilic cytoplasm, and peripheral palisading (*left side*), wet keratin and calcifications (*right side*) (H&E; 100×); (**b**) Adamantinomatous craniopharyngioma with cellular areas (*right side*) and wet keratin (*left side*) (H&E; 200×); (**c**) Papillary craniopharyngioma composed of fibrovascular areas *lined* by squamous epithelium merging to form solid sheets in some areas (H&E; 40×); (**d**) A composite neoplasm with intermingled areas of pituitary adenoma (*left side*) and adamantinomatous craniopharyngioma (*upper right*) (H&E; 200×)

is also a common site for pilocytic astrocytoma. Otherwise, frozen sections show features similar to those described above. Smear preparations reveal flat sheets of squamous epithelium and wet keratin.

Papillary craniopharyngioma also forms a well-circumscribed mass lesion, usually growing superiorly into the third ventricle. It typically does not have the grossly cystic appearance with the "machinery oil" fluid, but rather, it appears solid due to compact pseudopapillary architecture. It is relatively easily separated from the adjacent neuroglial tissue in contrast to adamantinomatous craniopharyngioma. Histologically, the fibrovascular stroma is lined by stratified squamous epithelium of variable thickness, forming solid sheets amongst the stromal fronds (Fig. 10.3c). Keratinization, calcification, prominent peripheral palisading of basaloid cells, and stellate reticulum are not present. Fibrovascular stroma can have chronic inflammatory infiltrate. Epithelium may contain scattered columnar, ciliated or mucinous cells.

Although clinicopathologic features of craniopharyngioma are distinctive enough and its diagnosis does not pose a problem in the great majority of cases, immunohistochemical characteristics can be useful in some situations. Craniopharyngiomas are positive for carcinoembryonic antigen (CEA) and various keratin subtypes; however, the differential expression of keratin subtypes has not been found useful in its differential diagnosis from xanthogranuloma and Rathke's cleft cyst, the two other cystic lesions in the region, due to significant overlap in expression patterns (Tateyama et al. 2001; Le et al. 2007). Of note is the identification of nuclear beta-catenin in 94% of adamantinomatous

craniopharyngiomas, while this feature was not observed in any of the lesions that enter the differential diagnosis, including papillary craniopharyngioma (Buslei et al. 2005).

Prognostic Factors

Although craniopharyngiomas are WHO Grade I neoplasms, prognostic data that can be obtained by pathologic evaluation are not well-detailed. Whether papillary craniopharyngioma implies a better prognosis is debatable. Lower recurrence rates, and therefore, better prognosis have been reported for papillary craniopharyngioma (Adamson et al. 1990; Tavangar et al. 2004); however, this has not been a consistent conclusion (Crotty et al. 1995). While the lower recurrence, where identified, was attributed to gross total recurrence (Weiner et al. 1994), others reported it as an independent factor (Tavangar et al. 2004). P53 immunoreactivity was significantly related to recurrence and regrowth (Tena-Suck et al. 2006). Cell proliferation index initially appeared promising to identify more aggressive craniopharyngiomas (Nishi et al. 1999; Izumoto et al. 2005); however, no correlation between proliferation index and recurrence rate was subsequently confirmed by others, whether it was similar between histologic types (Losa et al. 2004) or was higher in adamantinomatous than in papillary craniopharyngioma (Tena-Suck et al. 2006). In addition, immunohistochemical expressions of estrogen and progesterone receptors correlated with both lower Ki-67 proliferation index and lower recurrences (Izumoto et al. 2005). It has been suggested that higher microvessel density and vascular endothelial growth factor (VEGF) play a role in prognosis (Vidal et al. 2002). Malignant transformation is rare, is in the form of squamous cell carcinoma mainly after radiation treatment, and is associated with short survival (Rodriguez et al. 2007). Metastasis through cerebrospinal dissemination or inadvertent implantation to distant sites can be seen, but is a rare occurrence, and has been reviewed in detail (Frangou et al. 2009).

Origins of Pituitary Adenoma and Craniopharyngioma

Although a rare occurrence, pituitary adenoma and craniopharyngioma are known to coexist in some cases, either as separate areas in an apparent collision tumor (Karavitaki et al. 2008), or in an intermingled manner (Fig. 10.3d), an even rarer event (Gokden and Mrak 2009). Pituitary tissue and pituitary adenoma can clearly undergo squamous metaplastic change in response to irradiation (Nishioka et al. 2002) or necrosis (Kepes et al. 1982). In addition, nuclear positivity for beta-catenin has been shown by immunohistochemistry in the seemingly transitional squamoid areas of the pituitary adenoma in an intermingled pituitary adenoma and craniopharyngioma case (Gokden and Mrak 2009). These observations suggest, at least in some cases, the presence of a common precursor cell for both of these neoplasms and/or a potential in the cells of either or both of these neoplasms to undergo metaplastic transformation towards the other. This is not surprising given the embryologic origins of anterior pituitary from the Rathke's pouch and the apparent metaplastic development of the squamous cell nests in pars tuberalis from anterior pituitary cells (Asa et al. 1983).

References

Adamson TE, Wiestler OD, Kleihues P, Yasargil MG (1990) Correlation of clinical and pathological features in surgically treated craniopharyngiomas. J Neurosurg 73:12–17

Asa SL (1998) Tumors of the pituitary gland. Atlas of tumor pathology, 3rd Series, Fascicle 22, Series Editor Rosai J, AFIP, Washington, DC, pp 149–150

Asa SL, Kovacs K, Bilbao JM (1983) The pars tuberalis of the human pituitary: a histologic, immunohistochemical, ultrastructural and immunoelectron microscopic analysis. Virchows Arch A Pathol Anat Histopathol 399:49–59

Bunin GR, Surawicz TS, Witman PA, Preston-Martin S, Davis F, Bruner JM (1998) A descriptive epidemiology of craniopharyngioma. J Neurosurg 89:547–551

Buslei R, Nolde M, Hofmann B, Meissner S, Eyupoglu IY, Siebzehnrubl F, Hahnen E, Kreutzer J, Fahlbusch R (2005) Common mutations of beta-catenin in adamantinomatous craniopharyngioma but not in other

tumours originating from the sellar region. Acta Neuropathol 109:589–597

Crotty TB, Scheithauer BW, Young WF, Davis DH, Shaw EG, Muller GM, Burger PC (1995) Papillary craniopharyngioma: a clinicopathologic study of 48 cases. J Neurosurg 83:206–214

Daly AF, Tichomirowa MA, Beckers A (2009) The epidemiology and genetics of pituitary adenomas. Best Pract Res Clin Endocrinol Metab 23:543–554

Frangou EM, Tynan JR, Robinson CA, Ogieglo LM, Vitali AM (2009) Metastatic craniopharyngioma: case report and literature review. Childs Nerv Syst 25:1143–1147

Ghadially FN (1982) Ultrastructural pathology of the cell and matrix, 2nd edn. Butterworths, London, pp 288–289

Gokden M, Mrak RE (2009) Pituitary adenoma with craniopharyngioma component. Human Pathol 40:1189–1193

Horvath E, Kovacs K, Singer W, Smyth HS, Killinger DW, Erzin C, Weiss MH (1981) Acidophil stem cell adenoma of the human pituitary: clinicopathologic analysis of 15 cases. Cancer 47:761–771

Horvath E, Lloyd RV, Kovacs K, Sano T, Kontogeorgos G, Troillas J, Asa SL (2004) Plurihormonal adenoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds) World Health Organization classification of tumours. Pathology and genetics: tumours of endocrine organs. IARC Press, Lyon, p 35

Izumoto S, Suzuki T, Kinoshita M, Hashiba T, Kagawa N, Wada K, Fujimoto Y, Hashimoto N, Saitoh Y, Maruno M, Yoshimine T (2005) Immunohistochemical detection of female sex hormone receptors in craniopharyngiomas: correlation with clinical and histologic features. Surg Neurol 63:520–525

Karavitaki N, Scheithauer BW, Watt J, Ansorge O, Moschopoulos M, Llaguno AV, Wass JAH (2008) Collision lesions of the sella: co-existence of craniopharyngioma with gonadotroph adenoma and of Rathke's cleft cyst with corticotroph adenoma. Pituitary 11:317–323

Kepes JJ, Salyer J, Hiszczynskyj R (1982) Squamous metaplasia following necrosis of the adenohypophysis and of a chromophobe adenoma of the pituitary. Virchows Arch A Pathol Anat Histopathol 395:69–76

Kontogeorgos G, Watson RE Jr, Lindell EP, Barkan AL, Farrell WE, Lloyd RV (2004) Growth hormone producing adenoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds) World Health Organization classification of tumours. Pathology and genetics: tumours of endocrine organs. IARC Press, Lyon, pp 14–19

Kovacs K, Horvath E, Ryan N, Ezrin C (1980) Null cell adenoma of the human pituitary. Virchows Arch Pathol Anat Histolpathol 387:165–174

Le BH, Towfigi J, Kapadia SB, Lopes MBS (2007) Comparative immunohistochemical assessment of craniopharyngioma and related lesions. Endocr Pathol 18:23–30

Lloyd RV, Chandler WF, Kovacs K, Ryan N (1986) Ectopic pituitary adenomas with normal anterior pituitary glands. Am J Surg Pathol 108:546–552

Lloyd RV, Kovacs K, Young WF Jr, Farrell WE, Asa SL, Troillas J, Kontogeorgos G, Sano T, Scheithauer BW, Horvath E (2004) Pituitary tumors: introduction. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds) World Health Organization classification of tumours. Pathology and genetics: tumours of endocrine organs. IARC Press, Lyon, pp 10–13

Losa M, Vimercati A, Acerno S, Barzaghi RL, Mortini P, Mangili F, Terreni MR, Santambrogio G, Giovanelli M (2004) Correlation between clinical characteristics and proliferative activity in patients with craniopharyngioma. J Neurol Neurosurg Psychiatry 75:889–892

Meij BP, Lopes MBS, Ellegala DB, Alden TD, Laws ER Jr (2002) The long-term significance of microscopic dural invasion in 354 patients with pituitary adenomas treated with transsphenoidal surgery. J Neurosurg 96:195–208

Nishi T, Kuratsu J, Takeshima H, Saito Y, Kochi M, Ushio Y (1999) Prognostic significance of the MIB-1 labeling index for patient with craniopharyngioma. Int J Mol Med 3:157–161

Nishioka H, Hirano A, Haraoka J, Nakajima N (2002) Histological changes in the pituitary gland and adenomas following radiotherapy. Neuropathology 22:19–25

O'Hara BJ, Patau A, Miettinen M (1998) Keratin subsets and monoclonal antibody HBME-1 in chordoma: immunohistochemical differential diagnosis between tumors simulating chordoma. Human Pathol 29:119–126

Osamura RY, Sano T, Ezzat S, Asa SL, Barkan AL, Watson RE Jr, Lindell EP (2004) TSH producing adenoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds) World Health Organization classification of tumours. Pathology and genetics: tumours of endocrine organs. IARC Press, Lyon, pp 24–25

Pernicone PJ, Scheithauer BW, Sebo TJ, Kovacs KT, Horvath E, Yound WF Jr, Lloyd RV, Davis DH, Guthrie BL, Schoene WC (1997) Pituitary carcinoma: a clinicopathologic study of 15 cases. Cancer 79:804–812

Rodriguez FJ, Scheithauer BW, Tsunoda S, Kovacs K, Vidal S, Piepgras DG (2007) The spectrum of malignancy in craniopharyngioma. Am J Surg Pathol 31:1020–1028

Rushing EJ, Giangaspero F, Paulus W, Burger PC (2007) Craniopharyngioma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO classification of tumours of the central nervous system. IARC Press, Lyon, pp 238–240

Saeger W, Horvath E, Kovacs K, Nose V, Farrell WE, Lloyd RV, Watson RE Jr, Lindell EP (2004) Prolactin producing adenoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds) World Health Organization classification of tumours. Pathology and genetics: tumours of endocrine organs. IARC Press, Lyon, pp 20–23

Saeger W, Ludecke DK, Buchfelder M, Fahlbusch R, Quabbe HJ, Petersenn S (2007) Pathohistological classification of pituitary tumors: 10 years of experience with the German Pituitary Tumor Registry. Eur J Endocrinol 156:203–216

Sautner D, Saeger W (1991) Invasiveness of pituitary adenomas. Pathol Res Pract 187:632–636

Scheithauer BW, Kovacs KT, Laws ER Jr, Randall RV (1986) Pathology of invasive pituitary tumors with special reference to functional classification. J Neurosurg 65:733–744

Scheithauer BW, Laws ER Jr, Kovacs K, Horvath E, Randall RV, Carney JA (1987) Pituitary adenomas of the multiple endocrine neoplasia type 1 syndrome. Semin Diagn Pathol 4:205–211

Scheithauer BW, Gaffey TA, Lloyd RV, Sebo TJ, Kovacs KT, Horvath E, Yapicier O, Young WF Jr, Meyer FB, Kuroki T, Riehle DL, Laws ER Jr (2006) Pathobiology of pituitary adenomas and carcinomas. Neurosurgery 59:341–353

Selman WR, Laws ER Jr, Scheithauer BW, Carpenter SM (1986) The occurrence of dural invasion in pituitary adenomas. J Neurosurg 64:402–407

Shin JL, Asa SL, Woodhouse LJ, Smyth HS, Ezzat S (1999) Cystic lesions of the pituitary: clinicopathological features distinguishing craniopharyngioma, Rathke's cleft cyst, and arachnoid cyst. J Clin Endocrinol Metab 84:3972–3982

Surawicz TS, McCarthy BJ, Kupelian V, Jukich PJ, Bruner JM, Davis FG (1999) Descriptive epidemiology of primary brain and CNS tumors: results from the Central Brain Tumor Registry of the United States, 1990–1994. Neuro Oncol 1:14–25

Tateyama H, Tada T, Okabe M, Takahashi E, Eimoto T (2001) Different keratin profiles in craniopharyngioma subtypes and ameloblastomas. Pathol Res Pract 197:735–742

Tavangar SM, Larijani B, Mahta A, Hosseini SMA, Mehrazine M, Bandarian F (2004) Craniopharyngioma: a clinicopathological study of 141 cases. Endocr Pathol 15:339–344

Tena-Suck ML, Salinas-Lara C, Arce-Arellano RI, Rembao-Bojorquez D, Morales-Espinosa D, Sotelo J, Arrieta O (2006) Clinicopathological and immunohistochemical characteristics associated to recurrence/regrowth of craniopharyngiomas. Clin Neurol Neurosurg 108:661–669

Vidal S, Kovacs K, Lloyd RV, Meyer FB, Scheithauer BW (2002) Angiogenesis in patients with craniopharyngiomas: correlation with treatment and outcome. Cancer 94:738–745

Weiner HL, Wisoff JH, Rosenberg ME, Kupersmith MJ, Cohen H, Zagzag D, Shiminski-Maher T, Flamm ES, Epstein FJ, Miller DC (1994) Craniopharyngiomas: a clinicopathological analysis of factors predictive of recurrence and functional outcome. Neurosurgery 35:1001–1010

Familial Pituitary Adenomas: An Overview

11

Vladimir Vasilev, Adrian Daly, and Albert Beckers

Contents

Abstract

The recently recognized higher prevalence of pituitary tumours presents a challenge for endocrinologists and health-care resource providers in terms of diagnostics and therapy. The majority of pituitary tumors arise sporadically as anterior pituitary adenomas but approximately 5% can be attributed to a familial syndrome. The clinical and genetic characteristics of familial pituitary adenomas have been well portrayed in multiple endocrine neoplasia type 1 (MEN 1) and Carney complex (CNC). Recently, familial cases of pituitary tumours that were unrelated to MEN 1 or CNC were described under the clinical definition of familial isolated pituitary adenomas (FIPA). In 15–20% of FIPA patients, mutations in the *AIP* gene can be found. As clinical treatment in FIPA kindreds with *AIP* mutations and in MEN1 cases with pituitary adenomas can be challenging compared with sporadic cases, the issues of when and how best to screen subjects genetically, hormonally and radiologically have become increasingly prominent.

Introduction

Pituitary adenomas represent a diverse group of predominantly benign tumours whose comparatively high prevalence in general population continues to provoke significant interest among endocrinologists and neurosurgeons. According to the last report of the Primary Brain and Central

V. Vasilev • A. Daly • A. Beckers (✉)
Department of Endocrinology, Centre Hospitalier Universitaire de Liege, Domaine Universitaire du Sart-Tilman, 4000 Liege, Belgium
e-mail: albert.beckers@chu.ulg.ac.be

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_11, © Springer Science+Business Media Dordrecht 2013

Brain Tumour Registry of the United States (2010) they are the third most common form of brain neoplasia comprising 12.7% of all brain tumours by histology type and the second most common entity (25.4%) in young adults (20–34 years). Historically there has been a controversy concerning their prevalence in general population as autopsy and radiological series reported figures for incidentally-found pituitary adenomas as high as 14.4 and 22.5% respectively (Ezzat et al. 2004) while some older epidemiological studies estimated their prevalence rates as 19–28 cases per 100,000 inhabitants (Davis et al. 2001). Two recent community-based cross-sectional studies in the Belgian province of Liege (Daly et al. 2006b) and in Banbury, UK (Fernandez et al. 2010), however, suggest that clinically apparent pituitary adenomas may occur as frequently as 94 and 77.6 cases per 100,000 people respectively. These results indicate that the true prevalence of clinically relevant pituitary adenomas may have been underestimated previously.

The majority of pituitary adenomas occur sporadically and only 5% arise in a familial setting; familial acromegaly has been described for well over a century (Erdheim 1903). Hereditary pituitary adenomas develop as a part of the distinct endocrine tumour syndromes: Multiple Endocrine Neoplasia type 1 (MEN1) and Carney Complex (CNC). The culprit gene in MEN1, *MEN1*, was identified in 1997 (Chandrasekharappa et al. 1997) and by 2008 approximately 565 different mutations were reported (Lemos and Thakker 2008). In 1985 Carney et al. (1986) described a new multiple tumour syndrome (CNC) that presented infrequently with familial acromegaly. Genetic research has linked almost 70% of cases with Carney complex to mutations in the gene for type A regulatory subunit of protein kinase A (PRKAR1A) (Kirschner 2010). By the end of 1990s, however, significant numbers of patients had accumulated who had familial pituitary tumours without mutations in either *MEN1* or *PRKAR1A* genes. A new condition, termed Familial Isolated Pituitary Adenomas (FIPA) was coined to describe these families (Valdes Socin et al. 2000). Mutations in the *aryl hydrocarbon receptor interacting protein* (*AIP*) gene were reported in FIPA patients (Vierimaa et al. 2006), but account for about 15–20% of all patients with this disorder; therefore the search for other genetic causes is still ongoing.

Multiple Endocrine Neoplasia Type 1 (Men 1)

Multiple endocrine neoplasia type 1 (MEN 1) is a rare autosomal dominant condition with high penetrance and no sex predominance. It is characterized by the occurrence of primary hyperparathyroidism, enteropancreatic neuroendocrine tumours and pituitary adenomas; other associated non endocrine tumors can occur. Sporadic and familial forms have been described; the first presenting with pathology in at least two of the three principle endocrine glands and the second is defined as a MEN 1 case plus a first degree relative with one of these three tumours. Parathyroid adenomas are usually the first clinical manifestation of the disorder and affect as many as 95% of patients by the age of 50 (Falchetti et al. 2008). Diagnosed in 35–80% of patients, pancreatic islet cell tumours are the second most common presentation of MEN1 and are mostly characterized by excessive hormone production leading to marked clinical symptoms. Gastrinomas causing Zollinger-Ellison syndrome account for nearly a half of pancreatic lesions in MEN1 and due to multiple peptic ulcers and a large proportion of malignant tumours they represent one of the major causes for mortality and morbidity in this condition (Marini et al. 2006). The prevalence of pituitary tumours in MEN1 varies widely from 10 to 60% in the different studies and they are the first clinical manifestation of the disease in up to 25% of patients (Falchetti et al. 2008). However, only 2.7% of pituitary adenomas can be attributed to MEN 1 (Scheithauer et al. 1987). Pituitary pathology is much more prevalent in familial MEN 1 cases compared to non-familial ones. Also, women with MEN 1 have an increased predisposition to having a pituitary adenoma. Nearly all types of pituitary tumours have been reported, with prolactinomas being the most frequent, but the proportions of prolactin-secreting,

GH-secreting, ACTH-secreting and non-functional adenomas remain similar in MEN 1 cases and sporadic populations (Verges et al. 2002). Pituitary tumours in MEN 1, however, appear to be larger and more aggressive than their sporadic counterparts and macroadenomas comprise approximately 85% of them compared to only 42% of non-MEN 1 pituitary adenomas. This is probably one of the reasons why MEN 1 pituitary tumours are more likely to cause compression related symptoms. This tendency for aggressiveness is especially well observed in MEN 1 prolactinomas where the proportion of macroadenomas reaches 84% in contrast to the general population where microadenomas are the predominant prolactinoma phenotype (Daly et al. 2009). Response to therapy is also significantly lower in MEN 1 related pituitary tumours as only 42% of them achieve hormone normalization in contrast to sporadic functional pituitary adenomas where almost 90% of patients can be successfully treated (Verges et al. 2002). Apart from these three main types of neoplasms, over 20 other endocrine and non-endocrine tumours have been described in association with MEN 1. These include the less common carcinoid tumours and adrenal cortical tumours, usually non-secreting, as well as various inactive benign lesions such as lipomas, facial angiofibromas and collagenomas.

Genetic studies for loss of heterozigoty have linked the responsible gene to a locus on chromosome 11q13 (Larsson et al. 1988). The gene itself was cloned several years later (Chandrasekharappa et al. 1997) and consists of 10 exons, encoding a 610 amino acid protein, called menin. *MEN1* is generally considered to act as a tumour suppressor gene and its transcript – menin has been shown to interact with a variety of proteins that take part in transcriptional regulation, genome stability, cell proliferation and apoptosis. Localized predominantly in the nucleus, menin suppresses Jun- and NF-κB-mediated transcriptional activation, participates in the regulation of transforming growth factor-β (TGF-β) signaling pathways by interacting with Smad family of proteins, regulates the expression of cyclin-dependant kinase inhibitors genes p27 and p18 by being a component in histone methyltransferase complexes, inhibits cell proliferation through interacting with the activator of S-phase kinase (ASK), maintains stable gene expression by controlling genome stability and DNA replication and repair (Lemos and Thakker 2008). Further more, numerous menin binding sites in chromatin were identified, many of them being not only within promoter regions, but also inside 3' end of genes as well as introns. However, none of the numerous menin functions has been proven critical in MEN1 tumorigenesis. A recent study demonstrated overexpression of transcriptional factor HLXB9 in pancreatic islet cells in the absence of menin (Scacheri et al. 2006), implicating the possibility that preferential targeting of specific genes by menin in endocrine tissue may explain the tendency for endocrine tumour formation. A pituitary specific function of menin is the interaction with activin – a negative regulator of prolactin, growth hormone and corticotropine secretion and pituitary cell proliferation, mediated through the inhibition of *Pit-1* gene expression (Hendy et al. 2005).

Mutations in *MEN1* gene are spread throughout the whole coding sequence and include 41% frameshift deletions, 23% nonsense mutations, 20% missense mutations, 9% splice-site mutations, 6% in-frame deletions and 1% whole gene deletions (Lemos and Thakker 2008). The majority of them lead to synthesis of truncated protein. The impact of the lack of menin on MEN 1 tumorigenesis has been studied in specially developed knockout mouse models. Homozigous animals ($MEN1^{-/-}$) die early in embryonic phase with severe developmental defects in many organs while heterozygous mice ($MEN1^{+/-}$) develop parathyroid adenomas and carcinomas, pancreatic islet cells tumours, pituitary adenomas and various other types of neoplasia, thus providing a model for human MEN 1 disease. Pituitary pathology in these animals is confined mainly to prolactinomas and somatotropinomas with a large proportion of malignant cases (Lemos and Thakker 2008).

So far no evident genotype-phenotype correlations have been observed although few kindreds with prevailing prolactinomas have been reported in Canada and Tasmania. Mutations in *MEN1* are also detected in one third of sporadic enteropancreatic

tumours and 20% of sporadic parathyroid adenomas but they seem to be extremely rare in sporadic pituitary adenomas although loss of heterozygoty in locus 11q13 has been proven for up to 30% of them (Daly et al. 2009).

Multiple Endocrine Neoplasia Type 4 (Men 4)

Despite the large number of identified mutations in coding regions of *MEN 1* gene, DNA tests still fail to detect anomalies in more than 20% of patients with clinical characteristics of the disease. This may be due to mutations within introns or promoter regions or may also imply the involvement of other genes in the development of MEN 1-like conditions. Recently a mutation in *CDNK1B* gene, coding cycline-dependant kinase inhibitor $p27^{Kip1}$, was identified in rats with MEN 1-like symptoms (Pellegata et al. 2006). The animals presented with numerous neuroendocrine tumours like pheochromocytoma, medullary thyroid carcinoma, parathyroid adenomas, pancreatic hyperplasia and pituitary adenomas. In humans *CDNK1B* gene is located on chromosome 12p13 and its product $p27^{Kip1}$ plays an important role in cell cycle regulation by inhibiting cyclin/CDK complexes. Interestingly, pituitary adenomas originating from the intermediate lobe are the only tumours that develop spontaneously in homozygous $p27^{Kip1-/-}$ mice. Although mutations in human *CDKN1B* gene are confirmed in only five families the condition was accepted as a new syndrome and called MEN 4. The first registered case was documented in a German kindred exhibiting familial acromegaly, primary hyperparathyroidism, renal angiomyolipoma and testicular cancer among various members. Genetic testing revealed a nonsense mutation in *CDKN1B* gene in the absence of any detectable pathology in *MEN1* gene (Pellegata et al. 2006). Soon after, a heterozygous germline frameshift mutation of *CDKN1B* gene was reported in another MEN1-like case, negative for *MEN1* mutations, – a Dutch female patient with small-cell cervical carcinoma, ACTH-secreting pitui-tary adenoma and hyperparathyroidism. More recently, three novel mutations were identified in MEN1-like cases presenting with parathyroid and other endocrine tumours but without pituitary lesions (Agarwal et al. 2009). Mutations in *CDKN1B* gene are, however, found in less than 3% of patients with presumable MEN1 phenotypes, negative for *MEN1* muatations, which suggests that other genetic factors may also be involved. (Pellegata et al. 2006; Agarwal et al. 2009).

Carney Complex

Carney Complex (CNC) is another autosomal dominant condition that is associated though infrequently with familial pituitary pathology, mainly acromegaly. The disease is characterized by spotty skin pigmentations, myxomas, endocrine hyperactivity and schwannomas and since the description of the first cohort of patients in 1985 (Carney et al. 1986) more than 500 patients have been reported in the largest database (Boikos and Stratakis 2007). Approximately 70% of cases with CNC present in a familial trait with slight female predominance. Benign skin lesions are the most common clinical manifestation of the disease and include lentigenes, cutaneous or mucosal myxomas, blue nevi and café-au-lait spots. Cardiac myxomas are the most frequent non-cutaneous lesions in CNC and account for more than a half of the disease-specific mortality. Endocrine abnormalities are observed in approximately a third of CNC patients and are mainly due to Cushing's syndrome caused by primary pigmented nodular adrenocortical disease (PPNAD). Less common endocrine presentations include large cell calcifying sertoli cell tumours (LCCSCTs) and benign or malignant thyroid nodules (Boikos and Stratakis 2007). Pituitary adenomas occur with an incidence of 10–12% of CNC patients and usually cause acromegaly or gigantism depending on the age of onset but at least one family with prolactinomas has also been reported (Kirschner 2010). A distinguishing feature of GH-secreting tumours in CNC is multifocal hyperplasia of somatomammotrope cells amidst normal pituitary tissue. This finding, together with

the elevations in GH and IGF_1 levels as well as mild hyperprolactinemia that can be detected in almost 75% of patients, suggests that acromegaly in CNC may develop insidiously from pituitary hyperplasia to overt adenoma.

Inactivating mutations in the gene for type 1A regulatory subunit of protein kinase A (*PRKAR1A*), located on chromosome 17q22–24, have been identified in over 70% of patients with CNC. Unlike MEN 1, however, CNC is probably genetically heterogeneous as a second locus on chromosome 2p16 has been implicated although specific genetic alterations still have to be confirmed in affected families (Boikos and Stratakis 2007). As in MEN 1, no somatic mutations in *PRKAR1A* gene have been documented in sporadic pituitary tumours. Protein kinase A (PKA) is a cAMP-dependant protein kinase composed of two regulatory and two catalytic subunits and is a component of a wide scope of metabolic and regulatory pathways involved in cell proliferation, transcription and apoptosis. The majority of *PRKAR1A* gene mutations lead to premature stop codon generation and subsequent nonsense mRNA degradation. The loss of type 1A regulatory subunits disrupts the balance in PKA tetramer formation and induces release of free catalytic subunits resulting in increased cAMP-dependant kinase activity in affected tissues (Robinson-White et al. 2006). In pituitary cells the stimulation of GHRH receptor by its ligand leads to GH synthesis and release through the PKA pathway and its specific activation in CNC may result in GH hypersecretion in the absence of physiologic signals. Complete knockout of *PRKAR1A* gene in mice severely impairs cardiac morphogenesis and is lethal during early embryogenesis while heterozygous $PRKAR1A^{+/-}$ animals, despite exhibiting many of the tumour lesions observed in humans with CNC, fail to develop any significant pituitary pathology. Tissue-specific ablation of *PRKAR1A* gene in mice, however, induces gradual development of pituitary tumours with biochemical features, quite similar to CNC-related acromegaly in humans, e.g. GH hypersecretion with slow progression from hyperplasia to adenoma (Kirschner 2010).

Familial Isolated Pituitary Adenomas (FIPA)

Familial isolated pituitary adenomas (FIPA) represent a recently defined hereditary syndrome characterized by familial occurrence of pituitary tumours of any functional type in the absence of clinical and genetic features of MEN 1 and CNC (Beckers and Daly 2007). Since its first description at the end of the last century (Valdes Socin et al. 2000) active research and increased recognition has led to the identification of more than 200 families with this disorder (Chahal et al. 2010). However, FIPA is estimated to account only for 2.5% of all pituitary tumours, a proportion similar to MEN 1 (Daly et al. 2006a). Genealogical data from affected kindreds indicates autosomal dominant mode of inheritance with incomplete penetrance. Tumour phenotype within individual FIPA families may present in homogeneous manner with all affected members exhibiting the same adenoma type, or heterogeneously when different pituitary tumours arise within the kindred. All functional types of pituitary tumours may be associated with this condition but prolactin- or GH-secreting adenomas are almost inevitably present in affected families. Prolactin-secreting adenomas are the most common phenotype and comprise about 40% of all FIPA tumours Sexual predisposition, age of presentation and proportion of microadenomas are similar to sporadic prolactinomas. In heterogeneous FIPA families, however, they exhibit more aggressive behavior with significantly higher rates of suprasellar extension and cavernous sinus invasion. GH-secreting adenomas account for 30% of FIPA tumours and somatoprolactinomas are responsible for another 7% of them. They are equally distributed between homogeneous and heterogeneous families but, unlike FIPA prolactinomas, somatotropinomas are more aggressive when occurring in a homogeneous setting. In homogeneous FIPA, acromegaly is usually diagnosed 10 years earlier with tumours more frequently displaying extracellar growth compared to heterogeneous kindreds and sporadic populations (Beckers and Daly 2007). Acromegaly in FIPA patients also appears to respond poorly to

somatostatin analogue therapy (Leontiou et al. 2008). Non-secreting adenomas, predominantly associated with heterogeneous families, arise in 13% of FIPA patients and are also characterized by more aggressive evolution, being diagnosed earlier and exhibiting more invasive properties than sporadic adenomas. Gonadotropinomas, corticotropinomas and thyreotropinomas account for 4, 4 and 1% of FIPA tumours respectively and are usually associated with other adenoma types in heterogeneous kindreds although individual families with homogeneous presentation have been reported (Beckers and Daly 2007).

In 2006 a detailed genome-wide screening in search for potential genes involved in familial adenoma tumorigenesis implicated that inactivating mutations in the gene coding aryl hydrocarbon receptor interacting protein (*AIP*) could be responsible for familial acromegaly and prolactinomas in large Finnish kindreds (Vierimaa et al. 2006). Loss of heterozygoty at the *AIP* locus in tumour tissue from affected patients indicates that *AIP* gene may experience a tumour suppressor function. In the largest cohorts of FIPA patients mutations in *AIP* gene can be found in approximately 22% of all cases and in 40% of families with acromegaly occurring in a homogeneous FIPA setting (Beckers and Daly, 2007; Chahal et al. 2010). Though quite infrequently, patients with sporadic pituitary tumours also seem to harbour *AIP* mutations with the majority of them having acromegaly. Within FIPA families, *AIP* mutation carriers are significantly younger at diagnosis and tend to have larger tumours than patients with normal *AIP* sequence (Daly et al. 2007). Contrary to the overall female predominance in FIPA, they are mostly men (71%) and usually present with somatotropinomas or somatoprolactinomas (86%) (Cazabat et al. 2009). There also exists some discrepancy in terms of clinical characteristics and histology results in tumours positive for *AIP* mutations as somatotropinomas are stained not only for GH but in a third of cases for prolactin and infrequently for FSH (Beckers and Daly 2007).

The *AIP* gene is ubiquitously expressed in various tissues throughout the body and in normal pituitary it is associated with secretory granules in somatotrope and lactotrope cells. In sporadic pituitary adenomas, however, *AIP* is expressed in all tumour types but in prolacatinomas, non-secreting adenomas and corticotropinomas it can only be identified in the cytoplasm whereas in somatotropinomas it is localized within secretory vesicles (Leontiou et al. 2008). The exact pathophysiological mechanisms that are involved in pituitary tumorigenesis caused by *AIP* mutations still remain unknown. Homozygous *AIP* $^{-/-}$ knockout mice develop severe cardiovascular abnormalities that are incompatible with life while heterozygotes ($AIP^{+/-}$) exhibit no pituitary pathology. The gene itself consists of 6 exons and encodes a 330 aminoacid protein which C-terminal end is required for binding to the aryl hydrocarbon receptor (AhR). This receptor acts as ligand-inducible transcription factor and modulates cellular responses to various toxic environmental substances, such as dioxins (Beckers and Daly 2007). In the absence of ligands the AhR couples to a dimer of the 90 kDa heat shock protein (HSP90), acting as chaperone, and AIP and p23 proteins, acting as co-chaperons, to form a multiprotein complex in the cytoplasm (Kazlauskas et al. 1999). The activation of the complex by its xenobiotic ligand results in nuclear translocation where AhR binds to the aryl hydrocarbon receptor nuclear translocator (ARNT) and promotes the transcription of specific genes coding various drug metabolizing enzymes as well as other proteins such as the cyclin dependant kinase inhibitor $p27^{Kip1}$. The role of AIP in the regulation of AhR activity is not quite clear, but it seems to participate in the stabilization and the cytoplasmic retention of the complex by an yet unknown mechanism. Data on the effect of AhR activation on cell proliferation are controversial but recently it was shown that reduced AIP expression in pituitary adenomas, positive for AIP mutations, is associated with decreased AhR activity, suggesting an inhibitory function of AhR in pituitary tumorigenesis (Jaffrain-Rea et al. 2009). Further more, AIP overexpression in cell cultures including pituitary cell lines slows down cell proliferation rates (Leontiou et al. 2008). Another possible pathophysiological link between AIP and pituitary tumorigenesis lies in the interaction with two specific types of phosphodiesterases – PDE4A5

and PDE2A. These enzymes inactivate cyclic nucleotides like cAMP by disrupting the phosphodiester bond in their molecules and thus they may participate in the regulation of the various signaling pathways utilizing cAMP as intracellular second messenger, including the GHRH receptor cascade in pituitary cells. The interaction of AIP with PDE4A5, however, inhibits enzyme activity with resultant increase in cAMP concentration and it currently remains unclear if a tumour suppressor effect can be achieved in this way as cAMP overproduction is usually associated with pro-oncogenic outcomes. Binding to PDE2A interrupts the nuclear translocation of the AhR complex possibly by local reduction in cAMP levels (Ozfirat and Korbonits 2010). Very recently the tyrosine kinase receptor encoded by the *RET* protooncogene was identified as a novel binding partner of AIP in pituitary cells. Depending on the presence or the absence of its specific ligand – glial cell line-derived neurotropic factor (GDNF) – RET promotes cell growth and migration or induces apoptosis respectively. More over, the domain responsible for the proapoptotic activity is the same that is responsible for AIP interaction. This RET-AIP binding presumably prevents the formation of a complex between AIP and survivin – a recently recognized apoptosis inhibitor and cell cycle regulator. Without the stabilizing role of AIP, survivin is put to rapid degradation with consequent increase in apoptosis (Vargiolu et al. 2009). In vitro studies, however, have failed to confirm that some missense AIP mutations could disrupt the association with RET (Chahal et al. 2010) and although it may be tempting to accept a more essential role of such interaction in pituitary tumorigenesis its true relevance remains questionable. Apart from stabilizing the AhR complex, AIP may also bind to a set of nuclear receptors including the peroxysome proliferator-activated receptor α (PPARα), the glucocorticoid receptor, and β-thyroid hormone receptor 1 (TRβ1). Further more, it has been proposed a role in virus induced tumorigenesis as a potential partner of hepatitis B virus X antigen and Epstein-Barr virus-encoded nuclear antigen 3 (EBNA-3)(Ozfirat and Korbonits 2010). The outcomes of these interactions, however, still remain to be fully elucidated.

To date, more than 50 different mutations have been identified throughout the sequence of *AIP* gene and approximately 70% of them disrupt the C-terminal end of AIP polypeptide chain that is essential for protein-protein interactions. Nonsense and frameshift mutations lead to premature stop codons with resultant truncated protein while missense mutations tend to affect the TPR domains and the terminal α-helix. Several mutations like R304, R271 and R81 are reported in independent FIPA families from different centers indicating possible mutational hotspots in the *AIP* gene while the Q14X mutation, though common in Finnish FIPA kindreds, has not been identified elsewhere suggesting possible founder effect (Ozfirat and Korbonits 2010). So far no genotype-phenotype correlations have been reported in FIPA families harbouring AIP mutations although some observations may imply a less aggressive character for mutations with conserved C-terminal part of AIP (Cazabat et al. 2009). Families with marked heredity for pituitary tumours, however, may lack mutations in *AIP* gene which is a strong indicator that other genetic disruptions may also be involved in FIPA development.

Management of Familial Pituitary Adenomas

Treatment of pituitary tumours that arise in familial setting practically does not differ from the management of sporadic adenomas in terms of indications and therapeutic approaches. Physicians should bear in mind, however, that pituitary tumours developing as a part of MEN 1 and FIPA syndromes have more aggressive evolution, present earlier and often respond poorly to therapy. As MEN 1 has been recognized for quite some time consensus statements and guidelines for the investigation and management of this condition have been developed (Brandi et al. 2001). Genetic testing for mutations in *MEN 1* gene is warranted in patients who meet the clinical criteria for the disease and the identification of a mutation allows screening for carriers among the relatives. Mutation positive individuals should undergo annual biochemical assessment of prolactin, starting from the age of 5, total serum calcium from

the age of 8 and gastrin from age 20 as well as periodic (every 3–5 years) imaging tests to screen for relevant tumours. Mutation negative relatives can be surely excluded from further follow-up. When no mutation could be identified in the index case it may be appropriate to consider a haplotype or linkage analysis in an investigational laboratory provided sufficient number of affected family members is available. Testing for mutations in *CDKN1B* gene in MEN 1-like cases without *MEN 1* mutations is still reserved only for research purposes as MEN 4 seems to be extremely rare condition. Similarly, patients meeting the diagnostic criteria for CNC should be tested for germline mutations in *PRKAR1A* gene. Carriers should be followed-up for the different presentations of the syndrome and clinical, biochemical and imaging screening should be performed on a yearly basis. Regarding pituitary pathology, evaluation of GH, IGF_1 and prolactin secretion is appropriate. By definition FIPA is diagnosed in patients with hereditary pituitary tumours in the absence of mutations of *MEN 1* and *PRKAR1A* genes and as the number of reported families increase detailed family history is of essential importance in patients with pituitary adenomas. Although mutations of *AIP* gene are observed in only 20% of cases genetic testing in at least one affected member may be valuable from a clinical aspect as patients with mutations are associated with more aggressive disease. Identifying carriers among relatives is potentially beneficial for performing regular MRI and hormonal evaluation. In the absence of tumour on the imaging study surveillance may be carried on clinically and biochemically (IGF_1 and prolactin). Special attention should be paid to genetic counseling of FIPA patients and their relatives because of the relatively low prevalence of *AIP* mutations and the uncertain degree of penetrance. Genetic screening among sporadic pituitary adenomas may not be warranted in unselected cases but it may be considered in young patients with aggressive disease who are more likely to carry *AIP* mutations.

In conclusion, familial presentation of pituitary tumours although occurring in a small proportion of patients with pituitary adenoma provides a unique opportunity for investigation of genetic and molecular pathways of tumorigenesis. In recent years the scope of familial pituitary syndromes has expanded with the definition of FIPA and MEN4 in addition to the well-described MEN1 and CNC. More over, modern technology has made possible not only the elucidation of culprit genetic defects behind these disorders but also presents an important tool for identifying at-risk individuals among affected families. The in-dept understanding of the specific evolution and clinical characteristics of familial syndromes may provide basis for preclinical diagnosis, better prevention and appropriate management of individual patients. Still, much remains to be done, especially in FIPA where the majority of patients do not harbour *AIP* mutations and may possibly have defects in yet unidentified genes. In addition, it is unclear whether FIPA patients have also predisposition for other endocrine or non-endocrine tumours which could expand its definition beyond the pituitary. Further clinical studies in larger populations as well as the development of appropriate experimental models may help elucidate these issues.

References

Agarwal SK, Mateo CM, Marx SJ (2009) Rare germline mutations in cyclin-dependent kinase inhibitor genes in multiple endocrine neoplasia type 1 and related states. J Clin Endocrinol Metab 94:1826–1834

Beckers A, Daly AF (2007) The clinical, pathological, and genetic features of familial isolated pituitary adenomas. Eur J Endocrinol 157:371–382

Boikos SA, Stratakis CA (2007) Carney complex: the first 20 years. Curr Opin Oncol 19:24–29

Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia A, Lips CJ, Lombardi G, Mannelli M, Pacini F, Ponder BA, Raue F, Skogseid B, Tamburrano G, Thakker RV, Thompson NW, Tomassetti P, Tonelli F, Wells SA Jr, Marx SJ (2001) Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab 86:5658–5671

Carney JA, Hruska LS, Beauchamp GD, Gordon H (1986) Dominant inheritance of the complex of myxomas, spotty pigmentation, and endocrine overactivity. Mayo Clin Proc 61:165–172

Cazabat L, Guillaud-Bataille M, Bertherat J, Raffin-Sanson ML (2009) Mutations of the gene for the aryl hydrocarbon receptor-interacting protein in pituitary adenomas. Horm Res 71:132–141

Chahal HS, Chapple JP, Frohman LA, Grossman AB, Korbonits M (2010) Clinical, genetic and molecular

characterization of patients with familial isolated pituitary adenomas (FIPA). Trends Endocrinol Metab 21:419–427

Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J, Boguski MS, Agarwal SK, Kester MB, Kim YS, Heppner C, Dong Q, Spiegel AM, Burns AL, Marx SJ (1997) Positional cloning of the gene for multiple endocrine neoplasia-type 1. Science 276:404–407

Daly AF, Jaffrain-Rea ML, Ciccarelli A, Valdes-Socin H, Rohmer V, Tamburrano G, Borson-Chazot C, Estour B, Ciccarelli E, Brue T, Ferolla P, Emy P, Colao A, De Menis E, Lecomte P, Penfornis F, Delemer B, Bertherat J, Wemeau JL, De Herder W, Archambeaud F, Stevenaert A, Calender A, Murat A, Cavagnini F, Beckers A (2006a) Clinical characterization of familial isolated pituitary adenomas. J Clin Endocrinol Metab 91:3316–3323

Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A (2006b) High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. J Clin Endocrinol Metab 91:4769–4775

Daly AF, Vanbellinghen JF, Khoo SK, Jaffrain-Rea ML, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo AP, Tamburrano G, Raverot G, Barlier A, De Herder W, Penfornis A, Ciccarelli E, Estour B, Lecomte P, Gatta B, Chabre O, Sabate MI, Bertagna X, Garcia Basavilbaso N, Stalldecker G, Colao A, Ferolla P, Wemeau JL, Caron P, Sadoul JL, Oneto A, Archambeaud F, Calender A, Sinilnikova O, Montanana CF, Cavagnini F, Hana V, Solano A, Delettieres D, Luccio-Camelo DC, Basso A, Rohmer V, Brue T, Bours V, Teh BT, Beckers A (2007) Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. J Clin Endocrinol Metab 92:1891–1896

Daly AF, Tichomirowa MA, Beckers A (2009) The epidemiology and genetics of pituitary adenomas. Best Pract Res Clin Endocrinol Metab 23:543–554

Davis JR, Farrell WE, Clayton RN (2001) Pituitary tumours. Reproduction 121:363–371

Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2005-2009. Neuro-Oncol (2012) 14 (suppl 5): v1-v49.

Erdheim J (1903) Zur normalen und pathologischen Histologie der Glandula thyroidea, parathyroidea, und hypophysis. Beitr Pathol Anat 33:158–236

Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, McCutcheon IE (2004) The prevalence of pituitary adenomas: a systematic review. Cancer 101:613–619

Falchetti A, Marini F, Luzi E, Tonelli F, Brandi ML (2008) Multiple endocrine neoplasms. Best Pract Res Clin Rheumatol 22:149–163

Fernandez A, Karavitaki N, Wass JA (2010) Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clin Endocrinol (Oxf) 72:377–382

Hendy GN, Kaji H, Sowa H, Lebrun JJ, Canaff L (2005) Menin and TGF-beta superfamily member signaling via the Smad pathway in pituitary, parathyroid and osteoblast. Horm Metab Res 37:375–379

Jaffrain-Rea ML, Angelini M, Gargano D, Tichomirowa MA, Daly AF, Vanbellinghen JF, D'Innocenzo E, Barlier A, Giangaspero F, Esposito V, Ventura L, Arcella A, Theodoropoulou M, Naves LA, Fajardo C, Zacharieva S, Rohmer V, Brue T, Gulino A, Cantore G, Alesse E, Beckers A (2009) Expression of aryl hydrocarbon receptor (AHR) and AHR-interacting protein in pituitary adenomas: pathological and clinical implications. Endocr Relat Cancer 16:1029–1043

Kazlauskas A, Poellinger L, Pongratz I (1999) Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (Aryl hydrocarbon) receptor. J Biol Chem 274:13519–13524

Kirschner LS (2010) PRKAR1A and the evolution of pituitary tumors. Mol Cell Endocrinol 326(1–2):3–7

Larsson C, Skogseid B, Oberg K, Nakamura Y, Nordenskjold M (1988) Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature 332:85–87

Lemos MC, Thakker RV (2008) Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. Hum Mutat 29:22–32

Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, Chahal HS, Igreja SC, Jordan S, Rowe J, Stolbrink M, Christian HC, Wray J, Bishop-Bailey D, Berney DM, Wass JA, Popovic V, Ribeiro-Oliveira A Jr, Gadelha MR, Monson JP, Akker SA, Davis JR, Clayton RN, Yoshimoto K, Iwata T, Matsuno A, Eguchi K, Musat M, Flanagan D, Peters G, Bolger GB, Chapple JP, Frohman LA, Grossman AB, Korbonits M (2008) The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. J Clin Endocrinol Metab 93:2390–2401

Marini F, Falchetti A, Del Monte F, Carbonell Sala S, Gozzini A, Luzi E, Brandi ML (2006) Multiple endocrine neoplasia type 1. Orphanet J Rare Dis 1:38

Ozfirat Z, Korbonits M (2010) AIP gene and familial isolated pituitary adenomas. Mol Cell Endocrinol 326 (1–2):71–79

Pellegata NS, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, Hofler H, Fend F, Graw J, Atkinson MJ (2006) Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. Proc Natl Acad Sci U S A 103:15558–15563

Robinson-White A, Meoli E, Stergiopoulos S, Horvath A, Boikos S, Bossis I, Stratakis CA (2006) PRKAR1A Mutations and protein kinase A interactions with other signaling pathways in the adrenal cortex. J Clin Endocrinol Metab 91:2380–2388

Scacheri PC, Davis S, Odom DT, Crawford GE, Perkins S, Halawi MJ, Agarwal SK, Marx SJ, Spiegel AM, Meltzer PS, Collins FS (2006) Genome-wide analysis

of menin binding provides insights into MEN1 tumorigenesis. PLoS Genet 2:e51

Scheithauer BW, Laws ER Jr, Kovacs K, Horvath E, Randall RV, Carney JA (1987) Pituitary adenomas of the multiple endocrine neoplasia type I syndrome. Semin Diagn Pathol 4:205–211

Valdes Socin H, Poncin J, Stevens V, Stevenaert A, Beckers A (2000) Adenomes hypophysaires familiaux isoles non lies avec la mutation somatique NEM-1. Siuvi de 27 patients. Ann D Endocrinol 61:301

Vargiolu M, Fusco D, Kurelac I, Dirnberger D, Baumeister R, Morra I, Melcarne A, Rimondini R, Romeo G, Bonora E (2009) The tyrosine kinase receptor RET interacts in vivo with aryl hydrocarbon receptor-interacting protein to alter survivin availability. J Clin Endocrinol Metab 94:2571–2578

Verges B, Boureille F, Goudet P, Murat A, Beckers A, Sassolas G, Cougard P, Chambe B, Montvernay C, Calender A (2002) Pituitary disease in MEN type 1 (MEN1): data from the France-Belgium MEN1 multicenter study. J Clin Endocrinol Metab 87: 457–465

Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, Gundogdu S, De Menis E, Makinen MJ, Launonen V, Karhu A, Aaltonen LA (2006) Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science 312:1228–1230

Papillary Glioneuronal Tumor

12

Daniel C. Dim

Contents

Abstract

The recognition of papillary glioneuronal tumor as a distinct entity was clearly and well established with the detailed description of this tumor in the case series of nine such tumors by Komori et al. (Am J Surg Pathol 22: 1171–1183, 1998). Presently, the literature abounds with several dozen reports of this tumor, which in a nutshell, characterize it as a biphasic tumor of mixed glial and neuronal differentiation. These cerebral neoplasms are supratentorial, and relatively circumscribed solid lesions with a cystic component. Magnetic resonance imaging (MRI) and computed tomography (CT) reveal contrast-enhancing masses with negligible mass effect. The histopathology exhibits gliovascular pseudopapillae composed of single or multi-layered astrocytic cells enclosing often hyalinized blood vessels lying in a bed of aggregates of neurocytes, neurons, and ganglioid cells. Because of the overwhelming evidence from the various reported cases, the World Health Organization (WHO) which initially placed this tumor as a variant of ganglioglioma, eventually recognized it as a neoplasm worthy of placement in a separate category in a recent publication. These rare and low-grade tumors behave in a fashion characteristic of WHO grade I tumors. This review is an overview of the clinical, neuroimaging, and neuropathologic features of the neoplasm, and addresses the evidence supporting its classification as a distinct entity.

D.C. Dim (✉)
Department of Pathology, University of Missouri at Kansas City School of Medicine, 2411 Holmes Street, Kansas City, MO 64108, USA
e-mail: dimd@umkc.edu

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_12,

Introduction

Papillary glioneuronal tumor (PGNT) is a member of the diverse group of mixed glioneuronal tumors (GNTs) of the central nervous system (CNS), which are typically indolent tumors composed of glial cells mingled with neuronal cells of different differentiation (McLendon and Provenzale 2002). The other members of this heterogenous category of mixed GNTs include gangliogliomas, central and extraventricular neurocytomas, rosette-forming glioneuronal tumor of the fourth ventricle, and dysembryoplastic neuroepithelial tumors (DNET), and are usually associated with a favorable prognosis like PGNT (Kleihues and Cavenee 2000).

PGNT is a rare neoplasm and its epidemiology has not been established because less than 50 cases have been reported in the literature. PGNTs are supratentorial and generally affect the cerebral hemispheres in close proximity to the ventricles. Although the tumor has been reported in all regions of the cerebrum, the vast majority of cases to date have preference for the temporal lobe (Broholm et al. 2002; Komori et al. 1998; Prayson 2000). The tumor behavior is indolent and corresponds to WHO grade I lesions; however, there are exceptional cases that have manifested atypical features and recurrence (Ishizawa et al. 2006; Adam et al. 2007; Vaquero and Coca 2007; Newton et al. 2008; Javahery et al. 2009). The prominent presenting symptoms include headache and seizures, although other symptoms and signs related to presence of a mass in the brain may be noted. Tumors with similar morphology were previously described under different terms, namely pseudopapillary neurocytoma with glial differentiation (Kim and Suh 1997), and pseudopapillary ganglioneurocytoma (Komori et al. 1996). The progenitor stem cell of PGNT is still debated, but it is believed that they originate from multipotent cells committed to a biphasic glial and neuronal differentiation (Nakazato et al. 2007).

Clinical, Neuroimaging, and Neuropathological Features

The age of occurrence has a wide range with the youngest and oldest reported between 4 years and 75 years of age, respectively, with a mean of 27 years and no sex preference (Barnes et al. 2002; Tsukayama and Arakawa 2002; Dim et al. 2006; Louis et al. 2007). PGNT on imaging studies (Fig. 12.1) shows a relatively well-delineated cystic mass containing a contrast-enhanced mural nodule; low or normal intensity on T1-weighted images and high intensity on T2-weighted images (Stosic-Opincal et al. 2005). Some lesions could also have peripheral enhancement. There may be surrounding mass effect such as edema, but typically there is negligible or no mass effect, and calcification has been noted in some cases. Grossly, the tumor can be solid or solid with variable cystic deterioration including cystic mass or cystic with mural nodule. Hemorrhage and necrosis have been observed in only a few reported cases, but these features are a rarity (Adam et al. 2007; Newton et al. 2008; Javahery et al. 2009). The histologic characteristic of PGNT is that of two distinct components, astrocytic glial and a spectrum of neuronal cells. There is conspicuous pseudopapillary architecture composed of a single or pseudostratified layer of small, cuboidal glial cells with round to oval nuclei, finely speckled chromatin, and scant cytoplasm enclosing hyalinized blood vessels. Hyalinized vessels are commonplace in most glioneuronal tumors, but Lamszus et al. (2003) reported a PGNT case without conspicuous vascular hyalinization. These cells show cytoplasmic projections towards the vessel walls. The pseudopapillary architecture is dependent on dilapidation of the perivascular cells. Interspersed between the pseudopapillae are aggregations and small clusters of neurocytes, large ganglion cells, and intermediate-sized "ganglioid" cells in various combinations (Fig. 12.2). Some tumors exhibit islands of small oligidendroglial-like cells with prominent perinuclear halos or clusters of gemistocytes (Tanaka et al. 2005). The glial cells show absence of nuclear atypia and mitotic activity, while the neuronal cells show

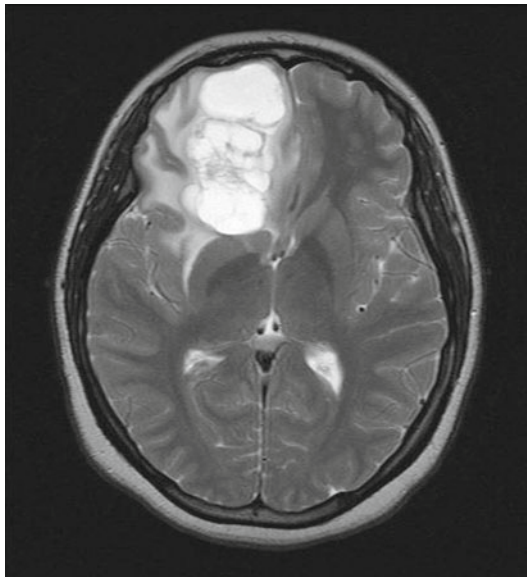

Fig. 12.1 Contrast-enhanced mass lesion on magnetic resonance imaging (MRI)

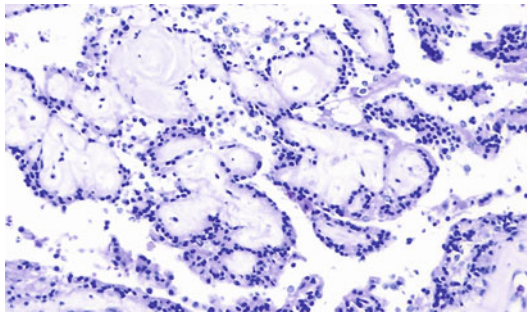

Fig. 12.2 Perivascular pseudopapillary tumor architecture

variance of shape and size. Rare cases exhibit microvascular proliferation, mitotic figures and necrosis (Newton et al. 2008; Javahery et al. 2009). The tumor mass infiltrates neuropil producing gliosis at the fringe with non-neoplastic brain tissue. Within the gliotic brain tissue are associated microcalcifications, hemosiderin-laden macrophages compatible with previous bleed, and Rosenthal fibers (Dim et al. 2006). The key cytologic features include ganglion cells with large, round, off-center nuclei, vesicular chromatin, conspicuous nucleoli, and abundant cytoplasm rich in Nissl's granules. There are also small to intermediate size cells with round nuclei, granular chromatin, indistinct nucleoli, and scant cytoplasm (Komori et al. 1998). Few cells with dark nuclei, sparse cytoplasm, and cytoplasmic processes may be noted.

Immunohistochemistry, Electron Microscopy, and Genetic Aberrations

Immunohistochemistry

The cuboidal glial cells enclosing the hyalinized vasocentric core to form the coat of the pseudopapillae are highlighted by GFAP (Fig. 12.3), vimentin, and S-100 protein. In some instances the pseudopapillae are lined by Olig2-positive cells as well as GFAP positive or negative astrocytes (Tanaka et al. 2005; Gelpi et al. 2007). The neurocytes in the interpapillary spaces are decorated by antibodies to synaptophysin, neurone specific enolase (NSE), class III B-tubulin, NeuN (Komori et al. 1998; Tanaka et al. 2005), and membrane staining for NCAM (Vajtai et al. 2006). Chromogranin is non-immunoreactive. Neurone filament protein (NFP) reactivity is restricted to ganglioid and ganglion cells (Komori et al. 1998; Dim et al. 2006).

Electron Microscopy

Ultrastructural evaluations of few PGNT cases have identified three cell types which are perivascular astrocytes, neurons, and a third unspecific cell of yet unknown differentiation. The perivascular astrocytes contain packs of intermediate filaments. Lying between the endothelial cells of the vessels and a collagen-rich adventitia is a thick basal lamina, which, in turn, is separated from the astrocytes by a thin basal lamina (Komori et al. 1998). Menigemistocytes and Olig2-expressing oligodendrocyte-like cells have also been noted (Ishizawa et al. 2006). The neuronal cells and the unclassified cells lie within the interpapillary spaces and differ in relative amounts between cases. The neuronal cells are of dissimilar size having oval nuclei with scant chromatin, free ribosomes, mitochondria, and microtubules. Infrequent synaptic complexes are sometimes present and neurosecretory granules are

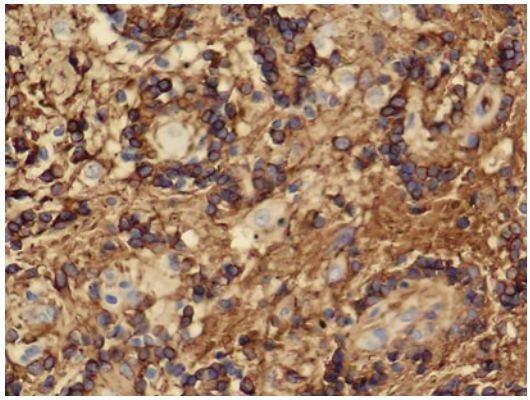

Fig. 12.3 Perivascular astrocytes showing positivity to glial fibrillary acidic protein (GFAP)

uncommon. The unclassified cells have irregular nuclei with coarse chromatin, minimal to moderate cytoplasm, variably developed mitochondria, ribosomes, and Golgi complexes. Other features of the unclassified cells can include intermediate filaments and microtubules.

Genetic Aberrations

A case reported by Faria et al. (2008) showed that conventional cytogenetics of PGNT in a 9 year-old girl with normal constitutional karyotype of 46,XX[17] was abnormal with gains and structural alterations affecting chromosome 7 with breakpoints at 7p22. The abnormal tumor karyotype was 47~48,XX,+der(7)t(7;7)(p22;?::7p22-7q11::7?)[cp7]. Comparative genomic hybridization showed a high-level amplification region at 7p14~q12. In addition, *EGFR* gene was not amplified with fluorescent in situ hybridization (FISH), as is noted in most low-grade gliomas and gangliogliomas. Tanaka et al. (2005) reported that in a series of 6 cases, there was no loss of 1p by FISH.

Discussion

PGNT was first described in the case report of Komori et al. (1996), and subsequently established as a distinct entity in the landmark case series by the same author (Komori et al. 1998). Tumors with similar morphology have been previously described under different names such as pseudopapillary neurocytoma with glial differentiation and pseudopapillary ganglioneurocytoma (Nakazato et al. 2007).

An acceptable hypothesis for the histogenesis of PGNT has not yet been advanced despite immunohistochemistry and electron microscopy studies. Nevertheless, it is presumed to arise from pluripotent cells with ability to undergo glioneuronal differentiation (Louis et al. 2007). It has been postulated that paraventricular cases may arise from subependymal matrix with the more superficial cases developing from secondary germinal layer (Komori et al. 1998). The reported cases follow a sporadic pattern of incidence, and there are no familial or syndrome-associated cases.

A review of the reported cases shows that the presenting clinical symptoms are commonly headaches and seizure but may include fever, nausea, vomiting, visual disturbances including diplopia and field defects, vertigo, gait and motor disturbances, memory loss, and behavioral changes (Celli et al. 2006). The period of symptoms before discovery of the tumor averaged 6.8 months in eight patients (Prayson 2000). A case mimicking a cavernous hemangioma presented with brain hemorrhage (Buccoliero et al. 2006). Some cases are asymptomatic, and the tumor only accidentally discovered during neuroimaging for other reasons (Williams et al. 2008). The differential diagnostic considerations of PGNT will include other tumors that present as single cystic masses with or without enhancing mural nodule including pilocytic astrocytoma and ganglioglioma. The distinct papillary architecture differentiates it from other tumors with similar histology like central neurocytoma, extraventricular neurocytoma, rosette-forming glioneuronal tumor of the fourth ventricle and dysembryoplastic neuroepithelial tumor. Papillary meningioma, a rare tumor variant with comparable architecture, should also be borne in mind. It displays a perivascular pseudopapillary pattern encompassing most of the tumor. Occurring largely in young patients, local invasion and invasion of the brain are common features. This tumor is listed in the WHO grade III category consequent upon their aggressive clinical behavior.

Prognosis and Predictive Factors

PGNT is usually characterized by a benign course and the morphology of most of them, including presence of cyst formation, no mitoses, hyalinized vessels, Rosenthal fibers and low proliferative index, is indeed a reflection of its benignity and indicate a favorable clinical outcome. Cases have been reported with variable atypical morphology such as necrosis, capillary endothelial proliferation, mitoses, and high proliferation activity exceeding 10 % (Ishizawa et al. 2006; Adam et al. 2007; Atri et al. 2007; Vaquero and Coca 2007; Newton et al. 2008; Javahery et al. 2009). However, most of these cases have not been associated with a more aggressive course. In the reported cases by Atri et al. (2007) and Newton et al. (2008), there seemingly was residual tumor for which conformal radiation and simultaneous chemotherapy were instituted, and the patients have continued to be neurologically stable. One wonders if the use of chemoradiotherapy made a difference. Treatment by gross total resection without recourse to adjuvant therapy is the mainstay of treatment, resulting in long-term survival without relapse. The longest surviving patients were followed up for 19 and 7 years, respectively, without reappearance of tumor (Epelbaum et al. 2006; Bouvier-Labit et al. 2000). There have been only three reported cases of recurrence in the English literature (Ishizawa et al. 2006; Javahery et al. 2009). In the two cases reported by Javahery et al. (2009), chemoradiotherapy (case 1) and repeat resection (case 2) were successful in controlling the disease. Review of all the cases reported in the literature clearly support the notion that gross total resection is the major factor and influence driving prognosis.

References

Adam C, Polivka M, Carpentier A, George B, Gray F (2007) Papillary glioneuronal tumor: not always a benign tumor? Clin Neuropathol 26:119–124

Atri S, Sharma MC, Sarkar C, Garg A, Suri A (2007) Papillary glioneuonal tumor: a report of a rare case and review of the literature. Childs Nerv Syst 23:349–353

Barnes NP, Pollock JR, Harding B, Hayward RD (2002) Papillary glioneuronal tumor in a 4-year-old. Pediatr Neurosurg 36:266–270

Bouvier-Labit C, Daniel L, Dufour H, Grisoli F, Figarella-Branger D (2000) Papillary glioneuronal tumour: clinicopathological and biochemical study of one case with 7-year follow-up. Acta Neuropathol 99:321–326

Broholm H, Madsen FF, Wagner AA, Laursen H (2002) Papillary glioneuronal tumor-a new tumor entity. Clin Neuropathol 21:1–4

Buccoliero AM, Giordano F, Mussa F, Taddei A, Genitori L, Taddei GL (2006) Papillary glioneuronal tumor radiologically mimicking a cavernous hemangioma with hemorrhagic onset. Neuropathology 26:206–211

Celli P, Caroli E, Giangaspero F, Ferrante L (2006) Papillary glioneuronal tumor: careport and literature review. J Neurooncol 80:185–189

Dim DC, Lingamfelter DC, Taboada EM, Fiorella RM (2006) Papillary glioneuronal tumor: a case report and review of the literature. Hum Pathol 37:914–918

Epelbaum S, Kujas M, Van Effenterre R, Poirier J (2006) Two cases of papillary glioneuronal tumours. Br J Neurosurg 20:90–93

Faria C, Miguens J, Antunes JL, Barroso C, Pimentel J, do Carmo Martins M, Moura-Nunes V, Roque L (2008) Genetic alterations in a papillary glioneuronal tumor. Case report. J Neurosurg Pediatr 1:99–102

Gelpi E, Preusser M, Czech T, Slavc I, Prayer D, Budka H (2007) Papillary glioneuronal tumor. Neuropathology 27:468–473

Ishizawa T, Komori T, Shibahara J, Ishizawa K, Adachi J, Nishikawa R, Matsutani M, Hirose T (2006) Papillary glioneuronal tumor with minigemistocytic components and increased proliferative activity. Hum Pathol 37:627–630

Javahery RJ, Davidson L, Fangusaro J, Finlay JL, Gonzalez- Gomez I, McComb JG (2009) Aggressive variant of a papillary glioneuronal tumor: report of 2 cases. J Neurosurg Pediatr 3:46–52

Kim DH, Suh YL (1997) Pseudopapillary neurocytoma of temporal lobe with glial differentiation. Acta Neuropathol 94:187–191

Kleihues P, Cavenee WK, eds (2000) Pathology and genetics of tumours of the central nervous system. IARC Press, Lyon. World Health Organization Classification of Tumours

Komori T, Scheithauer BW, Anthony DC, Scott RM, Okazaki H, Kobayashi M (1996) Pseudopapillary ganglioneurocytoma. J Neuropathol Exp Neurol 55:654–654

Komori T, Scheithauer BW, Anthony DC, Rosenblum MK, McLendon RE, Scott RM, Okazaki H, Kobayashi M (1998) Papillary glioneuronal tumor: a new variant of mixed neuronal-glial neoplasm. Am J Surg Pathol 22:1171–1183

Lamszus K, Makrigeorgi-Butera M, Laas R, Westphal M, Stavrou D (2003) September 2002: 24-year-old female with a 6-month history of seizures. Brain Pathol 13:115–117

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumors of the central nervous system. Acta Neuropathol 114:97–109

McLendon RE, Provenzale J (2002) Glioneuronal tumors of the central nervous system. Brain Tumor Pathol 19:51–58

Nakazato Y, Figarella-Branger D, Becker AJ, Scheithauer BW,Rosenblum MK (2007). In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds). Pathology and Genetics of Tumours of the Central Nervous System. IARC Press, Lyon. World Health Organization Classification of Tumours 113–114

Newton HB, Dalton J, Ray-Chaudhury A, Gahbauer R, McGregor J (2008) Aggressive papillary glioneuronal tumor: case report and literature review. Clin Neuropathol 27:317–324

Prayson RA (2000) Papillary glioneuronal tumor. Arch Pathol Lab Med 124:1820–1823

Stosic-Opincal T, Peric V, Gavrilovic S, Gavrilov M, Markovic Z, Sener RN (2005) Papillary glioneuronal tumor. Neuroradiology 185:265–267

Tanaka Y, Yokoo H, Komori T, Makita Y, Ishizawa T, Hirose T, Ebato M, Shibahara J, Tsukayama C, Shibuya M, Nakazato Y (2005) A distinct pattern of Olig2-positive cellular distribution in papillary glioneuronal tumors: a manifestation of the oligodendroglial phenotype? Acta Neuropathol 110:39–47

Tsukayama C, Arakawa Y (2002) A papillary glioneuronal tumor arising in an elderly woman: a case report. Brain Tumor Pathol 19:35–39

Vajtai I, Kappeler A, Lukes A, Amold M, Luthy AR, Leibundgut K (2006) Papillary glioneuronal tumor. Pathol Res Pract 202:107–112

Vaquero J, Coca S (2007) Atypical papillary glioneuronal tumor. J Neurooncol 83:319–323

Williams SR, Joos BW, Parker JC, Parker JR (2008) Papillary glioneuronal tumor: a case report and review of the literature. Ann Clin Lab Sci 38:287–292

Solitary Fibrous Tumors

13

Guilherme Geib and Tania W. Furlanetto

Contents

Abstract

Solitary fibrous tumors (SFT) are rare mesenchymal tumors that can affect central nervous system at any level. Histologically, they are characterized by round to spindle-shaped fibroblastic cells set in a collagenous matrix with variable amounts of hyalinized collagen bundles and strongly positive stain for CD34 on immunohistochemistry. Clinical presentation is related location, size of the tumor and affected structures in the CNS. Headache is the chief complaint in the majority of patients with intracranial tumors. When SFT involve the spinal cord, progressive limbs weakness, paresthesia, and radicular pain are the most consistent findings. On magnetic resonance imaging (MRI), these tumors are frequently isointense with normal brain parenchyma on T1-weighted images, hyperintense on T2-weighted-images, and show intense homogeneous enhancement after intravenous administration of gadolinium. Complete surgical resection is probably the only curative option and is regarded as the treatment of choice for all patients suited to it. Incomplete surgical resection is regarded as a bad prognosis factor for which radiation therapy may be an option.

Introduction

Solitary fibrous tumors (SFT) are rare spindle-cell neoplasms of mesenchymal origin. The first detailed histological description of SFT was made by Wagner (1870), and they were first recognized

G. Geib (✉) • T.W. Furlanetto
Internal Medicine Division, Hospital de Clínicas de Porto Alerge, 2350, Ramiro Barcelos, 90035-903, Porto Alerge, RS, Brazil
e-mail: ggeib@hcpa.ufrgs.br; furlanet@cpovo.net

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_13,

as a distinct soft tissue neoplasm by Klemperer and Rabin (1931) in the visceral pleura, the most common site of occurrence. However, the identity of SFT continued to be debated, with the tumor subsequently categorized under mesothelial neoplasms based on tissue culture submesothelial characteristics, until the general consensus of SFT as a distinct mesenchymal neoplasm finally emerged (Park and Araujo 2009).

These tumors have been described in many extra pleural sites including pericardium, peritoneum, lung, liver, upper respiratory tract, mediastinum, nasal cavities, thyroid and parathyroid glands, orbits, and the central nervous system (CNS). SFT in the CNS are usually dura-based, meningioma-like masses, that may be intracranial or spinal. Primary meningeal SFT was first described by Carneiro et al. (1996), and the first description of SFT in the spinal cord was made by Alston et al. (1997). Since then, approximately 100 cases of SFT have been reported both in the cranial and the spinal compartments of the CNS (Yilmaz et al. 2009). SFTs were included in the 2000 World Health Organization classification of CNS tumors in the category of mesenchymal neoplasm of the meninges (Kleihues and Cavanee 2000).

The cellular origin of SFT is still a matter of debate. SFTs were initially thought to originate from CD34-positive dura-based fibroblasts or dendritic cells (Carneiro et al. 1996; Cummings et al. 2001). However, the recent description of SFT arising in deep cortical structures argues against this hypothesis, and a possible origin from the mesenchyma of the cerebral vasculature has been proposed (Kim et al. 2004).

Pathology

SFT appears in gross pathologic examination as a firm and elastic well-circumscribed tumor, often with attached fragments of dura mater, and smooth, glistening pseudo-capsules. On cutting, their surface displays a variety of colors. Histologically, the tumors are composed of round to spindle-shaped fibroblastic cells set in a collagenous matrix with variable amounts of hyalinized collagen bundles. Cellularity ranges from hypercellular to lax areas. The vasculature shows a hemangiopericytoma-like growth pattern and vessels with thickened hyalinized walls. The mitotic rate is usually <4/10 high power field (HPF) (Insabato et al. 2009). Ultrastructural analysis has demonstrated features of pericytic, fibroblastic, and myofibroblastic differentiation with vascular prominence with intercapillary stroma containing various mesenchymal cells, including pericytes in close proximity to capillaries (Ide et al. 2005). Necrosis, marked nuclear hyperchromasia, and abundant mitosis are rarely observed. The features commonly proposed to be associated with aggressive SFT are increased cellularity, pleomorphism, increased mitotic activity (mitotic index >4/10 HPF), necrosis, hemorrhage, and atypical location (Mekni et al. 2009).

Using immunohistochemistry, SFTs are strongly positive for CD34 and variably positive for CD99, Bcl2, and vimentin. Some tumors express progesterone receptor in the nucleus of the cells. SFTs usually are negative for desmin, cytokeratins, and S-100 (Insabato et al. 2009). Immunohistochemical staining for Ki-67 (MIB-1) antibody ranges from less than 2 to 25% (Mekni et al. 2009). In addition, the staining for p53 and cyclin-D1 may be important to assess the biological behavior of the tumors (Mosquera and Fletcher 2009; Mekni et al. 2009). Only a few cases of SFT with anaplastic histological features were reported with invasion of parenchyma and bone, local recurrence, and distant metastasis (Mekni et al. 2009).

The main differential diagnoses are schwannoma, fibrous meningioma and hemangiopericytoma (HPC). Schwannomas are easily distinguished because of their microscopic features (nuclear pseudopalisading, wavy nuclei) and their strong immunoreactivity to S-100. Cellular whorls, storiform cell arrangements, and psammomas bodies are usually present in meningiomas. The cells in SFTs tend to be plumper than in meningiomas. Immunohistochemistry is also used for showing the complete absence of S-100 or EMA expression in SFTs as opposed to meningiomas. CD34 may be focally positive in meningiomas. Ultrastructural features of meningiomas include cytoplasmic interdigitations and well-formed desmosomes, which are lacking in SFTs. There is considerable overlap

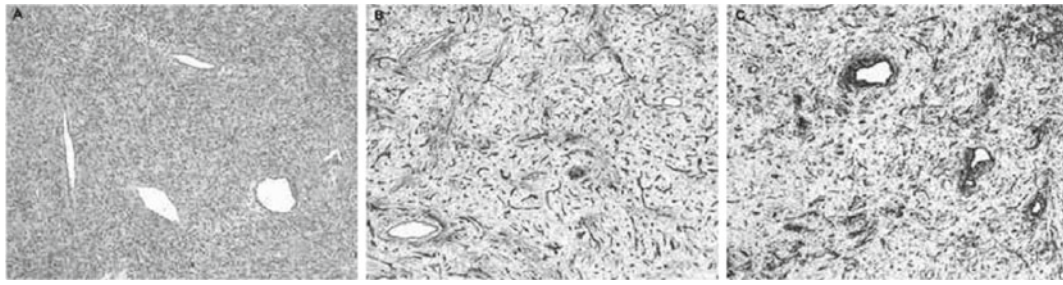

Fig. 13.1 Spindle cell tumor consistent with solitary fibrous tumor. Panel (**a**): Highly cellular spindle cell proliferation with a dense, hyalinized collagenous stroma and dilated vascular spaces, some showing a staghorn-like appearance. Areas of cellular pleomorphism and increased cellularity were present, but mitoses were not identified (H&E 50×); Panel (**b**): Strong immunoreactivity for CD34 (50×); Panel (**c**): Strong immunoreactivity for vimentin (50×) (From Furlanetto et al. 2009)

in the histological features of SFT and HPC. In a typical case of SFT, the distinction is based on the presence of frequent areas of hyalinization and collagen deposition, diffuse immunoreactivity for CD34, sparse reticulin staining around clusters of cells rather than abundant and fine reticulin around individual cells, and absence of basement membrane-like material at ultrastructural level, a hallmark of HPCs (Fig. 13.1).

Cytogenetics

Analyses of a limited number of SFT to date have not found any consistent characteristic cytogenetic abnormalities. A comparative genomic hybridization (CGH) analysis of three meningeal SFTs reported by Martin et al. (2002) showed one case with loss of chromosome 3 and two tumors with deletions of the region 3p21–p26. Other chromosomal losses included 4p15, 8q22–q24, 10, 11q14–q25, 17q11–q23, 20 and 21. Chromosomal gains were reported on 18p11–p13, 1p11–p36, and 20q11–q13. Cytogenetical abnormalities on chromosome 3 had not been previously described in SFTs from other primary sites.

Clinical and Imaging Features

SFTs can be present in any site of the CNS, involving the spinal cord in approximately one quarter of the patients (Tihan et al. 2003; Rodriguez et al. 2004; Caroli et al. 2004; Kim et al. 2004; Pizzolitto et al. 2004; Pakasa et al. 2005; Metellus et al. 2007; Furlanetto et al. 2009). When intracranial, they are more commonly found within the supratentorial space and the ventricular system, followed by infratentorial space, including posterior fossa structures, cerebellum and cerebellopontine angles. Tentorial SFT corresponds to a minority of cases (Hakan et al. 2009). Among the spinal SFT, the lesions were intradural in 73% and intramedullary in 27% of reported cases (Ciappetta et al. 2010).

The mean age at the time of diagnosis from 2 series of 18 patients each was, respectively, 47 and 56 years, with a sex ratio of approximately 1:1 (Tihan et al. 2003; Metellus et al. 2007). Clinical presentation is related to a few factors: location, size of the tumor and affected structures in the CNS. Headache is the chief complaint in the majority of patients with intracranial tumors. In some cases, SFT were found during evaluation for seizures. When SFT involve the spinal cord, progressive limbs weakness, paresthesia, and radicular pain were the most consistent findings. In a series, cranial nerve paresis and cerebellar ataxia were the most frequent symptoms for tumors at the infratentorial level; whereas, for supratentorial tumors, intracranial hypertension, epilepsy, and lateral hemianopsia were usually found (Metellus et al. 2007).

On computed tomography scan (CT), SFT appear usually as relatively well circumscribed, partially calcified heterogeneous masses, demonstrating variable degrees of enhancement upon

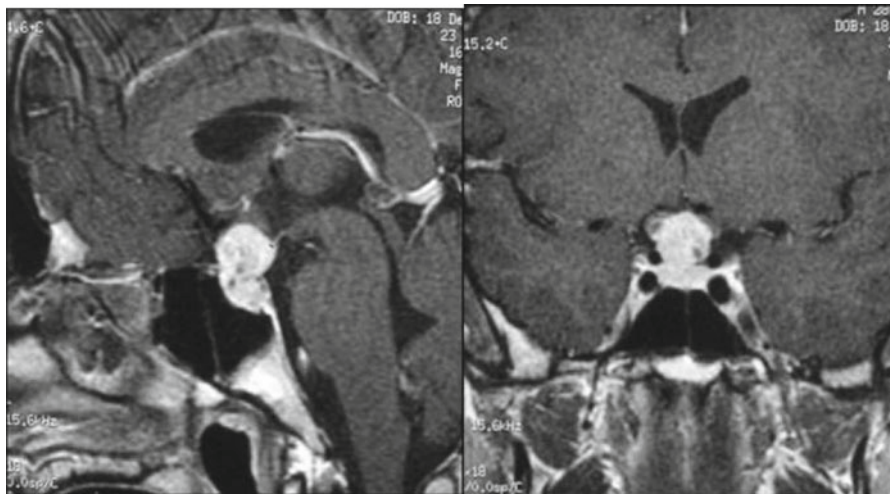

Fig. 13.2 MRI sagital (*left*) and coronal (*right*) T1WI views with IV Gadolinium showing, at the suprasellar level, a tumor above the pituitary gland with strong heterogeneous contrast enhancement. The pathological specimen was consistent with solitary fibrous tumor (From Furlanetto et al. 2009)

intravenous contrast infusion. On magnetic resonance imaging (MRI), these tumors are frequently isointense with normal brain parenchyma on T1-weighted images, hyperintense on T2-weighted-images, and show intense homogeneous enhancement after intravenous administration of gadolinium. In a series described by Mekni et al. (2009), the tumors averaged 5 cm in largest diameter. On imaging studies alone, these lesions were indistinguishable from other SNC tumors, like meningiomas or gliomas (Fig. 13.2).

Management

Despite lack of prospective data evaluating different treatment modalities for SFT, complete surgical resection is probably the only curative option and is regarded as the treatment of choice for all patients suited to it. In patients with incomplete resection, the role of postoperative radiation therapy remains uncertain. Management of patients who develop local or distant relapses has been challenging, since no clearly effective therapy exists. A new attempt of resection should be considered if technically feasible as it can lead to improvement in progression free survival.

Radiation therapy has been used in some cases, both as adjuvant therapy in patients at high risk for local recurrence or as primary therapy in unresectable tumors. Retrospective analyses of adjuvant radiation therapy have cautiously supported its utility, with studies showing at best a non-significant trend toward prolonged recurrence-free survival and overall survival (Park and Araujo 2009). Its use should not be recommended routinely in the adjuvant setting, especially in patients with completely resected tumors lacking adverse prognostic features. It should, therefore, be considered for those patients with aggressive tumors displaying adverse prognostic markers, until more data on its efficacy become available. Stereotaxic radiosurgery in recurrent unresectable SFTs appears to be a potentially promising therapeutic modality. The role of systemic therapy for advanced unresectable tumors remains unknown. Inhibition of angiogenesis has emerged as a potential promising therapy, but further studies are needed to determine its efficacy (Park and Araujo 2009).

Prognosis

The most important prognostic factor in SFT is complete surgical resection. Metellus et al. (2007) reported a 50% recurrence or progression rate in their series of 18 patients with a mean follow-up of 45 months. Incomplete surgical resection was

significantly associated with recurrence, while near 90% of patients with gross total resection were free from disease at follow-up. The histological criteria for aggressive extrapleural SFT, known to have a worse prognosis and a higher index of recurrences, are hypercellularity, moderate-to-marked cellular atypias, necrosis, and more than 4 mitoses per 10 HPF (Mekni et al. 2009). No study, to date, has defined criteria for malignant SFT in the CNS, so the same criteria are usually used.

References

Alston SR, Francel PC, Jane JA Jr (1997) Solitary fibrous tumor of the spinal cord. Am J Surg Pathol 21:477–483

Carneiro SS, Scheithauer BW, Nascimento AG, Hirose T, Davis DH (1996) Solitary fibrous tumor of the meninges: a lesion distinct from fibrous meningioma. A clinicopathologic and immunohistochemical study. Am J Clin Pathol 106:217–224

Caroli E, Salvati M, Orlando ER, Lenzi J, Santoro A, Giangaspero F (2004) Solitary fibrous tumors of the meninges: report of four cases and literature review. Neurosurg Rev 27:246–251

Ciappetta P, D'Urso PI, Cimmino A, Ingravallo G, Rossi R, Colamaria A, D'Urso OF (2010) Intramedullary solitary fibrous tumor of dorsal spinal cord. Neuropathology 30:273–278

Cummings TJ, Burchette JL, McLendon RE (2001) CD34 and dural fibroblasts: the relationship to solitary fibrous tumor and meningioma. Acta Neuropathol 102:349–354

Furlanetto TW, Pinheiro CF, Oppitz PP, de Alencastro LC, Asa SL (2009) Solitary fibrous tumor of the sella mimicking pituitary adenoma: an uncommon tumor in a rare location-a case report. Endocr Pathol 20:56–61

Hakan T, Turk CC, Aker FV (2009) Tentorial solitary fibrous tumour: case report and review of the literature. Neurol Neurochir Pol 43:77–82

Ide F, Obara K, Mishima K, Saito I, Kusama K (2005) Ultrastructural spectrum of solitary fibrous tumor: a unique perivascular tumor with alternative lines of differentiation. Virchows Arch 446:646–652

Insabato L, Siano M, Somma A, Gentile R, Santangelo M, Pettinato G (2009) Extrapleural solitary fibrous tumor: a clinicopathologic study of 19 cases. Int J Surg Pathol 17:250–254

Kim KA, Gonzalez I, McComb JG, Giannotta SL (2004) Unusual presentations of cerebral solitary fibrous tumors: report of four cases. Neurosurgery 54:1004–1009

Kleihues P, Cavanee WK (2000) World health organization classification of tumours: pathology and genetics: tumours of the nervous system world health organization

Klemperer P, Rabin CB (1931) Primary neoplasms of the pleura. A report of five cases. Arch Pathol 11:385–412

Martin AJ, Summersgill BM, Fisher C, Shipley JM, Dean AF (2002) Chromosomal imbalances in meningeal solitary fibrous tumors. Cancer Genet Cytogenet 135:160–164

Mekni A, Kourda J, Hammouda KB, Tangour M, Kchir N, Zitouna M, Haouet S (2009) Solitary fibrous tumour of the central nervous system: pathological study of eight cases and review of the literature. Pathology 41:649–654

Metellus P, Bouvier C, Guyotat J, Fuentes S, Jouvet A, Vasiljevic A, Giorgi R, Dufour H, Grisoli F, Figarella-Branger D (2007) Solitary fibrous tumors of the central nervous system: clinicopathological and therapeutic considerations of 18 cases. Neurosurgery 60:715–722

Mosquera JM, Fletcher CD (2009) Expanding the spectrum of malignant progression in solitary fibrous tumors: a study of 8 cases with a discrete anaplastic component – is this dedifferentiated SFT? Am J Surg Pathol 33:1314–1321

Pakasa NM, Pasquier B, Chambonniere ML, Morrison AL, Khaddage A, Perret AG, Dumollard JM, Barral FG, Péoc'h M (2005) Atypical presentations of solitary fibrous tumors of the central nervous system: an analysis of unusual clinicopathological and outcome patterns in three new cases with a review of the literature. Virchows Arch 447:81–86

Park MS, Araujo DM (2009) New insights into the hemangiopericytoma/solitary fibrous tumor spectrum of tumors. Curr Opin Oncol 21:327–331

Pizzolitto S, Falconieri G, Demaglio G (2004) Solitary fibrous tumor of the spinal cord: a clinicopathologic study of two cases. Ann Diagn Pathol 8: 268–275

Rodriguez F, Scheithauer BW, Ockner DM, Giannini C (2004) Solitary fibrous tumor of the cerebellopontine angle with salivary gland heterotopia: a unique presentation. Am J Surg Pathol 28:139–142

Tihan T, Viglione M, Rosenblum MK, Olivi A, Burger PC (2003) Solitary fibrous tumors in the central nervous system. A clinicopathologic review of 18 cases and comparison to meningeal hemangiopericytomas. Arch Pathol Lab Med 127:432–439

Wagner E (1870) Das tuberkelahnliche lymphadenom (der cytogene oder reticulirte tuberkel). Arch Heilk (Leipig) 11:497

Yilmaz C, Kabatas S, Ozen OI, Gulsen S, Caner H, Altinors N (2009) Solitary fibrous tumor. J Clin Neurosci 16:1578–1581

Pituitary Adenomas: MCM2 Protein as a Cell Proliferation Marker

14

Miriam da Costa Oliveira and Cristina Micheletto Dallago

Contents

Abstract

Pituitary adenomas occasionally show aggressive behavior with rapid growth and invasion of the surrounding tissues. The identification of biological markers able to recognize aggressive pituitary adenomas in early stages remains a challenge. We aimed to determine the expression of a new cell proliferation marker with clinical significance in several human neoplasms, Mcm2, in pituitary adenomas and to establish the relationship of the Mcm2 index with tumor extension and invasion. The proliferative index was determined in tumor specimens of 64 patients with acromegaly or clinically nonfunctioning pituitary adenoma using immunohistochemical methods for two antigens, Mcm2 and Ki-67. Fifty-four (86%) adenomas showed immunoreactivity to Mcm2. The median Mcm2 index was 0.91% (range 0–13.24%). Immunoreactivity to Ki-67 was observed in 61 adenomas (95%). The median Ki-67 index was 0.88% (range 0–7.39%). A significant positive correlation was found between log Mcm2 index and log Ki-67 index ($p < 0.001$). Mcm2 and Ki-67 detected a similar number of proliferating cells. Mcm2 index showed a significant association with tumor extension ($p = 0.02$), but not with tumor invasion and hormone phenotype. In conclusion, we demonstrated that Mcm2 was similar to Ki-67 in the identification of the proliferating cells in this sample.

M. Oliveira (✉) • C.M. Dallago
Neuroendocrinology Center, Complexo Hospitalar Santa Casa, Universidade Federal de Ciências da Saúde de Porto Alerge, Rua Dona Mimi Moro, 40, 90480-050 Porto Alerge, RS, Brazil
e-mail: mco@portoweb.com.br

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_14,

Introduction

Although anterior pituitary adenomas are usually benign and slow-growing tumors, occasionally they show more aggressive behavior, with rapid growth, recurrence or regrowth after surgical removal or invasion of the structures surrounding the sella turcica. The local invasion may cause hypopituitarism, visual field disturbances and symptoms of intracranial mass, as well as impair complete surgical removal and normalization of hormone levels in secreting tumors.

Due to the increase in morbidity and mortality associated with pituitary adenomas with aggressive behavior, in last years, several studies have been conducted aiming to identify biological markers able to recognize these tumors in early stages. The routine histological analysis and identification of mutations or altered expression of oncogenes and tumor suppressor genes seen in more common cancers (such as ras and p53), with little exception, were not proven to be capable of predicting exactly the aggressiveness of the pituitary adenomas (Losa et al. 1998).

In a variety of malignant neoplasms, the determination of cell proliferation indices seems to provide valuable prognostic information, being strongly correlated with clinicopathological parameters such as tumor recurrence and disease-free survival. In pituitary adenomas, however, there are still controversial whether conventional cell proliferation indices, such as Ki-67 and PCNA, have ability to identify tumor with aggressive behavior (Losa et al. 1998; Turner and Wass 1999). Some series have demonstrated that the Ki-67 proliferation index is significantly higher in invasive or recurrent anterior pituitary adenomas (Ekramullah et al. 1996; Thapar et al. 1996; Mastronardi et al. 2001; Jaffrain-Rea et al. 2002; Schreiber et al. 1999; Pizarro et al. 2004), while others have found no difference (Losa et al. 2000; Honegger et al. 2003; Tanaka et al. 2003). An additional problem in the application of Ki-67 as a proliferation marker is that the function of this protein remains unknown and there is evidence that it might be involved in other non-cell-cycle-related processes, such as ribosomal biosynthesis (Gonzalez et al. 2005; Mehrotra et al. 2006).

Recent studies have shown the potential use of the minichromosome maintenance (MCM) proteins as novel cell proliferation markers. The MCM proteins are a family of highly conserved proteins that are essential for initiation and regulation of the DNA replication in eukaryotic cells (Meng et al. 2001). They were first recognized in the yeast *Saccharomyces cerevisiae* as mutants defective in the maintenance of minichromosome, suggesting a role in plasmid replication or cell cycle progression (Tye 1999). In eukaryotes, six of these proteins (Mcm2-7) interact one another to form a hexameric complex that has an essential function as a replicative helicase (Giaginis et al. 2009).

The initialization of the DNA synthesis in eukaryotic cells is a multistep process that needs the binding to chromatin and the subsequent activation of numerous proteins in a highly ordered sequence. Briefly, the first step involves the binding of a complex of the proteins called "origin recognition complex" (ORC) to each replication origin. In the second step, the replication factors Cdc6 (cell division control protein 6) and Cdt1 (chromatin licensing and DNA replication factor 1) and subsequently the hexameric complex formed by proteins Mcm2-7 are recruited to replication origin. Then, the proteins ORC, Cdc6, Cdt1 and Mcm10 functionally interact with the MCM hexameric complex at replication origin, resulting in the formation of the prereplication complex (pre-RC). The last step is the activation of pre-RC complex by cyclin-dependent kinases; in this moment, the inactive MCM complex is converted into an enzymatically active helicase. Once activated, the MCM helicase allows the DNA replication machinery to access the binding sites on DNA, making the chromatin competent or licensed for the S phase of the cell cycle (Lei and Tye 2001; Gonzalez et al. 2003). The dissociation of the MCM proteins from chromatin irreversibly during S phase ensures that DNA replication occurs only once in each normal cell cycle (Meng et al. 2001; Gonzalez et al. 2005).

There is now substantial evidence that MCM proteins, particularly Mcm2 and Mcm5, could constitute prognostic tumor markers of greater

clinical significance than the conventional cell proliferation in several human neoplasms (Giaginis et al. 2009). Previous studies that evaluated the expression of MCM proteins in a variety of normal and malignant tissues from different systems have shown that these proteins detect more cells in cycle than the Ki-67 antigen (Gonzalez et al. 2003; Kodani et al. 2003; Korkolopoulou et al. 2005; Mehrotra et al. 2006; Gakiopoulou et al. 2007; Giaginis et al. 2009).

Like the other MCM proteins, the expression of Mcm2 is seen during all phases of the cell cycle and is absent in quiescent or terminally differentiated cells (Meng et al. 2001; Gonzalez et al. 2003; Giaginis et al. 2009). In normal and some reactive tissues, the expression of the MCM proteins is restricted to proliferative compartments. In contrast, in dysplastic and malignant tissues, MCM proteins are present in the majority of cells, and in carcinomas their frequency of expression is inversely correlated with the degree of tumor differentiation (Freeman et al. 1999). Some series have demonstrated that high levels of Mcm2 expression, over a cut-off of expression that varies *among the different types of tumors, was* significantly associated with poor prognosis in several tumors, including prostate and breast carcinomas (Meng et al. 2001; Gonzalez et al. 2003; Giaginis et al. 2009).

We aimed to detect and quantify the immunohistochemical expression of cell proliferation marker Mcm2 in normal pituitary tissues and in the two most prevalent types of pituitary adenomas of surgical management (clinically nonfunctioning and GH-secreting pituitary adenomas), and compare its ability to identify cells in the cell cycle with the classic marker Ki-67. We also aimed to establish the relationship of the Mcm2 index with tumor extension and invasion.

Determination of Cell Proliferation in Pituitary Tissues Using MCM2 Antigen: Methodology

For our analysis, tumor tissues were obtained from 64 patients who had undergone surgery for acromegaly (n=23) or clinically nonfunctioning pituitary adenoma (n=41) at a reference neurosurgery center located in southern Brazil. No patients with acromegaly had used somatostatin analogs or dopamine agonists prior to surgery. From the 64 patients, 32 were male. The median age of the patients at the time of surgery was 48.1 years, ranging from 20 to 66 years. Microadenomas were detected in three patients with acromegaly (4%). Among the 61 macroadenomas, 12 (19%) were intrasellar adenomas, 28 (44%) showed isolated suprasellar extension, and 21 (33%) were considered invasive. Among the 41 clinically nonfunctioning pituitary adenomas, 18 (44%) did not show immunoreactivity to any of the anterior pituitary hormones, 19 (46%) were immunoreactive to LH and/or FSH, two (5%) to GH and prolactin, one (2.5%) to gonadotropins and TSH, and one (2.5%) to ACTH. Among the 23 GH-secreting pituitary adenomas, ten (43.5%) were immunoreactive to GH, ten (43.5%) to GH and prolactin, two (9%) to GH and gonadotropins, and one (4%) was plurihormonal. Normal pituitary tissue surrounding adenomatous tissue removed from patients undergoing surgery and normal pituitary tissue obtained from autopsies were included (n=5). Standard hematoxylin-eosin stain was applied and immunohistochemical detection of pituitary hormones was performed for a routine diagnostic evaluation.

Ki-67 and Mcm2 immunostaining were performed using the streptavidin-biotin-peroxidase complex method. Primary antibodies used were the monoclonal mouse anti-human Mcm2 antibody (code NCL-MCM2; Novocastra Laboratories, Newcastle Upon Tyne/UK; diluted 1:30; overnight at 4°C) and the monoclonal mouse anti-human MIB-1 antibody (code M7240; DakoCytomation, Carpinteria, CA/USA; diluted 1:200; overnight at 4°C). Antigen retrieval was conducted using sodium citrate (steam heating at 92°C for 40 min). The endogenous peroxidase activity was blocked using a solution of 5% hydrogen peroxide and the nonspecific bindings using Dako® protein block solution. Color development was performed with diaminobenzidine. The negative control consisted of omitting of the primary antibody. Tonsil tissue was used as positive control.

The sections were examined under a standard light microscope. The entire section was assessed at low (100×) and high (400×) magnification, and the areas of tumors with the most intense staining were analyzed. The expression of the Ki-67 and Mcm2 antigens was evident as a dense brown precipitate restricted to the nuclei. The percentage of Ki-67 and Mcm2 positive nuclei (the Ki-67 labeling index (LI) and Mcm2 LI, respectively) was determined by manual counting of at least 500 nuclei of tumor cells at 400× magnification with the aid of a 10 × 10 square grid.

Statistical Analysis

Median and interquartile range were used to describe quantitative variables due to the asymmetry of the data, and proportions were used for categorical ones. Spearman's correlation coefficient was used to evaluate the correlation of measures of cell proliferation indices (Ki-67 and Mcm2), when comparing among themselves or with another variables (age and tumor extension). Mann–Whitney and Wilcoxon non-parametric tests were used for other comparisons. The level of significance used in the study was $p = 0.05$. Data were processed and analyzed with the aid of SPSS software, version 15.0.

Determination of Cell Proliferation in Pituitary Tissues Using MCM2 Antigen: Results

In this study, only one of five specimens of normal pituitary tissue evaluated showed cells with nuclear immunoreactivity to the Mcm2 antigen. In this case, 10 cells with positive nucleus were identified in a field at 400× magnification. The majority of the pituitary adenomas (86%) showed immunoreactivity to the Mcm2 antigen (Fig. 14.1). The mean ± SD Mcm2 LI was 1.73 ± 2.36%. The median (P25–P75) Mcm2 LI was 0.91% (0.41–2.10%), ranging from 0 to 13.24%. Immunoreactivity to Ki-67 was observed in 61 adenomas (95%). The mean ± SD Ki-67 LI was 1.30 ± 1.30%. The median (P25–P75) Ki-67 LI was 0.88% (0.45–1.85%), ranging from 0 to 7.39%. The antigen Mcm2 showed similar ability to identify cells in the cell cycle when compared with the classic marker Ki-67 ($p = 0.41$). We found a significant positive correlation between the logarithm of Ki-67 LI and the logarithm of Mcm2 LI ($p < 0.001$, $r = 0.59$). Arbitrarily using the threshold of 3% for the Mcm2 LI, the same value used for the Ki-67 LI to characterize an adenoma as "atypical" (2004 World Health Organization classification of tumors of the endocrine organs), ten adenomas showed an over value and nine of them were adenomas with extrasellar extension (Lloyd et al. 2004). In contrast, among the adenomas negative for Mcm2 ($n = 9$), only one was considered invasive.

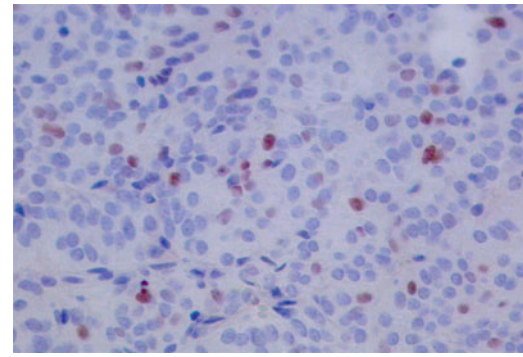

Fig. 14.1 Photomicrograph illustrating the expression of the Mcm2 protein by immunohistochemical method in pituitary adenoma. Original magnification: 400×

In our study, there was no significant association between the age or gender of the patients and the Ki-67 ($p = 0.10$ and $p = 0.48$, respectively) and Mcm2 LIs ($p = 0.15$ and $p = 0.69$, respectively). There were no statistically significant differences between clinically nonfunctioning and GH-secreting pituitary adenomas for Ki-67 and Mcm2 LIs ($p = 0.88$ and $p = 0.21$, respectively). The hormone phenotype did not significantly affect the Ki-67 and Mcm2 LIs ($p = 0.15$ and $p = 0.21$, respectively).

We found a significant positive correlation between tumor extension and the Ki-67 and Mcm2 LIs ($r = 0.32$, $p = 0.01$; and $r = 0.29$, $p = 0.02$, respectively). Adenomas restricted to sella shown a significantly lower Mcm2 LI (0.41%) than macroadenomas with isolated suprasellar

extension (1.18%) or invasive adenomas (1.10%). However, there was no significant correlation between tumor invasion and the Ki-67 and Mcm2 LIs ($p=0.08$ and $p=0.26$, respectively). In invasive adenomas ($n=21$), the median Mcm2 LI was 1.10%, while in non-invasive adenomas ($n=43$) it was 0.85%.

Discussion

Although the determination of cell proliferation indices seems to provide valuable prognostic information in a variety of tumors, there may be practical problems associated with using Ki-67 (or other IH method) in assessing cell proliferation. For example, the proliferation index is often found to be variable between different fields within the same tumor; because of heterogeneous distribution of proliferation cell. In addition, it is important note that the proliferation rate of the tissue, depend not only on the number of cells in the cell cycle but also the time taken to complete the cycle (a tumor could be proliferating rapidly and have a low LI, or be proliferating slowly but remain in G1 and so have a high LI). In any case, the current study aimed to perform a comparative assessment of two methods that are equally subject to this bias. As far as we know, the expression of cell proliferation marker Mcm2 in normal or adenomatous pituitary tissues has been rarely explored. Similarly to previous studies that determined the proliferative activity in pituitary tissues using conventional markers, we found absence of immunoreactivity to the Mcm2 antigen or much lower cell proliferation index in normal pituitary than in pituitary adenomas.

Unlike recent series that evaluated the Mcm2 expression in normal and neoplastic tissues of different systems, in our study the Mcm2 antigen did not identify a significantly greater number of cells in the cell cycle than the Ki-67 antigen (Gonzalez et al. 2003; Korkolopoulou et al. 2005; Mehrotra et al. 2006; Gakiopoulou et al. 2007). A reason given by the authors for the higher index of Mcm2 is the prior demonstration that Ki-67 protein is expressed during a shorter interval of the cell cycle than the Mcm2 protein. Although both proteins are present in all phases of the cell cycle, cells in early G_1 phase may not express Ki-67. Other reason cited is the demonstration that the Ki-67 expression in cycling cell might be downregulated by external factors, such as nutritional deprivation. A possible explanation for the variance in our findings is the histologically benign profile of the great majority of the pituitary adenomas, which are usually slow-growing tumors and therefore with much lower proliferative activity when compared with malignant neoplasms. Just as previous studies that compared the expression of Mcm2 and Ki-67 in neoplastic tissues of different systems, we found a significant positive correlation between the cell proliferation indices obtained by both antigens (Gonzalez et al. 2003; Korkolopoulou et al. 2005; Gakiopoulou et al. 2007). Regarding the findings described above, we agree that the Mcm2 protein was similar to the classic marker Ki-67 in the assessment of cell replication in normal and adenomatous pituitary tissues by the immunohistochemical method.

Several reports have demonstrated the lack of any relationship between cell proliferation indices of pituitary adenomas and sex or age of the patients (Turner and Wass 1999). Some researchers (Losa et al. 2000; Jaffrain-Rea et al. 2002; Tanaka et al. 2003; Gejman et al. 2008), but not all (Landolt et al. 1987; Knosp et al. 1989; Mastronardi et al. 2001; Paek et al. 2005; Fusco et al. 2008), found an inverse correlation between age and mean Ki-67 LI value. In our study, the proliferation indices of Ki-67 and Mcm2 were not significantly affected by the sex and age of the patients.

Some previous studies evaluated the relationship between secretory activity and proliferative capacity of the pituitary adenomas using conventional proliferation markers. Although some studies (Landolt et al. 1987; Thapar et al. 1996; Jaffrain-Rea et al. 2002) have shown a significantly higher Ki-67 LI in functioning adenomas than in nonfunctioning ones, other studies found no such difference (Knosp et al. 1989; Pizarro et al. 2004). Landolt et al. (1987) found higher values of Ki-67 LI in acromegalic patients. Other studies reported a higher Ki-67 LI in ACTH-secreting adenomas

(Shibuya et al. 1992; Mastronardi et al. 2001) and prolactinomas (Thapar et al. 1996). In our study, the Ki-67 and Mcm2 LIs showed no significant difference between clinically nonfunctioning adenomas and GH-secreting pituitary adenomas or between the hormone phenotypes.

Previous studies that analyzed the correlation between proliferative activity assessed by Ki-67 and tumor extension showed controversial results. Although some researchers (Paek et al. 2005; Gejman et al. 2008) have found no correlation, Jaffrain-Rea et al. (2002) demonstrated a significantly higher Ki-67 LI in macroadenomas than in microadenomas. In this study, we found a significant association between tumor extension and proliferative activity of tumors, determined by both cell proliferation markers. The Mcm2 LI was significantly lower in adenomas restricted to sella (0.41%) than in macroadenomas with isolated suprasellar extension (1.18%) or in invasive adenomas (1.10%).

The results of studies that evaluated the association of proliferative activity assessed by Ki-67 and tumor invasion are also controversial. Although some studies (Thapar et al. 1996; Mastronardi et al. 2001; Schreiber et al. 1999; Pizarro et al. 2004) have demonstrated a significantly higher Ki-67 LI in invasive adenomas than in non-invasive ones; other series, as well as ours, found no such difference (Losa et al. 2000; Honegger et al. 2003). Thapar et al. (1996) demonstrated a significant and progressive increment of the Ki-67 index from non-invasive (1.4%) to invasive adenomas (4.7%) and from those to pituitary carcinomas (12%). These authors also proposed a Ki-67 LI of 3% or more to distinguish non-invasive from invasive pituitary adenomas. Similar conclusion was reached by Mastronardi et al. (2001) that established a cut-off of 3.5% for invasive adenomas. In this study, we no found relationship between tumor invasiveness and Mcm2 LI. As in previous series, in this study we found an overlap of the values of proliferative indices between invasive and non-invasive adenomas (Thapar et al. 1996; Losa et al. 1998; Mastronardi et al. 2001). Thus, low proliferative indices were not found exclusively in non-invasive adenomas.

In conclusion, our study demonstrated a correlation between Mcm2 and Ki-67 antigens in the assessment of cell replication of clinically nonfunctioning and GH-secreting pituitary adenomas. Values of proliferative index above 3%, as obtained by Ki-67 or Mcm2 antigen, were associated with extrasellar extension of pituitary adenomas.

References

Ekramullah SM, Saitoh Y, Arita N, Ohnishi T, Hayakawa T (1996) The correlation of Ki-67 staining indices with tumour doubling times in regrowing non-functioning pituitary adenomas. Acta Neurochir (Wien) 138:1449–1455

Freeman A, Morris LS, Mills AD, Stoeber K, Laskey RA, Williams GH, Coleman N (1999) Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. Clin Cancer Res 5(8):2121–2132

Fusco A, Zatelli MC, Bianchi A, Cimino V, Tilaro L, Veltri F, Angelini F, Lauriola L, Vellone V, Doglietto F, Ambrosio MR, Maira G, Giustina A, degli Uberti EC, Pontecorvi A, De Marinis L (2008) Prognostic significance of the Ki-67 labeling index in growth hormone-secreting pituitary adenomas. J Clin Endocrinol Metab 93(7):2746–2750

Gakiopoulou H, Korkolopoulou P, Levidou G, Thymara I, Saetta A, Piperi C, Givaloes N, Vassilopoulos I, Ventouri K, Tsenga A, Bamias A, Dimopoulos MA, Agapitos E, Patsouris E (2007) Minichromosome maintenance 2 and 5 in non-benign epithelial ovarian tumours: relationship with cell cycle regulators and prognosis implications. Br J Cancer 97:1124–1134

Gejman R, Swearingen B, Hedley-Whyte ET (2008) Role of Ki-67 proliferation index and p53 expression in predicting progression of pituitary adenomas. Hum Pathol 39(5):758–766

Giaginis C, Georgiadou M, Dimakopoulou K, Tsourouflis G, Gatzidou E, Kouraklis G, Theocharis A (2009) Clinical significance of MCM-2 and MCM-5 expression in colon cancer: association with clinicopathological parameters and tumor proliferative capacity. Dig Dis Sci 54:282–291

Gonzalez MA, Pinder SE, Callagy G, Vowler SL, Morris LS, Bird K, Bell JA, Laskey RA, Coleman N (2003) Minichromosome maintenance protein 2 is a strong independent prognostic marker in breast cancer. J Clin Oncol 21(23):4306–4313

Gonzalez MA, Tachibana KK, Lasley RA, Coleman N (2005) Control of DNA replication and its potential clinical exploitation. Nat Rev Cancer 5:135–141

Honegger J, Prettin C, Feuerhake F, Petrick M, Schulte-Mönting J, Reincke M (2003) Expression of Ki-67 antigen in nonfunctioning pituitary adenomas: correlation with growth velocity and invasiveness. J Neurosurg 99:674–679

Jaffrain-Rea ML, Di Stefano D, Minniti G, Esposito V, Bultrini A, Ferretti E, Santoro A, Faticanti Scucchi L, Gulino A, Cantore G (2002) A critical reappraisal of MIB-1 labelling index significance in a large series of pituitary tumours: secreting versus non-secreting adenomas. Endocr Relat Cancer 9(2):103–113

Knosp E, Kitz K, Perneczky A (1989) Proliferation activity in pituitary adenomas: measurement by monoclonal antibody Ki-67. Neurosurgery 25:927–930

Kodani I, Osaki M, Shomori K, Araki K, Goto E, Ryoke K, Ito H (2003) Minichromosome maintenance 2 expression is correlated with mode of invasion and prognosis in oral squamous cell carcinomas. J Oral Pathol Med 32:468–474

Korkolopoulou P, Givalos N, Saetta A, Goudopoulou A, Gakiopoulou H, Thymara I, Thomas-Tsagli E, Patsouris E (2005) Minichromosome maintenance protein 2 and 5 expression in muscle-invasive urothelial cancer: a multivariate survival study including proliferation markers and cell cycle regulators. Hum Pathol 36:899–907

Landolt AM, Shibata T, Kleihues P (1987) Growth rate of human pituitary adenomas. J Neurosurg 67:803–806

Lei M, Tye BK (2001) Initiating DNA synthesis: from recruiting to activating the MCM complex. J Cell Sci 114:1447–1454

Lloyd RV, Kovacs K, Young WF Jr, Farrell WE, Asa SL, Trouillas J, Kontogeorgos G, Sano T, Scheithauer BW, Horvarth E (2004) Tumours of the pituitary gland. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds) *World Health Organization* classification of tumours. Pathology and genetics. Tumours of endocrine organs. IARC Press, Lyon, pp 10–13

Losa M, Franzin A, Mortini P, Terreni MR, Mangili F, Giovanelli M (1998) Usefulness of markers of cell proliferation in the management of pituitary adenomas. Clin Sci 95:129–135

Losa M, Barzaghi R, Mortini P, Franzin A, Mangili F, Terreni M, Giovanelli M (2000) Determination of the proliferation and apoptotic index in adrenocorticotropin-secreting pituitary tumors. Am J Pathol 156:245–251

Mastronardi L, Guiducci A, Puzzilin F (2001) Lack of correlation between Ki-67 labelling index and tumor size of anterior pituitary adenomas. BMC Cancer 1:12, Epub 2001 Aug 21

Mehrotra P, Gonzalez MA, Johnson SJ, Coleman N, Wilson JA, Davies BR, Lennard TWJ (2006) Mcm-2 and Ki-67 have limited potential in preoperative diagnosis of thyroid malignancy. Laryngoscope 116:1434–1438

Meng MV, Grossfeld GD, Williams GH, Dilworth S, Stoeber K, Mulley TW, Weinberg V, Carroll PR, Tlsty TD (2001) Minichromosome maintenance protein 2 expression in prostate: characterization and association with outcome after therapy for cancer. Clin Cancer Res 7(9):2712–2718

Paek K, Kim S, Song S, Choi S, Koh H, Youm J, Kim Y (2005) Clinical significance of Ki-67 labeling index in pituitary adenomas. J Korean Med Sci 20:489–494

Pizarro CB, Oliveira MC, Coutinho LB, Ferreira NP (2004) Measurement of Ki-67 antigen in 159 pituitary adenomas using the MIB-1 monoclonal antibody. Braz J Med Biol Res 37(2):235–243

Schreiber S, Saeger W, Ludecke DK (1999) Proliferation markers in different types of clinically non-secreting pituitary adenomas. Pituitary 1:213–220

Shibuya M, Saito F, Miwa T, Davis RL, Wilson CB, Hoshino T (1992) Histochemical study of pituitary adenomas with Ki-67 and anti-DNA polymerase α monoclonal antibodies, bromodeoxyuridine labeling, and nucleolar organizer region counts. Acta Neuropathol 84:178–183

Tanaka Y, Hongo K, Tada T, Sakai K, Kakizawa Y, Kobayashi S (2003) Growth pattern and rate in residual nonfunctioning pituitary adenomas: correlations among tumor volume doubling time, patient age, and MIB-1 index. J Neurosurg 98:359–365

Thapar K, Kovacs K, Scheithauer BW, Stefaneanu L, Horvath E, Pernicone PJ, Murray D, Laws ER Jr (1996) Proliferative activity and invasiveness among pituitary adenomas and carcinomas: an analysis using the MIB-1 antibody. Neurosurgery 38(1):99–107

Turner HE, Wass JAH (1999) Are markers of proliferation valuable in the histological assessment of pituitary tumours? Pituitary 1:147–151

Tye BK (1999) MCM protein in DNA replication. Annu Rev Biochem 68:649–686

Pituitary Adenomas: Role of Cyclin-Dependent Kinase Inhibitors

15

Katsuhiko Yoshimoto, Takeo Iwata, Noriko Mizusawa, Zhi Rong Qian, Shahidan Wan Nazatul Shima, Shinji Ono, and Kyoko Ishimoto

Contents

Abstract

Human pituitary adenomas are common tumors. In spite of extensive investigations, the molecular basis of human pituitary tumorigenesis remains elusive. The cell cycle is driven by cyclins-cyclin-dependent kinases (CDKs) complexes. Because CDK inhibitors (CKIs) serve as negative regulators of cell cycle, dysregulation in CKIs is recognized as critical factors in tumorigenesis. In recent years, extensive studies have demonstrated that somatic mutations, underexpression, and DNA methylation of the CKIs genes were frequently observed in various types of human cancers. Although the role of CKIs in human pituitary tumors has been elucidated to a limited extent, studies on knockout mice suggested that some CKIs are involved in tumorigenesis of murine pituitary gland. For example, knockout mice of p18^{Ink4c} and p27^{Kip1} develop both pituitary intermediate- and anterior-lobe tumors and intermediate-lobe tumors, respectively. Each of the INK4 and CIP/KIP family members shows unique pattern of gene expression, mutations and promoter methylation in human pituitary adenomas. Until now, changes of mRNA or protein levels of p16^{INK4A}, p18^{INK4C}, and p27^{KIP1} in pituitary adenomas have been reported. Non-functioning pituitary adenomas show reduced expression of p16^{INK4A} by epigenetic changes. In pituitary adenomas, mRNA and protein levels of p18^{INK4C} were reduced by unidentified mechanisms and protein levels of p27^{KIP1} are reduced by protein

K. Yoshimoto (✉) • T. Iwata • N. Mizusawa • S.W.N. Shima • S. Ono
Department of Medical Pharmacology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan
e-mail: yoshimoto@tokushima-u.ac.jp

Z.R. Qian
Department of Human Pathology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

K. Ishimoto
Department of Oral and Maxillofacial Prosthodontics, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_15,

modification. These changes of expression levels may contribute to pituitary tumorigenesis.

Introduction

Pituitary adenomas are the most commonly encountered intracranial tumors. Although pituitary adenomas are benign, it is associated with significant morbidity due to its critical location and oversecretion of pituitary hormones. A large number of studies have been conducted to elaborate the molecular and pathological basis of pituitary tumorigenesis. However, the mechanisms of tumorigenesis of pituitary adenomas are largely unknown.

The cell cycle is a tightly regulated process and is controlled at different stages by specific cyclins and cyclin-dependent kinases (CDK). A critical point in the cell cycle is the G1/S transition checkpoint frequently aberrated in human cancers. Cyclin-CDK complexes phosphorylate retinoblastoma (RB) protein, resulting in the progression of cells into the S phase. Activating somatic mutation (Arg24 to Cys) of the CDK4 gene observed in both hereditary and sporadic melanoma and somatic mutations or promoter hypermethylation of the RB1 gene are rare in human pituitary adenomas (Honda et al. 2003; Kirsch et al. 2009), whereas some conflicting results of hypermethylation of the RB1 promoter in pituitary adenomas have been reported (Ogino et al. 2005).

Cyclin-CDK complexes are inhibited by CDK inhibitors (CKIs), which play a crucial regulatory role at G1/S transition. Two families of CKIs based on their structural and functional similarities are defined. The INK4 family comprising $p16^{INK4A}$ (CDKN2A), $p15^{INK4B}$ (CDKN2B), $p18^{INK4C}$ (CDKN2C), and $p19^{INK4D}$ (CDKN2D) shows their negative regulatory activity against cell cycle progression by binding to CDK4 and CDK6 and interfering their association with cyclin D. On the other hand, the CIP/KIP family including $p21^{CIP1}$ (CDKN1A), $p27^{KIP1}$ (CDKN1B), and $p57^{KIP2}$ (CDKN1C) binds both cyclin and CDK, and shows a broad spectrum of inhibitory effects on cyclin/CDK complexes including cyclin D/CDK4, cyclin E/CDK2, and cyclin A/CDK2.

Recent studies showed that deregulation of CKIs contributed to development of various tumors, suggesting that CKIs act as tumor suppressors (Besson et al. 2008). However, the role of CKIs in human pituitary tumors has been elucidated to a limited extent. This review will describe the recent findings of CKIs associated to human pituitary tumorigenesis, with a particular focus on data of CKI's knockout mice, and gene expression, mutations, and promoter methylation profile of CKIs in human pituitary adenomas.

$p16^{INK4A}$ (CDKN2A)

The genes encoding $p16^{INK4A}$ and $p15^{INK4B}$ are located on chromosome 9p21.3. In a relative small 25 kb region, the locus includes three related genes of $p16^{INK4A}$, $p15^{INK4B}$, and $p14^{ARF}$. *CDKN2A* encodes two distinct proteins through alternative reading frames: $p16^{INK4A}$ and $p14^{ARF}$, which binds the p53-stabilizing protein MDM2. The $p16^{INK4A}/p14^{ARF}/p15^{INK4B}$ locus is frequently deleted in a wide spectrum of tumors. In addition, more frequent mutations of the $p16^{INK4A}$ gene have been reported in various malignancies.

$p16^{Ink4a}$ null mice develop lymphomas and sarcomas with low penetrance, but not pituitary tumors (Quereda and Malumbres 2009). However,reduced expression of $p16^{INK4A}$ is observed in all subtypes of human pituitary adenomas: the most frequent in non-functioning (NF) adenomas (Simpson et al. 1999; Seemann et al. 2001). Loss of heterozygosity (LOH) on the chromosome 9p21 is observed in only 6% of pituitary adenomas and the $p16^{INK4A}$ gene mutations are infrequent in pituitary adenomas (Yoshimoto et al. 1997; Kirsch et al. 2009), suggesting other mechanisms of reduced expression of $p16^{INK4A}$. Epigenetic inactivation by promoter methylation is one of the important mechanisms of gene silencing in human cancers. Promoter methylation implicated in aberrant gene expression is a hallmark of human pituitary tumorigenesis. Promoter hypermethylation is a common mechanism of $p16^{INK4A}$ inactivation in

various tumors including pituitary adenomas (Woloschak et al. 1997; Simpson et al. 1999; Seemann et al. 2001; Ogino et al. 2005; Yoshino et al. 2007). Methylation-associated silencing of the $p16^{INK4A}$ gene is more frequent in NF adenomas than other subtypes and is associated with loss of $p16^{INK4A}$ protein expression (Woloschak et al. 1997; Simpson et al. 1999; Seemann et al. 2001). These suggest subtype-specific deregulation of the $p16^{INK4A}$ gene in pituitary tumors. With regard to the role of epigenetic inactivation by $p16^{INK4A}$ methylation in tumorigenesis, conflicting concepts are proposed: One is involvement in an early event of pituitary tumorigenesis (Simpson et al. 1999) and another is involvement in progression of adenomas rather than an early event (Seemann et al. 2001).

$p15^{INK4B}$ (CDKN2B)

$p15^{INK4B}$ expression is shown to be downregulated in mitogen-stimulated lymphocytes. Although the $p15^{INK4B}$ gene concomitant with the $p16^{INK4A}$ gene is usually deleted in a large variety of tumors, mutations of the $p15^{INK4B}$ gene in human tumors are infrequent. Epigenetic silencing by hypermethylation of the $p15^{INK4B}$ gene has been demonstrated in mantle cell lymphoma, ovarian cancer, and acute myeloid leukemia (Hutter et al. 2006).

The major phenotype observed in $p15^{Ink4b}$ null mice is angiosarcomas with a long latency and low frequency, indicating that $p15^{Ink4b}$ has limited tumor-suppressing activities (Quereda and Malumbres 2009). In addition, the activity of the $p15^{Ink4b}$ gene is thought to provide a second line of defense against inactivation of $p16^{Ink4a}$, thereby serving as a backup of $p16^{Ink4a}$ (Quereda and Malumbres 2009).

In human pituitary adenomas, somatic mutations of the $p15^{INK4B}$ gene were not detected (Yoshimoto et al. 1997). Ogino et al. (2005) reported that the $p15^{INK4B}$ gene promoter was hypermethylated in 36% of pituitary adenomas. But they did not demonstrate the effect of promoter hypermethylation on $p15^{INK4B}$ expression. Further investigations are necessary to establish the correlation between protein or mRNA expression of $p15^{INK4B}$ and promoter hypermethylation of the gene in pituitary adenomas.

$p18^{INK4C}$ (CDKN2C)

The $p18^{INK4C}$ gene is located at chromosome 1p32, a region frequently altered in various tumors. Loss of $p18^{INK4C}$ protein expression has been reported in various human tumors such as testicular cancers, Hodgkin lymphomas, hepatocellular carcinomas, medulloblastomas, and glioblastomas and hypermethylation of the $p18^{INK4C}$ promoter was shown in Hodgkin lymphomas (Sánchez-Aguilera et al. 2004). Deletions of the $p18^{INK4C}$ gene are clearly less common than deletions of the $p16^{INK4A}/p14^{ARF}/p15^{INK4B}$ locus. Although $p18^{INK4C}$ gene mutations are rare in human cancers (Bostrom et al. 2001), van Veelen et al. (2009) reported the presence of somatic inactivating missense mutations of $p18^{INK4C}$ in human medullary thyroid carcinomas and pheochromocytomas.

Mice lacking of $p18^{Ink4c}$ display organomegaly, testicular tumors, pheochromocytomas, and intermediate-lobe pituitary tumors (Quereda and Malumbres 2009). In addition, hyperplasia in the anterior pituitary lobe is observed. Although mice lacking two INK4 proteins, $p15^{Ink4b}$ and $p18^{Ink4c}$, do not show an accelerated tumorigenesis including pituitary glands, collaboration of $p18^{Ink4c}$ with CIP/KIP family members confers decreased tumorigenesis. For example, mice lacking either $p18^{Ink4c}$ or $p27^{Kip1}$ slowly develop pituitary adenomas, whereas mice carrying simultaneous deletion of $p18^{Ink4c}$ and $p27^{Kip1}$ develop pituitary adenomas within 3 months (Quereda and Malumbres 2009). Furthermore, $p18^{Ink4c-/-}Pten^{+/-}$ or $p18^{Ink4c-/-}Men1^{+/-}$ mice develop anterior-lobe tumors with an accelerated rate compared to $Pten^{+/-}$ or $Men1^{+/-}$ mice, respectively (Quereda and Malumbres 2009). The above findings denote the important role of $p18^{INK4C}$ in pituitary tumorigenesis.

In our study, $p18^{INK4C}$ protein expression analysed by immunohistochemistry was lost or significantly reduced in 64% of human pituitary adenomas compared with levels in normal

pituitary glands (Hossain et al. 2009). $p18^{INK4C}$ mRNA levels were low in all adrenocorticotropic hormone (ACTH) adenomas and NF-follicle-stimulating hormone (FSH) and in 42, 70 and 66% of growth hormone (GH), prolactin, and subtype 3 adenomas, respectively. The results were consistent with a report of significant reduction of $p18^{INK4C}$ expression in ACTH adenomas (Morris et al. 2005). Furthermore, Kirsch et al. (2009) reported that immunohistochemistry revealed underexpression of $p18^{INK4C}$ protein in both hormonally active (42%) and inactive (36%) pituitary adenomas.

We analyzed the promoter methylation status of the $p18^{INK4C}$ gene in pituitary adenomas with low mRNA levels by bisulfite sequencing. Hypermethylation was detected in only 1 NF-FSH adenoma; the other adenomas showed no methylation in the promoter of the $p18^{INK4C}$ gene (Hossain et al. 2009). On the other hand, Kirsch et al. (2009) reported that about 40% of pituitary adenomas displayed $p18^{INK4C}$ promoter methylation by the method of methylation-specific PCR. The contrasting data on promoter methylation may be resulted from different methods. The bisulfite sequencing is the most straightforward means of detecting methylation status, which denotes an accurate map of the position of each methylated cytosine residue. Therefore, aberrant methylation seems not to be a responsible factor for the low levels of $p18^{INK4C}$ in pituitary adenomas. In our study, no somatic mutations except for a known single nucleotide polymorphism of the $p18^{INK4C}$ gene in 89 pituitary adenomas were detected (Hossain et al. 2009), consisting with the report by Kirsch et al. (2009).

$p19^{INK4D}$ (CDKN2D)

The $p19^{INK4D}$ gene is located at chromosome 19p13. $p19^{INK4D}$-overexpressing cells showed enhanced DNA repair activity after ultraviolet irradiation in neuroblastoma cells (Ceruti et al. 2005). The four proteins of the INK4 family share a similar structure; however, $p19^{INK4D}$ is shown to be an unstable protein in contrast to $p16^{INK4A}$, $p15^{INK4B}$, and $p18^{INK4C}$. Deletion of the $p19^{Ink4d}$ gene in mice neither develops tumors even after long observations nor accelerates tumor formation in $p18^{Ink4c}$ null mice (Quereda and Malumbres 2009), suggesting that $p19^{Ink4d}$ is not a tumor suppressor. Mutations and expression levels of the $p19^{INK4D}$ gene in human pituitary adenomas have not been reported.

$p21^{CIP1}$ (CDKN1A)

The $p21^{CIP1}$ gene, which is also referred to WAF1, is localized on chromosome 6p21.2. $p21^{CIP1}$ acts down stream of p53, and restricts cell cycle progression into S phase in the presence of DNA damage (Jung et al. 2010). In addition, $p21^{CIP1}$ binds regulatory proteins such as E2F1, c-Myc, STAT3 and p300/CBP other than CDKs (Roninson 2002).

Although $p21^{Cip1}$ null mice were initially presumed to be lack of apparent tumorigenesis, sarcomas and lymphomas occur in $p21^{Cip1}$ null mice bred on a 129Sv/C57BL6 50:50 background (Quereda and Malumbres 2009). This suggests that $p21^{Cip1}$ has the ability to function as a tumor suppressor. $Rb^{+/-}$ mice develop pituitary intermediate lobe tumors, pituitary tumor-transforming gene $(Pttg)^{-/-}$ rescues pituitary tumorigenesis in $Rb^{+/-}$ mice. $p21^{Cip1}$ deficiency restores abrogated pituitary tumor formation in $Rb^{+/-}Pttg^{-/-}$ knockout mice, suggesting that $p21^{Cip1}$ protein may contribute to decrease pituitary tumorigenesis in the $Rb^{+/-}Pttg^{-/-}$ mice (Chesnokova et al. 2008). In the anterior lobe of pituitary gland, deletion of the Pttg gene results in $p21^{Cip1}$ induction in GH-producing cells (Chesnokova et al. 2007). Interestingly, overexpression of Pttg in rat GH3 pituitary cells also enhanced $p21^{Cip1}$ (Chesnokova et al. 2008). Thus, both of overexpression and deficiency of Pttg enhanced $p21^{Cip1}$ expression.

PTTG levels in GH adenomas are high as compared with other subtype adenomas (Salehi et al. 2010). Elevated expression of $p21^{CIP1}$ protein in human GH adenomas is observed, while $p21^{CIP1}$ expression is very low in normal pituitary glands (Chesnokova et al. 2008). Furthermore, GH adenomas overexpressing PTTG exhibit

senescence as evidenced by increased $p21^{CIP1}$, ataxia-telangiectasia mutated kinase, and senescence-associated-β-galactosidase levels (Shen et al. 2005).

Inactivating mutations of the $p21^{CIP1}$ gene are rare in human cancers including pituitary adenomas (Roninson 2002). Promoter hypermethylation of the $p21^{CIP1}$ gene was detected only in 3% of pituitary adenomas (Yoshino et al. 2007). ACTH-, NF-FSH-, and null cell adenomas are mostly immunonegative for $p21^{CIP1}$ (Chesnokova et al. 2008). Recently, $p21^{CIP1}$ was reported to be directly downregulated by miR-17, miR-20a, miR-20b, miR-93, miR-106a, and miR-106b. Therefore, overexpression of these miRNAs leads to increased cell proliferation and accelerated G1/S transition (Jung et al. 2010). Involvement of these miRNAs in pituitary tumorigenesis remains elusive.

$p27^{KIP1}$ (CDKN1B)

The $p27^{KIP1}$ gene is located at chromosome 12p13. Dysregulated expression of $p27^{KIP1}$ is frequent in a wide variety of human malignancies and is considered as a prognostic marker for clinical outcome of human cancers (Besson et al. 2008). $p27^{KIP1}$ levels are regulated by transcription, translation, proteolytic degradation by the Scf-Skp2 E3 ubiquitin-protein ligase, cytoplasmic mislocalization etc. (Besson et al. 2008). The stability of $p27^{KIP1}$ as well as $p21^{CIP1}$ and $p57^{KIP2}$ is tightly regulated by ubiquitination and proteasome-mediated degradation during various stages of the cell cycle.

$p27^{Kip1}$ deficient mice display an increased body size and striking features of tumor development in several organs including the intermediate lobe of the pituitary glands and are susceptible to tumorigenesis by chemical carcinogens and irradiation (Quereda and Malumbres 2009). Even the loss of one allele of $p27^{Kip1}$ is predisposed to tumors in multiple tissues with a chemical carcinogens or irradiation (Quereda and Malumbres 2009). In addition, depletion of $p27^{Kip1}$, but not $p18^{Ink4c}$, cooperates with Cdk4 R24C mutation in pituitary tumor development (Quereda and Malumbres 2009).

Besson et al. (2007) generated knockin mice expressing a mutant $p27^{Kip1}$ protein, called p27(CK-), which is unable to interact with cyclins and CDKs. p27(CK-) dominantly causes hyperplasic lesions and tumors in multiple organs including the lung, retina, ovary, adrenals, spleen, lymphomas etc. In addition, p27(CK-) develops aggressive pituitary anterior-lobe tumors. This suggests that in addition to its role as a tumor suppressor, $p27^{Kip1}$ also functions as an oncogene.

$p27^{KIP1}$ protein expression is downregulated in human pituitary adenomas (Bamberger et al. 1999). The lower levels of $p27^{KIP1}$ protein in ACTH adenomas are consistent with development of intermediate lobe-derived pituitary tumors in $p27^{Kip1}$ null mice. In ACTH adenomas, an accentuated phosphorylation of $p27^{KIP1}$ leading to its increased degradation is observed (Korbonits et al. 2002). As observed in other tumors, downregulated expression of $p27^{KIP1}$ in pituitary adenomas does not result from inactivating mutations of the $p27^{KIP1}$ gene (Tanaka et al. 1997). Unexpectedly, allelotyping by microsatellite markers and fluorescence *in situ* hybridization revealed trisomy 12 in 24% of pituitary adenomas (Tanaka et al. 1997). Promoter hypermethylation of the $p27^{KIP1}$ gene is not detected in pituitary adenomas (Yoshino et al. 2007). In addition, $p27^{KIP1}$ is regulated by miR-221 and miR-222 (le Sage et al. 2007), suggesting that deregulated expression of miR-221 and miR-222 promotes tumorigenesis; however, information on levels of miR-221 and miR-222 in pituitary adenomas was unavailable. Recently, Landa et al. (2010) reported that reduced $p27^{KIP1}$ transcription depends on the genotype of the −79C>T (rs34330) variant as a novel mechanism underlying $p27^{KIP1}$ downregulation. Involvement of the allelic variant at −79 (C>T) in the $p27^{KIP1}$ gene in pituitary tumorigenesis remains elusive.

Although the majority of pituitary adenomas are sporadic, some arise as familial syndromes. Out of CKIs, the $p27^{KIP1}$ gene is the only identified gene responsible for heritable pituitary tumors. Pellegata et al. (2006) reported that a homozygous

germ-line mutation in $p27^{Kip1}$ causes a multiple endocrine neoplasia (MEN)-like syndrome (named MENX) in the rat, with a phenotypic overlap of both MEN1 and MEN2. The MENX-associated $p27^{Kip1}$ mutant protein was shown to be highly unstable. Furthermore, a germline nonsense mutation in the $p27^{KIP1}$ gene was identified in an MEN1-suspected patient with GH adenoma and parathyroid tumors. An inactivating $p27^{KIP1}$ germ-line mutation was also detected in a patient with hyperparathyroidism, an ACTH adenoma, and a neuroendocrine carcinoid tumor (Georgitsi et al. 2007). To date, germ-line mutations of $p27^{KIP1}$ have been reported only in five families with *MEN1*-negative MEN1 families (Agarwal et al. 2009).

$p57^{KIP2}$ (CDKN1C)

The $p57^{KIP2}$ gene is an imprinted gene located at the chromosomal locus 11p15.5, a region implicated in Beckwith-Wiedemann syndrome. The maternal allele is preferentially expressed (Pateras et al. 2009). $p57^{KIP2}$ is frequently downregulated both transcriptionally and translationally in many human cancers, and its decreased expression is correlated with aggressiveness in several malignancies (Pateras et al. 2009). Although LOH and increased methylation of the promoter are associated with decreased $p57^{KIP2}$ mRNA, somatic mutations of the $p57^{KIP2}$ gene were not reported in human tumors (Pateras et al. 2009).

Despite the similarities of structure between $p27^{Kip1}$ and $p57^{Kip2}$, the phenotypes of $p27^{Kip1}$ null mice and $p57^{Kip2}$ null mice quite differ. Whereas the null mice of $p21^{Cip1}$ or $p27^{Kip1}$ does not show gross defects in embryonic development, $p57^{Kip2}$ null mice show neonatal death, as well as developmental defects in multiple tissues (Quereda and Malumbres 2009). The developmental defects occur possibly due to delayed differentiation and increased apoptosis. Thus, the role of $p57^{Kip2}$ as tumor suppressor is largely obscure. Recently, Jin et al. (2008) demonstrated that the prostates of $p57^{Kip2}$ null mice grafted under the kidney capsule of athymic mice developed prostatic intraepithelial neoplasia, suggesting that $p57^{Kip2}$ is an important gene in prostate cancer tumorigenesis.

The critical role of $p57^{Kip2}$ plays in the maintenance of proper pituitary size; $p57^{Kip2}$ mutant pituitaries are hyperplasic during development, whereas $p57^{Kip2}$ overexpression produces a profound reduction in pituitary size (Quereda and Malumbres 2009).

In human pituitary adenomas, expression levels of $p57^{KIP2}$ gene have not been reported.

References

Agarwal SK, Mateo CM, Marx SJ (2009) Rare germline mutations in cyclin-dependent kinase inhibitor genes in multiple endocrine neoplasia type 1 and related states. J Clin Endocrinol Metab 94:1826–1834

Bamberger CM, Fehn M, Bamberger AM, Ludecke DK, Beil FU, Saeger W, Schulte HM (1999) Reduced expression levels of the cell-cycle inhibitor p27Kip1 in human pituitary adenomas. Eur J Endocrinol 140:250–255

Besson A, Hwang HC, Cicero S, Donovan SL, Gurian-West M, Johnson D, Clurman BE, Dyer MA, Roberts JM (2007) Discovery of an oncogenic activity in p27Kip1 that causes stem cell expansion and a multiple tumor phenotype. Genes Dev 21:1731–1746

Besson A, Dowdy SF, Roberts JM (2008) CDK inhibitors: cell cycle regulators and beyond. Dev Cell 14:159–169

Bostrom J, Meyer-Puttlitz B, Wolter M, Blaschke B, Weber RG, Lichter P, Ichimura K, Collins VP, Reifenberger G (2001) Alterations of the tumor suppressor genes CDKN2A (p16INK4a), p14ARF, CDKN2B (p15INK4b), and CDKN2C (p18INK4c) in atypical and anaplastic meningiomas. Am J Pathol 159:61–69

Ceruti JM, Scassa ME, Flo JM, Varone CL, Canepa ET (2005) Induction of p19INK4d in response to ultraviolet light improves DNA repair and confers resistance to apoptosis in neuroblastoma cells. Oncogene 24:4065–4080

Chesnokova V, Zonis S, Rubinek T, Yu R, Ben-Shlomo A, Kovacs K, Wawrowsky K, Melmed S (2007) Senescence mediates pituitary hypoplasia and restrains pituitary tumor growth. Cancer Res 67:10564–10572

Chesnokova V, Zonis S, Kovacs K, Ben-Sholomo A, Wawrowsky K, Bannykh S, Melmed S (2008) p21Cip1 restrains pituitary tumor growth. Proc Natl Acad Sci U S A 105:17498–17503

Georgitsi M, Raitila A, Karhu A, van der Luijt RB, Aalfs CM, Sane T, Vierimaa O, Mäkinen MJ, Tuppurainen K, Paschke R, Gimm O, Koch CA, Gündogdu S, Lucassen A, Tischkowitz M, Izatt L, Aylwin S, Bano G, Hodgson S, De Menis E, Launonen V, Vahteristo P, Aaltonen LA (2007) Germline CDKN1B/p27Kip1

mutation in multiple endocrine neoplasia. J Clin Endocrinol Metab 92:321–325

Honda S, Tanaka-Kosugi C, Yamada S, Sano T, Matsumoto T, Itakura M, Yoshimoto K (2003) Human pituitary adenomas infrequently contain inactivation of retinoblastoma 1 gene and activation of cyclin dependent kinase 4 gene. Endocr J 50:309–318

Hossain MG, Iwata T, Mizusawa N, Qian ZR, Shima SW, Okutsu T, Yamada S, Sano T, Yoshimoto K (2009) Expression of p18INK4C is down-regulated in human pituitary adenomas. Endocr Pathol 20:114–121

Hutter G, Scheubner M, Zimmermann Y, Kalla J, Katzenberger T, Hübler K, Roth S, Hiddemann W, Ott G, Dreyling M (2006) Differential effect of epigenetic alterations and genomic deletions of CDK inhibitors [p16(INK4a), p15(INK4b), p14(ARF)] in mantle cell lymphoma. Genes Chromosomes Cancer 45:203–210

Jin RJ, Lho Y, Wang Y, Ao M, Revelo MP, Hayward SW, Wills ML, Logan SK, Zhang P, Matusik RJ (2008) Down-regulation of p57Kip2 induces prostate cancer in the mouse. Cancer Res 68:3601–3608

Jung YS, Qian Y, Chen X (2010) Examination of the expanding pathways for the regulation of p21 expression and activity. Cell Signal 22:1003–1012

Kirsch M, Mörz M, Pinzer T, Schackert HK, Schackert G (2009) Frequent loss of the CDKN2C (p18INK4c) gene product in pituitary adenomas. Genes Chromosomes Cancer 48:143–154

Korbonits M, Chahal HS, Kaltsas G, Jordan S, Urmanova Y, Khalimova Z, Harris PE, Farrell WE, Claret FX, Grossman AB (2002) Expression of phosphorylated p27Kip1 protein and jun activation domain binding protein 1 in human pituitary adenomas. J Clin Endocrinol Metab 87:2635–2643

Landa I, Montero-Conde C, Malanga D, De Gisi S, Pita G, Leandro-García LJ, Inglada-Pérez L, Letón R, De Marco C, Rodríguez-Antona C, Viglietto G, Robledo M (2010) Allelic variant at -79 (C>T) in CDKN1B (p27Kip1) confers an increased risk of thyroid cancer and alters mRNA levels. Endocr Relat Cancer 17:317–328

le Sage C, Nagel R, Agami R (2007) Diverse ways to control p27Kip1 function: miRNAs come into play. Cell Cycle 6:2742–2749

Morris DG, Musat M, Czirjak S, Hanzely Z, Lillington DM, Korbonits M, Grossman AB (2005) Differential gene expression in pituitary adenomas by oligonucleotide array analysis. Eur J Endocrinol 153:143–151

Ogino A, Yoshino A, Katayama Y, Watanabe T, Ota T, Komine C, Yokoyama T, Fukushima T (2005) The p15INK4b/p16INK4a/RB1 pathway is frequently deregulated in human pituitary adenomas. J Neuropathol Exp Neurol 64:398–403

Pateras IS, Apostolopoulou K, Niforou K, Kotsinas A, Gorgoulis VG (2009) p57KIP2: "Kip"ing the cell under control. Mol Cancer Res 7:1902–1919

Pellegata NS, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, Höfler H, Fend F, Graw J, Atkinson MJ (2006) Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. Proc Natl Acad Sci U S A 103:11558–11563

Quereda V, Malumbres M (2009) Cell cycle control of pituitary development and disease. J Mol Endocrinol 42:75–86

Roninson IB (2002) Oncogenic functions of tumour suppressor p21Waf1/Cip1/Sdi1: association with cell senescence and tumour-promoting activities of stromal fibroblasts. Cancer Lett 179:1–14

Salehi F, Kovacs K, Scheithauer BW, Cantelmi D, Horvath E, Lloyd RV, Cusimano M (2010) Immunohistochemical expression of pituitary tumor transforming gene (PTTG) in pituitary adenomas: a correlative study of tumor subtypes. Int J Surg Pathol 18:5–13

Sánchez-Aguilera A, Delgado J, Camacho FI, Sánchez-Beato M, Sánchez L, Montalbán C, Fresno MF, Martín C, Piris MA, García JF (2004) Silencing of the p18INK4C gene by promoter hypermethylation in Reed-Sternberg cells in Hodgkin lymphomas. Blood 103:2351–2357

Seemann N, Kuhn D, Wrocklage C, Keyvani K, Hackl W, Buchfelder M, Fahlbusch R, Paulus W (2001) CDKN2A/p16 inactivation is related to pituitary adenoma type and size. J Pathol 193:491–497

Shen KC, Heng H, Wang Y, Lu S, Liu G, Deng CX, Brooks SC, Wang YA (2005) ATM and p21 cooperate to suppress aneuploidy and subsequent tumor development. Cancer Res 65:8747–8753

Simpson DJ, Bicknell JE, McNicol AM, Calyton R, Farrell WE (1999) Hypermethylation of the p16/CDKN2A/MTS1 gene and loss of protein expression is associated with nonfunctional pituitary adenomas but not somatotrophinomas. Genes Chromosomes Cancer 24:328–336

Tanaka C, Yoshimoto K, Yang P, Kimura T, Yamada S, Moritani M, Sano T, Itakura M (1997) Infrequent mutations of p27Kip1 gene and trisomy 12 in a subset of human pituitary adenomas. J Clin Endocrinol Metab 82:3141–3147

van Veelen W, Klompmaker R, Gloerich M, van Gastteren CJR, Kalkhoven E, Berger R, Lips CJ, Medema RH, Höppener JW, Acton DS (2009) P18 is a tumor suppressor gene involved in human medullary thyroid carcinoma and pheochromocytoma development. Int J Cancer 124:339–345

Woloschak M, Yu A, Post KD (1997) Frequent inactivation of the p16INK4a gene in human pituitary tumors by gene methylation. Mol Carcinog 19:21–24

Yoshimoto K, Tanaka C, Yamada S, Kimura T, Iwahana H, Sano T, Itakura M (1997) Infrequent mutations of p16INK4A and p15INK4B genes in human pituitary adenomas. Eur J Endocrinol 136:74–80

Yoshino A, Katayama Y, Ogino A, Watanabe T, Yachi K, Ohta T, Komine C, Yokoyama T, Fukushima T (2007) Promoter hypermethylation profile of cell cycle regulator genes in pituitary adenomas. J Neurooncol 83:153–162

Pituitary Tumorigenesis: Role of Regulation of Wee1 Kinase by microRNAs

16

Henriett Butz and Attila Patocs

Contents

Abstract

Mechanisms involved in pituitary tumorigenesis, especially of non-functional pituitary adenomas remain unclear. Various cell cycle inhibitors have been found to be underexpressed in pituitary tumors. However alterations of the G2/M checkpoint have not been revealed as a major player in the pathogenic process. Wee1 kinase, a nuclear protein that delays G2/M transition has been recently recognized as a tumor suppressor and has been found as a potential pathogenic factor in pituitary adenomagenesis. In this chapter after a brief summary of the cell cycle regulation, the biogenesis and function of miRs in pituitary adenomas, we review the role and function of Wee1 kinase focusing on its potential role in pituitary tumorigenesis. MicroRNAs posttranscriptionally regulating expression of Wee1 kinase and their expression and role in pituitary adenoma development are also discussed.

Cell Cycle

The cell cycle is a process by which cells grow, replicate their genome, and divide. The control of the cell cycle is executed by the interaction of a cyclically operating set of proteins that initiate and coordinate the progression of the cell through the cycle. Cell division is classified into the following phases: G1 phase (gap) in which the cell commits itself to entering the cell cycle. It is followed by S phase (synthesis of DNA) in which the genome is duplicating and then proceeds the G2 phase where

H. Butz (✉)
Faculty of Medicine, Second Department of Medicine, Semmelweis University, H-1088 Budapest, Hungary

Molecular Medicine Research Group, Hungarian Academy of Sciences
e-mail: henriettbutz@gmail.com

A. Patocs
Department of Laboratory Medicine, Semmelweis University, H-1088 Budapest, Hungary
e-mail: attilapatocs@yahoo.com

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_16,

the synthesized genetic material has the opportunity for repairing itself. Finally the cycle will end with M phase (mitosis) in which the cell with its genetic material segregates into daughter cells. In mammalian cells, the main proteins involved in this process are the cyclins, cyclin-dependent kinases (CDKs) and their inhibitors (CDK inhibitors or CKIs).

Cyclin-dependent kinases (CDKs) and their cyclin partners are accelerating and inducing cell cycle progression. CDK activity is modulated by fluctuations in the cellular concentration of their activators (cyclins) or inhibitors (CDK inhibitors or CKIs), which in turn are regulated by specific positive (transcriptional induction by mitogenic and anti-mitogenic pathways) and negative (i.e. proteolysis by the ubiquitin-proteosome system) signals. CDKs are activated by association with cyclins, by phosphorylation on the T-loop threonine by CDK-activating kinases (CAK), and by dephosphorylation on threonine 14/tyrosine 15 residues by the CDC25 phosphatases. In addition, CDK activity can be impeded by negative phosphorylation of threonine 14/tyrosine 15 residues mediated by WEE1-like kinases and by association with CKIs (Morgan 1995).

The specific inhibitors of CDKs (CKIs) also play major role in cell cycle regulation by mediating antimitogenic signals or checkpoint responses. They block CDK activation or impair the access of substrate/ATP. CKIs are divided into INK4 and Cip/Kip family. The INK4 family (p16INK4a, p15INK4b, p18INK4c, p19INK4d) inhibits progression through G1/S by binding CDK4 and CDK6. The Cip/Kip family (p21Cip1, p27Kip1, p57Kip2) could associate to CDK2 and CDK1 complexes and blocks their kinase activity, whereas their role in binding to CDK4–cyclin D or CDK6–cyclin D complexes is unclear (Malumbres and Barbacid 2005).

Biogenesis and Role of miRs in Tumorigenesis

MicroRNAs (miRs) are short (approx. 19–25 nt) non-coding RNA molecules that posttranscriptionally regulate gene expression via RNA interference by binding 3′UTR of protein coding mRNAs (Lagos-Quintana et al. 2001).

It is thought that 30–50% of all protein coding genes might to be controlled by miRs (Lewis et al. 2005). Their roles have been considered in development, cell proliferation, differentiation, apoptosis and also tumorigenesis including pituitary adenomas.

MiRs are encoded by genes located within introns of protein-coding genes or intergenicly. They can occur singly or clustered through the genome predicting a long transcript encompassing several coordinately expressed miRNAs. After transcribing by RNA polymerase II they come through a maturation process in the nucleus and cytoplasm. In the nucleus pri-miRs (primary transcript) are processed by an RNase III (Drosha) containing complex. Drosha asymmetrically cleaves both strands near the base of primary stem-loop into a ~60–70 nucleotide long precursor-miRNA (pre-miR) characterized by a hairpin secondary structure. Then the hairpin structured pre-miR molecule is transported to the cytoplasm by Exportin-5. Here pre-miRs are processed by an other RNase III enzyme (Dicer) that cleaves the pre-miR into a ~21 nucleotide long miR:miR* duplex. One strand of this RNA duplex (guide strand or matured miR) is incorporated into the miRNA induced silencing complex (miRISC) while the other strand (passenger strand or miR*) usually is degraded. In this complex miR interacts with its mRNA targets 3′UTR by base-pairing and repress the expression of its targets by three major processes: mRNA cleavage, mRNA degradation by deadenylation or inhibition of translation initiation (Krol et al. 2010) (Fig. 16.1).

Several investigations have been showed that the characteristic gene expression profiles for a given tumor have been negatively correlated with specific miRs expression. Based on their role in tumorigenesis miRs are classified as onco-miRs or tumorsuppressor-miRs depending on the targeted gene(s) function. In addition, expression pattern of miRs may be useful for evaluation of tumor grade and stage.

miRs in Pituitary Tumorigenesis

According to pituitary adenoma only a few miR-target gene interactions have been proved. Bottoni et al. (2007) found that miR-16-1 which is under-

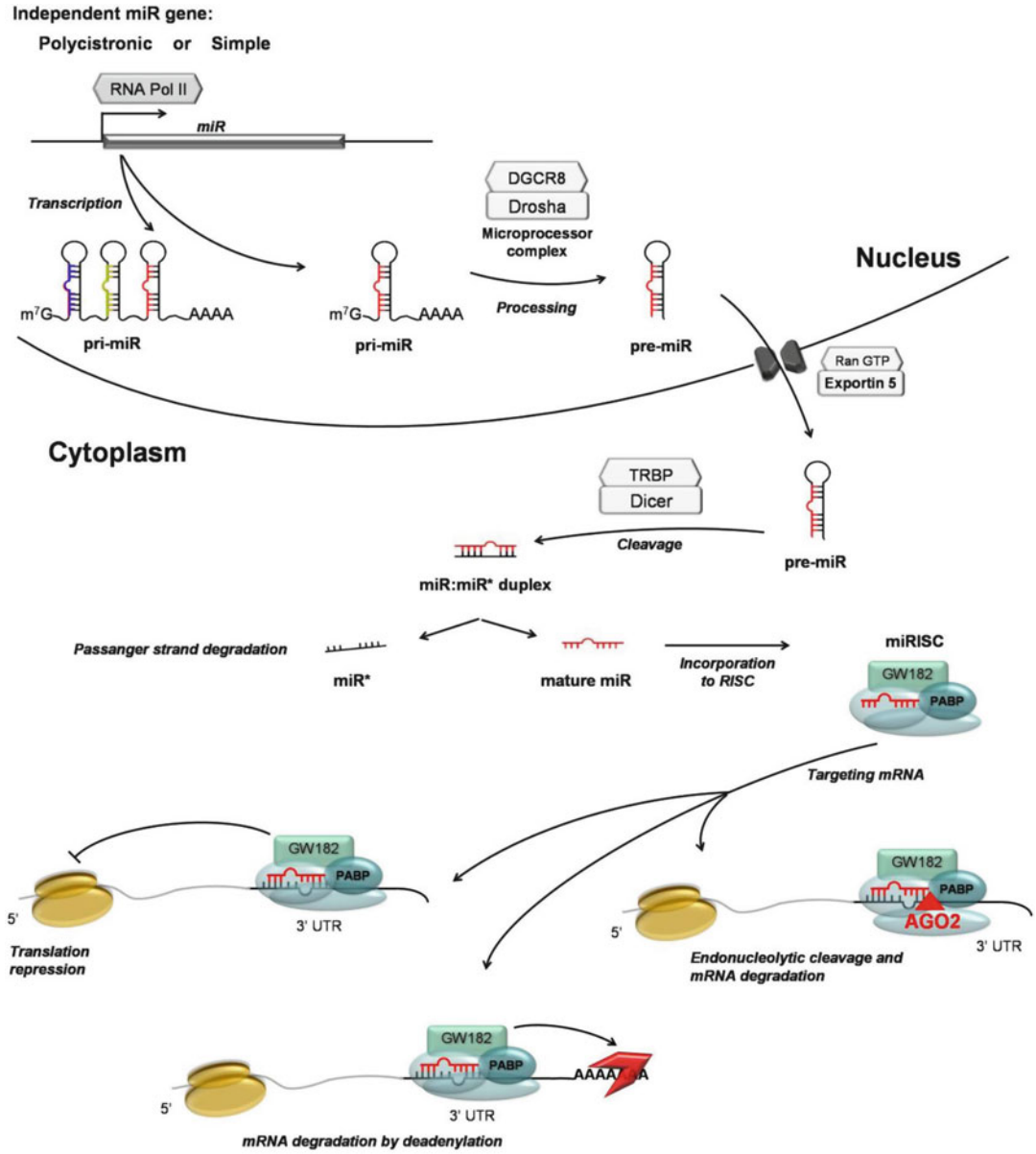

Fig. 16.1 Biogenesis of miRs. Detailed description is in the text

expressed in pituitary adenomas targets BCL2 protooncogene. BCL2 is overexpressed in a third of pituitary adenomas and in this manner miR-16-1 possibly influences its expression on protein level and thereby takes part in adenoma development. Using miR expression analysis performed by microarray they also identified 24 miRs which expressions are predictive in separation of normal from adenomatous tissues. Bottoni et al. (2007) described other 29 miRs which expression was characteristic for different hormone-producing phenotypes. They were able to identify specific miRs for ACTH-, GH-, PRL-producing tumors as well as for non-functioning histotype. Relationship with tumor size was also detected, miR-140, -99, -99b, -30b and 30c were found to be overexpressed in macroadenomas and miR-138-2 in microadenomas. The authors

suggested that the overexpressed miRs in adenomas (miR-150, miR-152, miR-191 and miR-192) may be connected to the regulation of cell proliferation based on previous results showing that inhibition of these miRs diminished cell growth (Cheng et al. 2005). Amaral et al. (2008) investigated ACTH secreting pituitary tumors and identified 8 underexpressed miRs with potential pathogenic role, but they could not present any relationship with clinical characteristics including tumor size, except for miR-141 which was lower expressed in patients with higher chance for remission.

Another miR, let-7 was found to be underexpressed in pituitary adenomas and one of its targets, HMGA2 was overexpressed in these tumors. Importantly their interaction was validated by several groups and Qian et al. (2009) demonstrated their negative correlation in pituitary adenomas. Our group analyzed the expression levels of Smad family members together with miRs in nonfunctioning pituitary tissues (NFPA). Smad proteins are key regulators of the activin/transforming growth factor-beta (TGF-b) signaling which play a prominent role in regulating pituitary tumor growth and prolactin secretion from pituitary lactotrope cells. Our results demonstrated that Smad3, Smad6 and Smad9 (=Smad8) were significantly underexpressed in NFPA compared to normal pituitary tissues, and expression of Smad3 but not Smad6 and Smad9 showed a tendency to correlate with tumor size ($P=0.08$). MiR expression profiles of the same samples combined with complex bioinformatical analysis revealed 19 miRs which were found targeting and negatively correlated with Smad3 expression in pituitary adenoma tissues (Butz et al. 2010). Of these miRs the role of miR-140 has been confirmed by reporter gene experiment (Pais et al. 2010) and it has already been connected to cell growth in lung carcinoma cell line (Cheng et al. 2005).

Another result of our study was the identification of already tumorigenesis-related miRs in pituitary adenoma development. We identified a specific subset of overexpressed miRs in pituitary tumors compared to normal tissue targeting Wee1 kinase and others involved in different mechanisms leading to tumor development. Thus, miR-155, an oncomir with pathogenetic role in hemopoietic malignancies (lymphomas, leukemia) and solid tumors (lung, colon, breast, cervical, thyroid cancer) (Faraoni et al. 2009); miR-516a-3p which has been suggested to be involved in glioblastoma development and has been found to be associated with aggressiveness of breast cancer; and miR-128a, a brain-enriched miR frequently detected as being downregulated in glioblastomas. In addition, its ectopic overexpression reduced neuroblastoma cell motility and invasiveness and its targets which include an oncogene/stem cell renewal factor, Bmi-1 and a transcription factor, E2F3a may reflect its tumorsuppressor role.

We found two other highly overexpressed miRs in NFPA, miR-93 and miR-20a. Although these miRs were potential candidates for targeting *Wee1* 3′UTR, we were unable to demonstrate their interaction with our in vitro luciferase assay suggesting that these miRs may affect tumorigenesis via other mechanisms (Butz et al. 2011). Indeed, both miRs are considered as oncomirs; miR-93 targets the tumor suppressor gene *FUS1* and its expression was found to be upregulated in breast and lung cancer, whereas miR-20a, a member of miR-17-92 cluster was reported as overexpressed in colorectal and gastric cancer. MiR-20a has validated targets such as p21, cyclin D1, and it prevents transcription of p19 via leukaemia/lymphoma related factor (LRF).

Ebi et al. (2008) working with lung cancer showed that miR-20a was overexpressed in lung cancer tissues and cell lines and O'Donnell et al. (2005) demonstrated that this miR directly targeted the transcription factor E2F1. In addition, E2F1 has been found to be overexpressed in 29 of 46 Rb(+/−) murine pituitary tissues and in 45 of 80 human pituitary tumors. Overall it has been hypothesized that overexpression of miR-20a together with miR-17-92 cluster may serve as a protective mechanism against DNA damage. Pickering et al. (2009) reported that miR17-5p/miR-20a is involved in accurate control of E2F1 (less than twofold change in E2F1 protein levels was enough to modify the timing of cell cycle progression) in regulation of S-phase entry.

Similarly to these previous reports, we also found miR-20a overexpressed in 15/18 of NFPA and 4/8 of GH ± P-producing pituitary adenomas, but the exact role of this miR in pituitary tumorigenesis needs further studies.

Alterations of Cell Cycle in Pituitary Adenomas

Alterations of several cell cycle-related genes have been associated with pituitary adenoma development. For example, promoter hypermethylation of CDK inhibitors (p14, p15, p16, p18, p21, p27, and retinoblastoma protein) and of cell growth suppressors MEG3a (maternally expressed gene 3a) and GADD45G (growth arrest- and DNA damaged inducible gene γ) have been previously described in pituitary tumors (Dworakowska and Grossman 2009). The p15, p16, p18 and p19 inhibit mainly Cyclin D-CDK4/6, while p21, p27 and p57 inhibit Cyclin E-CDK2 complex. These active complexes phosphorylate retinoblastoma protein that releases E2F transcription factor, which represents the committed step for cell division at G1/S transition. In addition, p21, p27 and p57 inhibit Cyclin A-CDK2 in S phase. Loss of these proteins is frequently detected in several solid tumors and p21, p27 and p57 transgenic knockout animals usually develop numerous neoplasms including pituitary tumors (Franklin et al. 2000). MEG3 activates p21 through p53 and suppress cell proliferation, so its functional loss leads to G1/S checkpoint disorder.

In nonfunctioning pituitary adenomas (NFPA), increased expression of high-mobility group A2 (HMGA2), cyclin B2, and cyclin D1 was observed. However alterations of the G2/M transition have not been clearly evaluated only underexpression of GADD45G was demonstrated. GADD45G hinders formation of cyclin B-CDK2 complex. Although recent results based on protein array examination suggest that deregulation of G2/M checkpoint may be involved in tumorigenetic process (Zhan and Desiderio 2010).

Function of Wee1 Kinase

G2/M checkpoint is destined for filtering damaged DNA synthesized during S phase. G2/M transition and thereby mitotic entry is regulated by two kinases; Wee1 and CDC25 having opposite effects. Wee-like kinases (Wee1, Wee2 and PKMYT) inactivate CDK1 by phosphorylation, while CDC25 works as a phosphatase. By its function Wee1 kinase has been described as a mitotic inhibitor (McGowan and Russell 1993), its overexpression causes G2 arrest by keeping CDK1 inactive. Although Wee1 inhibitors may represent suitable anti-tumor compounds, as in tumors where the G1 checkpoint control is defective by impairment of p53/Rb pathway, abrogation of the G2 checkpoint by Wee1 inhibition could render the tumor cells sensitive to DNA damaging drugs (Kawabe 2004), and hence targets for apoptosis.

Wee1 protein is predominantly a nuclear protein while Myt1 localizes associated to membrane. Both are active during interphase and are inactivated at G2/M transition. Wee1 is regulated by phosphorylation and binding to 14-3-3 proteins during interphase. It is also suggested that binding different isoforms of 14-3-3 has different consequences: 14-3-3β, -ε, -ζ promote Wee1 activity while -Θ inactivates the kinase through cytoplasmic sequestration (Katayama et al. 2005).

Wee1 has two sites (Tyr295 and 362) which are known autophosphorylation sites that enhance its activity while phosphorylation at Ser642 by Akt inactivates Wee1 at G2/M transition (Katayama et al. 2005). Through this phosphorylation G2/M transition is promoted. Wee1 degradation is also regulated by phosphorylation at sites: Ser53, Ser121 and Ser123. These sites could be phosphorylated by several kinases (Plk1: polo-like kinase, CK2: casein kinase 2 and CDK1 as a feedback regulation) and then the phosphorylated Wee1 binds to β-Trcp, an E3 ubiquitin ligase leading protein degradation also required for G2/M transition (Watanabe et al. 2005, 2004).

It has been reported that the DNA replication checkpoint stabilizes Wee1 within nucleus thereby delaying entry into mitosis until appropriate conditions have been met, and it would be degraded at the time of mitotic entry by CDC34 (Michael and Newport 1998). This proteolysis event was required for a timely entrance into mitosis and was inhibited when DNA replication was blocked.

In the case of DNA damage – for example malfunctioning DNA replication – DNA damage checkpoint regulation is induced, and as a part of it Wee1 is activated and CDC25 is inhibited by Checkpoint kinase1/2 (Chk 1/2). Checkpoint kinases are induced for incomplete replication and block mitotic entry. Beside CDC34, human Hsl7 homologue JBP1 (Janus kinase binding protein 1) also can mediate Wee1 degradation as a part of DNA damage response pathway. JBP1 can interact with Jak family kinases, presumably to control transcription, it is a positive regulator of p53 and a negative regulator of Cyclin E for associating specifically with its transcription start site (Jansson et al. 2008).

Wee1 and Its Targeting miRs in Pituitary Adenoma

Our group has showed that the expression of Wee1 kinase (both of total and phophorylated on Ser123 form) is diminished in growth hormone with and without prolactin producing (GH ± P) and also in hormonally inactive, non-functioning pituitary adenomas (NFPA). Despite the change of protein amount we could not prove difference in mRNA level of Wee1 gene between normal and adenoma samples suggesting that transcription of *Wee1* is not altered in these tissues. Based on these findings we hypothesized that the decreased Wee1 protein expression might be related to overexpression of Wee1-targeting miRs. Applying three computational target prediction methods several potential miRs' target sites in the 3′ UTR of *Wee1* were identified. To confirm the direct interactions between these miRs and the 3′ UTR of the *Wee1* reporter gene assay experiments were performed by cloning this region into a luciferase reporter system (Butz et al. 2010). Our results demonstrated that miR-128a, miR-155 and miR-516a-3p significantly repressed the luciferase activity, while the co-transfection of different combinations of these miRs produced no further decrease in luciferase activities suggesting that the effect of these miRs may be redundant on *Wee1* 3′UTR. By site directed mutagenesis binding sites for of miR-128a (between nucleotides 28–35 and 252–258) and miR-516a-3p (between nucleotides 40–46) have been validated (Fig. 16.2).

The direct interaction between miR-128a, miR-155 and miR-516a-3p and the 3′UTR of *Wee1* was further explored. HeLa cells were transiently transfected with pre-miR precursors, and expression of Wee1 was assessed by Western blot. A significant decrease of Wee1 protein was detected after pre-miR transfections accompanied by a diminished cell proliferation. All these results together demonstrated that miR-128a, miR-155 and miR-516a-3p directly targets the 3′UTR of *Wee1* and also confirms that tumor cells which have damaged G1 checkpoint may be sensitivity for Wee1 inhibition (Butz et al. 2011; Zhang et al. 1998; Koniaras et al. 2001). Underexpression of Wee1 in pituitary adenoma cells – similarly to other typical slow growing cells (for example non-transformed MCF10A and NIH/3T3 cells) may promote tumorigenesis by other mechanism. In these cells Wee1 inhibition would not lead to mitotic catastrophe instead will modify the fine-tuned timing of G2/M transition.

In a recent study Qi et al. (2009) experimentally validated two miRs (miR-195 and miR-372) targeting the *Wee1* 3′UTR in stem cells. In our study we found that miR-195 was moderately overexpressed in NFA, and down-regulated in GH-producing adenomas as compared to normal pituitary tissues. Our results again reflect the complexity of miR-related protein expression and suggest that miR-195 may be differentially involved in the pathomechanism of these two types of pituitary adenomas. All these results together may suggest that downregulation of the Wee1 by overexpressed miRs (miR-128a, miR-155, miR-195 and miR-516a-3p) may contribute to the tumorigenesis of the pituitary gland (Fig. 16.3).

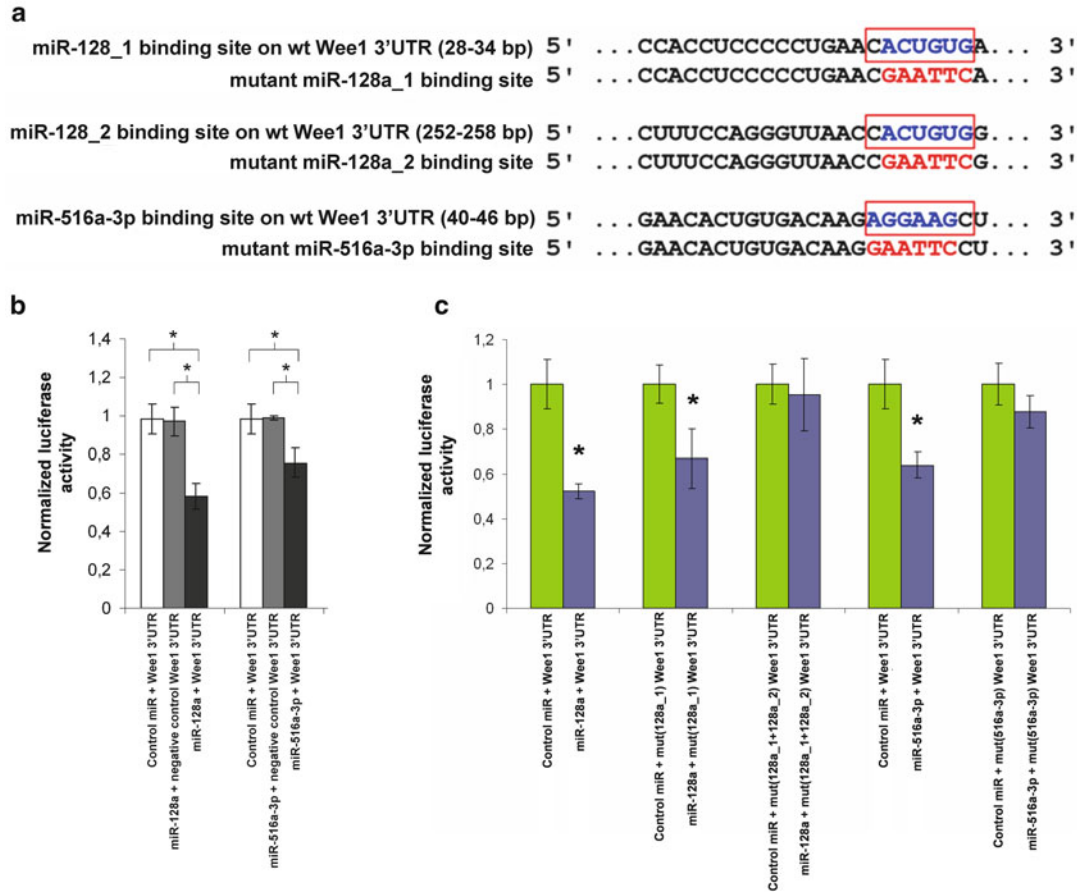

Fig. 16.2 Identification of the binding sites of miR-128a and miR-516a-3p at *Wee1* 3′UTR (Quote partially from Butz et al. 2010). *Panel A*: wild type and mutant binding sites of the predicted miRs in Wee1 3′UTR.; *Panel B*: Luciferase activity of HeLa cells after transfection with miR-128a and miR-516a-3p. *Open columns*: effect of the control miR precursor on wild type human *Wee1* 3′UTR. *Grey columns*: effect of the studied miR precursor on the negative control human *Wee1* 3′UTR. *Black columns*: effect of the studied miR precursor on wild type human *Wee1* 3′UTR. The tested miRs significantly inhibited the luciferase activity of vectors containing wild type *Wee1* 3′UTR. *Panel C*: Luciferase activity of HeLa cells after transfection with mutant *Wee1* 3′UTR. *Green columns*: effect of the control pre-miR precursor on the different types of *Wee1* 3′UTR. *Blue columns*: effects of the miR-128a and miR-516a-3p on the wild type and mutant *Wee1* 3′UTR. The predicted binding sites are functionally active (*Columns* represent mean, *vertical lines* illustrate SD; *$p<0.05$)

Role of Wee1 in Other Tumors

In contrast to data obtained mainly in basic research setup, only a few data is available for the role of Wee1 in patients suffering from different diseases.

Backert et al. (1999) using cDNA microarray identified Wee1 as a potential tumor suppressor in colon mucosa. They detected an underexpression of Wee1 in colon tumor cells compared to normal cells which may suggest that decreased expression or loss of Wee1 in colon carcinoma cells supports Wee1's tumor suppressor function. Another study including 79 patients with non small cell lung cancer (NSCLC) found that the loss of Wee1 confers an advantage to neoplastic cells by allowing faster progression through the cell cycle. Using immunohistochemistry they observed the absence of Wee1 expression in about two thirds of patients. Patients lacking

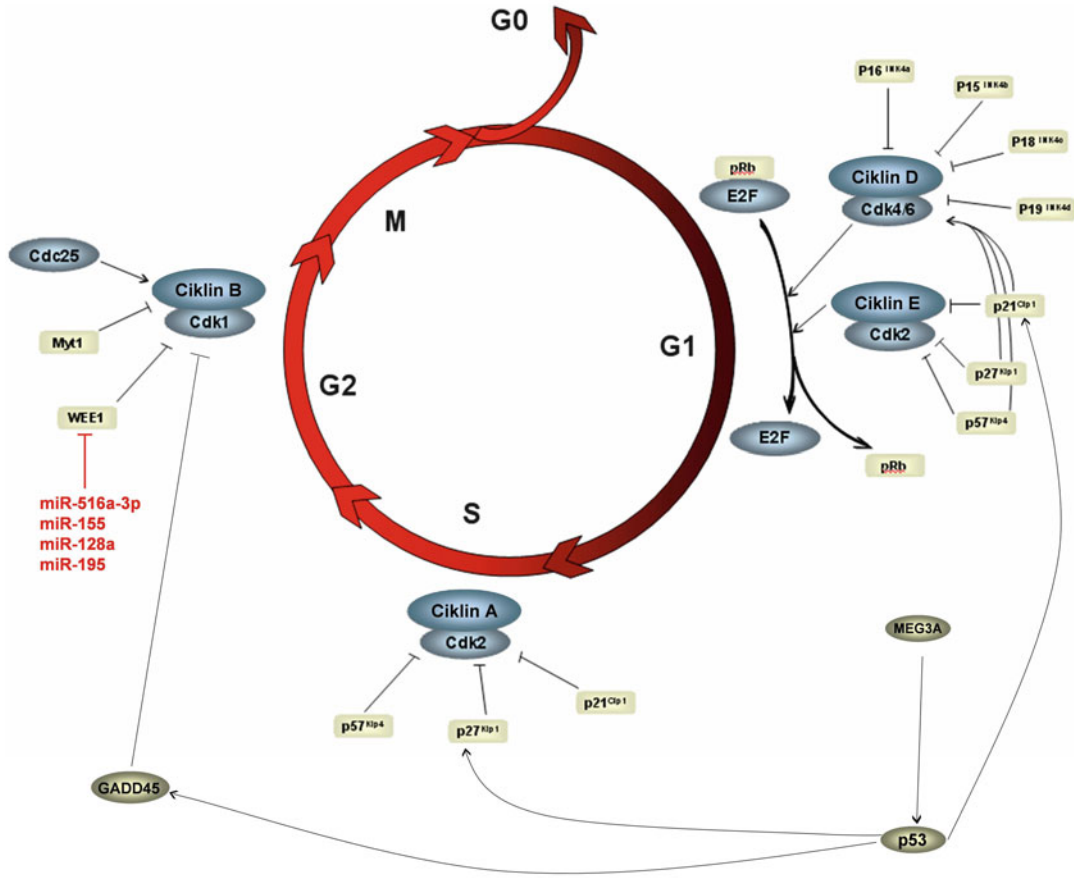

Fig. 16.3 Regulation of cell cycle in pituitary. Overexpressed miRs that are involved in adenomagenesis through inhibition of Wee1 is indicated in *red*

expression of Wee1 had a higher recurrence rate and a poorer prognosis, and tended to have higher Ki-index and proliferating cell nuclear antigen (PCNA-LI) levels. They concluded that Wee1 expression was a significant prognostic factor and Wee1 may be only a weak suppressor of tumor progression in NSCLC.

Interestingly it has been described in additional studies that tumors overexpressing Wee1 prevented themselves from mitotic catastrophe. Indeed, Wee1 inhibitors may represent suitable anti-tumor compounds, as in tumors where the G1 checkpoint control is defective by impairment of p53/Rb pathway, abrogation of the G2 checkpoint by Wee1 inhibition could render the tumor cells sensitive to DNA damaging drugs (Kawabe 2004), and hence targets for apoptosis. For example, Mir et al. (2010) reported that Wee1 has a gatekeeper role against mitotic catastrophe in glioblastoma. It was found that inhibition of Wee1 by siRNA or small molecular compound in cells exposed to DNA damaging agents result in abrogation of the G2 arrest, premature termination of DNA repair, and cell death. All these results together suggest that inhibition of Wee1 sensitizes glioblastoma to ionizing radiation in vivo.

For conclusion the inhibition of Wee1 kinase by a selective small molecule inhibitor significantly enhances the anti-tumor efficacy of DNA damaging agents, specifically in p53 negative tumors by abrogating S-G2 checkpoints, while normal cells with wild-type p53 are not severely damaged due to the intact function of the G1 checkpoint mediated by p53. That is

why the role of Wee1 is strongly context dependent (Mizuarai et al. 2009).

Future Perspectives

Very recently it was found that in particular conditions miRs could be suitable biomarkers of pathologic processes. For example miRs isolated from serum are sensitive biomarkers for early diagnosis of traumatic injury, muscular dystrophy or specific tumors (Zhang et al. 2011; Mizuno et al. 2011; Santarelli et al. 2011). They are acceptable markers because they are stable in plasma and in many cases their quantities show good correlation with pathogenic processes e.g. as diabetes, inflammation or tumors. They represent suitable tools for screening and monitoring of several conditions/diseases.

According to the therapeutical application of small RNAs the real challenge is their administration route. Hence miRNAs expression and their effects are cell-type specific is very important to introduce the miRNA with potential therapeutic role into the targeted tissue. However, the initial results of these attempts are encouraging. The first such experimental drug (Miravirsen) is currently in Phase II trial. This is an anti-miR against miR-122 which is essential for reproduction of the Hepatitis C virus (HCV). The targeted patient population HCV infected patients while the administration route is a subcutaneous injection. In preclinical studies there are other attempts which are focusing on introduction of miRs intravenously. For example, miR-34a is able to reduce the growth and metastatic potential of the CD44+ prostate cancer (Liu et al. 2011). Apart of miRs siRNA-s are also tryed to be used with therapeutic purposes. For delivery of these double stranded RNA molecules nanoparticules have been applied. In summary it can be imagined that in near future more and more novel miRs and siRNAs will be identified and will be tested for different processes and parallel these new findings represent start points for development of novel therapeutic approaches.

References

Amaral FC, Torres N, Saggioro F, Neder L, Machado HR, Silva WA Jr, Moreira AC, Castro M (2008) MicroRNAs differentially expressed in ACTH-secreting pituitary tumors. J Clin Endocrinol Metab 94:320–323

Backert S, Gelos M, Kobalz U, Hanski ML, Böhm C, Mann B, Lövin N, Gratchev A, Mansmann U, Moyer MP, Riecken EO, Hanski C (1999) Differential gene expression in colon carcinoma cells and tissues detected with a cDNA array. Int J Cancer 82:868–874

Bottoni A, Zatelli MC, Ferracin M, Tagliati F, Piccin D, Vignali C, Calin GA, Negrini M, Croce CM, Degli Uberti EC (2007) Identification of differentially expressed microRNAs by microarray: a possible role for microRNA genes in pituitary adenomas. J Cell Physiol 210:370–373

Butz H, Likó I, Czirják S, Igaz P, Khan MM, Zivkovic V, Bálint K, Korbonits M, Rácz K, Patócs A (2010) Down-regulation of Wee1 kinase by a specific subset of microRNA in human sporadic pituitary adenomas. J Clin Endocrinol Metab 95:E181–E191

Butz H, Likó I, Czirják S, Igaz P, Korbonits M, Rácz K, Patócs A (2011) microRNA profile indicates downregulation of the TGFβ pathway in sporadic nonfunctioning pituitary adenomas. Pituitary. 14(2):112–124. doi:10.1007/s11102-010-0268-x

Cheng AM, Byrom MW, Shelton J, Ford LP (2005) Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res 33:1290–1297

Dworakowska D, Grossman AB (2009) The pathophysiology of pituitary adenomas. Best Pract Res Clin Endocrinol Metab 23:525–541

Ebi N, Semba H, Tokunaga S, Takayama K, Wataya H, Kuraki T, Yamamoto H, Akamine S, Okamoto I, Nakanishi Y (2008) A phase II trial of gefitinib monotherapy in chemotherapy–naive patients of 75 years or older with advanced non-small cell lung cancer. J Thorac Oncol 3(10):1166–1171

Faraoni I, Antonetti FR, Cardone J, Bonmassar E (2009) miR-155 gene: a typical multifunctional microRNA. Biochim Biophys Acta 1792:497–505

Franklin DS, Godfrey VL, O'Brien DA, Deng C, Xiong Y (2000) Functional collaboration between different cyclin-dependent kinase inhibitors suppresses tumor growth with distinct tissue specificity. Mol Cell Biol 20:6147–6158

Jansson M, Durant ST, Cho EC, Sheahan S, Edelmann M, Kessler B, La Thangue NB (2008) Arginine methylation regulates the p53 response. Nat Cell Biol 10:1431–1439

Katayama K, Fujita N, Tsuruo T (2005) Akt/protein kinase B-dependent phosphorylation and inactivation of WEE1Hu promote cell cycle progression at G2/M transition. Mol Cell Biol 25:5725–5737

Kawabe T (2004) G2 checkpoint abrogators as anticancer drugs. Mol Cancer Ther 3:513–519

Koniaras K, Cuddihy AR, Christopoulos H, Hogg A, O'Connell MJ (2001) Inhibition of Chk1-dependent G2 DNA damage checkpoint radiosensitizes p53 mutant human cells. Oncogene 20:7453–7563

Krol J, Loedige I, Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11:597–610

Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. Science 294:853–858

Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15–20

Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, Patrawala L, Yan H, Jeter C, Honorio S, Wiggins JF, Bader AG, Fagin R, Brown D, Tang DG (2011) The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med 17:211–215

Malumbres M, Barbacid M (2005) Mammalian cyclin-dependent kinases. Trends Biochem Sci 30:630–641

McGowan CH, Russell P (1993) Human Wee1 kinase inhibits cell division by phosphorylating p34cdc2 exclusively on Tyr15. EMBO J 12:75–85

Michael WM, Newport J (1998) Coupling of mitosis to the completion of S phase through Cdc34-mediated degradation of Wee1. Science 282:1886–1889

Mir SE, De Witt Hamer PC, Krawczyk PM, Balaj L, Claes A, Niers JM, Van Tilborg AA, Zwinderman AH, Geerts D, Kaspers GJ, Peter Vandertop W, Cloos J, Tannous BA, Wesseling P, Aten JA, Noske DP, Van Noorden CJ, Würdinger T (2010) In silico analysis of kinase expression identifies WEE1 as a gatekeeper against mitotic catastrophe in glioblastoma. Cancer Cell 18:244–257

Mizuarai S, Yamanaka K, Itadani H, Arai T, Nishibata T, Hirai H, Kotani H (2009) Discovery of gene expression-based pharmacodynamic biomarker for a p53 context-specific anti-tumor drug Wee1 inhibitor. Mol Cancer 8:34

Mizuno H, Nakamura A, Aoki Y, Ito N, Kishi S, Yamamoto K, Sekiguchi M, Takeda S, Hashido K (2011) Identification of muscle-specific microRNAs in serum of muscular dystrophy animal models: promising novel blood-based markers for muscular dystrophy. PLoS One 6:e18388

Morgan DO (1995) Principles of CDK regulation. Nature 374:131–134

O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT (2005) c-Myc-regulated microRNAs modulate E2F1 expression. Nature 435:839–843

Pais H, Nicolas FE, Soond SM, Swingler TE, Clark IM, Chantry A, Moulton V, Dalmay T (2010) Analyzing mRNA expression identifies Smad3 as a microRNA-140 target regulated only at protein level. RN A 16:489–494

Pickering MT, Stadler BM, Kowalik TF (2009) miR-17 and miR-20a temper an E2F1-induced G1 checkpoint to regulate cell cycle progression. Oncogene 28:140–145

Qi J, Yu JY, Shcherbata HR, Mathieu J, Wang AJ, Seal S, Zhou W, Stadler BM, Bourgin D, Wang L, Nelson A, Ware C, Raymond C, Lim LP, Magnus J, Ivanovska I, Diaz R, Ball A, Cleary MA, Ruohola-Baker H (2009) microRNAs regulate human embryonic stem cell division. Cell Cycle 8:3729–3741

Qian ZR, Asa SL, Siomi H, Siomi MC, Yoshimoto K, Yamada S, Wang EL, Rahman MM, Inoue H, Itakura M, Kudo E, Sano T (2009) Overexpression of HMGA2 relates to reduction of the let-7 and its relationship to clinicopathological features in pituitary adenomas. Mod Pathol 22:431–441

Santarelli L, Strafella E, Staffolani S, Amati M, Emanuelli M, Sartini D, Pozzi V, Carbonari D, Bracci M, Pignotti E, Mazzanti P, Sabbatini A, Ranaldi R, Gasparini S, Neuzil J, Tomasetti M (2011) Association of MiR-126 with soluble mesothelin-related peptides, a marker for malignant mesothelioma. PLoS One 6:e18232

Watanabe N, Arai H, Nishihara Y, Taniguchi M, Watanabe N, Hunter T, Osada H (2004) M-phase kinases induce phospho-dependent ubiquitination of somatic Wee1 by SCFbeta-TrCP. Proc Natl Acad Sci U S A 101:4419–4424

Watanabe N, Arai H, Iwasaki J, Shiina M, Ogata K, Hunter T, Osada H (2005) Cyclin-dependent kinase (CDK) phosphorylation destabilizes somatic Wee1 via multiple pathways. Proc Natl Acad Sci U S A 102:11663–11668

Zhan X, Desiderio DM (2010) Signaling pathway networks mined from human pituitary adenoma proteomics data. BMC Med Genomics 3:13

Zhang CC, Boritzki TJ, Jackson RC (1998) An inhibitor of glycinamide ribonucleotide formyltransferase is selectively cytotoxic to cells that lack a functional G1 checkpoint. Cancer Chemother Pharmacol 41:223–228

Zhang Y, Liao Y, Wang D, He Y, Cao D, Zhang F, Dou K (2011) Altered expression levels of miRNAs in serum as sensitive biomarkers for early diagnosis of traumatic injury. J Cell Biochem 112(9):2435–2442. doi:10.1002/jcb.23168

Pituitary Tumor Cells: Role of PKCα, PKCδ and PKCε Expression

17

Juan Pablo Petiti and Alicia Inés Torres

Contents

Abstract

The PKC family is involved in a wide variety of cellular processes such as proliferation, senescence and cell death, which are determined by the specific subcellular targeting of these kinases.

In adenomatous pituitary cells, it has been observed that the enzyme activity and expression of PKCs were higher than in normal pituitary. The isozymes PKCα and PKCε are usually involved in tumorigenesis and are the most expressed in human pituitary adenomas. The specific PKCα and PKCε activation is closely associated with the tumoral pituitary cell proliferation and cell cycle progression through the ERK 1/2 pathway. By contrast, PKCδ has been shown to mediate anti-proliferative and apoptotic signals. In the regression of pituitary tumors triggered by bromocriptine the PKCδ /p38 pathway is involved in a non-apoptotic mechanism identified as parapoptosis.

Each individual PKC isozyme is undoubtedly an attractive target for therapeutic intervention, given its role in survival and cell death in pituitary tumors, processes that contribute to the onset and progression of the tumorigenesis. The combination of specific inhibitors of PKC and the "upstream-downstream" kinases of the signalling pathways with conventional antitumoral drugs could lead to a better tolerance and effectiveness in the regression of pituitary tumors.

J.P. Petiti (✉) • A.I. Torres
Centro de Microscopía Electrónica, Facultad de Ciencias Médicas, Universidad de Córdoba,
5000 Córdoba, Argentina
e-mail: jpetiti@cmefcm.uncor.edu

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_17,

Introduction

In all multicellular organisms, highly coordinated mechanisms are needed to maintain a stable number of cells in an organization of tissues and organs. There is a delicate balance between the rate of proliferation and cell death, and an increased number of cells can occur as a result of a change in the signal transduction pathways that regulate these two essential processes. This can take place at different levels: in receptors, in the signaling pathways, and in gene expression, with alterations at any of these levels possibly causing a normal cell to become cancerous (Kazanietz and Blumberg 1996).

Pituitary adenomas are common benign monoclonal neoplasms accouting for approximately 15% of intracranial tumors. Although most are manageable or have clinically indolent courses, several subsets of the tumor can cause significant morbidity and early mortality. In recent years, the demonstration that pituitary adenomas are monoclonal in origin has provided further evidence that pituitary neoplasia arises from the replication of a single mutated cell, in which growth results from either inappropriate expression, the activation of proto-oncogenes, or the inactivation of tumor suppressor genes. Among the common proto-oncogenes that have been analyzed in pituitary tumors are the proteins involved in proliferative signal transduction, such as Protein Kinase C (PKC) (Vandeva et al. 2010).

PKC isozymes have been grouped into three subclasses, according to their regulatory properties, which are defined by specific domains in the proteins. The conventional or classical PKCs include PKCα, βI, βII, and γ, and are activated by Ca^{2+} and/or by diacylglycerol (DAG) and phorbol esters. The novel PKCs, δ, ε, θ, and η, which are Ca^{2+} independent, can also be activated by DAG and phorbol esters. Finally, the atypical PKCs, which include PKCζ and PKCι, are unresponsive to both Ca^{2+} and DAG/phorbol esters (Ohno and Nishizuka 2002). The PKCs are the main mediators of phorbol ester tumor promoters, such as phorbol 12-myristate 13-acetate (PMA), with individual PKC isozymes displaying differential sensitivity (MacEwan et al. 1999).

The PKC family is involved in a wide variety of cellular processes such as proliferation, senescence and cell death (Ohno and Nishizuka 2002). This chapter will address the roles of PKCα and PKCε in pituitary tumor cells, given that both proteins are closely related to the regulation of cell proliferation. Moreover, these enzymes are usually involved in tumorigenesis (Griner and Kazanietz 2007) and are the most expressed isozymes in human pituitary adenomas (Jin et al. 1993).

Expression of PKCα and PKCε in Pituitary Cells

It has been demonstrated that PKCα, β, δ, ε, ζ and θ are expressed in the pituitary gland as well as in the tumor pituitary GH3 cells (MacEwan et al. 1999). The activations of PKCs in pituitary cells, has been associated with the regulation of cell proliferation (Petiti et al. 2008), and cell death (Palmeri et al. 2009), depending on the type of stimulus applied to the particular cell as well as the specific PKC isozyme.

In adenomatous pituitary cells, it has been observed that the enzyme activity and expression of PKCs were higher than in normal human or rat pituitaries. Furthermore, these levels were found to be significantly higher in invasive tumors than in non-invasive tumors (Alvaro et al. 1992). Immunohistochemical and *in situ* hybridization analysis of the calcium-dependent PKCα, β, and γ has revealed that PKCα was the predominant isozyme in normal and neoplastic human pituitary adenoma cells. In addition, the localization of the calcium-independent PKCδ, ε and ζ by *in situ* hybridization showed that PKCε mRNA was abundantly expressed in human pituitary adenomas (Jin et al. 1993).

Although PKC mutations are rare, a single point mutation in PKCα was observed in invasive pituitary tumors. It was also reported that this PKCα mutant was unable to bind tightly to cellular membranes and that it fails to transduce several antitumorigenic signals. In addition, it seems that the PKCα mutant can influence the progression of endocrine tumors by severing the transduction of extracellular

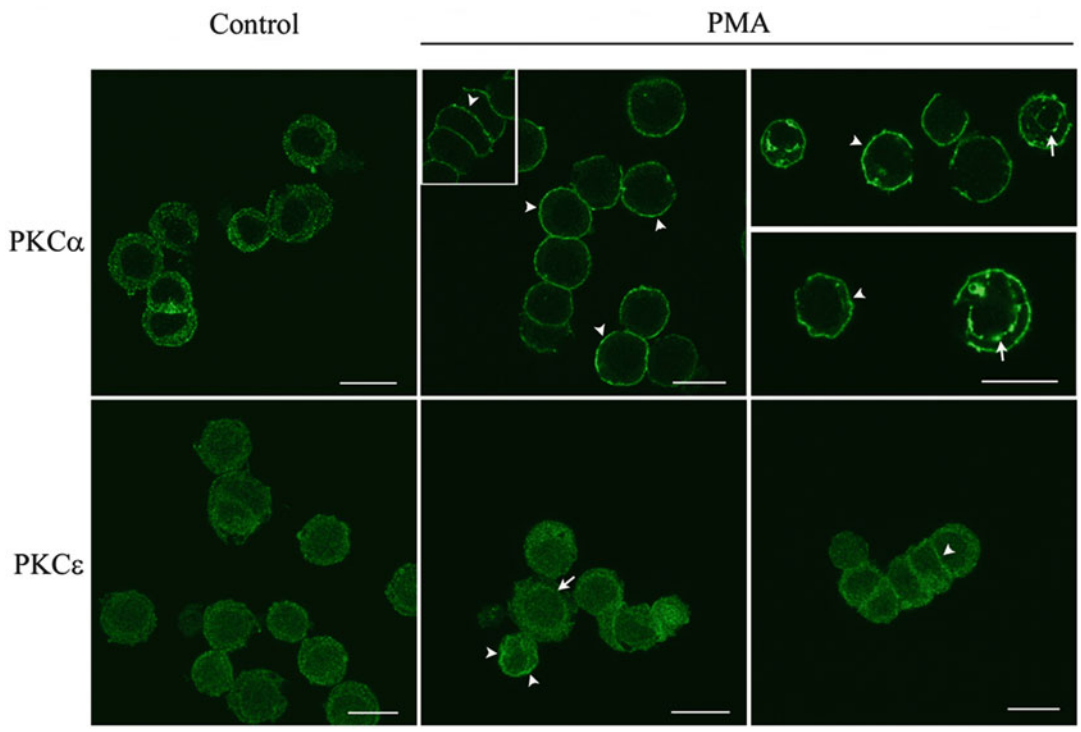

Fig. 17.1 Targeting of endogenous PKCα and PKCε in pituitary tumor GH3B6 cells. The cells were stimulated with or without PMA for 15 min, and the PKC isozymes localization was analyzed by confocal laser scanning microscopy. The phorbol ester stimuli induced PKCα and PKCε translocation from cytoplasm to plasma (*arrowhead*) and nuclear (*arrows*) membranes. *Inset*: the tumoral cells show the PKCα accumulated at the cell-cell contact after PMA incubation. The photographs correspond to a representative experiment from a total of three which had similar results. Bar = 20 μm (Petiti et al. 2009)

signals, which are pivotal in the suppression of tumor growth and in the enhancement of apoptosis of cancer cells (Zhu et al. 2005).

Subcellular Localization of PKCα and PKCε in Pituitary Cells

Over recent years, it has become clear that, upon activation by different stimuli, each PKC isozyme is translocated or targeted to specific intracellular compartments, including the plasma membrane, Golgi complex, mitochondria and cell nucleus (Akita 2002). Several reports have also demonstrated that PMA induces the translocation of PKC isozymes to a specific cellular compartment. For instance, in pancreatic cancer cells, phorbol ester treatment resulted in transient PKCα activation accompanied by the translocation of the enzyme into membrane and nuclear compartments, effects that were correlated with the regulation of cell cycle progression (Detjen et al. 2000). The novel PKCε has also been reported to translocate from the cytosol to the plasma membrane in CHO-K1 cells stimulated with PMA (Shirai et al. 1998).

The subcellular localization of specific PKC isozymes is a key component in determining their function and regulation. Recently, it has been reported that the proliferation of normal and tumoral lactotrophs is closely associated with the specific PKCα and PKCε plasma membrane localizations and with the activation of the ERK1/2 pathway after phorbol ester treatment. An interesting finding observed was the translocation of PKCα and PKCε to the nuclear membrane after PMA treatment, which was even more striking in tumoral lactotroph cells (Figs. 17.1

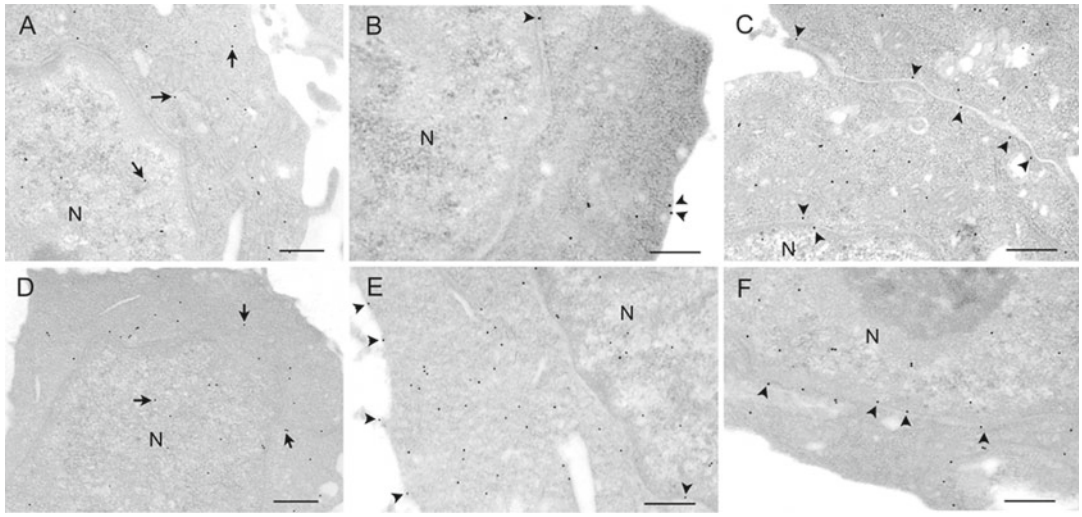

Fig. 17.2 Immuno-electron labelling for PKCα and PKCε in pituitary tumor GH3B6 cells. In control cells, PKCα (**a**) and PKCε (**d**) immunoreactivities are distributed throughout the cytoplasm and nucleus (*N*) but not associated with specific organelles (*arrows*). The PMA-treated tumoral cells display immunogold particles for PKCα (**b–c**) and PKCε (**e–f**) attached to the plasma membrane and nuclear envelope (*arrowhead*). Bar = 1 μm (Petiti et al. 2009)

and 17.2). This result may be associated with the mitogenic response to phorbol ester, suggesting the participation of these isozymes in this cellular process (Petiti et al. 2009).

These investigations indicate the existence of a close correlation between the subcellular localization of PKC isozymes with their different biological functions, revealing that these localizations are not only isotype or cell line specific, but also stimuli specific. The specific subcellular targeting of PKCα and PKCε could be indicative of the role of these kinases in the regulation of the activity of normal and tumoral lactotroph cells.

PKCα and PKCε in the Proliferation of Tumoral Pituitary Cells

Cell and tissue homeostasis depends on a highly coordinated network of signaling pathways, resulting in the spatial and temporal balance of proliferation, growth arrest, differentiation and apoptosis. The aberrant regulation of any of these biological processes may contribute to the onset and progression of tumorigenesis. This is critically connected with the participation of PKC isozymes (Basu and Sivaprasad 2007), which activate a wide variety of signaling cascades, depending on the cell type, the stimulus and the isoenzyme involved. The mitogen-activated protein kinase (MAPK) cascade, which involves Raf, mitogen-activated kinase effector kinase (MEK) and extracellular-signal regulated kinase (ERK) 1/2, it is ubiquitously expressed in mammalian cells and is implicated in the regulation of cell proliferation as well as cell death (Krishna and Narang 2008). There is also evidence that in various cell types the ERK/MAPK pathway can be activated in either a PKC-dependent or PKC-independent manner in response to different stimulus (Qiu and Leslie 1994).

Pituitary tumors may originate from either functional mutations or the overexpression of the ubiquitously expressed proto-oncogenes that are components of common proliferative pathways. Abnormalities in the expression of membrane and nuclear receptors, growth factors, transcription factors, and their signalling proteins have been proposed to play a relevant role in cell transformation and/or clonal expansion (Spada et al. 2007).

In previous studies, we have confirmed the involvement of PKC isozymes in the mitogenesis and cell death of normal and tumoral pituitary cells (Petiti et al. 2008; Palmeri et al. 2009). Also, we demonstrated that the activation of PKCs by phorbol ester was required to induce GH3B6 pituitary adenoma cell proliferation (Petiti et al. 2010). Although, the molecular mechanism by which PKCs may up-regulate cell proliferation in pituitary adenomas is still not well understood. The ERK1/2 pathway has emerged as a central regulator of cell proliferation, which controls both cell growth and cell cycle progression (Krishna and Narang 2008). Recently, it has been observed that ERK1/2 are downstream targets of PKCs, which mediate the proliferative response of GH3B6 cells to PMA (Petiti et al. 2010).

Despite PKCs having a clear role in tumorigenesis, it has been a challenging task to determine the relative contribution of the individual isozymes, in order to define their specific roles in this process (Griner and Kazanietz 2007). PKCε seems to be involved in tumor development, tumor cell invasion, and metastasis in several tissues (Basu and Sivaprasad 2007), and PKCα has been described to increase the cell proliferation in tumor cells, with this effect being mediated by ERK1/2 activation (Haughian and Bradford 2009). Therefore, a greater knowledge of the roles of the individual PKC isozymes in tumor development is necessary before being able to propose the use of PKC as a therapeutic target.

Related to the contribution of different isozymes in the proliferation of pituitary tumor cells, we reported that PKCα and PKCε regulate the proliferation of pituitary adenomatous cells, with the participation of these PKC isozymes on the GH3B6 cell growth being confirmed by the use of the PKCα (Gö6976) and PKCε (εV1-2) inhibitors, which were able to block the mitogenic activity induced by PMA (Petiti et al. 2010). In a previous investigation using normal pituitary cells, it was also observed that phorbol ester treatment induced the early plasma membrane translocation and activation of PKCε promoting cell proliferation (Petiti et al. 2008). However, using GH3B6 pituitary tumor cells it was demonstrated that besides the participation of PKCε, the isoenzyme PKCα also was involved in the regulation of cell proliferation induced by phorbol ester (Petiti et al. 2010). These above studies clearly demonstrate that PKCα and PKCε are attractive therapeutic targets for the control of pituitary adenoma growth. In support of this assumption, it has been previously reported in pituitary tumor HP75 cells that PMA increased the tumoral invasion, a process that was blocked by the inhibition of PKCα (Hussaini et al. 2007).

PKCδ Involved in Pituitary Tumor Cell Death

From the vast amount of PKC-related literature that has been generated, it is now clear that PKCs are implicated in modulating almost all aspects of tumorigenesis. However, many areas in the field still remain to be explored, particularly those related to heterogeneity in functional responses conferred by distinct PKC isozymes and the cell-type dependency of these effects. In this regard, there has been more focus on the opposing roles generally observed for PKCs in the proliferation, survival and promotion of a cancer-cell phenotype. For example, there is growing evidence to support a role for PKCα and PKCε in promoting cell survival (Basu and Sivaprasad 2007; Haughian and Bradford 2009). Whereas, PKCδ has been shown to convey anti-proliferative and apoptotic signals in various cell types. In addition, the suppression of PKCδ expression or activity induces a transformed phenotype or is associated with tumour growth (Jackson and Foster 2004).

It has been well established that numerous apoptotic stimuli can lead to PKCδ activation through proteolysis of the hinge region, thus generating a C-terminal catalytic fragment of 40 KDa. In GH3B6 pituitary adenoma cells was demonstrated that the proteolytic activation of PKCδ played an important role in the programmed cell death (Leverrier et al. 2002). The finding that PKCs are involved in apoptosis introduces the possibility of targeting PKCs

during the therapeutic intervention of diseases, where there is deregulation in the processes of cell survival and cell death such as cancer. Bromocriptine (Bc) has been used in the treatment of human PRL-secreting pituitary adenomas, with cytotoxic and antiproliferative effects on pituitary cells causing pituitary tumoral mass regression (Stefaneanu et al. 2000). Although prolactinomas respond to Bc, intolerance and resistance to dopaminergic agonists have also been reported (Delgrange et al. 2005). Related to this, focusing the search for treatments on those promoting cell death with minimum side effects has been the objective in several basic investigations in order to optimize the effectiveness of antitumoral treatment.

In a recent study, we identified the non-apoptotic mechanism parapoptosis to be the predominant cell death type involved in the regression of estrogen induced pituitary tumors by Bc treatment. In this prolactinoma experimental model, Bc induced a significant increase in the expression of the 78 kDa PKCδ holoenzyme, and its 40 kDa catalytic fragment in the nuclear fraction was correlated with the PKCδ translocation to the nuclear compartment of pituitary cells at different involutive and dying stages, indicative of PKCδ activation. Moreover, Bc treatment was directly related to the increase in the phosphorylation of the ERK1/2 and p38 levels in nuclear fraction. The occurrence of parapoptosis triggered by Bc involve the participation of the PKCδ, ERK 1/2, and p38 signaling pathways (Palmeri et al. 2009).

Further investigation into the molecular mechanisms of non-apoptotic cell death should yield fresh insights into their roles in physiological cell death and their potential for manipulation by new anti-tumoral drugs.

PKCα and PKCε Regulation of the Cell Cycle of Tumor Pituitary Cells

In recent years, it has become increasingly evident that PKCs can impact on the cell cycle in either a positive or negative way, depending on the stimuli, the cell type and isozyme specificity. Indeed, PKC isozymes have been shown to regulate the progression of cells from the G1 to S phase as well as the transition from the G2 to M phase (Fishman et al. 1998). Molecular analysis of human pituitary neoplasias has shown that cell cycle desregulation is significantly implicated in pituitary tumorigenesis, with an important number of genetic and epigenetic alterations occurring in pituitary tumors, involving specific regulators of the cell cycle (Quereda and Malumbres 2009).

Evidence has also accumulated supporting the idea that the effect of phorbol esters on cell proliferation is determined by the ability of PKC isozymes to regulate the cell cycle machinery. In pituitary tumor cells, it has been observed that activation of PKCα and PKCε increased the percentage of GH3B6 pituitary adenoma cells in the proliferative fraction (S + G2/M), suggesting that both PKC isozymes contribute to the regulation of the pituitary tumor cell cycle (Petiti et al. 2010). In recent years, it has also been demonstrated that the PKCε inhibition blocked G1/S transition by the accumulation of cells in the G0/G1 phase in non-small cell lung cancer cells (Bae et al. 2007). In T24 urinary bladder carcinoma cells, it was shown that PKC alpha inhibitor (Go6976) induces dephosphorylation of retinoblastoma, with this active form being able to arrest the cell cycle (Aaltonen and Peltonen 2010).

Taking into account that more than 80%of pituitary tumors display alterations in at least in one of the cell cycle regulators during G1/S transition (Simpson et al. 2001) and that PKCs are involved in tumor cell cycle regulation, the testing of selective PKC inhibitors as anticancer drugs could be proposed for clinical trials. In addition, a better knowledge of the specific genetic and epigenetic alterations in human patients is necessary in order to be able to select the best combination of current treatments or to propose new therapeutic approaches. Current and future genetic models should increase our understanding of the pituitary disorders and permit evaluation of new therapies before their use in the Clinic.

Targeting PKCα and PKCε in Tumor Cells

Signal transduction is a fundamental process of living cells. It encompasses a multitude of interacting and cross-talking molecular cascades and chain reactions that, when in good order, result in a cell and healthy organism. However, if these cascades are perturbed, in the wrong way, or are different due to mutations, then the pathways can go awry, and a disease state may ensue. Enormous progress has been made in deciphering these cascades and in correlating them with diseases. Novel drug targets have been identified within cells, as opposed to only surface receptors, which have been the mainstay of drug discovery for a long time (Persidis 1998).

Although targeted therapy has shown potent antitumor activity in a variety of solid tumors, including breast, colon, and lung cancer, resistance to these agents requires the appraisal of novel molecular targeted therapeutics, including drugs that interfere with signal transduction for cell survival or cell death such as the PKC signalling pathway (Serova et al. 2006).

The PKC family is undoubtedly an attractive target for therapeutic intervention, given its role in tumorigenesis and its potential for enhancing the cytotoxicity of existing drugs. Recently, it has been reported that enzastaurin (LY317615), a PKCβ-selective inhibitor, in combination with other drugs showed a good tolerance with preliminary evidence of anticancer activity, particularly in thyroid cancer (Hanauske et al. 2009).

PEP005 (ingenol-3-angelate) is a novel anticancer agent that was previously shown to modulate PKC, resulting in antiproliferative and proapoptotic effects in several human cancers (Benhadji et al. 2008). In Colo205 colon cancer cells, exposure to PEP005 induced translocation of PKCδ from the cytosol to the nucleus and other cellular membranes, and activation of Raf1, ERK1/2, c-Jun NH2-terminal kinase and p38. Interestingly this agent also reduced expression of PKCα. These data suggest that PEP005 -induced activation of PKCδ and reduced expression of PKCα resulted in the apoptosis and decrease of cells in the S phase of the cell cycle by mechanisms mediated by activation of Ras/Raf/MAPK signaling pathways (Serova et al. 2008).

Although the extrapolation of the drug effects from one cell type to another is not always justified, the action of PEP005 on PKC isozymes could be considered in the context of pituitary tumor cells, bearing in mind that in these cells PKCα is pro-mitogenic and PKCδ is related to cell death (Petiti et al. 2010; Palmeri et al. 2009). Therefore, the expected effects of this drug on pituitary tumors would be the proliferative cell inhibition by decreasing the expression of PKCα, and also the induction of cell death through the activation of PKCδ /p38.

To date, there are few reports that inform on the specific PKC isozymes implicated in pituitary tumorigenesis and drug resistance. Therefore, a deeper understanding of PKC family expression could help in producing predictive biomarkers to optimize the diagnosis and treatment of pituitary disorders. The combination of specific inhibitors of PKC and the "upstream-downstream" kinases of the signalling pathways with conventional cytotoxic could lead to a better tolerance and effectiveness in the regression of pituitary tumors. In addition, future studies about the effects of drugs that selectively inhibit PKCs/MAPK pathways might produce significant advances in the treatment of pituitary diseases.

References

Aaltonen V, Peltonen J (2010) PKCalpha/beta I inhibitor Go6976 induces dephosphorylation of constitutively hyperphosphorylated Rb and G1 arrest in T24 cells. Anticancer Res 30:3995–3999

Akita Y (2002) Protein kinase C-epsilon (PKC-epsilon): its unique structure and function. J Biochem 132:847–852

Alvaro V, Touraine P, Raisman VR, Bai-Grenier F, Birman P, Joubert D (1992) Protein kinase C activity and expression in normal and adenomatous human pituitaries. Int J Cancer 50:724–730

Bae KM, Wang H, Jiang G, Chen MG, Lu L, Xiao L (2007) Protein kinase C epsilon is overexpressed in primary human non-small cell lung cancers and functionally required for proliferation of non-small cell lung cancer cells in a p21/Cip1-dependent manner. Cancer Res 67:6053–6063

Basu A, Sivaprasad U (2007) Protein kinase Cepsilon makes the life and death decision. Cell Signal 19: 1633–1642

Benhadji KA, Serova M, Ghoul A, Cvitkovic E, Le Tourneau C, Ogbourne SM, Lokiec F, Calvo F, Hammel P, Faivre S, Raymond E (2008) Antiproliferative activity of PEP005, a novel ingenol angelate that modulates PKC functions, alone and in combination with cytotoxic agents in human colon cancer cells. Br J Cancer 99:1808–1815

Delgrange E, Sassolas G, Perrin G, Jan M, Trouillas J (2005) Clinical and histological correlations in prolactinomas, with special reference to bromocriptine resistance. Acta Neurochir (Wien) 147:751–757

Detjen KM, Brembeck FH, Welzel M, Kaiser A, Haller H, Wiedenmann B, Rosewicz S (2000) Activation of protein kinase C alpha inhibits growth of pancreatic cancer cells via p21(cip)-mediated G(1) arrest. J Cell Sci 113:3025–3035

Fishman DD, Segal S, Livneh E (1998) The role of protein kinase C in G1 and G2/M phases of the cell cycle (review). Int J Oncol 12:181–186

Griner EM, Kazanietz MG (2007) Protein kinase C and other diacylglycerol effectors in cancer. Nat Rev Cancer 7:281–294

Hanauske AR, Lahn M, Musib LC, Weigang-Köhler K, Yilmaz E, Graefe T, Kuenen B, Thornton D, McNealy P, Giaccone G (2009) Phase Ib safety and pharmacokinetic evaluation of daily and twice daily oral enzastaurin in combination with pemetrexed in advanced/metastatic cancer. Ann Oncol 20:1565–1575

Haughian JM, Bradford AP (2009) Protein kinase C alpha (PKCalpha) regulates growth and invasion of endometrial cancer cells. J Cell Physiol 220:112–118

Hussaini IM, Trotter C, Zhao Y, Abdel-Fattah R, Amos S, Xiao A, Agi CU, Redpath GT, Fang Z, Leung GK, Lopes MB, Laws ER (2007) Matrix metalloproteinase-9 is differentially expressed in nonfunctioning invasive and noninvasive pituitary adenomas and increases invasion in human pituitary adenoma cell line. Am J Pathol 170:356–365

Jackson DN, Foster DA (2004) The enigmatic protein kinase Cdelta: complex roles in cell proliferation and survival. FASEB J 18:627–636

Jin L, Maeda T, Chandler WF, Lloyd RV (1993) Protein kinase C (PKC) activity and PKC messenger RNAs in human pituitary adenomas. Am J Pathol 142:569–578

Kazanietz MG, Blumberg PM (1996) Protein kinase C and signal transduction in normal and neoplastic cells. In: Sirica AE (ed) Cellular and molecular pathogenesis. Raven, New York, pp 389–402

Krishna M, Narang H (2008) The complexity of mitogen-activated protein kinases (MAPKs) made simple. Cell Mol Life Sci 65:3525–3544

Leverrier S, Vallentin A, Joubert D (2002) Positive feedback of protein kinase C proteolytic activation during apoptosis. Biochem J 368:905–913

MacEwan DJ, Johnson MS, Mitchell R (1999) Protein kinase C isoforms in pituitary cells displaying differential sensitivity to phorbol ester. Mol Cell Biochem 202:85–90

Ohno S, Nishizuka Y (2002) Protein kinase C isotypes and their specific functions: prologue. J Biochem 132:509–511

Palmeri CM, Petiti JP, Sosa LV, Gutiérrez S, De Paul AL, Mukdsi JH, Torres AI (2009) Bromocriptine induces parapoptosis as the main type of cell death responsible for experimental pituitary tumor shrinkage. Toxicol Appl Pharmacol 240:55–65

Persidis A (1998) Signal transduction as a drug-discovery platform. Nat Biotechnol 16:1082–1083

Petiti JP, De Paul AL, Gutiérrez S, Palmeri CM, Mukdsi JH, Torres AI (2008) Activation of PKC epsilon induces lactotroph proliferation through ERK1/2 in response to phorbol ester. Mol Cell Endocrinol 283:77–84

Petiti JP, Gutiérrez S, Mukdsi JH, De Paul AL, Torres AI (2009) Specific subcellular targeting of PKCalpha and PKCepsilon in normal and tumoral lactotroph cells by PMA-mitogenic stimulus. J Mol Histol 40:417–425

Petiti JP, Gutiérrez S, De Paul AL, Andreoli V, Palmeri CM, Sosa LV, Bocco JL, Torres AI (2010) GH3B6 pituitary tumor cell proliferation is mediated by PKCalpha and PKCepsilon via ERK 1/2-dependent pathway. Cell Physiol Biochem 26:135–146

Qiu ZH, Leslie CC (1994) Protein kinase C-dependent and -independent pathways of mitogen-activated protein kinase activation in macrophages by stimuli that activate phospholipase A2. J Biol Chem 269:19480–19487

Quereda V, Malumbres M (2009) Cell cycle control of pituitary development and disease. J Mol Endocrinol 42:75–86

Serova M, Ghoul A, Benhadji KA, Cvitkovic E, Faivre S, Calvo F, Lokiec F, Raymond E (2006) Preclinical and clinical development of novel agents that target the protein kinase C family. Semin Oncol 33:466–478

Serova M, Ghoul A, Benhadji KA, Faivre S, Le Tourneau C, Cvitkovic E, Lokiec F, Lord J, Ogbourne SM, Calvo F, Raymond E (2008) Effects of protein kinase C modulation by PEP005, a novel ingenol angelate, on mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling in cancer cells. Mol Cancer Ther 7:915–922

Shirai Y, Sakai N, Saito N (1998) Subspecies-specific targeting mechanism of protein kinase C. Jpn J Pharmacol 78:411–417

Simpson DJ, Frost SJ, Bicknell JE, Broome JC, McNicol AM, Clayton RN, Farrell WE (2001) Aberrant expression of G(1)/S regulators is a frequent event in sporadic pituitary adenomas. Carcinogenesis 22:1149–1154

Spada A, Lania A, Mantovani G (2007) Hormonal signaling and pituitary adenomas. Neuroendocrinology 85:101–109

Stefaneanu L, Kovacs K, Scheithauer BW, Kontogeorgos G, Riehle DL, Sebo TJ, Murray D, Vidal S, Tran A, Buchfelder M, Fahlbusch R (2000) Effect of dopamine agonists on lactotroph adenomas of the human pituitary. Endocr Pathol 11:341–352

Vandeva S, Jaffrain-Rea ML, Daly AF, Tichomirowa M, Zacharieva S, Beckers A (2010) The genetics of pituitary adenomas. Best Pract Res Clin Endocrinol Metab 24:461–476

Zhu Y, Dong Q, Tan BJ, Lim WG, Zhou S, Duan W (2005) The PKCalpha-D294G mutant found in pituitary and thyroid tumors fails to transduce extracellular signals. Cancer Res 65:4520–4524

Pituitary Adenoma: Role of HMGA Proteins

18

Monica Fedele and Alfredo Fusco

Contents

Abstract

Pituitary tumors are in most cases monoclonal adenomas arising from the adenohypophysial cells, and represent about 15% of intracranial tumors. They, while benign tumors, show a significant morbidity related to the endocrinological symptoms of hypo- or hyper-secretion of hormones and/or mass effect of the tumor on adjacent brain structures. Despite their considerable social impact, yet relatively little is known about the molecular events underlying pituitary tumorigenesis. Recent studies have demonstrated that the *High Mobility Group A (HMGA)* gene family, including *HMGA1* and *HMGA2*, has a critical role in the development of pituitary adenomas. Indeed, the *HMGA2* gene is amplified and overexpressed in several pituitary adenomas, and, consistently, transgenic mice overexpressing either Hmga1 or Hmga2 develop pituitary adenomas. The overexpression of the HMGA proteins would induce pituitary adenomas mainly by enhancing the activity of the transcription factor E2F1 and by upregulating the expression of cyclin B2 and other genes involved in the regulation of the cell cycle.

Introduction

The tumors of the anterior pituitary gland account for up to 15% of diagnosed brain tumors, while occult adenomas are discovered in as many as 25% of unselected autopsies, and are the second most

M. Fedele (✉) • A. Fusco
Instituto di Endocrinologia Sperimentale (IEOS) del CNR, Naples c/o Dipartimento di Biologia e Patologia Cellulare e Molecolare, CNR and Università degli Studi di Napoli "Federico II", Naples, Italy
e-mail: mfedele@unina.it; alfusco@unina.it

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_18,

common type of intracranial tumor by histology in young adults in the 20–34 years age range. Although they are generally benign and rarely progress to carcinomas, a significant proportion of pituitary adenomas (PA) show invasive and/or recurrent growth characteristics (Heaney and Melmed 2004). For this and the clinical consequences of hyper- or hypo-secretion of hormones, as well as the mass effect on adjacent brain structures, PA have a significant social impact.

As recently reported in a large study in the province of Liege, Belgium, most of PA are prolactinomas (66.2%), followed by non-functional tumors (NFPA) (14.7%), somatotropinomas (13.2%) and corticotroph adenomas (5.9%) (Daly et al. 2006). With the sole exception of somatotroph adenomas, 40% of which are associated with the activating mutation of the *gsp* oncogene (Landis et al. 1989), little is known about the genetic defects leading to pituitary tumor formation, which likely involves multiple initiating and promoting factors. Indeed, a number of alterations in the expression of growth factors and their receptors, cell cycle regulation and signal transduction pathways, specific hormonal factors or other molecules with still unclear mechanism of action have been reported in human PA and sometimes supported by transgenic or knockout animal models (Asa and Ezzat 2009). However, it is not clear yet which pathogenic changes are specifically associated with the different PA sub-types, and whether these aberrations are an initial or late event in tumorigenesis. Here, we focus on the recently discovered role in PA development of a group of chromatinic proteins, the HMGA proteins.

The High Mobility Group A (HMGA) Proteins

The High Mobility Group A (HMGA) family is comprised of three low molecular weight proteins: HMGA1a, HMGA1b, and HMGA2 (formerly HMGI, HMGY, and HMGI-C, respectively). They are encoded by two distinct genes, *HMGA1* and *HMGA2*, which are located either on human chromosome 6p21 and on human chromosome 12q13–15, respectively. These proteins bind the minor groove of AT-rich DNA sequences through three short basic repeats, the so-called "AT-hooks" and orchestrate the assembly of nucleoprotein complexes through a high-grade network of protein-DNA and protein-protein interactions, playing key roles in chromatin architecture, gene transcription and replication. These functions have important rebounds in a wide spectrum of biological processes, ranging from embryonic development, cell differentiation and transformation, cell cycle progression, apoptosis, senescence, DNA repair, up to different aspects of cell physiopatholgy (Fusco and Fedele 2007; Hock et al. 2007).

Both *HMGA* genes are widely and abundantly expressed during embryogenesis, conversely the expression of *HMGA2* has not been detected in any of the several adult mouse and human tissues tested, is very low in CD34 positive hematopoietic stem cells, in mouse preadipocytic proliferating cells and in meiotic and post-meiotic cells (secondary spermatocytes and spermatids). As far as HMGA1 is concerned, its expression in adult tissues is much lower in comparison with that observed in embryonic tissues (Fusco and Fedele 2007). Overexpression of *HMGA1* and *HMGA2* represents a general feature of experimental and human malignancies. However, no mutations or rearrangements have been detected in human carcinomas apart from a few cases of hematological neoplasias where alterations of *HMGA2* have been reported. In malignant tumors of a wide range of tissues, including thyroid, colon, pancreatic, breast, lung and gastric cancer, a clear association between high HMGA1/A2 expression and a poor prognosis has been also observed, suggesting detection of HMGA proteins as both diagnostic molecular markers and predictors of poor postoperative survival (Fusco and Fedele 2007).

It is now clearly established that the overexpression of HMGA in different types of cancer is not casually associated to cell transformation, but plays a causal role in tumor development. Indeed, *in vitro* studies have shown that blocking the synthesis of HMGA proteins can either prevent neoplastic transformation or lead neoplastic cells to death. Furthermore, overexpression of HMGA proteins can transform cells *in vitro* and determine

the development of several forms of neoplasias *in vivo*, including lipomas, NK-T/NK and T cell lymphomas, uterine leyomiomas, fibroadenomas of the breast, salivary gland adenomas and mixed growth hormone/prolactin cell pituitary adenomas (Fedele et al. 2010).

The HMGA proteins have also a critical role in the generation of human benign tumors of mesenchymal origin in which the *HMGA2* gene is rearranged. The effects of such rearrangements are dysregulation of the *HMGA2* gene, its expression, truncation, or formation of fusion genes encoding chimeric transcripts containing the three AT-hooks of *HMGA2* and ectopic sequences from other genes. In some cases only few amino acids are fused to the HMGA2 DNA binding domains, suggesting that the loss of the *HMGA2* sequences rather than the acquisition of new sequences is important for HMGA2 oncogenic activity. Recent studies evidence that the loss of the 3′ untranslated region (3′UTR) contributes to the HMGA2 oncogenic activity. Indeed, the *HMGA2* 3′UTR contains target sequences for different microRNAs (miRs), such as let-7, miR-98, and miR-196. Since miRs can down-regulate gene expression at the post-transcription level by binding to sequences located in the 3′UTR region of the target genes and causing either cleavage of the mRNA or inhibition of protein synthesis, the truncation of the *HMGA2* gene, with the consequent loss of its 3′UTR, would result in increased HMGA2 protein levels that would have oncogenic activity (Mayr et al. 2007).

The HMGA Proteins in Pituitary Adenomas

In the late 1990s, cytogenetic analysis revealed that a small fraction of human pituitary adenomas showed an abnormal karyotype characterized by hyperdiploid or near triploid modal chromosome numbers and rare random structural abnormalities (Bettio et al. 1997). Subsequently, microsatellite and interphase FISH studies indicated that trisomy of chromosome 12, the chromosome where *HMGA2* gene is located, is pathogenetically important, and represents the most frequent cytogenetic alteration in human prolactinomas (Finelli et al. 2002). Using BAC probes encompassing the entire *HMGA2* locus, amplification and/or rearrangement of the *HMGA2* gene was observed in most of the prolactinomas analyzed, suggesting that the increased dosage of chromosome 12 might be a condition predisposing to selective overrepresentation of the *HMGA2* locus and/or rearrangement of the *HMGA2* gene (Finelli et al. 2002). In all the prolactinoma samples analyzed in this study, an induction of HMGA2 protein expression was detected, indicating a critical role of HMGA2 in human prolactinomas (Finelli et al. 2002). Overexpression of HMGA2 has been recorded also in human NFPA, which rarely harbor trisomy 12, suggesting a mechanism of activation different from that mainly operating in prolactinomas (Pierantoni et al. 2005).

In a recent study by Qian et al. (2009), the expression of HMGA2 and its possible association with let-7 miR expression were investigated in a series of 98 human PA of different histotypes. Nuclear staining of HMGA2 was observed in prolactinomas, silent ACTH adenomas and FSH/LH adenomas, whereas HMGA2 immunoreactivity was negative in 4 normal pituitary glands. High levels of expression of HMGA2 correlated with tumor grade, extent of invasion, tumor size, and higher Ki-67 proliferation index. Interestingly, HMGA2 overexpression was associated to down-regulation of let-7 expression that would account for the elevated HMGA2 protein levels and consequent pituitary cell transformation (Qian et al. 2009).

As far as HMGA1 is concerned, neither rearrangements nor amplification of the *HMGA1* locus have been detected in human pituitary adenomas (Fedele et al. 2010), but overexpression of the *HMGA1* gene has been described in different subtypes of human pituitary adenomas (Evans et al. 2008; De Martino et al. 2009) and transgenic mice overexpressing HMGA1 develop pituitary adenomas (Fedele et al. 2005). Also in this case, deregulation of a miR that targets HMGA1 might account for HMGA1 overexpression: indeed miR-16, which targets the *HMGA1* mRNA (Kaddar et al. 2009), is down-regulated in human prolactinomas (Bottoni et al. 2005).

Therefore, these data clearly indicate that HMGA overexpression is an important and frequent event in pituitary tumorigenesis.

HMGA-Transgenic Mice: Animal Models of Pituitary Tumorigenesis

The critical role of HMGA2 proteins in pituitary oncogenesis is validated by the phenotype of transgenic mice overexpressing the *HMGA2* gene under the transcriptional control of the cytomegalovirus promoter (Fedele et al. 2002). In fact, starting from 6 months of age, 85% of female animals developed pituitary adenomas secreting GH and prolactin. Significant higher prolactin and GH circulating levels were evidenced, in Hmga2 mice with respect to controls, by the age of 6 months, although clinical symptoms of the tumor appeared by the age of 12 months. This tumor was easily detachable from the surrounding brain tissue and not showing any evidences of gross infiltration of the brain or sphenoid bone. Nevertheless, by the age of about 12 months, the Hmga2 mice carrying a pituitary tumor, showed deformed skulls, several symptoms of head tilt (loss of equilibrium, excessive tear production and general behavioural changes suggestive of headache) and premature death (Fedele et al. 2002). The transgenic males developed the same phenotype with a lower incidence (40%) and a longer latency period (about 18 months). Similarly, mice expressing a truncated *Hmga2* transgene develop PA histologically undistinguishable from those developed by Hmga2 mice, but with an incidence close to 100% in the female gender (Fedele and Fusco 2010). Interestingly, the same phenotype is also observed in mice overexpressing *Hmga1* (Fedele et al. 2005) supporting the idea that both *HMGA* family genes could play a pivotal role in pituitary adenoma development.

Molecular Mechanisms in the HMGA-Mediated Pituitary Tumorigenesis

Since the HMGA are mainly involved in transcriptional regulation and their role depends on the interaction with other proteins, studies aimed at identifying the mechanisms by which these proteins could be involved in pituitary tumorigenesis have moved in two main directions: (a) the search for new HMGA-interactors that are already known to have an important role in the development of pituitary adenomas; (b) the search for downstream effectors of HMGA proteins by the analysis of the gene expression profiles of pituitary tumors induced by HMGA overexpression.

HMGA Proteins Enhances E2F1 Activity

Since mice carrying a germ-line mutation of one pRB allele are highly predisposed to develop pituitary tumors, and this occurs also in mice with impaired functioning of p27 or p18, both of which converge on pRB (Fedele and Fusco 2010), it was decided to investigate a possible role of HMGA2 protein in pRB pathway.

pRB controls cell cycle progression through its interaction with the E2F family of transcription factors. E2F1 is known to activate transcription of a number of genes required for the S phase of the cell cycle. Inactivation of transcription of E2F1 target genes entails recruitment of pRB by E2F1 to the gene promoters. This recruitment masks the activation domain of E2F1 and prevents its interaction with the general transcriptional machinery. In addition, pRB recruits class I histone deacetylase (HDAC1) proteins that repress transcription by removing acetyl groups from the histone octamer. Removal of the acetyl groups facilitates the condensation of nucleosomes into chromatin, which in turn blocks access to transcription factors and leads to gene repression.

E2F1 activity was highly increased in pituitary adenomas from Hmga2 mice compared to wild type glands and it has been shown that HMGA2 binds to pRB and prevents it from repressing the E2F1-responsive promoters mainly by displacing HDAC1 from pRB (Fedele et al. 2006). Subsequently, the critical role of E2F1 increased activity in the pituitary tumorigenesis of Hmga2 mice was confirmed by the drastic reduction in the incidence and severity of the pituitary tumor phenotype obtained by crossing the Hmga2 mice

with E2F1 knockout mice (Fedele et al. 2006). Therefore, the activation of E2F1 by HMGA2 is a critical event in pituitary tumorigenesis likely accounting for the development of the same pathology observed in pRB+/– mice. The crucial role of enhanced E2F1 activity in the onset and progression of pituitary prolactinomas seems also confirmed by the expression profile of non-invasive, invasive and aggressive-invasive prolactinomas (Wierinckx et al. 2007). Five genes involved in the cell cycle (PTTG, ASK, CCNB1, AURKB and CENPE), all of them regulated at transcription level by E2F1, were highly up-regulated in the aggressive-invasive prolactinomas, suggesting a correlation of the HMGA-dependent activation of E2F1 with invasion, progression and recurrence of pituitary tumors.

HMGA Proteins Up-Regulate CCNB2 and Other Cell Cycle-Related Genes

Cell cycle dysregulation appears to be the main pathogenetic event in the development of pituitary tumors. In fact, it has been estimated that more than 80% of human pituitary tumors show alterations at least in one of the regulators of the cell cycle (Malumbres and Barbacid 2001). These alterations are frequently represented by epigenetic events that target several cell cycle regulators, leading to overexpression of cyclins (mainly D1, D3, E, B1 and B2), as well as downregulation of CKIs (mainly $p16^{INK4A}$, $p15^{INK4B}$, $p27^{Kip1}$, and $p21^{Cip1}$) and pRB expression (Farrell and Clayton 2003). The alteration of cell cycle as a critical event in the induction of pituitary adenomas by the *HMGA2* gene is further confirmed by the direct role of HMGA proteins in the upregulation of cyclin B2 gene transcription, leading to overexpression of cyclin B2 in pituitary adenomas developed by mice carrying *Hmga* transgenes (De Martino et al. 2009). Indeed, *ccnb2* was one of the most up-regulated genes in pituitary adenomas from Hmga2 mice in comparison with normal pituitary glands from wild-type mice (De Martino et al. 2007) and both the HMGA proteins are able to bind the mouse *ccnb2* promoter region, enhancing its activity and driving up-regulation of *ccnb2* gene transcription (De Martino et al. 2009). Moreover, a direct correlation between *CCNB2* and *HMGA* expression levels was reported in a panel of human pituitary adenomas of different histotypes, supporting a direct role of HMGA proteins in the regulation of cyclin B2 protein also in human pituitary adenomas (De Martino et al. 2009).

Interestingly, the analysis of the mRNA expression profile of the pituitary adenomas arising in Hmga2 transgenic mice also revealed the overexpression of other genes, such as *ccnb1*, *ccnd3* and *cdc2*, coding for proteins involved in cell cycle regulation (De Martino et al. 2007). Some of them are frequently altered by epigenetic events in human pituitary adenomas (Farrell and Clayton 2003). Cyclin D1 is frequently overexpressed in all sub-types of human pituitary adenomas, with prevalence in NFPA. A cyclin D1 gene allelic imbalance has also been described in about 25% of analyzed adenomas (Hibberts et al. 1999). B-type cyclins have recently been described as overexpressed in many human pituitary adenomas, with prevalence in prolactinomas (Wierinckx et al. 2007; De Martino et al. 2009). Therefore, by acting as regulators of different steps of cell cycle progression, the HMGA proteins play a crucial role in the maintenance of the pituitary cell homeostasis.

HMGA Proteins Down-Regulate the MIA Gene

Consistent with the ability of HMGA proteins of either activate or repress many different genes, it is possible that other mechanisms may contribute to the role of HMGA2 in pituitary tumorigenesis. Among the genes most down-regulated in the gene chip microarray analysis above described, there was the *Mia/Cd-rap* gene, whose expression was essentially suppressed in all of the mouse pituitary adenomas analysed. This gene encodes a small, secreted protein that is expressed normally at the onset of chondrogenesis. Interestingly, it is secreted by malignant melanoma cells and elicits growth inhibition of melanoma cells *in vitro*. Moreover, in melanoma cells it has been shown that Mia/Cd-rap

interacts with components of the extracellular matrix, such as laminin and fibronectin, suggesting that it may have a function in regulating cellular motility and metastasis (Fedele et al. 2010). De Martino et al. (2007) reported that the HMGA proteins are able to bind the *Mia/Cd-rap* gene promoter and negatively regulate its activity. Moreover, the same authors reported that Mia/Cd-Rap overexpression in rat pituitary cells causes growth inhibition, suggesting a potential tumor suppressor role for the *Mia/Cd-rap* gene in pituitary tumorigenesis. Consistently, a subsequent study showed MIA as one of the most down-regulated genes in prolactinomas *versus* normal controls (Evans et al. 2008).

Other Possible Mechanisms

Since HMGA proteins play a plethora of different roles in different cells, it cannot be excluded that other mechanisms may contribute to the HMGA-mediated pituitary cell transformation. It is likely that the ability of the HMGA proteins to positively regulate interleukin-2 (IL-2) expression (Baldassarre et al. 2001) might have a role in enhancing pituitary cell proliferation. In fact, several evidences, including the presence of IL-2 receptors on pituitary cells and the up-regulation of c-fos following stimulation with IL-2, indicate that this cytokine acts as autocrine and paracrine factor of the anterior pituitary gland regulating both cell proliferation and hormone secretion. The up-regulation of the IL-2 pathway in pituitary adenomas of *Hmga1*- and *Hmga2*-transgenic mice seem to validate this hypothesis (Fedele, unpublished data).

A role in pituitary cell transformation by the HMGA proteins might be also the drastic increase in the HMGA-induced pituitary adenomas of Pit-1, a trancriptional factor that is critical in the development and proliferation of GH-, PRL- and TSH-secreting pituitary cells (Fedele and Fusco 2010). It could be speculated that HMGA2 leads to hyperplasia of cells expressing Pit-1 or alternatively that the abnormal growth of the embryonic cells secreting GH and PRL may induce Pit-1 expression. However, it has been recently demonstrated that HMGA proteins bind both Pit-1 and Pit-1-responsive DNA elements, thus positively modulating the *Pit-1* promoter, also synergistically cooperating with Pit-1 (Palmieri et al. 2012). Moreover, high expression levels of Pit-1 represents a constant feature of human pituitary GH and PRL secreting adenomas, and different evidences suggest a potential role for Pit-1 in cell proliferation, prevention of apoptotic death and pathogenesis of pituitary tumors (Fedele and Fusco 2010). Therefore, both the hypotheses are worth of consideration. Finally, since HMGA2 is a stem cell marker that participates in the maintenance of the stem cell self-renewal capacity (Nishino et al. 2008), it is reasonable to hypothesize that HMGA2 over-expression may contribute to the expansion of pituitary stem cells that may represent a potential start site of the transformation process (de Almeida et al. 2010).

Conclusions and Therapeutic Perspectives

All the data reported in the literature assess a critical role for overexpression of the HMGA proteins in pituitary tumorigensis. Indeed, overexpression of both HMGA1 and HMGA2 proteins, in some cases also associated with amplification or rearrangement of the *HMGA2* locus, has been found very frequently in human pituitary adenomas (Fusco and Fedele 2007), and consistently, transgenic mice overexpressing either *Hmga1* or *Hmga2* develop pituitary adenomas (Fedele et al. 2002, 2005).

The critical role of HMGA proteins in pituitary tumorigenesis is likely in their quite unique ability to activate multiple signals that act on different aspects of the biology of the pituitary cell involved in the neoplastic transformation. Due to this important role of the HMGA protein overexpression in the development of a significant number of prolactinomas, a therapy based on the suppression of the HMGA2 function may be envisaged for the treatment of recurrent prolactinomas. To design such a therapy different approaches may be taken in consideration: (a) restoration of the expression of miRs, having as targets HMGA1 and/or HMGA2, which are down-regulated in pituitary adenomas

in comparison with the normal pituitary; (b) inoculation of siRNA targeting the HMGA proteins; (c) Inhibition of HMGA protein activity by drugs. The availability of transgenic mice overexpressing either Hmga1 or Hmga2 will represent an excellent animal model to test the efficacy of this and other innovative therapies for pituitary adenomas.

References

Asa SL, Ezzat S (2009) The pathogenesis of pituitary tumors. Annu Rev Pathol 4:97–126

Baldassarre G, Fedele M, Battista S, Vecchione A, Klein-Szanto AJ, Santoro M, Waldmann TA, Azimi N, Croce CM, Fusco A (2001) Onset of natural killer cell lymphomas in transgenic mice carrying a truncated HMGI-C gene by the chronic stimulation of the IL-2 and IL-15 pathway. Proc Natl Acad Sci U S A 98:7970–7975

Bettio D, Rizzi N, Giardino D, Persani L, Pecori-Giraldi F, Losa M, Larizza L (1997) Cytogenetic study of pituitary adenomas. Cancer Genet Cytogenet 98:131–136

Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, degli Uberti EC (2005) miR-15a and miR-16-1 down-regulation in pituitary adenomas. J Cell Physiol 204:280–285

Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A (2006) High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. J Clin Endocrinol Metab 91:4769–4775

de Almeida JP, Sherman JH, Salvatori R, Quiñones-Hinojosa A (2010) Pituitary stem cells: review of the literature and current understanding. Neurosurgery 67:770–780

De Martino I, Visone R, Palmieri D, Cappabianca P, Chieffi P, Forzati F, Barbieri A, Kruhoffer M, Lombardi G, Fusco A, Fedele M (2007) The Mia/Cd-rap gene expression is downregulated by the high-mobility group a proteins in mouse pituitary adenomas. Endocr Relat Cancer 14:875–886

De Martino I, Visone R, Wierinckx A, Palmieri D, Ferraro A, Cappabianca P, Chiappetta G, Forzati F, Lombardi G, Colao A, Trouillas J, Fedele M, Fusco A (2009) HMGA proteins up-regulate CCNB2 gene in mouse and human pituitary adenomas. Cancer Res 69:1844–1850

Evans CO, Moreno CS, Zhan X, McCabe MT, Vertino PM, Desiderio DM, Oyesiku NM (2008) Molecular pathogenesis of human prolactinomas identified by gene expression profiling, RT-qPCR, and proteomic analyses. Pituitary 11:231–245

Farrell WE, Clayton RN (2003) Epigenetic change in pituitary tumorigenesis. Endocr Relat Cancer 10:323–330

Fedele M, Fusco A (2010) Role of the high mobility group A proteins in the regulation of pituitary cell cycle. J Mol Endocrinol 44:309–318

Fedele M, Battista S, Kenyon L, Baldassarre G, Fidanza V, Klein-Szanto AJ, Parlow AF, Visone R, Pierantoni GM, Outwater E, Santoro M, Croce CM, Fusco A (2002) Overexpression of the HMGA2 gene in transgenic mice leads to the onset of pituitary adenomas. Oncogene 21:3190–3198

Fedele M, Pentimalli F, Baldassarre G, Battista S, Klein-Szanto AJ, Kenyon L, Visone R, De Martino I, Ciarmiello A, Arra C, Viglietto G, Croce CM, Fusco A (2005) Transgenic mice overexpressing the wild-type form of the HMGA1 gene develop mixed growth hormone/prolactin cell pituitary adenomas and natural killer cell lymphomas. Oncogene 24:3427–3435

Fedele M, Visone R, De Martino I, Troncone G, Palmieri D, Battista S, Ciarmiello A, Pallante P, Arra C, Melillo RM, Helin K, Croce CM, Fusco A (2006) HMGA2 induces pituitary tumorigenesis by enhancing E2F1 activity. Cancer Cell 9:459–471

Fedele M, Palmieri D, Fusco A (2010) HMGA2: a pituitary tumour subtype-specific oncogene? Mol Cell Endocrinol 326:19–24

Finelli P, Pierantoni GM, Giardino D, Losa M, Rodeschini O, Fedele M, Valtorta E, Mortini P, Croce CM, Larizza L, Fusco A (2002) The high mobility group A2 gene is amplified and overexpressed in human prolactinomas. Cancer Res 62:2398–2405

Fusco A, Fedele M (2007) Roles of HMGA proteins in cancer. Nat Rev Cancer 7:899–910

Heaney AP, Melmed S (2004) Molecular targets in pituitary tumours. Nat Rev Cancer 4:285–295

Hibberts NA, Simpson DJ, Bicknell JE, Broome JC, Hoban PR, Clayton RN, Farrell WE (1999) Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. Clin Cancer Res 5:2133–2139

Hock R, Furusawa T, Ueda T, Bustin M (2007) HMG chromosomal proteins in development and disease. Trends Cell Biol 17:72–79

Kaddar T, Rouault JP, Chien WW, Chebel A, Gadoux M, Salles G, Ffrench M, Magaud JP (2009) Two new miR-16 targets: caprin-1 and HMGA1, proteins implicated in cell proliferation. Biol Cell 101:511–524

Landis CA, Masters SB, Spada A, Pace AM, Bourne HR, Vallar L (1989) GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. Nature 340:692–696

Malumbres M, Barbacid M (2001) To cycle or not to cycle: a critical decision in cancer. Nat Rev Cancer 1:222–231

Mayr C, Hemmann MT, Bartel DP (2007) Disrupting the pairing between *let-7* and *Hmga2* enhances oncogenic transformation. Science 315:1576–1579

Nishino J, Kim I, Chada K, Morrison SJ (2008) Hmga2 promotes neural stem cell self-renewal in young but not old mice by reducing p16Ink4a and p19Arf expression. Cell 135:227–239

Palmieri D, Valentino T, De Martino I, Esposito F, Cappabianca P, Wierinkx A, Vitiello M, Lombardi G, Colao A, Trouillas J, Pierantoni GM, Fusco A, Fedele M (2012) PIT1 upregulation by HMGA proteins has a role in pituitary tumorigenesis. Endocr Relat Cancer 19:123–135

Pierantoni GM, Finelli P, Valtorta E, Giardino D, Rodeschini O, Esposito F, Losa M, Fusco A, Larizza L (2005) High-mobility group A2 gene expression is frequently induced in non-functioning pituitary adenomas (NFPAs), even in the absence of chromosome 12 polysomy. Endocr Relat Cancer 12:867–874

Qian ZR, Asa SL, Siomi H, Siomi MC, Yoshimoto K, Yamada S, Wang EL, Rahman MM, Inoue H, Itakura M, Kudo E, Sano T (2009) Overexpression of HMGA2 relates to reduction of the let-7 and its relationship to clinicopathological features in pituitary adenomas. Mod Pathol 22:431–441

Wierinckx A, Auger C, Devauchelle P, Reynaud A, Chevallier P, Jan M, Perrin G, Fèvre-Montange M, Rey C, Figarella-Branger D, Raverot G, Belin MF, Lachuer J, Trouillas J (2007) A diagnostic marker set for invasion, proliferation, and aggressiveness of prolactin pituitary tumors. Endocr Relat Cancer 14:887–900

Pituitary Adenomas: Role of E-Cadherin in Tumor Invasion

19

Marianne S. Elston

Contents

Abstract

Whilst the majority of pituitary tumors are benign, local invasion is common and the currently available markers to determine pituitary tumor invasiveness are imperfect. Loss of the invasion-suppressor E-cadherin, at the cell membrane has been identified in a variety of human malignancies and has been associated with invasion in prolactinomas, although not for other pituitary tumor subtypes. More recently nuclear staining of E-cadherin has been identified in a number of tumors including pituitary adenomas. Whilst methylation of the promoter of E-cadherin is common in pituitary adenomas, preserved mRNA expression has been demonstrated in association with absent membranous staining supportive of protein movement rather than loss. Nuclear E-cadherin has been demonstrated to be common in nonfunctioning pituitary adenomas and a subset of growth hormone-secreting adenomas in which it is associated with tumor size and invasion. Nuclear E-cadherin may influence tumor growth through regulation of the p120-Kaiso signaling pathway and modulation of apoptosis. The presence of nuclear E-cadherin in pituitary adenomas may prove to be a useful marker in identifying invasive pituitary adenomas.

M.S. Elston (✉)
Department of Endocrinology, Waikato Hospital, Private Bag 3200, Hamilton, Waikato 3240, New Zealand
e-mail: marianne.elston@waikatodhb.health.nz

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_19,

Introduction

The vast majority of pituitary tumors are benign but still may result in significant morbidity and premature mortality through mass effect and hormonal dysfunction. One difficulty with pituitary tumors is determining which tumors will recur or become invasive following initial surgical resection and require additional intervention. Similar to many non-pituitary endocrine tumors, markers of invasiveness used in other tumor types are frequently less useful in pituitary tumors. The World Health Organization (WHO) classification includes a separate code for an atypical pituitary adenoma; that is one which has atypical morphological features suggestive of aggressive behavior which may include an elevated mitotic index, Ki-67 labeling index >3% and extensive nuclear staining for p53 immunoreactivity (DeLellis et al. 2004). However, as these pathological features are not consistent across clinically invasive pituitary adenomas, alternative markers to identify invasive pituitary tumors are required, both for prognosis and as potential therapeutic targets.

E-cadherin has been proposed as a marker associated with pituitary tumor invasion. Loss of E-cadherin at the cell surface has been associated with invasion and metastasis in a wide variety of human tumor types, including prolactin-secreting pituitary adenomas (Qian et al. 2002). Recently E-cadherin has been detected in the nucleus of some tumor types; this is only identifiable when performing immunohistochemical staining using an antibody directed against the cytoplasmic domain. Demonstration of nuclear E-cadherin is reported to be associated with invasion in tumors such as pancreatic neuroendocrine tumors (Chetty et al. 2008). Similarly, nuclear staining for E-cadherin has been identified in pituitary tumors and found to be associated with tumor invasion (Elston et al. 2009; Fougner et al. 2010). This chapter will focus on the identification of altered E-cadherin expression in pituitary adenomas and its role in pituitary tumor invasion.

Markers of Invasion in Pituitary Tumors

Pituitary carcinomas diagnosed by central nervous system metastases or extracranial spread are rare with an estimated incidence of approximately 0.2% of pituitary tumors (Ragel and Couldwell 2004). Although the overwhelming majority of pituitary tumors are benign, some of these adenomas are locally invasive and grow into surrounding venous sinuses, bone, dura, and brain tissue. Reported rates of invasive pituitary tumors vary considerably depending on the method used to determine invasiveness. For example, dural invasion is common even in tumors considered to be non-invasive (up to 69% of microadenomas and 94% of macroadenomas when assessed histologically) (Selman et al. 1986) and so is not predictive of an aggressive tumor. Markers of invasiveness used in other tumor types are frequently less useful in endocrine tumors including pituitary tumors. Histology alone is unable to reliably differentiate between benign, invasive and malignant pituitary adenomas because features such as atypia, nuclear pleomorphism, necrosis and mitotic index are not consistently increased in invasive tumors compared with those which are non-invasive.

Whilst the WHO classification markers of an atypical pituitary adenoma (DeLellis et al. 2004) may point to a tumor being more invasive they are not uniformly demonstrated in invasive lesions. For example, whilst more aggressive pituitary tumors frequently have a higher Ki-67 labeling index, cut-offs differ between studies, the labeling index may vary according to tumor subtype and, for functional tumors, as to whether medical pre-treatment was given. For these reasons Ki-67 labeling index does not uniformly correlate with tumor invasion. Consequently, alternative markers to identify invasive tumors are required. E-cadherin has been demonstrated to be associated with invasion in a number of human tumors and therefore may provide a useful marker for invasion in pituitary tumors.

E-Cadherin Structure and Function

E-cadherin is a member of the classic cadherin family of transmembrane glycoproteins. The classic cadherin family includes E-cadherin, N-cadherin and P-cadherin, and are a subgroup of the larger cadherin superfamily containing over 30 members. E-cadherin, N-cadherin and P-cadherin are the most widely studied of the classic cadherins and are named after the tissues in which they were first described – epithelial, neural and placental, respectively. The classic cadherins contain three domains – an extracellular domain containing five cadherin repeats, a single transmembrane domain and a highly conserved cytoplasmic domain (Fig. 19.1). Homophilic interactions between the extracellular domains of E-cadherin molecules results in calcium-dependent cell-cell adhesion. The cytoplasmic domain of E-cadherin has two functional components – a juxtamembrane domain (JMD) and a catenin-binding domain (CBD). E-cadherin is encoded by the gene *CDH1*, located on chromosome 16q22.1. The *CDH1* promoter contains a CpG island which extends into the first exon and intron. *CDH1* contains 16 exons and is transcribed into a 4,851 bp mRNA. This is translated into an 882 amino acid, 135 kDa precursor protein which is rapidly processed into the mature 120 kDa molecule.

E-Cadherin as a Cell Adhesion Molecule

E-cadherin acts at the adherens junction as part of a multiprotein complex important in cell-cell adhesion and signaling. The CBD of E-cadherin's cytoplasmic domain is linked to the actin cytoskeleton via β-, γ- and α-catenin and p120 protein. Whilst β-catenin is an important part of this cell adhesion complex it is also the central mediator of the canonical Wnt signaling pathway (Fig. 19.2). In this pathway, binding of a Wnt ligand to the frizzled-lipoprotein-related protein receptor complex results in disassembly of the β-catenin destruction complex [which is comprised of adenomatous polyposis coli (APC), AXIN, glycogen synthase kinase 3β (GSK3β) and casein kinase 1 (CKI)]. This Wnt signaling leads to cytoplasmic accumulation and nuclear translocation of unphosphorylated β-catenin. Nuclear β-catenin can then increase the transcription of genes involved in cell proliferation such as MYC and cyclin D1. Retention of β-catenin at

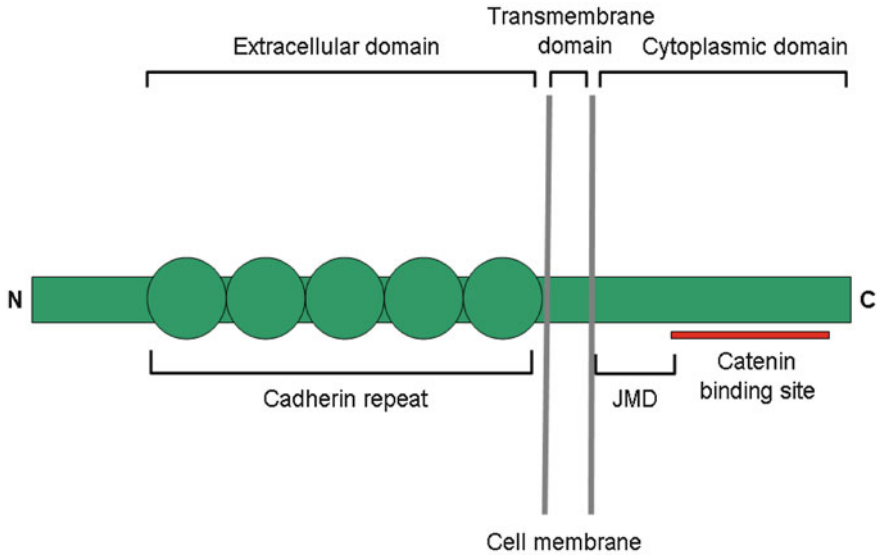

Fig. 19.1 Schematic structure of E-cadherin. *N* N-terminus, *C* C-terminus, *JMD* juxtamembrane domain

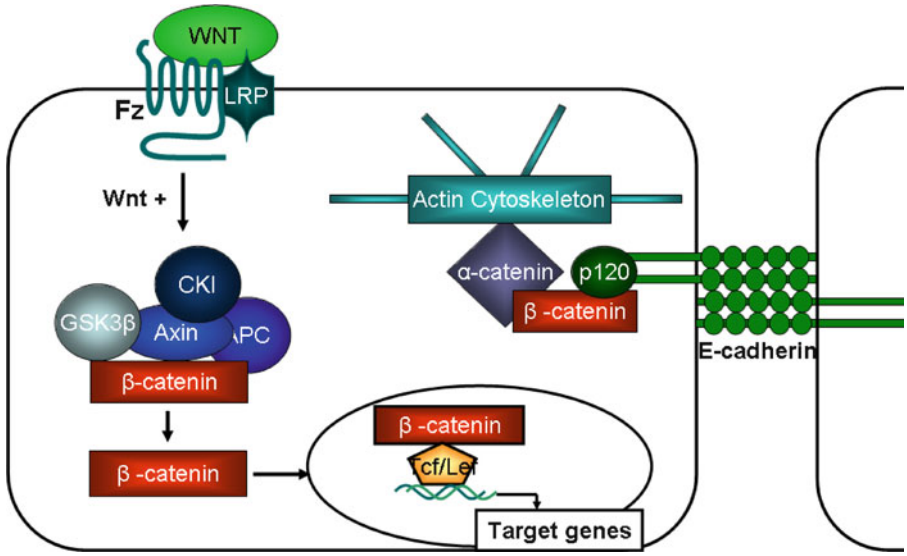

Fig. 19.2 Simplified schematic illustrating the dual roles of β-catenin. On the *left* the pivotal role of β-catenin as the central mediator of the Wnt-β-catenin signaling pathway is shown. On the *right* the role of β-catenin in cell adhesion is demonstrated in association with other cell adhesion complex members E-cadherin, p120, α-catenin and the actin cytoskeleton. *Fz* frizzled, *LRP* lipoprotein-related protein, *GSK3β* glycogen synthase kinase 3, beta, *CKI* casein kinase 1, *APC* adenomatous polyposis coli, *TCF* T-cell factor, *LEF* lymphoid enhancer factor

the cell membrane as part of the cell adhesion complex with E-cadherin helps prevent excess cytoplasmic availability of β-catenin.

Normal E-cadherin expression is important in maintaining cell polarity and epithelial tissue integrity. Loss of E-cadherin expression is associated with epithelial-mesenchymal transition (EMT) whereby epithelial tissue changes its phenotype from one of epithelial morphology (with expression of typical epithelial markers such as E-cadherin and cytokeratin), to one in which there is loss of cell-cell contact and the development of cell motility and expression of mesenchymal markers (such as N-cadherin and vimentin). This loss of cell adhesion and increased motility is associated with tumor cell invasion. Re-expression of E-cadherin in cell culture models is associated with reversion to a more benign phenotype consistent with E-cadherin acting to suppress invasion (Vleminckx et al. 1991). Membranous E-cadherin protein levels are regulated by the Armadillo family member, p120 which is required for stabilization of E-cadherin at the cell membrane. P120 binds to the JMD and loss of p120 results in internalization of E-cadherin due to interaction of clathrin adaptor proteins with an internalization sequence in the cytoplasmic tail of E-cadherin.

Altered E-cadherin gene and/or protein expression may also be secondary to a number of other potential mechanisms. Loss of heterozygosity of the region containing the *CDH1* locus is common in many cancers including breast and gastric cancer and may provide one hit towards silencing E-cadherin gene expression. *CDH1* contains a CpG island in the promoter which extends into the first exon and first intron. A CpG island is an area of DNA rich in cytosine and guanine (CpG) dinucleotides and approximately half of all human genes contain a CpG island in the promoter and first exon. Methylation of CpG islands in the promoter of a gene is a well-recognized mechanism of gene silencing. Most CpG dinucleotides in CpG islands, especially those in the promoter of genes, are unmethylated, however, in cancers normal methylation patterns are altered and many CpG sites that should be methylated

become unmethylated and vice versa. E-cadherin has previously been demonstrated to be methylated in a number of human tumors including breast cancer. Transcriptional repression of *CDH1* may also occur secondary to transcriptional repressors such as Snail homolog 1 (SNAI1), Slug (SNAI2), ZEB1, ZEB2, E47, TCF4 and TWIST1 (Peinado et al. 2007). Some of these transcriptional repressors are targets of the microRNA (miR) family miR200 therefore alterations in miR expression may also alter E-cadherin protein levels (Hurteau et al. 2007). Additionally, induction of phosphorylation of E-cadherin protein may result from the actions of factors such as IGF1R and c-MET resulting in ubiquitination and degradation (Christofori 2006). E-cadherin may also be cleaved by various metalloproteinases, caspase and γ-secretase (Christofori 2006; Ferber et al. 2008).

Germline mutations in *CDH1* are responsible for loss of E-cadherin protein expression in hereditary diffuse gastric cancer (Guilford et al. 1998) and somatic *CDH1* mutations have been identified in lobular breast cancer (Berx et al. 1995). There is also a common single nucleotide polymorphism within the E-cadherin promoter (−160 C/A) which alters transcription factor binding and so influences gene transcription. Carriers of the A allele have increased rates of certain cancers such as lung, prostate and gastric (Li et al. 2000).

Membranous E-Cadherin Immunostaining in Pituitary Tumors

Normal anterior pituitary tissue demonstrates a strong cytoplasmic membrane staining pattern for E-cadherin (Tsuchiya et al. 2006) (Fig. 19.3a). Pituitary adenomas have been reported to demonstrate loss of cytoplasmic membrane staining (Fig. 19.3b) however the reported frequency of absent staining has varied markedly between studies ranging from 0 to 100% (Kawamoto et al. 1997; Schwechheimer et al. 1998). These discordant findings are at least in part due to differences in the tumors included in the studies. Tumor size has been shown to correlate with E-cadherin membrane staining with microadenomas demonstrating higher rates of staining than macroadenomas (Qian et al. 2007). E-cadherin cytoplasmic membrane status appears to vary according to tumor subtype and GH-secreting adenomas with prominent fibrous bodies have been demonstrated to show reduced cytoplasmic membrane staining compared to GH-secreting adenomas without prominent fibrous bodies (Xu et al. 2002; Nishioka et al. 2003; Qian et al. 2007). In addition, pre-treatment of GH-secreting adenomas with somatostatin analogues has been demonstrated to be associated with lower E-cadherin levels, as measured by Western blotting, although not when assessed by immunohistochemistry

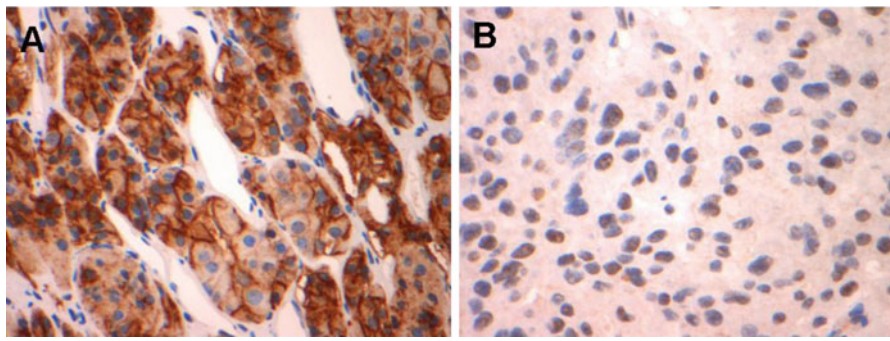

Fig. 19.3 E-cadherin immunohistochemistry using an antibody (clone 36/E, BD-Transduction Laboratories, Franklin Lake, NJ, USA) recognizing the cytoplasmic domain. Both images are counterstained with hematoxylin. (**a**) Normal pituitary Original magnification ×600. Strong membranous and weak cytoplasmic staining is demonstrated with absent nuclear staining. (**b**) Non-functioning pituitary adenoma. Original magnification ×600. Nuclear staining is demonstrated with absent cytoplasmic membrane staining

(Fougner et al. 2010). Non functioning pituitary adenomas (NFAs) have also been reported with frequent loss of cell membrane E-cadherin staining (Qian et al. 2007; Elston et al. 2009). Invasive prolactinomas, classified by the modified Hardy criteria, have been demonstrated to show reduced cell membrane E-cadherin staining compared to non-invasive adenomas (Qian et al. 2002). However, with the exception of prolactinomas, E-cadherin cell membrane staining has not correlated with invasion in most studies (Kawamoto et al. 1997; Qian et al. 2002; Xu et al. 2002; Yamada et al. 2007; Elston et al. 2009).

Membranous E-Cadherin and Beta-Catenin

Loss of other members of the cell adhesion complex, notably β-catenin may be associated with tumor invasion. The presence of membranous β-catenin in pituitary adenomas has identified to be weak or absent in approximately 16% of tumors (Tziortzioti et al. 2001; Elston et al. 2008). Absent membranous β-catenin has been shown to correlate with tumor invasion (Elston et al. 2008). Loss of cell membrane β-catenin staining has been reported to be associated with loss of E-cadherin cytoplasmic membrane staining in both invasive prolactinomas (Qian et al. 2002) and in other tumor subtypes (Elston et al. 2009). Loss of membranous β-catenin is only rarely associated with nuclear β-catenin (Tziortzioti et al. 2001; Elston et al. 2008) suggesting that a mechanism other than activation of the canonical Wnt signaling pathway is likely to be the cause of tumor invasion.

Mechanism of Altered Membranous E-Cadherin Expression in Pituitary Adenomas

As outlined earlier there are a number of potential mechanisms by which cell-surface expression of E-cadherin may be lost in tumors. One proposed mechanism is down-regulation of E-cadherin gene (*CDH1)* expression via methylation of the CpG island in its promoter. In a study assessing eight prolactinomas with decreased E-cadherin membrane staining no hypermethylation was found when assessed by methylation-specific PCR (MSP) (Qian et al. 2002). However, hypermethylation was identified using MSP in 6/16 GH-secreting tumors with prominent fibrous bodies although not in 10 GH-secreting tumors without fibrous bodies (Xu et al. 2002). In a larger series, assessing both clinically functioning and nonfunctioning adenomas, hypermethylation of the promoter was identified in 25/69 tumors using MSP (Qian et al. 2007). Of the 25 tumors with hypermethylation, E-cadherin staining was absent or significantly decreased in 22, however almost half of the unmethylated tumors also showed decreased E-cadherin staining (Qian et al. 2007). This suggests that promoter methylation is likely to be only one mechanism involved in the decreased cytoplasmic membrane staining of E-cadherin.

Absent E-cadherin cell membrane staining is associated with germline mutations in *CDH1* in hereditary diffuse gastric cancer (Guilford et al. 1998) and somatic mutations in *CDH1* have been identified in lobular breast cancer (Berx et al. 1995). No somatic E-cadherin mutations have yet been identified in pituitary adenomas (Elston et al. 2009).

Whilst a number of studies have assessed E-cadherin protein immunostaining only two previous studies have assessed gene expression in pituitary adenomas. In the first study *CDH1* mRNA expression was readily detected in 12/14 pituitary adenomas although concomitant protein levels were not reported (Howng et al. 2002). Preserved *CDH1* mRNA expression was confirmed by a second study by a different group in which 30 adenomas which had mRNA expression assessed also had cytoplasmic membrane staining performed (Elston et al. 2009). All tumors except one had detectable mRNA expression despite the majority of tumors having absent or markedly decreased cytoplasmic membrane staining. Given these findings of detectable mRNA expression in the presence of absent cytoplasmic membrane staining whilst methylation of the E-cadherin promoter appears to frequently be

identified, a protein-based mechanism appears to be a more likely explanation for the apparent reduction in E-cadherin at the cytoplasmic membrane in these adenomas than reduced gene expression.

Nuclear E-Cadherin Expression in Pituitary Adenomas

E-cadherin has been detected in the nucleus associated with loss of its cytoplasmic membrane staining in pancreatic, Merkel cell and esophageal tumors, suggesting that nuclear accumulation of this protein occurs during neoplasia (Chetty and Serra 2008b; Chetty et al. 2008). Commercial antibodies have been generated against different domains of E-cadherin (most commonly either the extracellular domain or the cytoplasmic domain). Detection of nuclear staining is highly dependent on the antibody used as nuclear staining is only detectable when immunohistochemistry is performed using an antibody directed against the cytoplasmic domain. Two studies have assessed nuclear staining of E-cadherin in pituitary adenomas using an antibody against the cytoplasmic domain. Elston et al. (2009) demonstrated nuclear E-cadherin staining in 38/44 (86%) of pituitary adenomas and in 0/8 normal pituitary tissue samples (Fig. 19.3). In this study, tumor subtype was strongly correlated with nuclear staining such that NFAs demonstrated nuclear staining in 26/28, whereas clinically functioning tumors were more likely to have absent or only weak nuclear staining but only small numbers of functioning tumors were assessed. No prolactinomas were included in this study. Furthermore, nuclear E-cadherin staining correlated with tumor invasion, as graded according to the modified Hardy criteria, and Ki-67 labeling index. Fougner et al. (2010) studied 83 GH-secreting adenomas, 29 of which had been treated preoperatively with somatostatin analogues and identified nuclear staining in 9/80 tumors. Nuclear staining correlated with tumor size and tumors demonstrating nuclear E-cadherin positivity demonstrated less shrinkage in response to somatostatin analogues than those which had negative staining (Fougner et al. 2010).

Correlation Between Membranous and Nuclear E-Cadherin Staining

In the two studies in which the immunohistochemical status of E-cadherin in pituitary adenomas was assessed using antibodies to both the extracellular and cytoplasmic domains an inverse correlation between nuclear and cytoplasmic membrane staining was demonstrated (Elston et al. 2009; Fougner et al. 2010). Given the absent membrane staining is typically associated with preserved mRNA expression; these findings suggest that E-cadherin is translocated to the nucleus during pituitary neoplasia, possibly following cleavage of its extracellular domain.

Mechanism of Nuclear E-Cadherin Translocation

The mechanism by which E-cadherin is translocated to the nucleus is currently unclear. In a study of solid pseudopapillary tumors of the pancreas, nuclear localization of E-cadherin was demonstrated in association with the presence of nuclear β-catenin leading the authors to suggest that these two proteins may be physically associated in this process (Chetty and Serra 2008a). However, in pancreatic endocrine tumors, frequent nuclear E-cadherin staining was found despite absence of nuclear β-catenin suggesting that nuclear β-catenin is not necessarily required for nuclear accumulation of E-cadherin (Chetty et al. 2008). In that particular study, aberrant E-cadherin staining was associated with absent/decreased β-catenin cytoplasmic membrane staining (Chetty et al. 2008). Similar findings have been reported for pituitary adenomas in that whilst absent or decreased β-catenin cytoplasmic membrane staining is associated with decreased cytoplasmic membrane E-cadherin staining the majority of tumors with absent or decreased membranous E-cadherin staining have preserved β-catenin cytoplasmic membrane staining (Elston et al. 2009). Nuclear β-catenin staining appears to be an uncommon finding in pituitary adenomas (Tziortzioti et al. 2001;

Elston et al. 2008) supporting the findings from pancreatic neuroendocrine tumors in that movement of E-cadherin into the nucleus is not dependent on β-catenin. More recently it has been demonstrated that the cytoplasmic fragment of E-cadherin is cleaved by a γ-secretase and translocates into the nucleus assisted by p120 (Ferber et al. 2008). In pituitary tumors p120 has been studied only in prolactinomas where it has been demonstrated to have an exclusively membranous staining pattern (Qian et al. 2002). Prolactinomas have yet to be assessed for the presence of nuclear E-cadherin.

Mechanism of Action of E-Cadherin in Tumor Invasion

E-cadherin has been termed an "invasion suppressor" and may promote tumor invasion through loss of cell-cell contact thereby encouraging cell migration or through effects on cell signaling pathways (Christofori 2006). Potential mechanisms for altered E-cadherin expression in tumor invasion include: loss of cell adhesion, interference of cell adhesion by a cleaved extracellular fragment of E-cadherin, increased cytoplasmic availability of β-catenin, and activation of pathways involved in proliferation by the cleaved cytoplasmic fragment of E-cadherin.

Nuclear accumulation of E-cadherin may result from cleavage of E-cadherin by γ-secretase, releasing a 38 kDa soluble C-terminal fragment which then translocates into the nucleus assisted by p120 (Ferber et al. 2008). In the nucleus p120 interacts with the transcriptional repressor Kaiso preventing it from binding to DNA (Ferber et al. 2008). Ferber et al. (2008) demonstrated that the C-terminal fragment of E-cadherin regulates p120-Kaiso-mediated signaling pathway by binding to DNA and that p120 is required for DNA binding. In addition to modulation of the p120-Kaiso pathway, the nuclear cytoplasmic fragment of E-cadherin may also regulate apoptosis (Ferber et al. 2008). In esophageal squamous cell carcinomas the presence of nuclear E-cadherin staining was demonstrated to be associated with elevated nuclear cyclin D1 levels and in functional studies the cytoplasmic domain of E-cadherin was shown to induce cyclin D1 promoter activity (Salahshor et al. 2008). Cyclin D1 has been demonstrated to be elevated in pituitary adenomas, in particular NFAs (Hibberts et al. 1999; Jordan et al. 2000; Elston et al. 2008), and it is possible that in addition to the other known mechanisms such as gene duplication, this increase in cyclin D1 is contributed to by nuclear E-cadherin acting through the p120-Kaiso signaling pathway.

In conclusion, E-cadherin is an "invasion suppressor" and may promote tumor invasion through loss of cell-cell contact thereby encouraging cell migration or, via nuclear translocation of a cytoplasmic fragment, through effects on cell signaling pathways such as the p120-Kaiso pathway. In pituitary tumors E-cadherin cell membrane staining is associated with tumor size and subtype. Absent membranous staining has been variably demonstrated and appears only to be consistently associated with invasion for prolactinomas. Whilst methylation of the *CDH1* promoter has been demonstrated in pituitary adenomas the mechanism by which absent membranous staining in pituitary adenomas occurs appears to be predominantly protein-based and is likely to be secondary to cleavage of the cytoplasmic domain which then translocates into the nucleus whereby it may be detected using an antibody directed against the cytoplasmic domain. Nuclear E-cadherin staining has been identified in a subset of pituitary tumors where it appears to be associated with tumor size, invasive status and, for GH-secreting adenomas, response to somatostatin analogues. If these findings are confirmed in further larger studies E-cadherin may prove to be an additional marker to help identify invasive pituitary adenomas.

References

Berx G, Cleton-Jansen AM, Nollet F, de Leeuw WJ, van de Vijver M, Cornelisse C, van Roy F (1995) E-cadherin is a tumour/invasion suppressor gene mutated in human lobular breast cancers. EMBO J 14:6107–6115

Chetty R, Serra S (2008a) Membrane loss and aberrant nuclear localization of E-cadherin are consistent features of solid pseudopapillary tumour of the pancreas.

An immunohistochemical study using two antibodies recognizing different domains of the E-cadherin molecule. Histopathology 52:325–330

Chetty R, Serra S (2008b) Nuclear E-cadherin immunoexpression: from biology to potential applications in diagnostic pathology. Adv Anat Pathol 15:234–240

Chetty R, Serra S, Asa SL (2008) Loss of membrane localization and aberrant nuclear E-cadherin expression correlates with invasion in pancreatic endocrine tumors. Am J Surg Pathol 32:413–419

Christofori G (2006) New signals from the invasive front. Nature 441:444–450

DeLellis R, Lloyd R, Heitz P, Eng C (2004) World health organization classification of tumours. Pathology and genetics of tumours of endocrine organs. IARC Press, Lyon

Elston MS, Gill AJ, Conaglen JV, Clarkson A, Shaw JM, Law AJ, Cook RJ, Little NS, Clifton-Bligh RJ, Robinson BG, McDonald KL (2008) Wnt pathway inhibitors are strongly down-regulated in pituitary tumors. Endocrinology 149:1235–1242

Elston MS, Gill AJ, Conaglen JV, Clarkson A, Cook RJ, Little NS, Robinson BG, Clifton-Bligh RJ, McDonald KL (2009) Nuclear accumulation of e-cadherin correlates with loss of cytoplasmic membrane staining and invasion in pituitary adenomas. J Clin Endocrinol Metab 94:1436–1442

Ferber EC, Kajita M, Wadlow A, Tobiansky L, Niessen C, Ariga H, Daniel J, Fujita Y (2008) A role for the cleaved cytoplasmic domain of E-cadherin in the nucleus. J Biol Chem 283:12691–12700

Fougner SL, Lekva T, Borota OC, Hald JK, Bollerslev J, Berg JP (2010) The expression of E-cadherin in somatotroph pituitary adenomas is related to tumor size, invasiveness, and somatostatin analog response. J Clin Endocrinol Metab 95:2334–2342

Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392:402–405

Hibberts NA, Simpson DJ, Bicknell JE, Broome JC, Hoban PR, Clayton RN, Farrell WE (1999) Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. Clin Cancer Res 5:2133–2139

Howng SL, Wu CH, Cheng TS, Sy WD, Lin PC, Wang C, Hong YR (2002) Differential expression of Wnt genes, beta-catenin and E-cadherin in human brain tumors. Cancer Lett 183:95–101

Hurteau GJ, Carlson JA, Spivack SD, Brock GJ (2007) Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. Cancer Res 67:7972–7976

Jordan S, Lidhar K, Korbonits M, Lowe DG, Grossman AB (2000) Cyclin D and cyclin E expression in normal and adenomatous pituitary. Eur J Endocrinol 143:R1–R6

Kawamoto H, Mizoue T, Arita K, Tominaga A, Eguchi K, Kurisu K (1997) Expression of epithelial cadherin and cavernous sinus invasion in human pituitary adenomas. J Neurooncol 34:105–109

Li LC, Chui RM, Sasaki M, Nakajima K, Perinchery G, Au HC, Nojima D, Carroll P, Dahiya R (2000) A single nucleotide polymorphism in the E-cadherin gene promoter alters transcriptional activities. Cancer Res 60:873–876

Nishioka H, Haraoka J, Akada K (2003) Fibrous bodies are associated with lower GH production and decreased expression of E-cadherin in GH-producing pituitary adenomas. Clin Endocrinol (Oxf) 59:768–772

Peinado H, Olmeda D, Cano A (2007) Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer 7:415–428

Qian ZR, Li CC, Yamasaki H, Mizusawa N, Yoshimoto K, Yamada S, Tashiro T, Horiguchi H, Wakatsuki S, Hirokawa M, Sano T (2002) Role of E-cadherin, alpha-, beta-, and gamma-catenins, and p120 (cell adhesion molecules) in prolactinoma behavior. Mod Pathol 15:1357–1365

Qian ZR, Sano T, Yoshimoto K, Asa SL, Yamada S, Mizusawa N, Kudo E (2007) Tumor-specific downregulation and methylation of the CDH13 (H-cadherin) and CDH1 (E-cadherin) genes correlate with aggressiveness of human pituitary adenomas. Mod Pathol 20:1269–1277

Ragel BT, Couldwell WT (2004) Pituitary carcinoma: a review of the literature. Neurosurg Focus 16:E7

Salahshor S, Naidoo R, Serra S, Shih W, Tsao MS, Chetty R, Woodgett JR (2008) Frequent accumulation of nuclear E-cadherin and alterations in the Wnt signaling pathway in esophageal squamous cell carcinomas. Mod Pathol 21:271–281

Schwechheimer K, Zhou L, Birchmeier W (1998) E-cadherin in human brain tumours: loss of immunoreactivity in malignant meningiomas. Virchows Arch 432:163–167

Selman WR, Laws ER Jr, Scheithauer BW, Carpenter SM (1986) The occurrence of dural invasion in pituitary adenomas. J Neurosurg 64:402–407

Tsuchiya B, Sato Y, Kameya T, Okayasu I, Mukai K (2006) Differential expression of N-cadherin and E-cadherin in normal human tissues. Arch Histol Cytol 69:135–145

Tziortzioti V, Ruebel KH, Kuroki T, Jin L, Scheithauer BW, Lloyd RV (2001) Analysis of beta-catenin mutations and alpha-, beta-, and gamma-catenin expression in normal and neoplastic human pituitary tissues. Endocr Pathol 12:125–136

Vleminckx K, Vakaet L Jr, Mareel M, Fiers W, van Roy F (1991) Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell 66:107–119

Xu B, Sano T, Yoshimoto K, Yamada S (2002) Downregulation of E-cadherin and its undercoat proteins in pituitary growth hormone cell adenomas with prominent fibrous bodies. Endocr Pathol 13:341–351

Yamada S, Ohyama K, Taguchi M, Takeshita A, Morita K, Takano K, Sano T (2007) A study of the correlation between morphological findings and biological activities in clinically nonfunctioning pituitary adenomas. Neurosurgery 61:580–584, discussion 584–585

Pituitary Tumorigenesis: Role of the Wnt Signaling Pathway

20

Marianne S. Elston

Contents

Abstract

Wnt signaling is important in the regulation of normal embryological development of the pituitary gland. Many human tumors have been identified as having altered Wnt signaling and recently the Wnt signaling pathways have also been identified as being perturbed in pituitary adenomas. Whilst data on the presence of nuclear β-catenin, the central mediator of the canonical Wnt signaling pathway, have been conflicting, altered expression of the Wnt signaling pathway inhibitors, WIF1 and members of the SFRP family appear to be common in pituitary adenomas and are likely to contribute to pituitary tumorigenesis. The mechanism by which this occurs is yet to be elucidated but is likely to occur through one of the non-canonical Wnt signaling pathways. Targeting Wnt signaling may prove to be a novel therapeutic option for pituitary tumors. However, due to our incomplete understanding of the roles of the different Wnt pathways in pituitary tumors and the crosstalk between pathways, such therapy needs to be carefully studied due to the potential for unexpected deleterious or long-term consequences.

Introduction

Clinically diagnosed pituitary adenomas have a prevalence of ~1/1,000 (Daly et al. 2006). Although more than 99% of these pituitary tumors are benign, they may still result in significant morbidity and even premature mortality due to mass effect and/or

M.S. Elston (✉)
Department of Endocrinology, Waikato Hospital, Private Bag 3200, Hamilton, Waikato, 3240, New Zealand
e-mail: marianne.elston@waikatodhb.health.nz

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_20,

hormonal dysfunction. The majority of pituitary tumors are sporadic but the molecular pathogenesis of these relatively common tumors is still not understood. New techniques in modern molecular biology, such as microarray analysis, have identified several previously unrecognized pathways, such as the Wnt signaling pathways, that are dysregulated in pituitary tumors. Wnt signaling is known to be important in pituitary development and dysregulation of Wnt signaling has been well characterized in other human tumor types, in particular colorectal cancer, although until relatively recently these pathways have received little attention in pituitary tumors. This chapter briefly reviews the Wnt signaling pathways and focuses on the potential role of Wnt signaling in pituitary tumorigenesis.

Overview of the Wnt Signaling Pathways

The term "Wnt" is derived from the mouse *Wnt1* gene which was initially termed *Int-1* as it is the preferred integration site for the Mouse Mammary Tumor Virus (MMTV) in virally induced breast tumors (Nusse and Varmus 1982). *Int-1* was identified as the mammalian homologue of the Drosophila gene *wingless* therefore the term *Wnt1* was coined as the amalgamation of *wingless* and *Int-1*. Wnts are a highly conserved family of secreted glycoproteins which are important for the normal development of both invertebrates and vertebrates. There are 19 known *WNT* genes in mammals and these are typically classified into those associated with the so-called canonical, or Wnt-β-catenin, pathway (Wnt1, Wnt2, Wnt2B etc.) and those associated with non-canonical pathways (Wnt5A, Wnt5B, Wnt11 etc.). In addition, some Wnt ligands appear to influence either pathway type depending on the cellular context. Wnt target genes are often tissue-specific, and a large number of downstream Wnt signaling targets have been identified including genes involved in proliferation such as *MYC*, *cyclin D1 (CCND1), p21,* and *PITX2*.

Wnt signaling is regulated by the balance between the various Wnt ligands, their receptors, and the Wnt antagonists. Two main classes of Wnt antagonists inhibit Wnt signaling. These two groups of Wnt inhibitors can be classified according to the mechanism by which inhibition occurs. The first group, which includes the five member secreted frizzled-related protein (SFRP) family and Wnt inhibitory factor 1 (WIF1), bind directly to the Wnt ligand and inhibit it binding to the frizzled receptor complex. These inhibitors therefore theoretically may inhibit either the canonical or non-canonical pathways. The second group of Wnt inhibitors includes the Dickkopf (DKK) and Sclerostin (SOST) families which inhibit canonical Wnt signaling by binding directly to the lipoprotein receptor-related protein (LRP) 5 or LRP6 co-receptor.

Three major Wnt signaling pathways have been described: the Wnt-β-catenin, or canonical, pathway; the Wnt/Jun N-terminal kinase (JNK); and the Wnt-calcium pathway. Of these pathways by far the best characterized is the Wnt-β-catenin pathway (Fig. 20.1) which has been implicated in the pathogenesis of a number of human tumor types. The canonical pathway has been most intensively investigated in colon cancer in which mutations occur in one or more of the key pathway components; *Adenomatous polyposis coli* (*APC*), *CTNNB1,* the gene encoding β-catenin, or *AXIN1*, in almost all colorectal tumors (Morin et al. 1997). These mutations result in failure of phosphorylation and ubiquitin-mediated destruction of β-catenin which therefore accumulates in the cytoplasm. Beta-catenin can then translocate into the nucleus to influence the transcription of target genes such as cyclin D1, resulting in increased cell proliferation.

The Wnt/JNK pathway was initially discovered due to its role in planar cell polarity in *Drosophila*. Binding of Wnt ligands such as Wnt11 and Wnt5A to the frizzled receptor leads to the recruitment of small GTPases such as RhoA and cdc42 resulting in activation of JNK or rho kinase. Wnt5A, in addition to acting as a ligand in the Wnt/JNK pathway, also stimulates the Wnt-calcium pathway resulting in intracellular calcium release and activation of protein kinase C, calmodulin kinase II and calcineurin. Whilst the Wnt-β-catenin pathway has been well established to have a pivotal role in tumorigenesis, these non-canonical pathways have been less well-defined and evidence of their role in cancer development has only relatively recently emerged (Jessen 2009).

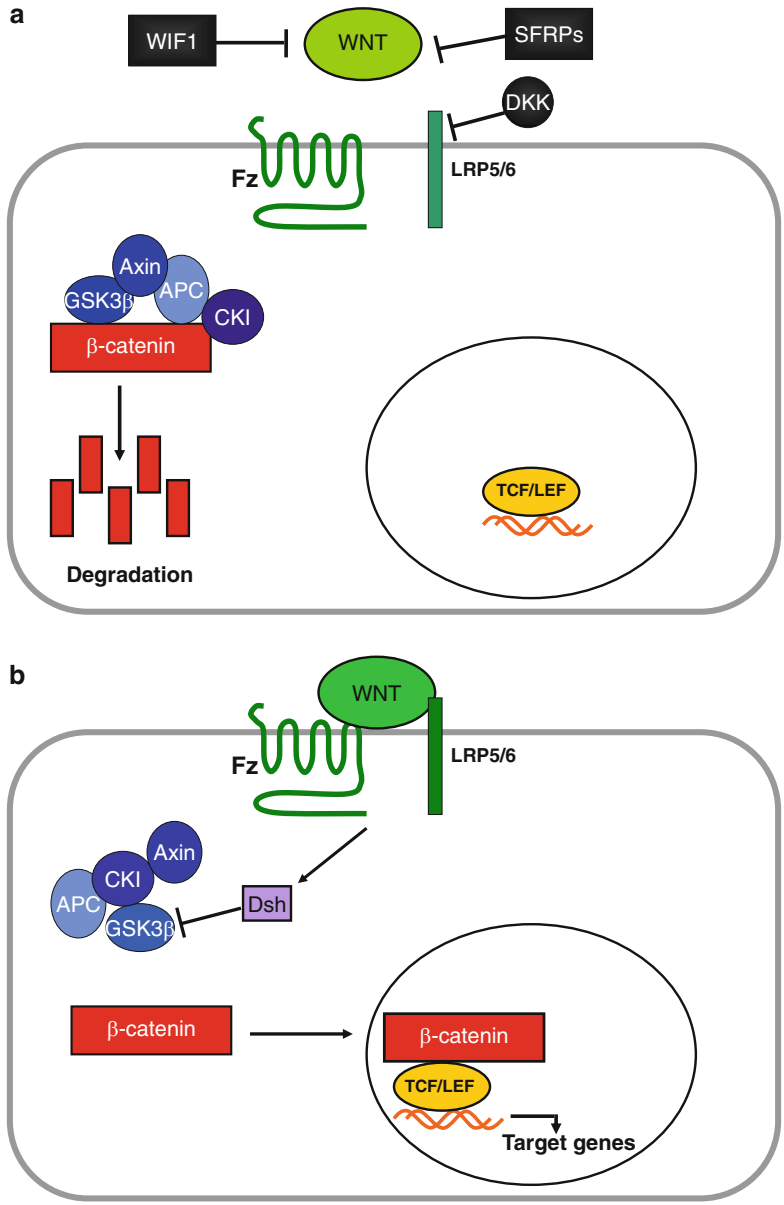

Fig. 20.1 Summary of the Wnt-beta-catenin pathway. (**a**) The Wnt inhibitors, Wnt inhibitory factor 1 (*WIF1*) and the secreted frizzled-related proteins (*SFRPs*) block Wnt signaling by binding to the Wnt ligand preventing activation of the frizzled (*Fz*) lipoprotein receptor-related protein (*LRP*) receptor complex. The Dickkopf (*DKK*) family binds to the *LRP5/6* co-receptor. In the absence of Wnt signaling, *β-catenin* is phosphorylated by the destruction complex of Adenomatous polyposis coli (*APC*), *Axin*, glycogen synthase kinase 3, beta (*GSK3β*) and casein kinase 1 (*CK1*). Phosphorylated beta-catenin is recognized by the E3 ubiquitin ligase complex and following ubiquitination is rapidly degraded by the proteosome. Wnt target genes are repressed by the binding of Groucho to the T-cell factor (*TCF*)/lymphoid enhancer binding factor (*LEF*) family of transcription factors. (**b**) Binding of the Wnt ligand to the Fz/LRP5/6 receptor complex activates Dishevelled (*Dsh*) resulting in phosphorylation of the LRP co-receptor by CKI and GSK3β which results in recruitment and disassembly of the degradation complex thereby beta-catenin remains unphosphorylated. Beta-catenin thus accumulates in the cytoplasm and translocates into the nucleus where it can affect the transcription of target genes (Reprinted from Elston and Clifton-Bligh (2010), Copyright 2010, with permission from Elsevier)

Wnt Signaling and Pituitary Development

Wnt signaling is important in normal pituitary development. The pituitary gland, with dual embryonic origin from oral and neural ectoderm, forms under the control of extrinsic developmental signals such as members of the Wnt signaling families, bone morphogenic protein-4 (BMP4), and members of the fibroblast growth factor (FGF) family as well as intrinsic signals from the oral ectoderm such as Sonic hedgehog (Treier et al. 1998). Various Wnt ligands have been demonstrated to be expressed during pituitary development, in particular Wnt4 and Wnt5a. *Wnt4* has been demonstrated to be expressed in Rathke's pouch (Treier et al. 1998). Decreased α-subunit, thyrotroph and somatotroph cell populations have been reported in *Wnt4*$^{-/-}$ mice as compared to wild-type littermates (Treier et al. 1998; Potok et al. 2008). Wnt5a expression has been reported throughout the ventral diencephalon (Treier et al. 1998). *Wnt5a*$^{-/-}$ mice have abnormal branching of the dorsal pouch although the anterior lobe was reported to appear similar to wild-type morphologically (Potok et al. 2008). Disruption of *Wnt5a* expression also results in altered FGF and BMP expression in the ventral diencephalon which is consistent with cross-talk between these pathways in pituitary development (Potok et al. 2008). In addition to Wnt4 and Wnt5a a number of other Wnt pathway members have also been detected during murine pituitary development. These include the Wnt ligands *Wnt2b*, *3*, *10b*, *11*, and *16*; the Frizzled receptors, *Fzd 1*, *2*, *3*, *4*, *6*, *8*; and the Wnt inhibitors *Wif1*, *Wnt inhibitor in the surface ectoderm* (*Wise*), and *Sost* (Potok et al. 2008).

Beta-Catenin and Pituitary Tumorigenesis

The central mediator of the canonical Wnt signaling pathway is β-catenin and until recently this was the main Wnt pathway member studied in pituitary tumors. Activation of the canonical Wnt signaling pathway results in nuclear accumulation of β-catenin which is readily detectable by immunohistochemistry. This may occur due to a number of mechanisms such as loss of Wnt pathway inhibitors, abnormalities in the destruction complex such as inactivating mutations in AXIN1, or activating mutations in *CTNNB1*. Of the seven published papers reporting on the presence/absence of nuclear β-catenin immunohistochemistry in pituitary adenomas, five did not identify nuclear β-catenin in any pituitary adenomas (Qian et al. 2002; Xu et al. 2002; Buslei et al. 2005; Elston et al. 2008; Miyakoshi et al. 2008) and a sixth described only infrequent staining (2/154 cases) (Tziortzioti et al. 2001). This contrasts to the findings of Semba et al. who reported nuclear staining in 21 of the 37 adenomas studied (Semba et al. 2001). The reason for these conflicting results is unclear. In an attempt to clarify this issue immunohistochemistry was performed in a group of 24 pituitary tumors using the anti-ABC clone 8E7 (Upstate cell signaling solutions, Lake Placid, NY, USA) (Elston, unpublished data). This antibody recognizes amino acid residues 36–44 of the β-catenin molecule and is specific for the active form of β-catenin (dephosphorylated on serine 37 or threonine 41) (anti-ABC). Unphosphorylated β-catenin accumulates if there is activation of the pathway; therefore an antibody which detects this particular form of β-catenin may be more sensitive in detecting nuclear accumulation than a less specific antibody. Nuclear staining was identified in all tumors, however normal pituitary tissue also had scattered cells showing staining although this was significantly less than seen in tumors (Elston, unpublished data). Whilst these findings are suggestive of activation of the Wnt-β-catenin pathway the validity of these findings is unclear as a recent report suggested that the anti-ABC antibody 8E7 also cross-reacts with a widely expressed, variably accessible, nuclear antigen which is not β-catenin (Maher et al. 2009). Therefore, on balance, there does not appear to be convincing evidence for frequent nuclear accumulation of β-catenin in pituitary adenomas to date.

Mutations in Beta-Catenin Destruction Complex Members

The study by Semba et al. which reported the presence of nuclear staining for β-catenin in 57% of pituitary tumors also included an analysis of exon 3 of *CTNNB1*, the gene which encodes β-catenin (Semba et al. 2001). Somatic mutations in exon 3, which is the hot spot for activating mutations, were identified in 4/21 samples studied (Semba et al. 2001). These findings have not been confirmed by other groups. Tziortzioti et al. (2001) did not identify any mutations in a 23 sample cohort (which included two samples with nuclear β-catenin accumulation on immunohistochemistry). Findings from an additional 113 patients have been reported and no *CTNNB1* mutations identified (Xu et al. 2002; Buslei et al. 2005; Oikonomou et al. 2005; Lee et al. 2009). Mutations in other key pathway genes (*GSK3β, APC, AXIN1*) have also been investigated but not identified (Buslei et al. 2005; Sun et al. 2005). In addition, normal expression of the APC protein has been reported (Sun et al. 2005). Based on these studies it appears that mutations in the genes encoding β-catenin and the destruction complex members do not play a significant role in pituitary tumorigenesis.

Wnt Inhibitor Expression in Pituitary Tumors

Microarray analysis provides a snapshot of gene expression at one particular point in time by allowing assessment of the expression of thousands of genes simultaneously and potentially may identify genes which may not be selected for study by other means. It was by this technique that altered expression of Wnt pathway antagonists in pituitary tumors were first identified suggesting that, despite the lack of nuclear β-catenin in pituitary tumors, Wnt signaling may play an important role in pituitary tumorigenesis. *SFRP1* was the first Wnt inhibitor identified as having altered expression in pituitary tumors in a study where 11 nonfunctioning adenomas (NFAs) were compared to 8 normal pituitary samples (Moreno et al. 2005). This group identified elevated expression of *SFRP1* in NFAs although this gene was not selected for further validation. In addition they also identified increased expression of *PITX2* and *CCND1*, two Wnt- β-catenin target genes. SFRP1 has previously been reported to act as either a Wnt antagonist or agonist (Rubin et al. 2006) but given the two elevated Wnt target genes the authors concluded that these results were most probably consistent with increased Wnt/β-catenin signaling in NFAs (Moreno et al. 2005). Most studies have demonstrated that the *SFRP* family members are putative tumor suppressor genes which has been shown in a number of human tumors including gastrointestinal, breast, cervical and bladder cancers (Rubin et al. 2006). However *SFRP1* has been reported to have anti-apoptotic effects in uterine leiomyomas (Rubin et al. 2006) suggesting that the net effect of *SFRP1* expression may well be context dependent. Elevation of *SFRP1* expression in NFAs has been confirmed by other groups (Altenberger et al. 2006; Shorts-Cary et al. 2007) although only one group has also assessed *SFRP1* mRNA expression using real-time quantitative RT-PCR (qPCR) which showed that whilst elevated *SFRP1* mRNA expression was present in a significant subset of NFAs (22/31) this was not significant for the entire cohort of NFAs nor in the other pituitary tumor subtypes studied (growth hormone (GH)-, adrenocorticotropin (ACTH)- and thyroid-stimulating hormone- secreting adenomas) (Elston et al. 2008).

In addition to *SFRP1*, on review of microarray data from other studies a number of other *SFRP* family members have also been shown to demonstrate altered gene expression. In a 2005 microarray study (Morris et al. 2005) *SFRP4* was under-expressed in NFAs, GH- ACTH- and prolactin (PRL)-secreting adenomas (−15.4-fold, −12.7-fold, −3.2-fold and −7.8-fold, respectively) (data accessible at NCBI GEO database accession GSE 2175). These findings have also been confirmed by other groups (Altenberger et al. 2006; Shorts-Cary et al. 2007; Elston et al. 2008) and it appears that under-expression of *SFRP4* in pituitary tumors occurs across all subtypes, both

functioning and nonfunctioning. Similar findings have also been reported for *SFRP2* (Shorts-Cary et al. 2007; Elston et al. 2008) and *SFRP3* (better known as *FRZB* [frizzled motif associated with bone development]) (Altenberger et al. 2006; Elston et al. 2008). This contrasts to *SFRP5* which was shown to have borderline elevation in only one study (Shorts-Cary et al. 2007) whereas when assessed by qPCR no difference in mRNA expression was identified in either functioning or NFAs as compared to normal pituitary tissue (Elston et al. 2008).

WIF1 inhibits Wnt signaling by the same mechanism as the SFRP family – by binding to the Wnt ligand thus preventing it interacting with the frizzled/LRP receptor complex. A number of different cancers and cell lines have been shown to have decreased *WIF1* expression including lung and gastrointestinal cancers (Mazieres et al. 2004; Taniguchi et al. 2005). *WIF1* expression has recently been demonstrated to be significantly decreased across all pituitary adenoma subtypes (apart from prolactinomas which were not studied) as compared to normal pituitary tissue (Elston et al. 2008). Reduced expression of *WIF1* has been confirmed on review of microarray data from two other groups (Altenberger et al. 2006) (personal communication Greisa Vila, Anton Luger) and (Shorts-Cary et al. 2007) (personal communication Aaron Knox, Margaret Wierman), and more recently shown in a study of prolactinomas (Jiang et al. 2010). Validation of the microarray findings for *WIF1* were confirmed using qPCR in a larger group of tumors (Elston et al. 2008). Additionally, WIF1 protein expression was assessed by immunohistochemical staining and was also identified as being decreased in 76% of pituitary adenomas with the majority of tumors that showed preserved staining being clinically functioning adenomas (Elston et al. 2008) Restoration of WIF1 expression in cancer cell lines has been shown to decrease colony formation and decrease cell growth (Taniguchi et al. 2005). These tumor suppressor effects of *WIF1* have been demonstrated to persist even in the presence of downstream canonical Wnt pathway mutations (He et al. 2005). *In vitro* studies in pituitary tumors have demonstrated that, similar to other human tumors, *WIF1* is also likely to be a tumor suppressor gene in pituitary tumors, as transfection of *WIF1* into the GH3 cell line has been demonstrated to result in decreased cell growth and colony formation (Elston et al. 2008). Functional studies assessing the role of other Wnt pathway inhibitors in pituitary have yet to be reported. A common mechanism for *WIF1* underexpression is methylation of its promoter (Mazieres et al. 2004). Methylation of the CpG island in the promoter of *WIF1* has also been demonstrated in pituitary tumors although this was predominantly limited to NFAs (Elston et al. 2008). Somatic mutations in *WIF1* were not identified in the one study which assessed a group of 12 pituitary adenomas (Elston, unpublished data).

In contrast to the findings for the SFRP family and WIF1 there is less evidence for dysregulation of Wnt inhibitors which bind to the LRP5 or LRP6 co-receptors within the ternary complex with the Wnt ligand and Frizzled receptor (Fig. 20.1). These inhibitors are theoretically specific for the canonical Wnt-β-catenin pathway rather than affecting both canonical and non-canonical pathways. This group includes DKK1-4 and a DKK3-related protein termed Soggy (also known as DKKL1). In addition, Sclerostin domain containing 1 (SOSTDC1), a known bone morphogenetic protein antagonist, also acts as a Wnt antagonist by binding to LRP6. Similarly, SOST has also been reported to inhibit Wnt signaling by binding to LRP receptors. DKK2 (Shorts-Cary et al. 2007; Elston et al. 2008) and DKK3 expression (Shorts-Cary et al. 2007) have been found to be reduced in pituitary adenomas on microarray analysis but validation of these findings by another method such as qPCR has not yet been performed. Similarly, SOSTDC1 has been demonstrated to be under-expressed on the array data (Altenberger et al. 2006; Elston et al. 2008) but again these findings have yet to be validated.

Wnt Pathway Target Gene Expression in Pituitary Adenomas

Increased Wnt signaling due to loss, or decreased expression, of Wnt pathway inhibitors would be expected to result in increased expression of Wnt pathway target genes. However, assessment of

Wnt target genes is complicated by the presence of the different Wnt signaling pathways, the tissue specificity and sheer number of potential target genes as well as determining which pathway(s) is altered by the Wnt inhibitor affected. Increased expression in pituitary adenomas of cyclin D1 (a well-known Wnt-β-catenin target gene) has been demonstrated by a number of groups (Hibberts et al. 1999; Elston et al. 2008). Whilst this increased expression of cyclin D may be due to increased Wnt signaling activity this gene may also be elevated by a number of other mechanisms such as gene duplication (Hibberts et al. 1999) and possibly as a result of aberrant expression of E-cadherin in the nucleus (Elston et al. 2009). As detailed above, *PITX2* has also been reported to be elevated in pituitary tumors (Moreno et al. 2005). *PTTG1* which is known to be over-expressed across all pituitary adenoma subtypes (Zhang et al. 1999), may also be regulated by the Wnt-β-catenin pathway as it has TCF4 binding sites in the promoter (Zhou et al. 2005). Some of the other known Wnt-β-catenin target genes have demonstrated altered expression which varies according to tumor subtype. For example, P21 has been demonstrated to show increased immunohistochemical staining in clinically functioning adenomas; in particular GH-secreting adenomas however most NFAs demonstrated low levels of staining (Neto et al. 2005). Abnormal *MYC* expression has also been demonstrated in a subset of pituitary adenomas (Wang et al. 1996). Given that nuclear β-catenin expression does not appear to be commonly detectable in pituitary adenomas, although increased expression of some Wnt-β-catenin target genes has been demonstrated which can be considered consistent with activation of the canonical Wnt pathway in pituitary adenomas this is far from conclusive as alternative mechanisms may be responsible for the altered gene expression. Support for the non-canonical Wnt pathways being involved in pituitary tumorigenesis comes from a recent study assessing Wnt signaling in estrogen-induced lactotroph proliferation (Giles et al. 2011). In this rat model, estrogen treatment was demonstrated to induce Wnt4 mRNA expression. Similar findings were also found at both the mRNA and protein level in the GH3 cell line. However addition of Wnt-conditioned culture media to GH3 cells failed to result in activation of canonical Wnt signaling or nuclear expression of β-catenin but altered calcium signaling was identified suggestive of a non-canonical Wnt pathway process. Further work remains to be done in this exciting area to clarify which of the Wnt pathways are involved in pituitary tumorigenesis.

In conclusion, there appears to be convincing evidence for dysregulation of the Wnt pathway inhibitors WIF1 and members of the SFRP family (with the exception of SFRP5) in pituitary adenomas. Functional studies on WIF1 have demonstrated that, similar to other tumor types WIF1 appears to be acting as a tumor suppressor gene in the pituitary although there is a paucity of functional data for the other SFRP family members. Wnt inhibitors specific to the canonical pathway are less convincingly altered in pituitary tumors. It is unclear by which pathway WIF1 and the SFRP members are acting, and given the lack of nuclear β-catenin, these genes may be acting through a non-canonical pathway. These pathways have not been as well characterized as the Wnt-β-catenin pathway but do appear to be involved in tumorigenesis in other organs. Further work is needed to determine the exact mechanisms by which these genes may be involved in pituitary tumorigenesis, in particular their effects on non-canonical signaling. Wnt pathway inhibitors may be potential therapeutic targets although cross-talk between signaling pathways is well known, for example interaction of the Wnt and Hedgehog pathways through SFRP1 and the commonality of GSK3β to Wnt and other signaling pathways such as the AKT/PI3 kinase pathway. Until we have a greater understanding of which pathway/s the Wnt inhibitors are acting through in pituitary adenomas and of the interactions of Wnt signaling pathways with other pathways, manipulation of these mechanisms with the use of specific targets in pituitary tumors remains premature.

References

Altenberger T, Bilban M, Auer M, Knosp E, Wolfsberger S, Gartner W, Mineva I, Zielinski C, Wagner L, Luger A (2006) Identification of DLK1 variants in pituitary- and

neuroendocrine tumors. Biochem Biophys Res Commun 340:995–1005

Buslei R, Nolde M, Hofmann B, Meissner S, Eyupoglu IY, Siebzehnrubl F, Hahnen E, Kreutzer J, Fahlbusch R (2005) Common mutations of beta-catenin in adamantinomatous craniopharyngiomas but not in other tumours originating from the sellar region. Acta Neuropathol (Berl) 109:589–597

Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A (2006) High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. J Clin Endocrinol Metab 91:4769–4775

Elston MS, Clifton-Bligh RJ (2010) Identification of Wnt family inhibitors: a pituitary tumor directed whole genome approach. Mol Cell Endocrinol 326(1–2):48–54

Elston MS, Gill AJ, Conaglen JV, Clarkson A, Shaw JM, Law AJ, Cook RJ, Little NS, Clifton-Bligh RJ, Robinson BG, McDonald KL (2008) Wnt pathway inhibitors are strongly down-regulated in pituitary tumors. Endocrinology 149:1235–1242

Elston MS, Gill AJ, Conaglen JV, Clarkson A, Cook RJ, Little NS, Robinson BG, Clifton-Bligh RJ, McDonald KL (2009) Nuclear accumulation of e-cadherin correlates with loss of cytoplasmic membrane staining and invasion in pituitary adenomas. J Clin Endocrinol Metab 94:1436–1442

Giles A, Madec F, Friedrichsen S, Featherstone K, Chambers T, Harper CV, Resch J, Brabant G, Davis JR (2011) Wnt signaling in estrogen-induced lactotroph proliferation. J Cell Sci 124:540–547

He B, Reguart N, You L, Mazieres J, Xu Z, Lee AY, Mikami I, McCormick F, Jablons DM (2005) Blockade of Wnt-1 signaling induces apoptosis in human colorectal cancer cells containing downstream mutations. Oncogene 24:3054–3058

Hibberts NA, Simpson DJ, Bicknell JE, Broome JC, Hoban PR, Clayton RN, Farrell WE (1999) Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. Clin Cancer Res 5:2133–2139

Jessen JR (2009) Noncanonical Wnt signaling in tumor progression and metastasis. Zebrafish 6:21–28

Jiang Z, Gui S, Zhang Y (2010) Analysis of differential gene expression by fiber-optic BeadArray and pathway in prolactinomas. Endocrine 38:360–368

Lee CI, Hsu MY, Chou CH, Wang C, Lo YS, Loh JK, Howng SL, Hong YR (2009) CTNNB1 (beta-catenin) mutation is rare in brain tumours but involved as a sporadic event in a brain metastasis. Acta Neurochir (Wien) 151:1107–1111

Maher MT, Flozak AS, Hartsell AM, Russell S, Beri R, Peled ON, Gottardi CJ (2009) Issues associated with assessing nuclear localization of N-terminally unphosphorylated beta-catenin with monoclonal antibody 8E7. Biol Direct 4:5

Mazieres J, He B, You L, Xu Z, Lee AY, Mikami I, Reguart N, Rosell R, McCormick F, Jablons DM (2004) Wnt inhibitory factor-1 is silenced by promoter hypermethylation in human lung cancer. Cancer Res 64:4717–4720

Miyakoshi T, Takei M, Kajiya H, Egashira N, Takekoshi S, Teramoto A, Osamura RY (2008) Expression of Wnt4 in human pituitary adenomas regulates activation of the beta-catenin-independent pathway. Endocr Pathol 19:261–273

Moreno CS, Evans CO, Zhan X, Okor M, Desiderio DM, Oyesiku NM (2005) Novel molecular signaling and classification of human clinically nonfunctional pituitary adenomas identified by gene expression profiling and proteomic analyses. Cancer Res 65:10214–10222

Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW (1997) Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275:1787–1790

Morris DG, Musat M, Czirjak S, Hanzely Z, Lillington DM, Korbonits M, Grossman AB (2005) Differential gene expression in pituitary adenomas by oligonucleotide array analysis. Eur J Endocrinol 153:143–151

Neto AG, McCutcheon IE, Vang R, Spencer ML, Zhang W, Fuller GN (2005) Elevated expression of p21 (WAF1/Cip1) in hormonally active pituitary adenomas. Ann Diagn Pathol 9:6–10

Nusse R, Varmus HE (1982) Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 31:99–109

Oikonomou E, Barreto DC, Soares B, De Marco L, Buchfelder M, Adams EF (2005) Beta-catenin mutations in craniopharyngiomas and pituitary adenomas. J Neurooncol 73:205–209

Potok MA, Cha KB, Hunt A, Brinkmeier ML, Leitges M, Kispert A, Camper SA (2008) WNT signaling affects gene expression in the ventral diencephalon and pituitary gland growth. Dev Dyn 237:1006–1020

Qian ZR, Li CC, Yamasaki H, Mizusawa N, Yoshimoto K, Yamada S, Tashiro T, Horiguchi H, Wakatsuki S, Hirokawa M, Sano T (2002) Role of E-cadherin, alpha-, beta-, and gamma-catenins, and p120 (cell adhesion molecules) in prolactinoma behavior. Mod Pathol 15:1357–1365

Rubin JS, Barshishat-Kupper M, Feroze-Merzoug F, Xi ZF (2006) Secreted WNT antagonists as tumor suppressors: pro and con. Front Biosci 11:2093–2105

Semba S, Han SY, Ikeda H, Horii A (2001) Frequent nuclear accumulation of beta-catenin in pituitary adenoma. Cancer 91:42–48

Shorts-Cary L, Xu M, Ertel J, Kleinschmidt-Demasters BK, Lillehei K, Matsuoka I, Nielsen-Preiss S, Wierman ME (2007) Bone morphogenetic protein and retinoic acid-inducible neural specific protein-3 is expressed in gonadotrope cell pituitary adenomas and induces proliferation, migration, and invasion. Endocrinology 148:967–975

Sun C, Yamato T, Kondo E, Furukawa T, Ikeda H, Horii A (2005) Infrequent mutation of APC, AXIN1, and GSK3B in human pituitary adenomas with abnormal accumulation of CTNNB1. J Neurooncol 73:131–134

Taniguchi H, Yamamoto H, Hirata T, Miyamoto N, Oki M, Nosho K, Adachi Y, Endo T, Imai K, Shinomura Y (2005) Frequent epigenetic inactivation of Wnt inhibi-

tory factor-1 in human gastrointestinal cancers. Oncogene 24:7946–7952

Treier M, Gleiberman AS, O'Connell SM, Szeto DP, McMahon JA, McMahon AP, Rosenfeld MG (1998) Multistep signaling requirements for pituitary organogenesis in vivo. Genes Dev 12:1691–1704

Tziortzioti V, Ruebel KH, Kuroki T, Jin L, Scheithauer BW, Lloyd RV (2001) Analysis of beta-catenin mutations and alpha-, beta-, and gamma-catenin expression in normal and neoplastic human pituitary tissues. Endocr Pathol 12:125–136

Wang DG, Johnston CF, Atkinson AB, Heaney AP, Mirakhur M, Buchanan KD (1996) Expression of bcl-2 oncoprotein in pituitary tumours: comparison with c-myc. J Clin Pathol 49:795–797

Xu B, Sano T, Yoshimoto K, Yamada S (2002) Downregulation of E-cadherin and its undercoat proteins in pituitary growth hormone cell adenomas with prominent fibrous bodies. Endocr Pathol 13: 341–351

Zhang X, Horwitz GA, Heaney AP, Nakashima M, Prezant TR, Bronstein MD, Melmed S (1999) Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas. J Clin Endocrinol Metab 84:761–767

Zhou C, Liu S, Zhou X, Xue L, Quan L, Lu N, Zhang G, Bai J, Wang Y, Liu Z, Zhan Q, Zhu H, Xu N (2005) Overexpression of human pituitary tumor transforming gene (hPTTG), is regulated by beta-catenin /TCF pathway in human esophageal squamous cell carcinoma. Int J Cancer 113:891–898

The Role of Aryl Hydrocarbon Receptor (AHR) and AHR-Interacting Protein (AIP) in the Pathogenesis of Pituitary Adenomas

21

Marie-Lise Jaffrain-Rea and Albert Beckers

Contents

M.-L. Jaffrain-Rea (✉)
Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, via Vetoio, Neuromed Institute Coppito 2, L'Aquila 67100, Italy
e-mail: jaffrain.ml@libero.it

A. Beckers
Department of Endocrinology, Centre Hospitalier Universitaire de Liege, Domaine Universitaire du Sart-Tilman, Liege 4000, Belgium

Abstract

Pituitary adenomas (PA) are common endocrine neoplasia, generally presenting as sporadic diseases, with a multifactorial pathogenesis including somatic mutational events in cancer-related genes. However, genetic predisposition can currently be recognized in >5% of affected patients, mostly involving the Multiple Endocrine Neoplasia type 1 (*MEN1*) gene and the more recently identified Aryl hydrocarbon receptor Interacting Protein (*AIP*) gene, both being tumor-suppressor genes. Germline mutations in the AIP gene can be observed in a FIPA (Familial Isolated Pituitary Adenoma) context, but also in a minority of young patients with an apparently sporadic disease. Although the role of AIP in the pathogenesis of PA remains largely unknown, it is known to be mainly expressed by somatototrophs, with a frequent loss of expression in most AIP-mutated PA and in invasive somatotropinomas. The best characterized function of AIP is to stabilize the Aryl hydrocarbon Receptor, also known as the dioxin receptor, in the cytoplasm, but multiple interactions of AIP with other proteins involved in endocrine signalling and the regulation of cell cycle and apoptosis have been reported. In this chapter, current knowledge about the possible role of AhR and additional AIP-related proteins in pituitary tumorigenesis will be analysed.

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_21,

Introduction

Pituitary adenomas (PA) are among the most frequent endocrine tumors, with a clinical prevalence approaching 1/1,000 inhabitants in recent studies (Beckers 2010). PA are typically benign, but extrasellar extension is frequent and invasive features towards surrounding structures (i.e., the dura, sellar bone, cavernous sinus) increase with tumor size. Malignant evolution is extremely rare and defined by extra-pituitary dissemination. PA are classified into secreting and non-secreting according to the presence or absence, respectively, of pituitary hormone hypersecretion. Their pathogenesis is multifactorial and, despite common monoclonality suggesting the presence of an initiating molecular event, they are usually sporadic, with a complex pathogenesis including genetic and epigenetic events and a variety of alterations in intra- and extra-cellular signalling (Asa and Ezzat 2009).

Inherited genetic susceptibility is being increasingly recognized and is currently estimated to involve at least 5% of PA patients. Arguments suggesting inherited susceptibility are: (1) the presence of extra-pituitary manifestations suggestive of a syndromic disease, namely the Multiple Endocrine Neoplasia type 1 syndrome (MEN1), which accounts for nearly 3% of PA, rarely the Carney's complex (CNC) or McCune Albright syndrome (MAS), (2) the presence of an isolated familiarity for PA, which was first recognized for patients with acromegaly (Isolated Familial Somatotropinoma – IFS), subsequently shown to potentially involve all PA phenotypes (Familial Isolated Pituitary Adenoma- FIPA) and also represents 2–3% of PA patients, (3) an early onset of the disease, especially in children and adolescents, who represent about only 10% of PA patients but are more likely to be syndromic or familial. We have recently reviewed the clinical presentation, molecular genetics and screening implications of the syndromic conditions cited hitherto (Jaffrain-Rea et al. 2010a) and we will focus our attention on the most relevant aspects of the FIPA syndrome and on the potential pathogenetic implications of the *Aryl hydrocarbon receptor Interacting Protein (AIP)* gene, which currently explains a significant subset of FIPA kindreds and has been involved in a minority of early onset PA with an apparently sporadic presentation.

Familial Isolated Pituitary Adenomas (FIPA), the *AIP* Gene and the Expanding Spectrum of *AIP*-Related Diseases

Familial tumors offer unique opportunities to characterize genes involved in their pathogenesis and, indirectly, in the normal physiology of the corresponding tissue. Familial forms of pituitary tumors occurring in association with hyperparathyroidism and pancreas endocrine neoplasia have defined the MEN1 syndrome and contributed to the identification of a disease locus in 11q13, leading in 1997 to the identification of the MEN1 gene, a major tumor suppressor gene. As recently reviewed by Thakker (2010), hundreds of inactivating mutations of the MEN1 gene could then be identified in MEN1 kindreds, genetic mice models have further supported the role of the MEN1 gene in disease susceptibility, and the molecular mechanisms involved in MEN1-related tumorigenesis are being progressively elucidated. Kindreds with isolated somatotropinomas (IFS) were already recognized to occur outside of the MEN1 syndrome and later confirmed to be unrelated to *MEN1* gene mutations. Nonetheless, linkage to the 11q13 region could be established in some cases by Galdelha et al. (1999), with documented LOH in the corresponding tumors suggesting the presence of a second tumor suppressor gene in this region. Recently, two important publications have lead to significant progress in the elucidation of inherited predisposition to PA linked to a new tumor suppressor gene in 11q13: (1) Vierimaa et al. (2006) identified germline inactivating mutations of the *AIP* gene in two large Finnish kindreds with GH/PRL-secreting PA and in an Italian IFS family; with somatic LOH in 11q13 being confirmed in the corresponding tumors, (2) Daly et al. (2006) characterized 64 kindreds with Familial Isolated Pituitary Adenomas (FIPA)

collected internationally, comprising both homogenous and heterogeneous families – i.e. expressing a single or multiple PA phenotypes, respectively –, the homogenous somatotropinoma/IFS subgroup representing ~ 30% of the whole series. Soon after, the same group reported germline mutations in the *AIP* gene in 15% of FIPA kindreds, and up to 50% of those with homogeneous somatotropinomas/IFS, respectively (Daly et al. 2007).

Clinical Presentation of Patients with a Germline AIP Mutation

The great interest in *AIP* as a new player in pituitary tumorigenesis has lead to a number of genetic studies on FIPA and sporadic PA allowing, within a 4 years period, to delineate the most common characteristics of AIP^{mut} patients and tumors, and to evaluate their therapeutic outcome. In a recent collaborative work collecting 96 AIP^{mut} patients worldwide (Daly et al., 2010), 60% cases occurred in a documented FIPA setting and 40% were apparently sporadic, respectively. Somatotropinomas were by far the most prevalent phenotype (~80%), followed by prolactinomas (~15%), NFPA, and rare corticotrophinomas and thyreotrophinomas. The median age at diagnosis and at first symptoms were 23 and 18 years overall, implying that half of the patients developed a symptomatic disease during childhood or adolescence. In this study, 75 AIP^{mut} somatotropinomas were compared to 232 *non-*AIP^{mut} somatotropinomas confirmed genetically, providing final evidence that the presence of germline *AIP* changes in acromegalics was associated with a much earlier age at diagnosis (20 years earlier) and a more aggressive course of the disease. The early onset translated into an unusual rate of overt or incipient gigantism (32 *vs* 6% in control cases). A male predominance was observed (>60%) and all giant were males. Somatotropinomas in the AIP^{mut} group were typically macroadenomas (>90%) and, as compared with non-AIP^{mut} somatotropinomas, they had significantly larger maximal tumor diameter. At diagnosis, AIP^{mut} acromegalics had higher median plasma GH levels and presented twice more frequently with PRL hypersecretion than non-AIP^{mut} patients. Disease control in AIP^{mut} somatotropinomas was also more difficult to achieve, with a lower decrease in GH/IGF-1 on somatostatin analogues therapy and a more frequent need for multiple surgeries and/or radiotherapy. Prolactinomas in AIP^{mut} patients were also recognized essentially in males (>75%), with a median age at diagnosis of 22 years, ~50% occurred in a familial setting and most were large and invasive, with an unusual rate of resistance to dopamine-agonist therapy (50%).

Molecular Genetics of AIP

Ozfirat and Korbonits (2010) have reviewed nearly 50 *AIP* mutations identified so far by international cohorts, reported by the Finnish, Belgian and British groups and additional family case reports. Most mutations are distributed through the entire coding sequence (6 exons), 60–70% are truncating – with a similar frequency for nonsense and frameshift mutations, followed by splice site mutations – and >20% are missense. The remaining alterations include large deletions and some changes of uncertain biological significance, such as rare polymorphisms and intron variants not expected to alter splicing. Promoter mutations have also been exceptionally reported. Some mutations have been encountered more frequently, such as AIP^{Q14X}- a founder mutation in Finland-, AIP^{R304X} – the most frequently reported in Europe, with a partial founder effect in Italy -, AIP^{R304Q} – which further indicates codon 304 as a hot mutational spot-, AIP^{R271W}, and AIP^{R81X}. Codon K241 may represent an additional hot spot, with both AIP^{K241E} and AIP^{K241X} changes being reported.

The penetrance of germline *AIP* mutations is incomplete. In FIPA kindreds, due to a still limited knowledge on large AIP^{mut} kindreds, it is currently but imprecisely estimated around 30% (15–45%). Preliminary data on familial screening in apparently sporadic patients indicate that *de novo* germline *AIP* mutations are very rare, but most AIP^{mut} relatives are unaffected. At the moment, *AIP* can

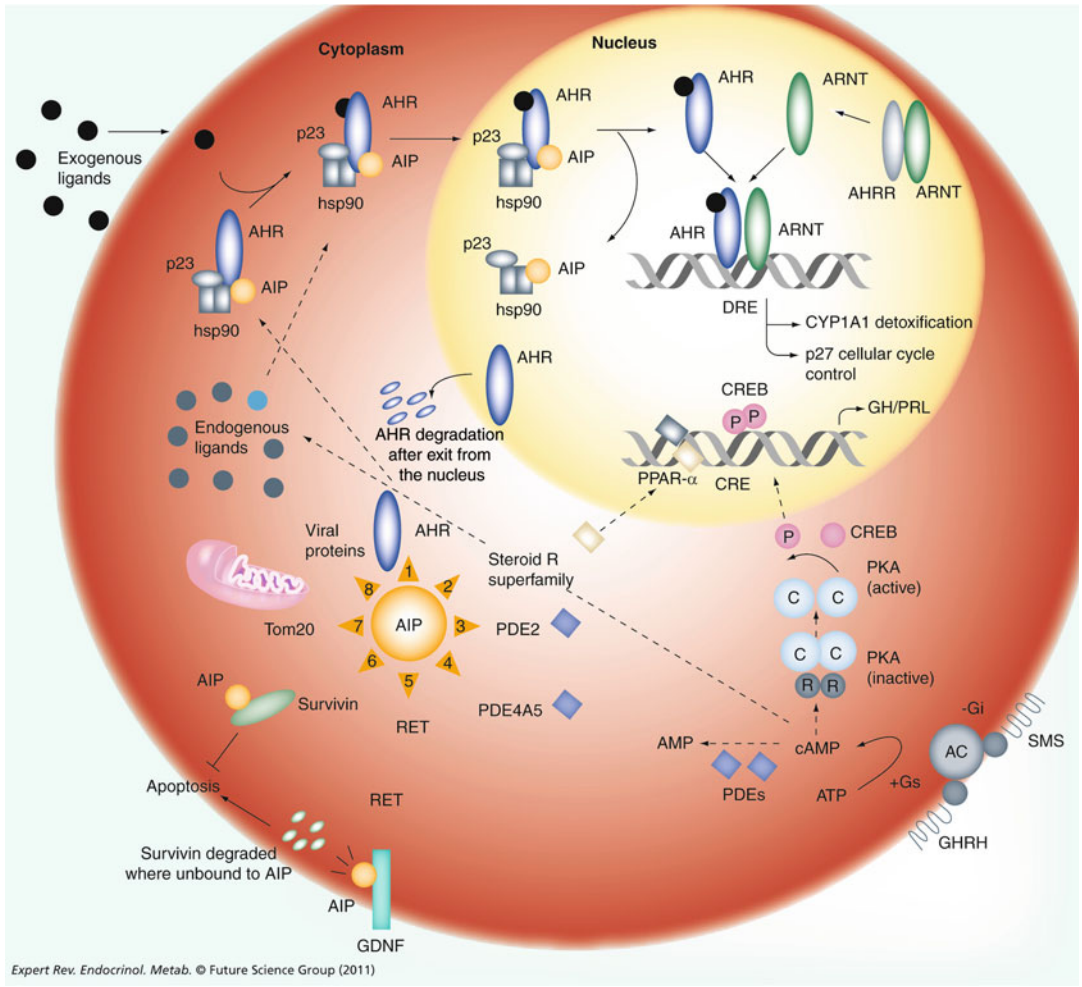

Fig. 21.1 AIP molecular interactions and related pathways. AIP is involved in multiple protein–protein interactions and potential molecular pathways: (1) the transcription factor AhR (Aryl hydrocarbon Receptor, or dioxin receptor) is stabilized by AIP in a cytoplasmic AhR/AIP/hsp90/p23 core complex; upon activation by exogenous AhR moves to the nucleus, heterodimerizes with Ah Receptor Nuclear Translocator (ARNT) and exerts direct transcriptional effects through Dioxin Responsive Elements (DRE) – activation by endogenous ligands (including cAMP) may elicit a different transcriptional response – (2) members of the steroid receptor superfamily include the transcription factor PPARα, the thyroid receptor TRβ1 and the glucocorticoid receptor, (3,4) Phosphodiesterases PDE2 and PDE4A5 are involved in the regulation of the cAMP concentration. cAMP is produced from ATP by the Adenyl Cyclase (AC), activates the Protein Kinase A (PKA) by binding its regulatory subunits (RR) and releasing its catalytic subunits (CC); this results in Ser-133 phosphorylation of the cAMP Responsive Elements Binding protein (CREB) and enhanced transcriptional activity through cAMP Responsive Elements (CRE), which are present in GH and PRL gene promoters; (5) the RET proto-oncogene is the tyrosine kinase receptor for the Glial cell line-Derived Neurotrophic Factor (GDNF) family of ligands; binding of AIP to RET prevents the formation of AIP/survivin complexes (6), in the absence of RET, AIP binds survivin, preventing its degradation thus protecting cells from apoptosis. (7) Tom 20 are proteins involved in mitochondrial import and (8) Viral proteins include the Hepatitis B virus X antigen and the Epstein Barr Virus (*EBV*)-encoded nuclear antigen-3 (EBNA-3). *See text for references* (NB Authorization to reproduce this figure from Jaffrain-Rea et al. (2010) has been obtained from Expert Reviews in Endocrinology and Metabolism)

be therefore considered either as a FIPA gene, which can be inherited in a dominant manner with a variable but clinically relevant penetrance, or as a PAP gene, with a low penetrance. These views are not necessarily conflicting since, similarly to disease severity, disease penetrance may vary

according to the biological effects of distinct mutations on target cells and the presence of genetic or environmental modifiers. The contrast between the frequent severity of the disease and its incomplete or low penetrance differentiates most *AIP*^{mut} kindreds from MEN1 kindreds and should be taken into account for genetic counselling (Jaffrain-Rea et al. 2010) (Fig. 21.1).

Lessons from an AIP$^{+/-}$ Mice Model

The homozygous *AIP*$^{-/-}$ knockout mice model reported by Lin et al. (2007) displayed severe developmental abnormalities and embryonic lethality but no recognized pituitary phenotype. However, the pituitary gland was not studied. Recently, the heterozygous *AIP*$^{+/-}$ mice model developed by Raitila et al. (2010) proved to develop pituitary tumors with a full penetrance. Such a high penetrance might be partially explained by the spontaneous development of PA in this strain of mice, since up to 40% of wild-type controls were also affected – mostly by prolactinomas – between 6 and 18 months-old. In contrast, 100% of *AIP*^{mut} animals developed PA – 80% somatotropinomas – from 3 to 15 months-old, with *AIP*^{mut} tumors being more aggressive and displaying a higher proliferative index than spontaneous tumors. These observations clearly support the tumor suppressing role of AIP in somatotrophs.

AIP, AHR and Other AIP-Related Proteins in the Pathogenesis of Pituitary Adenomas

Pituitary Expression of AIP

The interest towards the pituitary expression of AIP in the normal pituitary and its biological significance was born with its identification as a new pituitary tumor suppressing gene. Studies by Leontiou et al. (2008) and Jaffrain-Rea et al. (2009) have clearly shown an abundant expression of AIP in normal human pituitaries and indicated a topographic distribution largely overlapping that of GH-secreting cells, other AIP-expressing cells being mostly lactotrophs. This phenotypic characterization was confirmed by ultrastructural studies which also localized AIP in hormone-secreting granules. In contrast with the restricted expression of AIP in the normal pituitary, both groups have observed a potential, though heterogeneous, expression of AIP in all PA phenotypes. According to Real-Time RT-PCR analysis and immunohistochemical studies, the highest levels of AIP expression were observed in somatotropinomas, but also, unexpectedly, in non-functioning pituitary adenomas (NFPA). In *AIP*^{mut} tumors, AIP expression was typically down-regulated, due to hemizigosity, but the mutated protein could be readily detected in most cases. Intriguingly, we also noticed that AIP down-regulation was frequent in aggressive somatotropinomas, regardless of *AIP* mutations, and in the vast majority of prolactinomas, including microprolactinomas. These findings support a potential role for AIP in the pathogenesis of sporadic somatotropinomas, and suggest that, in addition to germline mutati ons, currently unrecognized mechanisms are responsible for AIP loss of expression in tumorous somatotrophs and lactotrophs.

The AIP Protein: From Structure to Function

The AIP protein, also known as ARA9/XAP2, is composed in humans of 330 aminoacids, with a high degree of conservation among species. Its expression starts in the embryo, and is nearly ubiquitous in the adult. From a functional point of view, its N-terminal half contains a FK506 binding peptidyl-prolyl cis-trans isomerase (PPI) domain sharing elevated homology with immunophilins of the FKBP52 class, whereas its C-terminal half contains three tetratricopeptide (TPR) domains, typically involved in protein-protein interactions, and a C-terminal α-helix (Carver et al. 1998). Therefore, AIP looks like a complex regulatory protein, able to indirectly modulate a number of cellular pathways and functions. Already characterized protein partners of AIP include: (1) the Aryl Hydrocarbon Receptor (AhR, also known as the "dioxin recep-

tor") itself, a member of the bHLH/PAS (basic Helix-Loop-Helix/Per-Arnt-Sim) family of transcription factors involved in cell response to polycyclic aromatic hydrocarbons but also in developmental processes and the regulation of cell cycle and differentiation, which unliganded form is stabilized in the cytoplasm in a multimeric AIP/AhR/Hsp90 complex; (2) the phosphodiesterases PDE4A5 and PDE2A, which are implicated in the cAMP signaling pathway; (3) the anti-apoptotic factor survivin and Ret, which prevents the formation of the AIP/survivin complex; (4) members of the steroid receptor superfamily such as PPAR-α, the β-thyroid hormone receptor 1 (TRβ1) and the glucocorticoid receptor; (5) additional proteins among which viral proteins and proteins involved in mitochondrial import (Ozfirat and Korbonits 2010 and Jaffrain-Rea et al. 2010a).

The molecular pathways involved in AIP-related pathogenesis have not been elucidated yet, but *in vitro* experiments on the rat lactosomatotroph cell line GH_3 have clearly shown that overexpression of the wild-type *AIP* gene reduces its proliferation rate, whereas the transfection of some AIP^{mut} genes inhibits this effect (Leontiou et al. 2008) and *AIP* gene silencing had a pro-proliferative effect (Heliövaara et al. 2009). This is in agreement with the proliferative index observed in the $AIP^{+/-}$ mice model cited hitherto (Raitila et al. 2010). Because most *AIP* mutations described so far may theoretically disrupt one or more functional interactions of AIP, we will now analyse which mechanisms could be the most attractive for pituitary tumorigenesis.

AIP and the Regulation of AHR Signalling

The best characterized function of AIP is to interact with the AhR/dioxin receptor and contributes to its stabilization in a AIP/AhR/hsp90/p23 cytoplasmic complex. The classical pathway for AhR activation is initiated by the binding of exogenous ligands such as dioxin and aromatic hydrocarbons. This stimulates the nuclear translocation of AhR and its heterodimerization with the closely related ARNT (AhR Nuclear Translocator, also known as the Hypoxia-Inducible Factor 1β, HIF1β). The AhR/ARNT complex then binds DNA consensus sequences known as XRE (xenobiotic-) /DRE(dioxin-response elements) localized in the promoter of responsive genes, recruits coactivator molecules and stimulates the transcription of a number of genes, including detoxifying enzymes which mediate the toxic response and genes involved in cell cycle control. The activated AhR is then quickly exported to the cytosol where it is degradated by the proteasome, hence preventing constitutive activity. There is also accumulating evidence for nucleo-cytoplasmic shuttling of AhR in the absence of exogenous ligands. Such findings, together with the ancestral and highly conserved expression of AhR, and the developmental abnormalities and diseases observed in the AhR null mice, clearly point to endogenous functions, which likely represent the key role of AhR during evolution and are supported by the report of an increasing number of potential endogenous activators of AhR. The complex control of gene expression by AhR and the potential crosstalk of AhR-related pathways with other signalling pathways, including hormone signalling, have been recently reviewed in details by Beischlag et al. (2008) and Puga et al. (2009), respectively. We will therefore attempt to evaluate their potential relevance to pituitary tumorigenesis.

AHR and the Pituitary Gland

The AhR is widely expressed in endocrine tissues, and its activation by exogenous dioxin-related compounds has been shown to potentially modulate pituitary function. Elango et al. (2006) reported that *in vitro* exposure of rainbow trout pituitary cells to 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) induces GH and prolactin (PRL) secretion, in part through AhR-mediated transcriptional effects. Dioxin is also able to interfere with both the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axis, but no clear-cut effect has been reported on normal adult corticotrophs or gonadotrophs *in vitro*.

Surprisingly, dioxin exposure appears to reduce the incidence of spontaneous PA in the rat, but not in humans.

We have recently studied the expression of AhR in the normal human pituitary and in a subset of human PA, including *AIP*^{mut} tumors, by Real-Time RT-PCR and immunohistochemistry (Jaffrain-Rea et al. 2009). The expression of AhR was lower than observed for AIP, but tended to have a broader phenotypic distribution. We used two anti-AhR antibodies, one monoclonal directed against a N-terminal epitope of the protein, near the DNA-binding domain, and one polyclonal against the C-terminal half of the protein, respectively. The first one showed a selective cytoplasmic immunostaining, which was significantly correlated with AIP expression, thereby supporting a role for AIP in the stabilization of AhR in the pituitary as reported in other tissues. Accordingly, cytoplasmic AhR expression was down-regulated in most *AIP*^{mut} PA and in *non-AIP*^{mut} PA displaying a low AIP expression. Nuclear immunostaining was best revealed by the C-terminal antibody, possibly due to epitope masquerading using the N-terminal antibody, but restricted to a minority of cases – mostly somatotropinomas and some AIP-expressing NFPA-. In contrast with Heliovaara et al. (2009) who reported nuclear AhR immunostaining in a subset of *AIP*^{mut} PA, this was not observed in our series. These data suggest that AhR signalling might be differentially regulated in PA, depending on phenotype and AIP status.

Down-regulation of the AhR partner ARNT has been observed in ~50% of the human *AIP*^{mut} PA studied by Heliovaara et al. (2009), and further supported by studies in the *AIP*$^{+/-}$ mice model developed by the same group (Raitila et al. 2010). In this model, AIP expression was lost in almost all PA arising in *AIP*$^{+/-}$ animals, due to LOH, and accompanied by loss of ARNT and/or its homologue ARNT2 in most cases (>90%). In contrast, AIP, ARNT and ARNT2 expression were maintained in all PA arising in the control mice. The biological significance of these findings remains unclear, since AhR expression was not reported in this model and an alternative partner of ARNT/ARNT2, the Hypoxia-Inducible Factor HIF1α, was similarly expressed in *AIP*-proficient and *AIP*-deficient PA, respectively.

AHR, the Cell Cycle and Tumorigenesis

Molecular mechanisms linking AhR to cell cycle regulation are of great pathological interest since AhR has been potentially implicated in cancer in several ways: (1) dioxin is a well-recognized carcinogen – with no specific site of tumor induction – and most effects of dioxin and related compounds are mediated by AhR; (2) overexpression of AhR has been reported in human cancers, such as breast cancer and melanoma. On the other hand, AhR down- regulation has also been observed in a minority of tumors (i.e. acute lymphocytic leukaemia) and exogenous stimulation of AhR has variable effects on cell proliferation. Indeed, in most cases a growth-suppressing effect of exogenous AhR activation is observed, and this is mediated by an induction of the CDKI p27^{Kip1} through AhR/ARNT binding on a DRE element. ARNT is absolutely required for this effect, and pRB has also been proposed as a co-activator. The interaction of AhR with pRb has been well documented: AhR interacts with hypophosphorylated pRb through at least two distinct pRb-binding domains and synergizes with pRb to repress the transcription of E2F-induced genes such as cyclin E, cdk2, DNA polymerase α and S-phase enzymes. Thus, AhR activation may arrest the cell cycle in G1/S. Because this effect requires pRb, it is lost in abnormal pRb-deficient cells. On the other hand, because E2F-1 is a pro-apoptotic member of the E2F family, which is able to arrest cells in G2/M, AhR can also inhibit the pro-apoptotic response by binding E2F-1 and promote cell survival. This occurs in the presence of DNA damage, when E2F-1 is stabilized in its active for by ATM/ATR and Chk2 phosphorylation. This dual control on the cell cycle has been reported by Marlowe and Puga (2005) as the "ying-yang" effect of AhR. There is also considerable evidence that endogenous AhR has a pro-proliferative potential. A possible explanation to this phenomenon has been provided recently on breast cancer cell lines, showing that endogenous

AhR forms a complex with cyclinD/cdk4 enhancing pRb phosphorylation, whereas stimulation by exogenous ligands disrupts such interaction and promotes pRb effects on the repression of E2F-induced genes (Barhoover et al. 2010). Therefore, it has become more and more evident that the final effect of AhR activation on cell growth, differentiation and apoptosis depends on a series of factors, including the presence or the absence of exogenous ligands, and cell phenotype, status and environment (Puga et al. 2009). On the other hand, the induction of early response oncogenes by dioxin and related compounds, which participate in their tumorigenic effects, does not appear to be dependent on AhR/ARNT transcription. To summarize, AhR generally induces growth arrest in the presence of exogenous ligands, whereas in the absence of exogenous ligands, it may exert an anti-apoptotic, prosurvival effect.

It is worth noting that the activation of AhR is also regulated by phosphorylation in its C-terminal half, which provides a further level of crosstalk between AhR and extracellular signalling linked to cell growth, differentiation and apoptosis. This phenomenon has been recently reviewed by Henkovà et al. (2008). Briefly, mitogen-activated protein kinases (MAPKs), which can be activated by a variety of growth factors, cytokines, and cellular stressful events including genotoxic and oxidative stress, are able to differentially modulate AhR subcellular localization, transcriptional activity and protein stability. Conversely, exogenous ligands of AhR have also been found to activate MAPKs. Whether the interplay between MAPKs and AhR signalling is relevant to pituitary tumorigenesis warrants further investigation, since MAPKs – especially the Extracellular Regulated Kinases ERK1/2, which can directly associate with AhR – have been involved in pituitary tumorigenesis (Cakir and Grossman 2009).

Finally, it should be noticed that the transcription factors RelA and RelB, which are involved in the inflammatory response but also in the regulation of cell survival and apoptosis induced by cytokines, are also able to dimerize with AhR, and cross-talk has been established between exogenous AhR activation and NFkB signalling (Beischlag et al. 2008).

AHR, AIP and the Modulation of Nuclear Endocrine Signalling

Among the multiple cross-talks involving AhR signalling, is able the best characterized is endocrine disruption, which is believed to account for the reported effects of dioxin and related compounds on thyroid function, sexual development and function, fertility, and some endocrine-related cancers such as testicular cancer. Several lines of evidence suggest that AhR is able to mediate off-target or non-DNA binding dependent transcription. The potential role of AIP in the endocrine disrupting effects of AhR is unclear, but AIP itself has been reported to interfere with steroid receptor activity – i.e. inhibition of glucocorticoid receptor activity through direct AIP/GR/Hsp90 interaction (Laenger *et al.* 2009) -, further enhancing the complexity of endocrine modulation by the AIP/AhR system.

AHR and Endocrine Disruption

Dioxin is a major endocrine disruptor. It reduces estrogen signalling in many ways including (1) increased estrogen metabolism through the CYP1a/b and CYP19/aromatase enzymes, (2) increased ERα degradation, (3) reduced ER transcriptional activity due to DRE upstream to ERE in target gene promoters, direct competition on ERE binding or for the recruitment of common co-activators (squelching). Also, both AhR and ARNT are able to directly interact with the ER**,** and ER may be recruited on active DRE elements and enhance AhR transcription, whereas ER signalling in itself is reduced (Beischlag et al. 2008). The endocrine disrupting effects of dioxin-related compounds as industrial pollutants have been widely studied in fish species because of their impact on sexual development and fertility. Elango et al. (2006) have observed, in the rainbow trout pituitary, that both estradiol and TCDD were able to stimulate GH and PRL transcription in a dose-dependent manner. The estrogenic effects of TCDD were observed only in the absence of E2 and strongly reduced by the addition of an AhR antagonist. This can be explained

by binding of the activated AhR/ARNT to the unliganded ERα/β, which results in the recruitment of unliganded ER and the co-activator p300 to ERE, with subsequent activation of gene transcription and oestrogenic effects. In contrast, in the presence of both TCDD and E_2, PRL transcription was lower than in controls, confirming endocrine disruption (transrepression). Additional nuclear partners of AhR have been reported, including the androgen receptor and TR, potentially interfering with endocrine signalling.

The observation that AIP^{mut} PA are more frequent and more severe in male patients – gigantism and resistant macroprolactinomas have been reported in males – is intriguing and yet unexplained. Studies from our laboratory have shown that PA express sex steroid hormones receptors with a differential pattern according to phenotype and patient's gender and gonadal function (Jaffrain-Rea et al. 1996), and that sex steroids are able to modulate their proliferation *in vitro* accordingly (Caronti et al. 1995). In particular, ER expression was higher in male prolactinomas and 17β-estradiol had a proliferative effect on most ER-expressing PA. It is tempting to hypothesize that abnormal crosstalk between sex steroids and AhR signalling in AIP-deficient cells might contribute to gender-related variations in tumor phenotype.

Interactions of AIP and AHR with PPARα

AIP is able to interact with PPARα which, unlike other type II steroid receptors, may be present in a latent form and form a complex with AIP and hsp90 (Sumanasekera et al. 2003). Like AhR, PPARα can be activated by endogenous or exogenous ligands, and exerts complex transcriptional effects which are highly dependent on cell type and environment. The transcriptional effects of PPAR agonists are mediated through PPAR/RXR heterodimers binding to the consensus sequences PPREs. A stimulating effect of PPARα agonists on PRL transcription and secretion has been reported in GH_4C_1 cells, which is believed to be indirect and dependent on Pit-1 activation and recruitment of co-activators (Tolon et al. 1998). Interestingly, AIP inhibits the transcriptional activity of PPARα, suggesting that loss of AIP function or expression in Pit-1-dependent cells may contribute to AIP-related pathogenesis. In addition, PPARs are affected by dioxin and related compounds in an AhR-dependent manner, and PPARα agonists may either potentiate or repress CYP1A genes transcription according to the cellular context (Beischlag et al. 2008). The potential effects of the AhR alterations reported in pituitary cells on PPARα signalling are unknown.

AIP, AHR and the Modulation of the cAMP Pathway

The cAMP-protein kinase A (PKA) pathway is essential for somatotrophs and lactotroph cells. In somatotrophs, it stimulates hormone secretion and cell proliferation and is positively and negatively regulated by the hypothalamic Growth-Hormone Releasing Hormone (GHRH) and somatostatin (SMS), respectively, whereas lactotrophs are under physiological inhibition by hypothalamic dopaminergic signalling. Constitutive activation of the cAMP pathway has been involved in the pathogenesis of sporadic pituitary tumorigenesis and inherited forms of somatolactotroph adenomas and/or hyperplasia in the setting of Carney complex or McCune Albright syndrome (Boikos and Stratakis 2007). Transgenic GHRH mice develop somatotroph hyperplasia and adenomas, and in humans activating mutations of the Gsalpha subunit gene (GNAS1) are the most common somatic mutations observed in somatotropinomas. Conversely, somatostatin analogues and dopamine-agonists are widely used in the pharmacological treatment of human somatotropinomas and prolactinomas, respectively. Upon ligand activation, specific receptors for these drugs – the somatostatin receptors SSTR 1,2,3,5 and the dopamine-agonist receptor D2R – inhibit the adenylate cyclase/cAMP/ PKA pathway, resulting in reduced hormone secretion and, to a variable extent, tumor shrinkage. Whether the unusual rate of pharmacological resistance observed in AIP^{mut} PA is due to some alteration in

cAMP-related pathways remains to be defined, but both AIP and AhR have been reported to modulate cAMP signalling in non pituitary models and some findings could be potentially extended to pituitary cells.

On one hand, cAMP is considered as a non-ligand endogenous activator of AhR, able to stimulate its translocation to the nucleus similarly to dioxin, although in this case nuclear AhR does not appear to dimerize with ARNT or induce CYP1A transcription, but rather forms a complex with yet unidentified proteins. Thus, cAMP itself or some event downstream cAMP may modulate the response to AhR and act as a repressor rather than an activator of classical AhR-dependent gene expression. On the other hand, AIP has been shown to interact with some phosphodiesterases (PDEs), which inactivate cyclic nucleotides, and this may modulate cAMP concentration and/or AhR nuclear translocation and transcriptional activity. Such mechanisms have been recently reviewed by de Oliveira and Smolenski (2009). Briefly, a direct interaction of AIP with the isoforms PDE4A5 and PDE2 has been demonstrated, which is mediated by its TPR domains. PDE4 phosphodiesterases are involved in cAMP degradation and induced by PKA (and ERK). Binding to AIP appears specific of the PDE4A5 isoform, and results in a dramatic decrease in its enzymatic activity. As shown by Leontiou et al. (2008) and further supported by work from the same group (Igreja et al. 2010) a number of germline AIP mutations have proven to lose the ability to interact with PDE4A5. Intriguingly, disruption of AIP/PDE4A5 interaction should lead to reduced intracellular cAMP concentrations. PDE2 is induced by cGMP and involved in the regulation of cAMP and cGMP concentrations. Interaction with AIP has no effect on its enzymatic activity, but PDE2 inhibits nuclear AhR translocation, likely as a result of local regulation of cAMP concentration. The effects of *AIP* mutations on AIP/PDE2 interaction have not been determined yet. Potential alterations in the cAMP pathway in the presence of abnormal AIP or AhR expression and function should be further investigated.

AIP and the RET/Survivin Interaction

It was recently reported by Vargiolu et al. (2009) that AIP is able to interact with survivin, an anti-apoptotic protein, and the tyrosine kinase receptor Ret. In this model, interaction of AIP with Ret prevents the stabilization of survivin by AIP. This represents an interesting potential link between AIP and cell survival, and an anti-apoptotic role of AIP may be hypothesized for example in NFPA expressing AIP. The Ret receptor is activated by the Glial-Derived Neurotrophic Factor (GDNF) forming a complex with the GDNF-Receptor α (GFRα1). Japon et al. (2002) have previously shown that Ret and its 2-ligand system GDNF and GFRα1 are expressed in the normal pituitary, essentially by somatotrophs, and invariably detected in somatotropinomas. The biological function of Ret in somatotrophs has then been investigated by the same group, using *in vitro* experiments and a Ret knock-out mice model, clearly showing that Ret was able to regulate the number of somatotroph cells through a Pit-1/p53/apoptotic pathway (Cañibano et al. 2007). Although Vargiolu et al. (2009) have tested the effects of some missense AIP mutations on RET binding and found no noticeable change, truncating mutations were not studied. It is tempting to hypothesize that some AIP mutations may disrupt its interaction with Ret, thereby allowing AIP to promote pituitary cell survival through survivin stabilization and/or abnormal Ret signalling.

Conclusion and Future Perspectives

The discovery of AIP as a predisposing gene for pituitary adenomas has opened a new field in the study of pituitary tumorigenesis, offering a broad spectrum of potentially related abnormalities in molecular pathways involved in endocrine signalling as well as the control of cell proliferation and apoptosis. Some of them involve completely new players in pituitary biology – such as AhR and related molecules –, others are more familiar pathways – such as the

cAMP-PKA pathway – to be revised at the light of new potential mechanisms of crosstalk in the pituitary gland. At the moment, the pathogenic role of the best characterized AIP partner, AhR has not been definitively proven in pituitary cells, and endocrine disruption and pathways such as PPAR and Ret signalling represent additional attractive candidates. The potential influence of AIP in the pharmacological response to the widely used somatostatin analogues and dopamine-agonist drugs should be evaluated and may provide new insights in the comprehension of pharmacological resistance in PA Preliminary data from our laboratory indicate that pre-operative treatment with SSA is associated with a higher AIP expression in sporadic, but not in AIPmut, somatotropinomas (Jaffrain-Rea et. al, 2010 b). Interestingly, unlike the *MEN1* gene, which seems poorly involved in sporadic pituitary pathogenesis, AIP may also play a significant role in the pathogenesis of a subset of PA, regardless of *AIP* mutations. In addition, inherited predisposition linked to the *AIP* gene is characterized by an incomplete or low penetrance, which strongly supports the need for additional factors to initiate or promote pituitary tumorigenesis and stimulates research work aimed to their identification. Another open issue remains the pituitary specificity of *AIP*-related neoplasia, since additional tumors have been occasionally observed in AIP^{mut} patients, and LOH in 11q13 with loss of the wild-type AIP allele was recently reported by Toledo et al. (2010) in an adrenocortical carcinoma operated on in an acromegalic patient with a truncating familial *AIP* mutation. Because AIP has a rather ubiquitous expression, the signalling pathways which are potentially disrupted by *AIP* mutations may be relevant for tumorigenesis in other tissues. The mice model developed by Raitila et al. (2010), genetic studies aimed at the identification of modifier loci in AIP^{mut} kindreds, new genomic and proteomic tools applied to AIP^{mut} tumors and *in vitro* models, should help provide significant insights into AIP-related pathogenesis in a the next future.

References

Asa SL, Ezzat S (2009) The pathogenesis of pituitary tumors. Ann Rev Pathol 4:97–126

Barhoover MA, Hall JM, Greenlee WF, Thomas RS (2010) Aryl hydrocarbon receptor regulates cell cycle progression in human breast cancer cells via a functional interaction with cyclin-dependent kinase 4. Mol Pharmacol 77:195–201

Beckers A (2010) Higher prevalence of clinically relevant pituitary adenomas confirmed. Clin Endocrinol (Oxf) 72:290–291

Beischlag TV, Morales JL, Hollingshead BD, Perdew GH (2008) The aryl hydrocarbon receptor complex and the control of gene expression. Crit Rev Eukaryot Gene Expr 18:207–250

Boikos SA, Stratakis CA (2007) Molecular genetics of the cAMP-dependent protein kinase pathway and of sporadic pituitary tumorigenesis. Hum Mol Genet 16(Spec No 1):R80–R87

Cakir M, Grossman AB (2009) Targeting MAPK (Ras/ERK) and PI3K/Akt pathways in pituitary tumorigenesis. Expert Opin Ther Targets 13:1121–1134

Cañibano C, Rodriguez NL, Saez C, Tovar S, Garcia-Lavandeira M, Borrello MG, Vidal A, Costantini F, Japon M, Dieguez C, Alvarez CV (2007) The dependence receptor Ret induces apoptosis in somatotrophs through a Pit-1/p53 pathway, preventing tumor growth. EMBO J 26:2015–2028

Caronti B, Paladini G, Caldelaro C, Bevilacqua MG, Petrangeli E, Esposito V, Tamburrano G, Gulino A, Jaffrain-Rea ML (1995) Effects of gonadal steroids on the growth of human pituitary adenomas in vitro. Tumor Biol 16:353–364

Carver LA, La Pres JJ, Jain S, Dunham EE, Bradfield CA (1998) Characterization of the Ah receptor – associated protein ARA9. J Biol Chem 50:33580–33587

Daly AF, Jaffrain-Rea ML, Ciccarelli A, Valdes-Socin H, Rohmer V, Tamburrano G, Borson-Chazot C, Estour B, Ciccarelli E, Brue T, Ferolla P, Emy P, Colao A, De Menis E, Lecomte P, Penfornis F, Delemer B, Bertherat J, Wemeau JL, De Herder W, Archambeaud F, Stevenaert A, Calender A, Murat A, Cavagnini F, Beckers A (2006) Clinical characterization of familial isolated pituitary adenomas. J Clin Endocrinol Metab 91:3316–3323

Daly AF, Vanbellinghen JF, Khoo SK, Jaffrain-Rea ML, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo AP, Tamburrano G, Raverot G, Barlier A, De Herder W, Penfornis A, Ciccarelli E, Estour B, Lecomte P, Gatta B, Chabre O, Sabate MI, Bertagna X, Garcia Basavilbaso N, Stalldecker G, Colao A, Ferolla P, Wemeau JL, Caron P, Sadoul JL, Oneto A, Archambeaud F, Calender A, Sinilnikova O, Montanana CF, Cavagnini F, Hana V, Solano A, Delettieres D, Luccio-Camelo DC, Basso A, Rohmer V, Brue T, Bours V, Teh BT, Beckers A (2007) Aryl

hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. J Clin Endocrinol Metab 92:1891–1896

Daly AF, Tichomirowa MA, Petrossians P, Heliövaara E, Jaffrain-Rea ML, Barlier A, Naves LA, Ebeling T, Karhu A, Raappana A, Cazabat L, De Menis E, Montañana CF, Raverot G, Weil RJ, Sane T, Maiter D, Neggers S, Yaneva M, Tabarin A, Verrua E, Eloranta E, Murat A, Vierimaa O, Salmela PI, Emy P, Toledo RA, Sabaté MI, Villa C, Popelier M, Salvatori R, Jennings J, Longás AF, Labarta Aizpún JI, Georgitsi M, Paschke R, Ronchi C, Valimaki M, Saloranta C, De Herder W, Cozzi R, Guitelman M, Magri F, Lagonigro MS, Halaby G, Corman V, Hagelstein MT, Vanbellinghen JF, Barra GB, Gimenez-Roqueplo AP, Cameron FJ, Borson-Chazot F, Holdaway I, Toledo SP, Stalla GK, Spada A, Zacharieva S, Bertherat J, Brue T, Bours V, Chanson P, Aaltonen LA, Beckers A (2010) Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. J Clin Endocrinol Metab 95:E373–E383

De Oliveira SK, Smolenski A (2009) Phosphodiesterases link the aryl hydrocarbon receptor complex to cyclic nucleotide signaling. Biochem Pharmacol 77:723–733

Elango A, Shepherd B, Chen TT (2006) Effects of endocrine disrupters on the expression of growth hormone and prolactin mRNA in the rainbow trout pituitary. Gen Comp Endocrinol 145:116–127

Galdelha MR, Prezant TR, Une KR, Glick RP, Moskal Ii SF, Vaisman M, Melmed S, Kineman RD, Frohman LA (1999) Loss of heterozygosity on chromosome 11q13 in two families with acromegaly/gigantism is independent of mutations of the multiple endocrine neoplasia type I gene. J Clin Endocrinol Metab 84:249–256

Heliövaara E, Raitila A, Launonen V, Paetau A, Arola J, Lehtonen H, Sane T, Weil RJ, Vierimaa O, Salmela P, Tuppurainen K, Mäkinen M, Aaltonen LA, Karhu A (2009) The expression of AIP-related molecules in elucidation of cellular pathways in pituitary adenomas. Am J Pathol 175:2501–2507

Henklovà P, Vrzal R, Ulrichovà J, Dvoràk Z (2008) Role of mitogen-activated protein kinases in aryl hydrocarbon signaling. Chem Biol Interact 172:93–104

Igreja S, Chahal HS, King P, Bolger GB, Srirangalingam U, Guasti L, Chapple JP, Trivellin G, Gueorguiev M, Guegan K, Stals K, Khoo B, Kumar AV, Ellard S, Grossman AB, Korbonits M, International FIPA Consortium (2010) Characterization of aryl hydrocarbon receptor interacting protein (AIP) mutations in familial isolated pituitary adenoma families. Hum Mut 31:950–960

Jaffrain-Rea ML, Petrangeli E, Ortolani F, Fraioli B, Lise A, Esposito V, Spagnoli LG, Tamburrano G, Frati L, Gulino A (1996) Cellular receptors for sex steroids in human pituitary adenomas. J Endocrinol 151:175–184

Jaffrain-Rea ML, Angelini M, Gargano D, Tichomirowa MA, Daly AF, Vanbellinghen JF, D'Innocenzo E, Barlier A, Giangaspero F, Esposito V, Ventura L, Arcella A, Theodoropoulou M, Naves LA, Fajardo C, Zacharieva S, Rohmer V, Brue T, Gulino A, Cantore G, Alesse E, Beckers A (2009) Expression of aryl hydrocarbon receptor (AHR) and AHR-interacting protein in pituitary adenomas: pathological and clinical implications. Endocr Relat Cancer 16:1029–1043

Jaffrain-Rea ML, Daly AF, Angelini M, Petrossians P, Bours V, Beckers A (2010a) Genetic susceptibility in pituitary adenomas: from pathogenesis to clinical implications. Exp Rev Endocrinol Metab 6(2):195–214

Jaffrain-Rea ML, Angelini M, Tichomirowa MA, Theodoropoulou M, Daly AF, Barlier A, Naves LA, Fajardo C, Zacharieva S, Rohmer V, Brue T, Ventura L, Esposito V, Giangaspero F, Alesse E, Beckers A (2010 b). Factors associated with AIP expression in somatotropinomas and the possible influence of somatostatin analogues. 15th Congress of the European Neuroendocrine Association, Liège, 22-25 September 2010, Belgium.

Japón MA, Urbano AG, Sáez C, Segura DI, Cerro AL, Diéguez C, Alvarez C (2002) Glial-derived neurotropic factor and RET gene expression in normal human anterior pituitary cell types and in pituitary tumors. J Clin Endocrinol Metab 87:1879–1884

Laenger A, Lang-Rollin I, Kozany C, Zschocke J, Zimmermann N, Rüegg J, Holsboer F, Hausch F, Rein T (2009) XAP2 inhibits glucocorticoid activity in mammalian cells. FEBS Let 583:1493–1498

Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, Chahal HS, Igreja SC, Jordan S, Rowe J, Stolbrink M, Christian HC, Wray J, Bishop-Bailey D, Berney DM, Wass JA, Popovic V, Ribeiro-Oliveira A Jr, Gadelha MR, Monson JP, Akker SA, Davis JR, Clayton RN, Yoshimoto K, Iwata T, Matsuno A, Eguchi K, Musat M, Flanagan D, Peters G, Bolger GB, Chapple JP, Frohman LA, Grossman AB, Korbonits M (2008) The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. J Clin Endocrinol Metab 93:2390–2401

Lin BC, Sullivan R, Lee Y, Moran S, Glover E, Bradfield CA (2007) Deletion of the aryl hydrocarbon receptor-associated protein 9 leads to cardiac malformation and embryonic lethality. J Biol Chem 282:35924–35932

Marlowe JL, Puga A (2005) Aryl hydrocarbon receptor, cell cycle regulation, toxicity, and tumorigenesis. J Cell Biochem 96:1174–1184

Ozfirat Z, Korbonits M (2010) AIP gene and familial isolated pituitary adenomas. Mol Cell Endocrinol 326:71–79

Puga A, Ma C, Marlowe JL (2009) The aryl hydrocarbon receptor cross-talks with multiple signal transduction pathways. Biochem Pharmacol 77:713–722

Raitila A, Lehtonen HJ, Arola J, Heliövaara E, Ahlsten M, Georgitsi M, Jalanko A, Paetau A, Aaltonen LA, Karhu A (2010) Mice with inactivation of aryl hydrocarbon receptor-interacting protein (Aip) display complete penetrance of pituitary adenomas with aberrant ARNT expression. Am J Pathol 177:1969–1976

Sumanasekera WK, Tien ES, Turpey R, Van den Heuvel JP, Perdew GH (2003) Evidence that peroxisome proliferator-activated receptor alpha is complexed with the 90-kDa heat shock protein and the hepatitis virus B X-associated protein 2. J Biol Chem 278:4467–4473

Thakker RV (2010) Multiple endocrine neoplasia type 1 (MEN1). Best Pract Res Clin Endocrinol Metab 24:355–370

Toledo RA, Mendonca BB, Fragoso MC, Soares IC, Almeida MQ, Moraes MB, Lourenço-Jr DM, Alves VA, Bronstein MD, Toledo SP (2010) Isolated familial somatotropinoma: 11q13-LOH and gene/protein expression analysis suggests a possible involvement of AIP also in non-pituitary tumorigenesis. Clinics (Sao Paulo) 65:407–415

Tolon RM, Castillo AI, Aranda A (1998) Activation of the prolactin gene by peroxisome proliferator-activated receptor α appears to be DNA binding-independent. J Biol Chem 41:26652–26661

Vargiolu M, Fusco D, Kurelac I, Dirnberger D, Baumeister R, Morra I, Melcarne A, Rimondini R, Romeo G, Bonora E (2009) The tyrosine kinase receptor RET interacts in vivo with aryl hydrocarbon receptor-interacting protein to alter survivin availability. J Clin Endocrinol Metab 94:2571–2578

Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, Gundogdu S, De Menis E, Makinen MJ, Launonen V, Karhu A, Aaltonen LA (2006) Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science 312:1228–1230

Pituitary Tumors: Role of Pituitary Tumor-Transforming Gene-1 (PTTG1)

22

Cuiqi Zhou

Contents

Abstract

Human pituitary tumor transforming gene-1 (PTTG1) is a novel oncogene. Its overexpression occurs in a wide variety of human tumors including pituitary adenomas. Several factors and signalling pathways regulate pituitary PTTG1 expression, including estrogen, epidermal growth factor, octamer-binding transcription factor 1 and Rb/E2F1. PTTG1 is extensively involved in physiologic and oncogenic functions. Molecular and clinical studies implicate diverse roles of PTTG1 in cell cycle regulation, genetic instability, cell proliferation, pituitary tumor angiogenesis and senescence. Both transgenic and knockout mouse models support a causal role for PTTG1 in the development of pituitary tumors. Here, we review the current knowledge of the regulatory mechanisms and the biological and pathophysiological roles of PTTG1 in pituitary tumors.

Introduction

The pituitary gland is composed of two anatomically, functionally and phylogenetically distinct parts: anterior pituitary and posterior pituitary. The anterior pituitary is the central regulator of the endocrine system, coordinating signals from the hypothalamus centrally and endocrine organs peripherally. Pituitary tumors are common benign monoclonal adenomas which arise from cells of the anterior pituitary gland. These monoclonal

C. Zhou (✉)
Department of Medicine, Cedars-Sinai Medical Center, David Geffen School of Medicine, University of California, Los Angeles, CA 90048 USA
e-mail: zhouc@cshs.org

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_22,

tumors arise from highly differentiated cells expressing hormone peptide products including growth hormone (GH), prolactin, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These tumors may be functional and actively secrete hormones leading to characteristic clinical features including acromegaly, Cushings disease and hyperprolactinemia. Commonly, they may be nonfunctional leading primarily to hypogonadism and compressive pituitary failure. These benign neoplasms account for ~15% of all intracranial tumors, and malignant transformation very rarely occurs. The proximal pathogenesis of pituitary tumors remains elusive. Disrupted growth factors, aberrant peripheral hormone feedback control, tumor suppressor gene inactivation and oncogene activation [e.g., pituitary tumor-transforming gene-1 (PTTG1)] are all possibly responsible for pituitary tumorigenesis.

Pituitary tumor-transforming gene-1 (PTTG1) was originally isolated from rat pituitary tumor cells by mRNA differential PCR display (Pei and Melmed 1997). Subsequently, human PTTG1 was isolated from a fetal liver cDNA library (Zhang et al. 1999b). Human PTTG1 has been identified as a vertebrate securin, mediating sister chromatid separation during mitosis (Zou et al. 1999). PTTG1 also functions as a proto-oncogene and facilitates cell-cycle progression, maintains chromosomal stability, and mediates transformation *in vitro* and tumorigenesis *in vivo*. PTTG1 abundance or loss of function both result in abnormal mitosis and chromosomal instability. In contrast to restricted normal tissue expression, PTTG1 is overexpressed in pituitary, thyroid, breast, esophageal and colorectal tumors (Vlotides et al. 2007). PTTG1 overexpression correlates with tumor invasiveness, metastases, differentiation, recurrence and prognosis. PTTG1 plays a role in the preliminary stages of pituitary tumor initiation. PTTG1 expression in pituitary is induced by oestrogen in a rat model, and precedes oestrogen-induced hyperplasia and adenoma formation, indicating that oestrogen-induced PTTG1 expression is coincident with early pituitary lactotroph transformation (Heaney et al. 1999). The present chapter delineates the regulatory and functional mechanisms that underlie PTTG1 action in pituitary tumors.

Structure and Distribution

Gene and Protein Structure

Human PTTG1 was characterized from a fetal liver cDNA library, using a 0.6-kb rat Pttg1 cDNA as a screening probe (Zhang et al. 1999b). It was shown to have 85% homology with the coding region of rat Pttg1. At the time, human PTTG1 was independently cloned and characterized by other groups, and identical sequences were submitted to GenBank (Vlotides et al. 2007). Human PTTG1 is located on chromosome 5 (5q35.1) and contains five exons and four introns. PTTG1 mRNA is 1.3 kb with an open reading frame of 609 nucleotides encoding a protein of 202 amino acids.

PTTG1 is a multi-domain protein consisting of a transactivation domain which is required for ubiquitin-mediated proteolysis, and a DNA-binding domain. The N-terminus of PTTG1 contains a destruction (D) box and a KEN box, both are involved in PTTG1 degradation. The C-terminus contains two proline-rich motifs (^{163}P-P-S-P^{166} and ^{170}P-S-P-P^{173}) that form a Src-homology (SH)-3-binding domain and enable PTTG1 to interact with SH-3-containing proteins. These SH-3 binding domains are critical for PTTG1 transforming ability, proliferative effect and transactivation activity (Boelaert et al. 2004; Pei 2000; Zhang et al. 1999b).

Family Members

Southern blot analysis of human genomic DNA revealed the presence of two additional genes homologous to human PTTG1 in the genome, PTTG2 and PTTG3. Both of PTTG2 and PTTG3 genes are intronless and present on different chromosomes. The PTTG2 gene is located on chromosome 8 (8q13.1), whereas the PTTG3 gene is present on chromosome 4 (4p15.1) (Chen et al. 2000). PTTG2 contains a 576-bp coding

sequence, encoding a 191-amino acid protein, with a 91% homology to PTTG1 at the amino acid level. PTTG3 contains a 609-bp coding sequence and encodes a 202-amino acid protein with 89 and 84% homology to PTTG1 and PTTG2, respectively. Similar to PTTG1, both PTTG2 and PTTG3 contain two conserved proline-rich motifs at the C terminus. Low levels of PTTG2 are detected in normal pituitary, brain, placenta, small intestine, colon, liver, spleen, thymus, prostate, testis and ovary, as well as pituitary adenomas, the primary fibroblast cell line LL-24, and in five cancer cell lines. By contrast, PTTG3 mRNA is extremely low or absent in these tissues (Chen et al. 2000). The physiologic role and the significance of PTTG2 and PTTG3 remain unclear.

Expression Profile

In normal adult human tissues, PTTG1 mRNA expression is strong in testis and thymus and weak in colon, small intestine, brain, placenta and pancreas. In human fetal tissue, PTTG1 mRNA expression was detected solely in liver (Pei and Melmed 1997). The high level of PTTG1 mRNA expression has been detected in a variety of malignant tumor cell lines, including promyelocytic leukemia HL-60, HeLa cell S3, chronic myelogenous leukemia K-562, lymphoblastic leukemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma SW480, lung carcinoma A549, and melanoma G361 (Zhang et al. 1999b).

Subcellular Localization

PTTG1 localizes both to the cytoplasm and to the nucleus; however, the ratio of cytoplasmic versus nuclear localization remains controversial. Dominguez et al. (1998) showed that human PTTG1 was mainly present in the cytoplasm (85%) in Jurkat cells by subcellular fractionation. Zhang et al. (1999a) and Saez et al. (1999) exhibited the predominant expression of PTTG1 in cytoplasm by in situ hybridization and immunohistochemistry respectively. Similarly, Stratford et al. (2005) reported the predominant cytoplasmic localization of PTTG1 in HCT116 cells transfected with EGFP-tagged PTTG1. On the other hand, Yu et al. (2000) demonstrated predominant nuclear localization of PTTG1 during interphase in JEG-3 cells when transfected with wild type PTTG1, a flag-tagged PTTG1, or an EGFP-tagged PTTG1. In the same study, live imaging of EGFP-tagged PTTG1 during mitosis revealed co-localization with microtubule asters in prophase and pro-metaphase, aggregation into distinct granules during anaphase, and diminished telophase expression. Mu et al. (2003) reported cell type-dependent PTTG1 subcellular distribution, which was predominantly nuclear in HeLa, Cos-7 and DU145 cells, but diffuse nuclear and cytoplasmic localization in A549, DLD-1 and NIH3T3 cells. While differential PTTG1 localization may be due to the variations in cell lines and tumor types examined, cell cycle-dependent expression of PTTG1 may also account for the reported differences.

Translocation of PTTG1 from cytoplasm to nucleus may be mediated by PTTG1 binding factor (PBF), which contains a nuclear localization signal (Chien and Pei 2000). The mitogen-activated protein kinase (MAPK) pathway may also be involved in PTTG1 translocation. The expression of a constitutively active form of MAP kinase kinase (MEK1) facilitates PTTG1 translocation to the nucleus in Cos-7 cells (Pei 2000). Recently, Wierinckx et al. (2007) found only nuclear PTTG1 expression to be predictive of aggressive pituitary tumor behaviour, indicating that PTTG1 subcellular localization appears to be a significant factor in determinating its tumorigenic role.

Protein Phosphorylation and Degradation

PTTG1 phosphorylation has been observed in a cell cycle-dependent manner (Ramos-Morales et al. 2000; Romero et al. 2001). Phosphorylated PTTG1 is upregulated in rapidly dividing cells and levels peak during mitosis, implying that PTTG1 phosphorylation plays a crucial role in

the control of cell division. Cdc2 is responsible for PTTG1 phosphorylation during mitosis, mainly at Ser165, which is highly conserved in the rat and mouse homologues (Ramos-Morales et al. 2000). It was reported that expression of constitutively phosphorylated PTTG1 results in increased cell proliferation and transformation ability (Boelaert et al. 2004). Mitogen-activated protein (MAP) kinase phosphorylates PTTG1 and stimulates its transactivation activity (Pei 2000). PTTG1 interacts with a MAP kinase kinase (MEK1) via SH3 domain between amino acids 51 and 54, and activation of this pathway facilitates nuclear translocation of PTTG1. PTTG1 also physically interacts with Phosphoinositol-3-kinase (PI3K), suggesting that PI3K signalling is involved in PTTG1 phosphorylation (Chamaon et al. 2005). Moreover, PTTG1 binds to Ku, the regulatory subunit of the DNA-dependent protein kinase (DNA-PK), and the DNA-PK catalytic subunit phosphorylates PTTG1 (Romero et al. 2001). However, the significance of DNA-PK-mediated PTTG1 phosphorylation still remains unclear.

PTTG1 is rapidly degraded at the end of metaphase (Zou et al. 1999). PTTG1 degradation ensues as a result of ubiquitination by anaphase-promoting complex (APC), an E3 ubiquitin ligase. PTTG1 degradation is catalyzed by fzy (fizzy/cdc20) and fzr (fizzy-related/cdh1/hct1). Both fzy and fzr induce the APC to ubiquitinate PTTG1. PTTG1 degradation is mediated by an RXXL destruction (D) box and a KEN box, and is inhibited only when both sequences are mutated (Zur and Brandeis 2001).

PTTG1 Expression in Pituitary Tumors

Multiple studies have demonstrated that PTTG1 is expressed at high levels in pituitary tumors. In normal pituitary, PTTG1 expression is restricted (Saez et al. 1999; Zhang et al. 1999a) and displays an estrous cycle-dependent expression pattern (Heaney et al. 2002). Saez et al. (1999) showed elevated PTTG1 mRNA expression in 12 pituitary adenomas (6 non-functioning, 4 GH, 1 PRL and 1 ACTH hormone), while only little PTTG1 was detected in normal pituitary by Northern blot. In addition, immunostaining showed cytoplasmic PTTG1 immunoreactivity in 32 of 36 pituitary adenomas; nuclear staining was only observed in a few cells.

Several findings indicate the potentially predictive value of PTTG1 as a marker of aggressive pituitary tumor behavior. Using comparative RT-PCR study in 54 pituitary adenomas, more than 50% increase of PTTG1 mRNA expression was observed in 21 of 30 non-functioning, all 13 GH, 9 of 10 PRL, and one ACTH-secreting tumors examined, with more than tenfold increases of PTTG1 expression evident in some tumors. Higher PTTG1 expression was observed in hormone-secreting tumors which had invaded the sphenoid bone compared with tumors confined to the pituitary fossa. However, there was no correlation shown between tumor stage and PTTG1 levels in non-functioning adenomas. Therefore, PTTG1 abundance is a molecular marker for invasiveness in hormone-secreting pituitary tumors. Interestingly, a recent study of 25 PRL-secreting pituitary tumors showed that although PTTG1 expression did not *per se* predict aggressive behavior, nuclear PTTG1 staining was one of the histological features (numerous mitoses, high Ki-67 index, and nuclear labelling of PTTG1 and P53) distinguishing aggressive–invasive PRL tumors from those behaving less aggressively (Wierinckx et al. 2007). Furthermore, PTTG1 expression correlates with pituitary tumor recurrence. Filippella et al. (2006) reported mainly nuclear PTTG1 immunoreactivity in approximately 90% of 45 pituitary tumors tested, whereas PTTG1 protein was not detected in normal pituitary tissue. PTTG1 expression showed a strong correlation with Ki-67 immunopositivity, and was higher in recurrent tumors. A PTTG1 score of 3.3% was the best cut-off for distinguishing between persistent/recurrent and non-recurrent tumors (sensitivity, 60%; specificity, 76%). In 27 out of the 45 patients for which 1-year follow-up was available, a cut-off of 2.9% for both PTTG1 and Ki-67 positivity predicted recurrence versus non-recurrence, Ki-67 being the superior predictor. PTTG1 overexpression is correlated with angiogenesis in human pituitary adenomas. In 101 pituitary adenomas, PTTG1 was significantly correlated with vascular endothelial growth

factor (VEGF) expression and blood vessel numbers. The analysis of CD34 immunopositivity and blood vessel distribution in tumors showed a high correlation between PTTG1 expression and microvascular density in GH-secreting pituitary adenomas. It is particularly noteworthy that immunohistochemical double staining indicated co-localization of VEGF in many PTTG1-positive tumor cells (Minematsu et al. 2006).

Other studies have investigated a potential correlation between PTTG1 expression and pituitary tumor subtype. Hunter et al. (2003) studied PTTG1 mRNA levels in 40 pituitary tumors, and showed elevated expression of PTTG1 in GH-secreting adenomas (2.7-fold) compared with non-functioning adenomas, suggesting cell type-dependent expression of PTTG1. Several reports showed that, although PTTG1 was upregulated in all histological subtypes, its expression was highest in ACTH-secreting and non-functioning pituitary tumors (McCabe et al. 2003; Saez et al. 1999; Zhang et al. 1999a). However, Minematsu et al. (2006) examined 101 pituitary adenomas and revealed that PTTG1 mRNA elevation in most pituitary adenoma subtypes did not have a statistically significant difference. At present, PTTG1 association with pituitary tumor subtype is elusive and needs further investigation.

Regulatory Mechanisms

Understanding mechanisms regulating PTTG1 expression in pituitary tumors could elucidate the process of tumor progression and help identify subcellular antitumor targets. The precise regulatory mechanism for PTTG1 expression remains unclear in pituitary tumors, but several possible pathways have been implicated including estrogen, epidermal growth factor, Oct-1 and Rb/E2F1 and so on.

Estrogen

Estrogen is an important regulator of pituitary PTTG1 expression (Heaney et al. 1999, 2002; Yin et al. 2001). Estrogen is mitogenic for lactotrophs and gonadotrophs, and high doses of estrogen induce rat lactotroph hyperplasia and adenoma formation. Estrogen treatment of rat pituitary somato-lactotroph GH3 cells dose-dependently induced Pttg1 mRNA expression at 12 h, peaking at 24 h (Heaney et al. 1999). Estrogen induces Pttg1 mRNA expression by acting upon an estrogen-response element (ERE) in the Pttg1 promoter region. Furthermore, in Fischer 344 rats, pituitary PTTG1 expression was induced by estrogen and preceded estrogen-induced hyperplasia and adenoma formation (Heaney et al. 1999). In a study by Yin et al. (2001) in two out of the four rat strains examined, estrogen increased pituitary Pttg1 mRNA levels in parallel with estrogen induced pituitary tumor development. Estrogen-induced pituitary Pttg1 levels increased concomitantly with the proestrus serum estradiol surge, and were blocked by antiestrogen coinfusion *in vivo* (Heaney et al. 2002). These results indicate that estrogen-induced PTTG1 expression is coincident with early pituitary lactotroph transformation and suggest a role for PTTG1 in the preliminary stages of tumour initiation.

Epidermal Growth Factor (EGF)

Epidermal growth factor (EGF) is an important growth factor that regulates pituitary development, hormone synthesis and cell proliferation. In pituitary folliculostellate cells (TtT/GF), Vlotides et al. (2006) demonstrated that EGF caused up to threefold induction of Pttg1 mRNA expression, enhanced proliferating cell nuclear antigen (PCNA), and increased entry of G0/1-arrested cells into S-phase. EGF-induced Pttg1 expression and cell proliferation was abolished by preincubation of TtT/GF cells with EGFR inhibitors AG1478 and gefitinib. Phosphatidylinositol 3 kinase (PI3K), protein kinase C (PKC) and MAPK pathways were involved in EGF-induced Pttg1 expression, as well as PCNA mRNA expression and entry into S-phase. EGF-induced Pttg1 expression was cell cycle dependent, peaking at the S-G2 transition and declining thereafter. Therefore, PTTG1 is a target for EGFR-mediated paracrine regulation of pituitary cell growth.

Octamer-Binding Transcription Factor 1 (Oct-1, POU2F1)

The transcription factor Oct-1 (also called POU2F1) is a member of the POU homeodomain family and is ubiquitously expressed in adult tissues. Zhou et al. (2008) detected concordant overexpression of Oct-1 and PTTG1 in 79 human pituitary tumor specimens by using confocal immunofluorescence imaging. Oct-1 specifically bound to the human PTTG1 promoter, and increased PTTG1 transcriptional activity up to 2.5-fold. The transactivation was abrogated by co-transfection of an inactive Oct-1 form lacking the POU domain or by utilizing mutated PTTG1 promoters. Oct-1 elevated PTTG1 mRNA and protein expression, and the induction was attenuated by Oct-1 siRNA. These findings show that Oct-1 induces PTTG1 transcriptional activity and expression, thus offering a mechanism for pituitary tumor PTTG1 abundance.

Rb/E2F1 Pathway

Retinoblastoma protein (Rb) controls the G1/S cell phase transition and enables cell growth by targeting key transcription factors including the E2Fs. Rb/E2F1 is dysregulated in murine and human pituitary tumors. PTTG1 is required for pituitary tumorigenesis and PTTG1 deletion attenuates pituitary tumor development in $Rb^{+/-}$ mice. It has been reported that human PTTG1 acts as a direct E2F1 target. E2F1 and PTTG1 were concordantly overexpressed in 29 of 46 $Rb^{+/-}$ murine pituitary tissues and also in 45 of 80 human pituitary tumors. E2F1 specifically bound the PTTG1 promoter. E2F1 overexpression dose-dependently induced PTTG1 transcription and enhanced PTTG1 mRNA and protein levels in p53-devoid cells. The presence of endogenous p53/p21 constrained the induction, whereas knocking down either p53 or p21 restored E2F1-induced PTTG1 transactivation and expression. Moreover, suppressing endogenous Rb elevated both E2F1 and PTTG1 protein levels, whereas attenuated E2F1 expression resulted in suppressed PTTG1. These results elucidate a mechanism for abundant tumor PTTG1 expression, whereby Rb inactivation releases E2F1 to induce PTTG1 expression. This signalling pathway may underlie the requirement of PTTG1 for pituitary tumorigenesis (Zhou et al. 2009).

Other Regulatory Factors and Pathways

Several factors and signalling pathways regulating PTTG1 expression have been implicated in some tumor models other than those in the pituitary (Vlotides et al. 2007). For example, an effect of insulin and insulin-like growth factor (IGF-I) on PTTG1 expression has been demonstrated in glioma (LN405 and U87MG) and breast (MCF-7) cancer cell lines. In astrocytic tumors, PTTG1 expression is induced by epidermal growth factor, transforming growth factor-α, hepatocyte growth factor and insulin-like growth factor-1. In Leydig testicular cancer cells, PTTG1 is upregulated by extracellular Ca^{2+} acting via the calcium-sensing receptor. In esophageal and colorectal cancers, PTTG1 abundance is correlated with aberrant activation of β-catenin/T-cell factor (TCF). In hepatocellular carcinoma, inactivation of tumor suppressor Kruppel-like factor (KLF6) is associated with increased PTTG1 expression (Lee et al. 2010).

Functions

PTTG1 and Cell Cycle Regulation

Securin Function in Mitosis

Equal chromosome segregation during mitosis is maintained by the separation of sister chromatids in a controlled manner. As a mammalian securin protein, PTTG1 is an important component of the spindle checkpoint controlling faithful chromatid separation. The spindle checkpoint blocks anaphase entry by inhibiting the anaphase-promoting complex (APC). The APC, also called cyclosome, is an ubiquitin ligase (E3) complex consisting of different subunits that ubiquitinate mitotic cyclins, securin and other cell cycle pro-

teins. Cohesin holds sister chromatids together during metaphase and dissociates at the onset of anaphase by a protein called separase. The premature activation of separase is prevented by the binding of securin. At metaphase to anaphase transition, once chromosome bi-orientation is complete, APC is activated and targets securin for proteasomal degradation. Securin destruction releases separase, which subsequently mediates degradation of the cohesin complex, and equal separation of sister chromatids proceeds to diploid daughter cells.

The mitotic spindle checkpoint is critical for preventing aneuploidy, which in turn can lead to tumorigenesis. The role of PTTG1 in aneuploidy has been investigated in HCT116 colorectal cancer cells, but not in pituitary cells. Jallepalli et al. (2001) knocked out PTTG1 in HCT116 cells by using homologous recombination and showed that PTTG1 is required for chromosomal stability. PTTG1 knockout cells exhibited a high rate of chromosome loss, similar to those observed in naturally occurring cancers. Even after prolonged incubation in nocodazole or colcemid, no evidence for chromatid separation in PTTG1$^{-/-}$ cells was observed. In addition, they showed that the deletion of PTTG1 blocked anaphase. Time lapse experiments and immunofluorescence microscopy experiments exhibited that cells lacking PTTG1 had impaired chromatid separation, and resulted in abnormal anaphase completion and aneuploidy.

G1/S Phase Transition

In addition to PTTG1 securin function, a recent report showed that PTTG1 acts coordinately with Sp1 to induce cyclin D3 expression and promote G1/S phase transition (Tong et al. 2007). PTTG1 interacted with the transcription factor Sp1 and a PTTG1-Sp1 complex colocalized on the CCND3 promoter. Suppression of either Sp1 or PTTG1 with siRNA resulted in attenuated G1/S transition, with increased G1 and decreased S phase. Cotransfection of Sp1 siRNA and PTTG1 plasmid or PTTG1 siRNA and Sp1 plasmid reversed the effect of the other. Transfecting Sp1 elevated CCND3 mRNA and protein expression, whereas co-transfection of Sp1 and PTTG1 siRNA neutralized the CCND induction. With the use of p21$^{-/-}$ HCT116 cells, PTTG1-mediated G1/S phase transition was shown to be p21-independent.

G2/M Phase Transition

PTTG1 has a well established role in binding and inhibiting separase and thereby ensuring the appropriate timing of sister chromatid separation. Anaphase promoting complex (APC) activity is low during G2 and prophase, when cells prepare to enter M-phase. Cyclin B accumulates during G2 and prophase when its rate of destruction by the APC is low. PTTG1 is also the APC substrate that is ubiquitinated and destroyed concomitantly with cyclin B. Marangos and Carroll (2008) found that PTTG1 regulates M-phase entry by modulating cyclin B stability. In mouse oocytes, excess PTTG1 caused stabilization of cyclin B and precocious entry into M-phase. Depletion of PTTG1 increased cyclin B degradation, resulting in delayed progression into M-phase. This effect required APC activity and was reversed by expression of wild-type PTTG1. These findings reveal a novel role for PTTG1 at the G2-M transition, whereby the competition between APC degradation and PTTG1 protection of cyclin B allows for M phase timing.

Mouse Models

Knockout Mouse Model

Wang et al. (2001) developed mice lacking the murine Pttg1 gene by homologous recombination. It is known that loss of yeast or drosophila securin is lethal. However, mice lacking Pttg1 were surprisingly viable and fertile; but they had testicular and splenic hypoplasia, thymic hyperplasia, and thrombocytopenia. Pttg1$^{-/-}$ mouse embryo fibroblasts exhibited aberrant cell cycle progression with prolonged G2-M phase and binucleated and multinucleated nuclei with increased aneuploidy. Pttg1$^{-/-}$ mouse embryo fibroblast metaphases contained quadriradial, triradial, and chromosome breaks, as well as premature centromere division (Wang et al. 2001). These findings indicate PTTG1 functions to

maintain chromosome stability, cell cycle progression, and appropriate cell division. The non-fatal phenotype of Pttg1$^{-/-}$ knockout mouse suggests more than one mechanism for sister chromatid separation. It is likely that other mechanisms compensate for the loss of PTTG1 and its functions in the cell cycle control. In subsequent studies, Wang et al. (2003) showed that the reduction of pancreatic islet mass and the decrease in β cell numbers in Pttg1$^{-/-}$ male mice resulted in diabetes type I in their late adulthood. Though the IGF-1 and thyroid hormone levels were normal in these mice, some additional non-genetic factors may have contributed to hyperglycemia development in these mice in addition to PTTG1 loss. In contrast, Pttg1$^{-/-}$ female mice exhibited normal plasma glucose levels, suggesting that estrogen might be protective for islet maintenance and β cell proliferation. Pttg1$^{-/-}$ mice do not develop tumors, but exhibit pituitary hypoplasia, whereas Rb$^{+/-}$ mice develop pituitary tumors with high penetrance. Therefore Pttg1$^{-/-}$ mice were crossbred with Rb$^{+/-}$ mice to test PTTG1 actions in pituitary tumorigenesis. Pttg1 deletion rescued enhanced tumor development caused by Rb heterozygosity. The development of pituitary tumors was delayed in Rb$^{+/-}$ Pttg1$^{-/-}$ mice. This effect was mediated by induction of p21 following PTTG1 knockout (Chesnokova et al. 2005).

Transgenic Mouse Model

To understand the role of PTTG1 in pituitary tumorigenesis, transgenic PTTG1 was targeted to the mouse pituitary driven by alpha-subunit glycoprotein promoter (Abbud et al. 2005). These transgenic mice showed plurihormonal focal pituitary transgene expression with LH-, TSH-, and unexpected GH-cell focal hyperplasia and adenoma, associated with increased serum LH, GH, testosterone, and/or IGF-I levels. MRI revealed both pituitary and prostate enlargement at 9–12 months. Urinary obstruction caused by prostatic hyperplasia and seminal vesicle hyperplasia, with renal tract inflammation, resulted in death by 10 months in some animals. Pituitary PTTG1 expression resulted in plurihormonal hyperplasia and hormone-secreting microadenomas with profound peripheral growth-stimulatory effects on the prostate and urinary tract. These findings provide evidence for early pituitary plasticity, whereby PTTG1 overexpression results in a phenotype switch in early pituitary stem cells and promotes differentiated polyhormonal cell focal expansion.

The oncogenic function of PTTG1 was further confirmed by crossbreeding Rb$^{+/-}$ mice with PTTG1 transgenic mice. The mice bearing a single Rb mutant allele developed pituitary tumors with high penetrance. Crossbreed Rb$^{+/-}$ with PTTG1 transgenic mice showed enlarged pituitary glands and a 3.5-fold increase in the frequency of tumors originating from α-subunit-expressing cells. The bitransgenic mice with enlarged pituitary glands developed pituitary tumors earlier compared to Rb$^{+/-}$ mice. Confocal microscopic experiments revealed an alteration in the chromatin pattern similar to malignant cells. Increases in pituitary hyperplasia in PTTG1 overexpressing cells were observed in these bitransgenic mice when compared to Rb$^{+/-}$ mice, supporting a role of PTTG1 in pituitary gland tumorigenesis (Donangelo et al. 2006).

PTTG1 and Cell Proliferation

The effects of PTTG1 on cell proliferation are complicated, as studies have reported contradictory findings. PTTG1 is a cell-transforming oncogene, therefore one would expect a pro-proliferative action; on the other hand, functioning as a securin protein, PTTG1 overexpression is expected to reduce cell proliferation by arrest of mitosis and inhibition of sister chromatid separation. In *ex vivo* pituitary tumor specimens, a correlation has been demonstrated between PTTG1 expression and cell proliferation. PTTG1 expression correlated with proliferation marker Ki-67 or proliferating cell nuclear antigen (PCNA) respectively in pituitary adenomas (Filippella et al. 2006). Furthermore, Pttg1 knockout mice exhibited a reduction in β-cell islet mass and a decrease in cell proliferation (Wang et al. 2003). In Rb$^{+/-}$ Pttg1$^{-/-}$ mice, PTTG1 ablation led to decreased pituitary cell proliferation (Chesnokova et al. 2005). These findings support the pro-proliferation role of PTTG1. On the contrary,

the anti-proliferative function of PTTG1 has also been illustrated in animal and cell transfection studies (Mu et al. 2003; Pei and Melmed 1997). A clinical study showed that although PTTG1 expression was associated with cell proliferation in the normal pituitary, no correlation was detected in 101 pituitary adenomas (Minematsu et al. 2006). Interestingly, the phosphorylation status of PTTG1 was involved in pro- or anti-proliferative effect of PTTG1 overexpression (Boelaert et al. 2004). In present, whether PTTG1 promotes or suppresses cell proliferation remains elusive. Further studies to illuminate PTTG1 roles in proliferation would be helpful to understand pituitary tumor progression.

PTTG1 and Angiogenesis

PTTG1 is involved in angiogenesis, at least partly, by induction of fibroblast growth factor 2 (FGF-2, also called bFGF) and VEGF (McCabe et al. 2002; Minematsu et al. 2006). Ishikawa et al. (2001) showed that the conditioned medium collected from NIH3T3 cells transfected with human PTTG1 induced angiogenesis. As a transactivator of growth factors, high PTTG1 expression induces FGF-2, VEGF and other proangiogenic genes. Both PTTG1 and FGF-2 are upregulated in pituitary tumors (Heaney et al. 1999). Zhang et al. (1999b) reported that transfecting PTTG1 in NIH3T3 cells resulted in increased bFGF mRNA expression and secretion. A point mutation of the putative SH3-binding site within the C-terminal region of PTTG1 abrogated this bFGF production. Pei (2001) demonstrated that PTTG1 transactivated bFGF by interacting with PBF. One clinical study detected higher expression levels of PTTG1, PTTG1 binding factor (PBF), and FGF-2 and its receptor FGFR1 mRNAs in pituitary adenomas as compared to normal tissues in 121 pituitary tumors. In addition, FGFR1 expression was higher in hormone-secreting pituitary tumors that invade bone (the sphenoid bone) than in those that do not (Heaney et al. 1999). These data suggest a causative association between PTTG1 and FGF-2 in pituitary adenomas. Moreover, a significantly positive correlation was found between VEGF and PTTG1 mRNA expressions, as well as between PTTG1 and KDR (kinase insert domain receptor; VEGF receptor) mRNA in pituitary adenomas. VEGF and PTTG1 showed co-localization (Minematsu et al. 2006). PTTG1 is important in regulating angiogenic genes. Therefore, further investigation of the role of PTTG1 in angiogenesis is essential for developing a stage-specific as well as a targeted cancer therapy.

PTTG1 and Senescence

Senescence refers to premature irreversible cell proliferation arrest. Senescent cells maintain cellular function and viability, but are devoid of proliferative potential. Senescence may thus function as a natural surveillance protecting against tumorigenesis. Furthermore, in benign tumors such as pituitary tumors, senescence may prevent malignant transformation. Mechanisms leading to senescence mainly converge to the p53/p21 and p16/pRb pathways.

PTTG1 Deletion Results in Pituitary Tumor Senescence

Pttg1-deficient pituitary cells exhibit intracellular p53 accumulation and p21 induction. p21 induction triggers senescence, which facilitates Rb hypophosphorylation and tumor growth arrest. High pituitary p21 levels observed in the absence of PTTG1 were associated with suppressed Cdk2 activity, decreased RB phosphorylation and cyclin A expression, all required for cell cycle progression (Chesnokova et al. 2005, 2007). Pttg1 deletion also led to extensive pituitary cell aneuploidy, which activated DNA damage signaling pathways triggering ARF/p53/p21 senescence evidenced by increased SA-β-gal activity in Pttg1$^{-/-}$ pituitary gland (Chesnokova et al. 2008).

PTTG1 Overexpression Results in Pituitary Tumor Senescence

Pttg1 deletion caused p21 induction has been observed mostly in GH-producing mouse Pttg1$^{-/-}$

pituitary cells (Chesnokova et al. 2007). p21 was also induced in Pttg1-transfected GH3 pituitary cells and in human GH-secreting pituitary adenomas exhibiting high PTTG1 levels (Chesnokova et al. 2008). Rat GH3 cells were transiently transfected with EGFP–PTTG1 plasmids, and immunocytochemistry showed enhanced p21 protein expression in these cells as compared with vector-transfected cells. Transfected cells were also sorted by flowcytometry, and increased p21 mRNA levels were observed in EGFP–PTTG1-positive GH3 cells relative to controls (Chesnokova et al. 2008). PTTG1 is abundantly expressed in human pituitary tumors. A strong positive correlation between PTTG1 and p21 expression was observed in 56 of 72 pituitary adenomas of various phenotypes. GH-secreting pituitary adenomas expressed markers of senescence including high levels of p21 and SA-β-gal activity. In 23 of 26 GH-producing pituitary adenomas accompanied with high PTTG1 levels, senescence was evidenced by increased p21 and SA-beta-galactosidase. Thus, either deletion or overexpression of PTTG1 promotes pituitary cell aneuploidy and p53/p21-dependent senescence, particularly in GH-secreting cells. p21 elevation in aneuploid pituitary cells may constrain pituitary tumor growth, thus accounting for the very low incidence of pituitary carcinomas.

Summary

PTTG1 is a highly interesting multi-domain and multi-functional protein. It is overexpressed in most human tumors including pituitary adenoma, and is transcriptionally regulated by various factors and signalling pathways. PTTG1 is involved in a wide array of physiologic and oncogenic functions. Molecular and clinical studies implicate diverse roles of PTTG1 in cell cycle regulation, genetic instability, cell proliferation, pituitary tumor angiogenesis and senescence. Transgenic mouse models of both PTTG1 overexpression and inactivation support a causal role for PTTG1 in the development of pituitary tumors. Further investigation of the PTTG1 pathway and its role as a biomarker and therapeutic target are warranted.

References

Abbud RA, Takumi I, Barker EM, Ren SG, Chen DY, Wawrowsky K, Melmed S (2005) Early multipotential pituitary focal hyperplasia in the alpha-subunit of glycoprotein hormone-driven pituitary tumor-transforming gene transgenic mice. Mol Endocrinol 19:1383–1391

Boelaert K, Yu R, Tannahill LA, Stratford AL, Khanim FL, Eggo MC, Moore JS, Young LS, Gittoes NJ, Franklyn JA, Melmed S, McCabe CJ (2004) PTTG's C-terminal PXXP motifs modulate critical cellular processes in vitro. J Mol Endocrinol 33:663–677

Chamaon K, Kirches E, Kanakis D, Braeuninger S, Dietzmann K, Mawrin C (2005) Regulation of the pituitary tumor transforming gene by insulin-like-growth factor-I and insulin differs between malignant and non-neoplastic astrocytes. Biochem Biophys Res Commun 331:86–92

Chen L, Puri R, Lefkowitz EJ, Kakar SS (2000) Identification of the human pituitary tumor transforming gene (hPTTG) family: molecular structure, expression, and chromosomal localization. Gene 248:41–50

Chesnokova V, Kovacs K, Castro AV, Zonis S, Melmed S (2005) Pituitary hypoplasia in pttg-/- mice is protective for Rb+/− pituitary tumorigenesis. Mol Endocrinol 19:2371–2379

Chesnokova V, Zonis S, Rubinek T, Yu R, Ben-Shlomo A, Kovacs K, Wawrowsky K, Melmed S (2007) Senescence mediates pituitary hypoplasia and restrains pituitary tumor growth. Cancer Res 67:10564–10572

Chesnokova V, Zonis S, Kovacs K, Ben-Shlomo A, Wawrowsky K, Bannykh S, Melmed S (2008) p21(Cip1) restrains pituitary tumor growth. Proc Natl Acad Sci USA 105:17498–17503

Chien W, Pei L (2000) A novel binding factor facilitates nuclear translocation and transcriptional activation function of the pituitary tumor-transforming gene product. J Biol Chem 275:19422–19427

Dominguez A, Ramos-Morales F, Romero F, Rios RM, Dreyfus F, Tortolero M, Pintor-Toro JA (1998) hpttg, a human homologue of rat pttg, is overexpressed in hematopoietic neoplasms. Evidence for a transcriptional activation function of hPTTG. Oncogene 17:2187–2193

Donangelo I, Gutman S, Horvath E, Kovacs K, Wawrowsky K, Mount M, Melmed S (2006) Pituitary tumor transforming gene overexpression facilitates pituitary tumor development. Endocrinology 147:4781–4791

Filippella M, Galland F, Kujas M, Young J, Faggiano A, Lombardi G, Colao A, Meduri G, Chanson P (2006) Pituitary tumour transforming gene (PTTG) expression correlates with the proliferative activity and recurrence status of pituitary adenomas: a clinical and immunohistochemical study. Clin Endocrinol (Oxf) 65:536–543

Heaney AP, Horwitz GA, Wang Z, Singson R, Melmed S (1999) Early involvement of estrogen-induced pituitary tumor transforming gene and fibroblast growth factor expression in prolactinoma pathogenesis. Nat Med 5:1317–1321

Heaney AP, Fernando M, Melmed S (2002) Functional role of estrogen in pituitary tumor pathogenesis. J Clin Invest 109:277–283

Hunter JA, Skelly RH, Aylwin SJ, Geddes JF, Evanson J, Besser GM, Monson JP, Burrin JM (2003) The relationship between pituitary tumour transforming gene (PTTG) expression and in vitro hormone and vascular endothelial growth factor (VEGF) secretion from human pituitary adenomas. Eur J Endocrinol 148:203–211

Ishikawa H, Heaney AP, Yu R, Horwitz GA, Melmed S (2001) Human pituitary tumor-transforming gene induces angiogenesis. J Clin Endocrinol Metab 86:867–874

Jallepalli PV, Waizenegger IC, Bunz F, Langer S, Speicher MR, Peters JM, Kinzler KW, Vogelstein B, Lengauer C (2001) Securin is required for chromosomal stability in human cells. Cell 105:445–457

Lee UE, Ghiassi-Nejad Z, Paris AJ, Yea S, Narla G, Walsh M, Friedman SL (2010) Tumor suppressor activity of KLF6 mediated by downregulation of the PTTG1 oncogene. FEBS Lett 584:1006–1010

Marangos P, Carroll J (2008) Securin regulates entry into M-phase by modulating the stability of cyclin B. Nat Cell Biol 10:445–451

McCabe CJ, Boelaert K, Tannahill LA, Heaney AP, Stratford AL, Khaira JS, Hussain S, Sheppard MC, Franklyn JA, Gittoes NJ (2002) Vascular endothelial growth factor, its receptor KDR/Flk-1, and pituitary tumor transforming gene in pituitary tumors. J Clin Endocrinol Metab 87:4238–4244

McCabe CJ, Khaira JS, Boelaert K, Heaney AP, Tannahill LA, Hussain S, Mitchell R, Olliff J, Sheppard MC, Franklyn JA, Gittoes NJ (2003) Expression of pituitary tumour transforming gene (PTTG) and fibroblast growth factor-2 (FGF-2) in human pituitary adenomas: relationships to clinical tumour behaviour. Clin Endocrinol (Oxf) 58:141–150

Minematsu T, Suzuki M, Sanno N, Takekoshi S, Teramoto A, Osamura RY (2006) PTTG overexpression is correlated with angiogenesis in human pituitary adenomas. Endocr Pathol 17:143–153

Mu YM, Oba K, Yanase T, Ito T, Ashida K, Goto K, Morinaga H, Ikuyama S, Takayanagi R, Nawata H (2003) Human pituitary tumor transforming gene (hPTTG) inhibits human lung cancer A549 cell growth through activation of p21(WAF1/CIP1). Endocr J 50:771–781

Pei L (2000) Activation of mitogen-activated protein kinase cascade regulates pituitary tumor-transforming gene transactivation function. J Biol Chem 275:31191–31198

Pei L (2001) Identification of c-myc as a down-stream target for pituitary tumor-transforming gene. J Biol Chem 276:8484–8491

Pei L, Melmed S (1997) Isolation and characterization of a pituitary tumor-transforming gene (PTTG). Mol Endocrinol 11:433–441

Ramos-Morales F, Dominguez A, Romero F, Luna R, Multon MC, Pintor-Toro JA, Tortolero M (2000) Cell cycle regulated expression and phosphorylation of hpttg proto-oncogene product. Oncogene 19:403–409

Romero F, Multon MC, Ramos-Morales F, Dominguez A, Bernal JA, Pintor-Toro JA, Tortolero M (2001) Human securin, hPTTG, is associated with Ku heterodimer, the regulatory subunit of the DNA-dependent protein kinase. Nucleic Acids Res 29:1300–1307

Saez C, Japon MA, Ramos-Morales F, Romero F, Segura DI, Tortolero M, Pintor-Toro JA (1999) hpttg is overexpressed in pituitary adenomas and other primary epithelial neoplasias. Oncogene 18:5473–5476

Stratford AL, Boelaert K, Tannahill LA, Kim DS, Warfield A, Eggo MC, Gittoes NJ, Young LS, Franklyn JA, McCabe CJ (2005) Pituitary tumor transforming gene binding factor: a novel transforming gene in thyroid tumorigenesis. J Clin Endocrinol Metab 90:4341–4349

Tong Y, Tan Y, Zhou C, Melmed S (2007) Pituitary tumor transforming gene interacts with Sp1 to modulate G1/S cell phase transition. Oncogene 26:5596–5605

Vlotides G, Cruz-Soto M, Rubinek T, Eigler T, Auernhammer CJ, Melmed S (2006) Mechanisms for growth factor-induced pituitary tumor transforming gene-1 expression in pituitary folliculostellate TtT/GF cells. Mol Endocrinol 20:3321–3335

Vlotides G, Eigler T, Melmed S (2007) Pituitary tumor-transforming gene: physiology and implications for tumorigenesis. Endocr Rev 28:165–186

Wang Z, Yu R, Melmed S (2001) Mice lacking pituitary tumor transforming gene show testicular and splenic hypoplasia, thymic hyperplasia, thrombocytopenia, aberrant cell cycle progression, and premature centromere division. Mol Endocrinol 15:1870–1879

Wang Z, Moro E, Kovacs K, Yu R, Melmed S (2003) Pituitary tumor transforming gene-null male mice exhibit impaired pancreatic beta cell proliferation and diabetes. Proc Natl Acad Sci USA 100:3428–3432

Wierinckx A, Auger C, Devauchelle P, Reynaud A, Chevallier P, Jan M, Perrin G, Fevre-Montange M, Rey C, Figarella-Branger D, Raverot G, Belin MF, Lachuer J, Trouillas J (2007) A diagnostic marker set for invasion, proliferation, and aggressiveness of prolactin pituitary tumors. Endocr Relat Cancer 14:887–900

Yin H, Fujimoto N, Maruyama S, Asano K (2001) Strain difference in regulation of pituitary tumor transforming gene (PTTG) in estrogen-induced pituitary tumorigenesis in rats. Jpn J Cancer Res 92:1034–1040

Yu R, Ren SG, Horwitz GA, Wang Z, Melmed S (2000) Pituitary tumor transforming gene (PTTG) regulates placental JEG-3 cell division and survival: evidence from live cell imaging. Mol Endocrinol 14:1137–1146

Zhang X, Horwitz GA, Heaney AP, Nakashima M, Prezant TR, Bronstein MD, Melmed S (1999a) Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas. J Clin Endocrinol Metab 84:761–767

Zhang X, Horwitz GA, Prezant TR, Valentini A, Nakashima M, Bronstein MD, Melmed S (1999b) Structure, expression, and function of human pituitary tumor-transforming gene (PTTG). Mol Endocrinol 13:156–166

Zhou C, Tong Y, Wawrowsky K, Bannykh S, Donangelo I, Melmed S (2008) Oct-1 induces pituitary tumor transforming gene expression in endocrine tumors. Endocr Relat Cancer 15:817–831

Zhou C, Wawrowsky K, Bannykh S, Gutman S, Melmed S (2009) E2F1 induces pituitary tumor transforming gene (PTTG1) expression in human pituitary tumors. Mol Endocrinol 23:2000–2012

Zou H, McGarry TJ, Bernal T, Kirschner MW (1999) Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. Science 285:418–422

Zur A, Brandeis M (2001) Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis. EMBO J 20:792–801

Pituitary Adenomas: Endoscopic Endonasal Transphenoidal Technique

23

Savas Ceylan and Ihsan Anik

Contents

Abstract

Endoscopic endonasal transsphenoidal surgery has gained increasing acceptance by neurosurgeons. In many centers throughout the world, this technique is now routinely used for the same indications as the conventional microsurgical technique. In 1963, Guiot and colleagues (La Presse Medicale 71:1225–1228, 1963) first proposed the use of an endoscope as part of a transnasorhinoseptal microsurgical approach. Endoscopic pituitary surgery differs from microscopic surgery, because it requires a steep learning curve for endoscopic skills and two-dimensional visualization. Gaining experience with the endoscope, some changes of the standard approaches have been performed satisfactorily for pituitary adenoma in parasellar and suprasellar location.

Treatment of pituitary adenomas invading the cavernous sinus is one of the great challenges in neurosurgical practice. Expanded surgical techniques should be performed for the removal of the cavernous sinus component in pituitary adenomas invading the cavernous sinus. Extended approaches are essential for reaching the area from lamina cribrosa to the cranio-cervical junction. Extended transsphenoidal approach was originally described by Weiss (Transnasal transsphenoidal approach. In: Apuzzo MLJ (ed) Surgery of the third ventricle, Williams and Wilkins, Baltimore, pp 476–494, 1987). Expanded and extended endoscopic approaches were reported overtime. The endoscopic transsphenoidal approach has been

S. Ceylan (✉) • I. Anik
Department of Neurosurgery, School of Medicine, Kocaeli University, Eski Istanbul Yolu 10.Km, 41380 Umuttepe, Izmit, Kocaeli, Turkey
e-mail: ssceylan@yahoo.com

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_23,

reported in the literature as a useful tool to treat sellar and parasellar lesions. Improved visualization with 0° and angled endoscopes allow the surgeon to identify anatomic landmarks. Angled endoscopes especially provide great advantages on the removal of tumor remnants at superior and lateral recesses. Different treatment modalities should be considered to achieve remission during the follow-up period in patients with secretuar pituitary adenomas.

Introduction

A microsurgical transsphenoidal approach has been well established as the standard surgical treatment for pituitary adenomas, although the visualization of suprasellar and lateral areas with the microscope is difficult. Guiot et al. (1963) were the first in the literature to report the use of an endoscope during microsurgical transsphenoidal surgery. Some authors have described an endoscope-assisted technique, to complement the microscope in the early or late stages of a traditional procedure. Most pituitary surgeons are now using an endoscope to visualize the hidden anatomical corners as supplementary visualizing tools during microscopic transsphenoidal pituitary surgery.

The first purely endoscopic pituitary surgery was reported by Jankowski et al. (1992). Pure endoscopic endonasal transsphenoidal surgery has been described in detail by Jho (2001). Recently, endoscopic endonasal transsphenoidal surgery has been progressively accepted by neurosurgeons (Cappabianca et al. 2004). It is performed completely via the endoscope, without the use of a nasal speculum. The angled-lens endoscopic view provides direct visualization of the suprasellar area and cavernous sinus. Extended transsphenoidal is originally described by Weiss (1987). Expanded and extended endoscopic approaches were reported overtime (Cavallo et al. 2005; Jho and Ha 2004; Kassam et al. 2005; Frank et al. 2006a). Extended approaches are essential for reaching the area from lamina cribrosa to the cranio-cervical junction. In our Neurosurgery Department (Kocaeli University School of Medicine, Kocaeli, Turkey); 350 endoscopic transsphenoidal approach were performed during the 13 years. Among these, 300 endoscopic transsphenoidal approaches were performed for pituitary tumors. The main problem in the initial stage of endoscopic transsphenoidal approach is the learning curve.

Learning Curve

Endoscopic pituitary surgery differs from microscopic surgery, since it requires a steep learning curve for endoscopic skills and two-dimensional visualization. Whether it has been performed with microscope or endoscope, experience is the important point in reduction of the complications and in the effectiveness of the surgical procedure in pituitary surgery. For neurosurgeons who are accustomed to the microscope, there is a new learning curve that must be overcome for endoscopic pituitary surgery.

In a study of surgical experience and complications in the transsphenoidal microscopic surgery by neurosurgeons in the United States, those having performed 200 cases and over were assessed as being experienced (Ciric et al. 1997). In other reports, even though a controlled study on the learning curve and experience was not presented for the reported endoscopic series of under 200, complication rates were comparable with those of an experienced microscopic group (Cappabianca et al. 2002). The main disadvantage of endoscopic pituitary surgery is the lack of standard training programs. In endoscopic transsphenoidal surgery, the learning curve is an important disadvantage for the neurosurgeon who is already well trained in microscopic surgery. In endoscopic pituitary surgery, the number of cases for the learning curve was calculated as 70–80 (Cappabianca et al. 2002). Additionally, in their study Sonnenburg et al. (2004) purposed to investigate the existence of learning curve for considering complications, revision surgery, length of stay and intraoperative blood loss.

In our recent study, we classified the first 40 cases as early and 38 cases as late period and found out that some changes on standard endoscopic approaches were possible on late-period cases (Koc et al. 2006). Considering our cases, we have seen that the operation time, the amount of resection and the number of the reoperations for residual tumor, gradually improved in time with increased experience. In the initial period, even though endoscopic surgery of microadenomas was relatively easy, effective management of some macroadenomas with parasellar and suprasellar locations was difficult.

Gaining experience with the endoscope, some changes of the standard approaches are performed satisfactorily for pituitary adenoma in parasellar and suprasellar location. The approaches for lesions extending into the ipsilateral cavernous sinus required extensive endoscopic experience and are certainly not suited for beginners.

Surgical Instruments

In our clinical series, surgeries were performed with the use of a 4-mm diameter endoscope with a 0°-angled lens and also a 30°- or 45°-angled endoscope for visualization of the parasellar and suprasellar areas. C-arm fluoroscopy was used in very few cases, only in selected ones. Navigation was used in some macroadenomas and in extended and expanded approaches.

In our department, endoscope holder was used in the initial cases however later on endoscope was used on free-hand and external irrigation was sufficient.

Fibrin glue was used for sella floor repairment in some of the selected patients. We used a purely endoscopic endonasal approach to the sella, which was performed via an anterior sphenoidotomy, through the existing sphenoid ostium, without the use of a transsphenoidal retractor or any postoperative nasal packing, and with a rigid diagnostic endoscope as the sole visualizing tool. After tumor removal was thought to be complete, a 30°- or 45°-angled endoscope was administered, which allowed complete resection of invasive adenomas by visualization of parasellar and suprasellar tumor extension. Angled endoscopes especially provide great advantages on removal of tumor remnants at superior and lateral recesses.

Surgical Approaches

Three different approaches can be classified in endoscopic transsphenoidal surgery:

1. Standard endoscopic transsphenoidal approach
2. Expanded cavernous sinus approach
3. Extended endoscopic transsphenoidal approach

Standard Surgical Technique

Generally mononostril approach is used for micro and macroadenomas. It has three phases: nasal, sphenoidal and sellar phase (Fig. 23.1).

Choice of nostril: In the comparable width of nasal cavity, considering septum deviation and pneumatized turbinates, the contralateral nostril was used for located paramedian adenomas. The size and shape of nasal cavity were measured with MR and computed tomography (CT) scan. Jho and Carrau (1997) suggested to work through both nostrils in cases with narrow nasal cavity in their established clinical series. An endoscope held with the non-dominant hand was inserted through one nostril and surgical instruments were inserted through the other. We think that the use of the contralateral nostril may facilitate endoscopic surgery in selected cases. In recent years as well as expand and extended approaches, we preferred binostril approach for the macroadenomas providing better manipulation.

Resection of turbinate: Jho and Ha (2004) recommend turbinate resection during the ascending phase of the learning curve. At the beginning, since we were unaccustomed with nasal anatomy and the nasal passage, resection of the lower half of the middle turbinate in first-group patients was performed if the nasal cavity was very narrow. Hence, it provided a larger operating cavity. Turbinate resection was not performed in late-period cases, except cavernous and extended approaches. In the standard approach, to create an adequate surgical space, the head of the middle

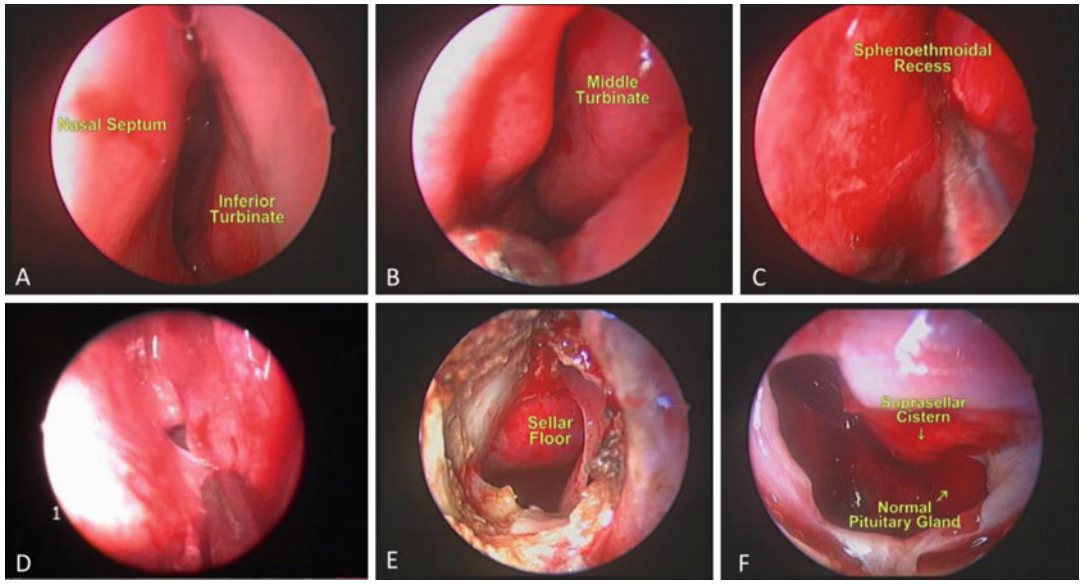

Fig. 23.1 Steps of the standard endoscopic transsphenoidal approach. Demonstrating; the nasal phase (**a**, **b**, **c**, **d**), Sphenoidal phase (**e**), and sellar phase (**f**)

turbinate was carefully pushed laterally hence surgical area between nasal septum and middle turbinate became wider.

Anterior sphenoidotomy: Resection of the sphenoid rostrum was performed and the anterior wall of the sphenoidal sinus was opened by drill and ronguer about 15-mm in size. The use of C-arm fluoroscopy was progressively decreased, but was required in case of conchal-type sphenoid sinus, when anatomical landmarks, such as the clivus, carotid prominence, opto-carotid recess, could not be distinguished. Besides, widespread use of neuronavigation also decreased the need of C-arm in these conditions. Improved visualization with 0° and angled endoscopes allow the surgeon to identify anatomic landmarks, carotid prominence, optic chiasm etc.

Sellar opening: The opening of the sellar floor was performed with a microdrill. In microadenomas, according to the location of the tumor, the half of sellar floor was opened. In these cases, adenomectomy was performed just only via appropriate sella opening floor without removing the midline septum of the sphenoid sinus (Hemisphenoidotomy approaches). Dural openings can be made in different ways. In macroadenoma cases, wider sella opening is performed, at least 1 cm opening should be made. Wide sellar floor opening provides increased intrasellar maneuver of the endoscope leading suprasellar dissection and visualization of lateral remnant parts thus make available total resection. In recent years capsular tumor dissection is gaining importance. The main property of endoscopic technique on capsular dissection is that it provides more information than microscopic techniques.

Histological psuedocapsule enveloping the pituitary tumors was defined in the early 1900, however classification characteristics of the pseudocapsule were not delineated until now. Pseudocapsule was termed by Oldfield and Vortmeyer (2006) as the boundary between the adenoma and surrounding pituitary tissue, that is made by the condensation of the basement membranes of the compressed peritumoral cell lands.

Microsurgical pseudocapsule was defined by Lee et al. (2009) as a peritumoral structure that can be distinguished both from tumor and normal gland under microscope intraoperatively.

Capsule structure was tried to be distinguish via endoscope prospectively in micro and macroadenomas for 40 of last 70 patients (Fig. 23.2). In microadenomas a capsule formation was seen due to compressed pituicytes (Fig. 23.2). In cases with macro and giant adenomas, a pseudocapsule formation was seen as circumscribed in some

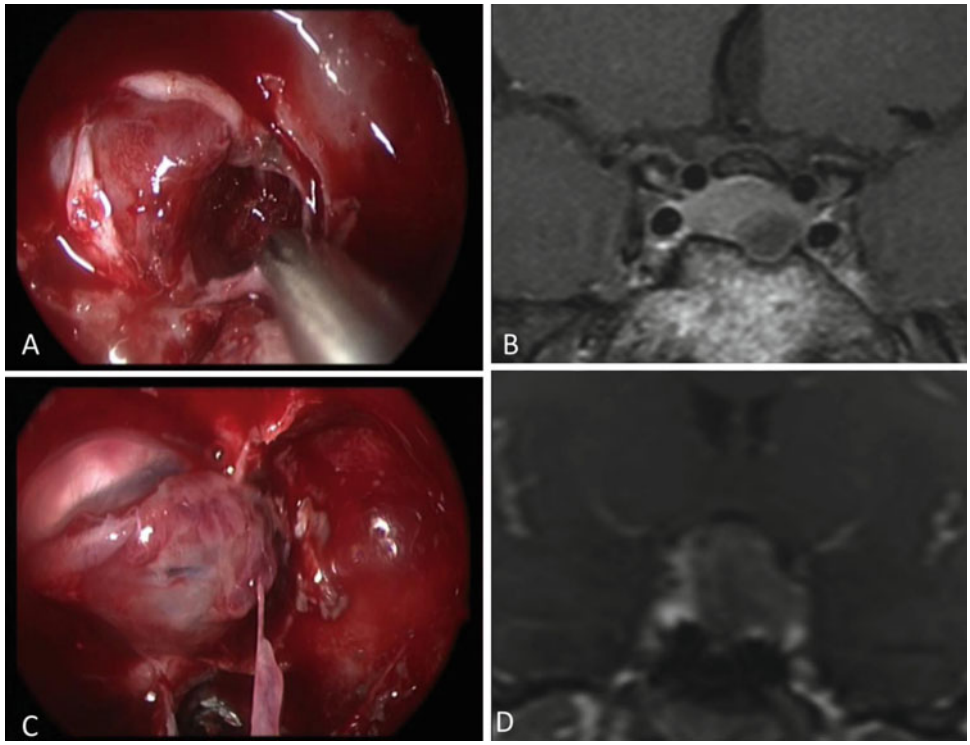

Fig. 23.2 (**a**, **b**) Intraoperative view of microcapsular dissectin and MRI of the patient demonstrating the microadenoma (**c**, **d**): Intraoperative view of macrocapsular dissectin and MRI of the patient demonstrating the macroadenoma

cases or irregular in other cases. This pseudocapsule is used in dissections of suprasellar and other neighboring structures. We believe that pseudocapsula should be removed for total resection. Suprasellar cistern should be seen in total and symmetric surgical area. Nowadays in each macroadenoma case, following gently opening of the dura and controlling the presence of pseudocapsule, usually the soft central part of the adenoma is removed via aspirator and various curets and total removal is tried by the dissection of the hard outer layer with covering pseudocapsula (Fig. 23.2). However, administrating microdoppler both on dural incision and on sellar phase tumour resection would be useful to prevent carotid injury, the most dramatic complication in the sellar phase.

Expanded Cavernous Sinus Approach

Pituitary adenomas usually compress surrounding structures, commonly resulting in sella enlargement and suprasellar extension. Invasion of the cavernous sinus account 6–10% of all pituitary adenomas. However, in more recent reports of extended microscopic and endoscopic approaches, authors have reported cavernous sinus invasion in >10% of lesions (Dolenc 1989; Kitano et al. 2008).

There are two major problems about pituitary adenomas invading CS. First is to define extension and invasion (Fig. 23.3). We defined the criteria for cavernous sinus invasion endoscopically. According to these criteria's invasion is identified and extension is distinguished. We performed an intraoperative evaluation of cavernous sinus invasion considering;

1. Visualization of the medial wall defect.
2. Visualization of at least one of the intra cavernous ICA segments (anterior vertical, horizontal, posterior bend, paraclival carotid artery) (Fig. 23.3).
3. Visualization of minor tumoral extensions through small focal pit holes of the medial wall of CS.

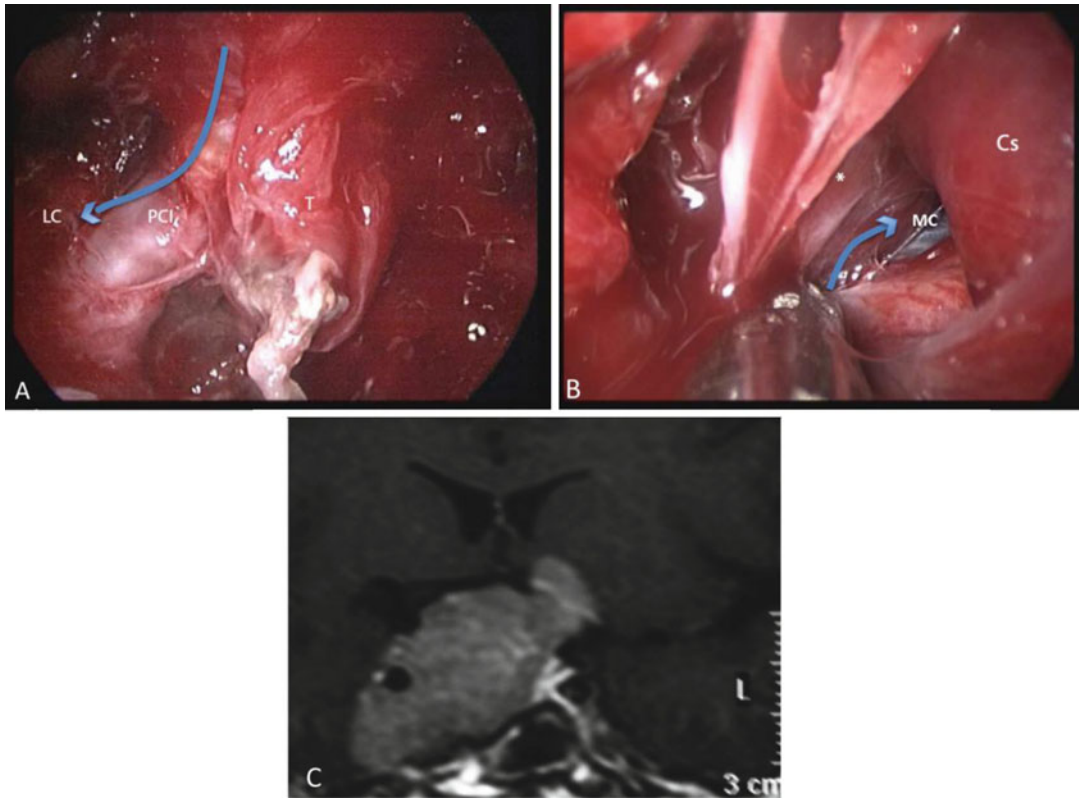

Fig. 23.3 44 year-old male with nonsecretory adenoma (**a**): Invasion on one side and displacement (**b**) on the other side were seen in the same case and was excellently viewed by the endoscopic technique. (**c**): Preoperative Gd-enhanced, T1-weighted MR images of the patient. *LC* lateral corridor, *MC* medial corridor, *Cs* carotid siphon, *Pcl* paraclival carotid artery, *T* fibrotic tumor, (*Blue arrows*): Demonstrating the area of lateral and medialcorridors

4. Carotid artery segments were confirmed by micro doppler and CS invasion was evaluated during the tumor removal in cases of inability to view the CS endoscopically due to CS hemorrhage.

Second problem is the difficulty in demonstrating medial wall via preoperative imaging modalities. There is no neuroimaging method that can show the medial wall precisely. In the literature, different results of the anatomic studies were published about the structure of the CS medial wall. Studies on the structure of the medial wall reported as one layer by some authors, as two layers in another or thin, loose, and histological defects are reported by the other authors (Chi and Lee 1980; Songtao et al. 2009; Yasuda et al. 2004; Yokoyama et al. 2001). Thus some authors reported that tumor histology is responsible for CS invasion while some authors insist on histological defects of the medial wall (Peker et al. 2005).

In the endoscopic cavernous sinus approach, the medial and lateral surgical corridors formed by neurovascular structures have been described (Figs. 23.3 and 23.4), (Cavallo et al. 2005). One endoscopic surgical corridor is medial to the intracavernous carotid artery, and another wider corridor is lateral to it. The medial corridor to the ICA is formed by the C-shaped segment of the intracavernous sinus–carotid artery, and is bordered posteriorly by the dorsum sellae and the posterior petroclinoid fold. The lateral corridor to the ICA (triangular area) is demarcated by the intracavernous tract of the ICA posteriorly, by the vidian nerve inferiorly, and by the medial pterygoid process anteriorly.

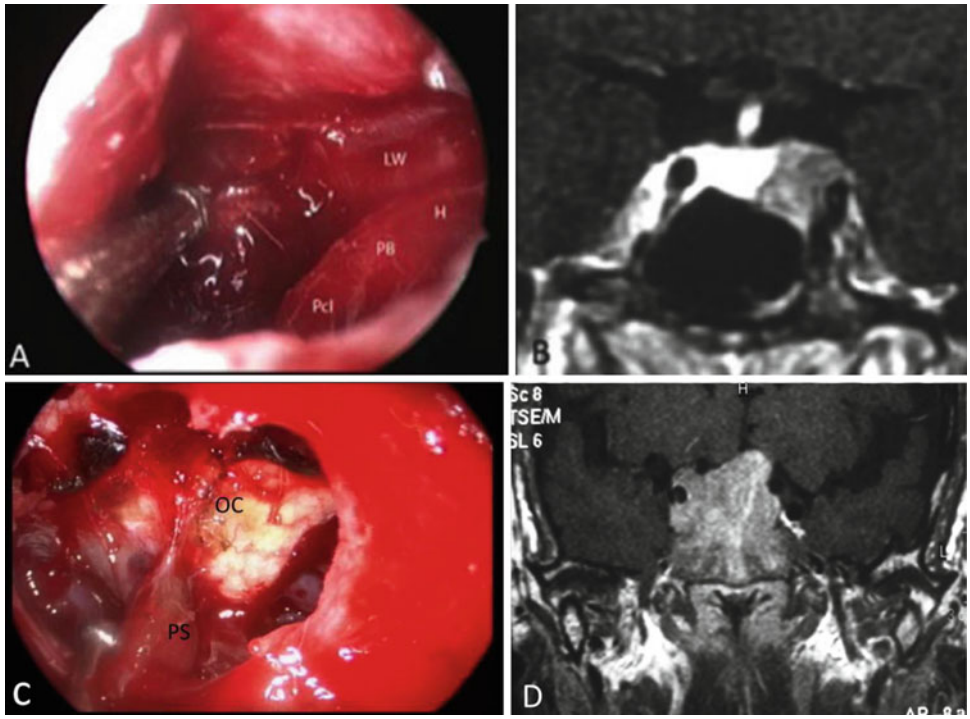

Fig. 23.4 (**a**, **b**) 18 year-old female with GH secreting adenoma. (**a**) Visualization of intracavernous ICA segments. Preoperative (**b**) Gd-enhanced, T1-weighted MR images of the patient. *LC* lateral corridor, *H* horizontal segment of the cavernous carotid artery, *Pb* posterior bend of the cavernous carotid artery, *Pcl* paraclival carotid artery (**c**, **d**) 53 year-old female with invasive adenoma (Extended Approach). Intraoperative view of the chiasm, the stalk. *OC* optic chiasm, *PS* pituitary stalk

In recently published anatomic studies, it is reported that the superior part of the medial wall was thinner while the inferior part to be thicker (Peker et al. 2005; Songtao et al. 2009). This confirms why pituitary adenomas are usually located at the medial corridor and demonstrate CS invasion through the superior part. Most pituitary adenomas extend into the cavernous sinus through the medial corridor (Cavallo et al. 2005). The ideal lesions for the endoscopic endonasal approach are soft tumors such as pituitary adenomas, which are unlikely to infiltrate the ICA and show a mediolateral growth pattern resulting in lateral displacement of the ICA. In contrast, hard, solid tumors such as meningiomas that are known to infiltrate the ICA may limit the indications for the endoscopic endonasal approach.

Treatment of pituitary adenomas invading the cavernous sinus is one of the great challenges in neurosurgical practice. Different treatment modalities should be considered to achieve remission during the follow-up period in these patients. Pituitary adenomas without cavernous sinus invasion can be removed without expanding the surgical area to the cavernous sinus: this is the standard endoscopic transsphenoidal approach. More reliable surgical techniques should be performed for the removal of the cavernous sinus component in pituitary adenomas invading the cavernous sinus.

Various microscopic approaches, including transfacial, transsphenoidal, transmaxillary, transmaxillosphenoidal, transethmoidal, and transsphenoethmoidal exposures have been proposed to remove lesions involving the anterior portion of the cavernous sinus (Das et al. 2001). These approaches are limited by a deep, narrow surgical corridor that does not allow adequate exposure of neurovascular structures.

Some authors proposed different endoscopic cavernous approaches in adenomas extending into the cavernous sinus. Cavernous approaches, taking tumoral invasion in account, require different modifications of the standard approach as endonasal transethmoidal (far lateral), ethmoidopterygosphenoidal route, and extended approach (Alfieri and Jho 2001; Cavallo et al. 2005).

The endoscopic transsphenoidal approach allowed us to inspect part of the parasellar and middle cranial fossa areas of the cavernous sinus. The size, shape, and pneumatization of the sphenoid sinus are important factors in the lateral approach to the cavernous sinus. In patients with a presellar or conchal-type sphenoid sinus, it is difficult to define surgical landmarks. Greater exposure of the lateral recess of the sphenoid sinus allows less extensive pterygoid process drilling. Depending on the anatomical variations of the nasion, and only in cases in which we used the medial corridor, we preferred a contralateral approach in the initial cases. We used combined approaches rather than a defined cavernous sinus approach in the last cases. In these approaches, resection of anterior and posterior ethmoid cells and total or partial removal of the middle turbinate with medial pterygoid process resection (Ceylan et al. 2010).

The medial corridor approach is adequate for Knosp Grades 2 and 3 lesions; however, the tumor should be removed by opening the lateral corridor in Grade 4 and ventral cases (Knosp et al. 1993). In these cases, both corridors should be used.

The transcranial approach to lesions of the cavernous sinus is regarded as overly aggressive despite recent advances in cranial base surgery. In the literature, low rates have been reported for complete resection via the transcranial approach to pituitary adenomas involving cavernous sinus. Some authors have described their experience with the microscopic transsphenoidal approach to the cavernous sinus (Dusick et al. 2005; Pamir et al. 2006). Couldwell et al. (1997) noted that the surgical exposure provided by a microscopic transsphenoidal approach is very limited because of the narrow midline corridor around the sella.

Kitano et al. (2008) described an extended transsphenoidal microscopic approach in 36 patients with pituitary adenomas and extension into the cavernous sinus. This technique was accomplished by extending the submucosal dissection of the nasal septum to the lateral wall of the nasal cavity using a modified speculum making the operative field up to 30 mm. In their cases, an extensive surgical corridor was provided via posterior ethmoidectomy, and in some cases by resection of the middle turbinate and posterior maxillary sinus wall. The authors reported that the narrow and deep operating field, which poses the risk of ICA injury during a blind procedure, is a major limitation of the microscopic extended transsphenoidal approach. They also noted that endoscopic procedures to cavernous sinus lesions might not be a standard procedure because of the difficulty in controlling unexpected massive hemorrhages from the venous plexus or ICA. In our series, hemorrhaging in three patients was controlled with clip placement (silver surgiclip) for intercavernous venous bleeding, hemostatic agents and continual irrigation were used for cavernous sinus bleeding. When these events occurred, surgery was continued; we do not consider such hemorrhagic events to contraindicate the use of the endoscopic transsphenoidal approach.

Frank and Pasquini (2006) performed the middle meatal and ethmoid–pterygosphenoid approaches. They described radical tumor removal via an endoscopic endonasal transsphenoidal approach in 21 of 35 patients with nonfunctioning adenomas, and hormonal remission in 13 of 30 patients with functioning adenomas. However, it is advisable for these techniques to be performed by otolaryngologists in endoscopic sinus surgery or by neurosurgeons together with otolaryngologists in the nasal step of cavernous sinus surgery. In our department, such approaches were performed without the help of otolaryngologists after the first 40 cases (Koc et al. 2006). A median approach to wide resection may be preferred over the middle meatal approach in adenomas involving the cavernous sinus.

The use of a speculum in the transsphenoidal approach results in a narrow surgical field, but the purely endoscopic approach we used without a speculum provides a wide, panoramic visualization that allows the surgeon room to work

freehand in the cavernous sinus. This approach also offers advantages for recurrent cases in which mucosal dissection was not used. Peripheral vision achievable with the endoscope has led to the development of surgical approaches that allow adequate exposure of the cavernous sinus with a reduction in surgical morbidity. Over the past decade, the use of endoscopy in transsphenoidal surgery has allowed widening of the surgical field, bringing it within the cavernous sinus.

Choice of Treatment Strategy

Primary treatment is resection in nonfunctional macroadenomas with visual deterioration, adenomas causing acromegaly, and Cushing disease. Remission has been achieved in ~60% of patients with acromegaly who underwent transsphenoidal surgery. If remission is not achieved after surgery, both medical treatment and stereotactic radiosurgery can be recommended. Medical treatment is never curative and requires long-term administration, making it costly (Kitano et al. 2008). Stereotactic radiosurgical success in hormone-secreting pituitary adenomas has been reported in ~80% of cases; however, the success rate is lower in pituitary adenomas with cavernous infiltration compared with noninfiltrating lesions, both in terms of volume reduction and biological remission rates (Pamir et al. 2007).

Endoscopic techniques allow easy approach to the medial and inferior walls of the cavernous sinus, and total excision can be reached in about 65% of cases. In hormone-secreting adenomas, remission can be reached in ~57–75% of cases, in 57% of GH-secreting adenomas, in 75% of prolactinomas, and in 75% of ACTH-secreting adenomas; thus, resection should be the first choice of treatment (Ceylan et al. 2010). However, when total resection is unsuccessful or total remission is not achieved, GKS should be added. In GH-secreting adenomas, combined medical treatment should be used during initiation of radiosurgery. Combined medical and stereotactic radiosurgery treatment should be the first choice in elderly and high-risk patients.

Extended Approaches

Extended endoscopic transsphenoidal surgical techniques were described by several authors in the literature (Frank et al. 2006a, b; Kassam et al. 2005). Endoscopic extended approaches can be used for midline skull base lesions from lamina cribrosa to foramen magnum. Also these approaches can be used in macro and giant adenomas demonstrating supra, infra and parasellar invasion (Fig. 23.4).

Extended Approach Is Performed for 25 Cases with Adenomas in Our Series

Surgical approaches were performed through both nostrils in all cases. After removing the middle turbinate of appropriate nasion (mostly preferred right nasion) the posterior nasal septum was removed. Mucosa of nasal septum was incised to form a flap with its pedicle for closure. The middle turbinate of the contralateral nostril was pushed laterally providing binostril access (Ceylan et al. 2009). Extended approach was performed by two surgeons through both nostrils providing the surgeons to use two or three more surgical instruments together with the endoscope.

This provides the surgeons with a deep perspective and a closer view during the intradural maneuvers. Wider anterior sphenoidotomy than a standard sellar lesion have been performed to reach to the suprasellar area. In our cases, resection of anterior and posterior ethmoid cells, total or partial removal of middle turbinate with medial pterygoid process resection has been performed according to tumor localization and requirements. Surgical procedures have been continued according to the lesion localization.

Closure Methods

The most important problem in endoscopic approach is the CSF leakage. To avoid this various closure techniques are defined and performed. These include closure with standard techniques,

closure with expanded and extended techniques. Different materials and methods have been suggested for sella floor closure in a transsphenoidal approach. For microadenomas, reconstruction of the anterior sella was performed with autogenous bone. Bone pieces derived from the rostrum of sphenoid, middle turbinate and sphenoid septum provided bone lamella for sella repair. In macroadenomas, gelfoam (Upjohn, Kalamazoo, Mich.), but not fat graft, was used to obliterate the dead space. Sella floor repair was difficult in some macroadenomas with much eroded sella. If no CSF leak was seen intraoperatively, sellar floor repair was enforced with fibrin glue after repairing with bone.

Effective dural closure should be performed to avoid postoperative CSF fistula in extended approaches (Hadad et al. 2006; Snyderman et al. 2007) and in macroadenomas in which CSF leak occurred intraoperatively. In our cases, a multilayer technique has been used to close the suprasellar defect following the closure of the sella floor and posterior sphenoid sinus wall. After the removal of the lesion, a single layer of surgical and fat graft was positioned inside the residual cavity. A dural graft was covered over the fat graft and positioned in the intradural space and a bone graft was used considering the borders of bone which is the solid barrier between the intra- and extradural compartments. Reconstruction have been continued by multiple layers of dural grafts and by mucosal flap with its pedicle slipped from the original nasal septum. Surgical glues have been used to fill the sphenoid cavity and hold the repair in place. A Fogarty catheter (12–14 French) was inflated in the posterior nasal cavity just in front of the opened sphenoid sinus in cases where a flap with pedicle was used.

Postoperative Evaluation

All patients with adenomas were evaluated with dynamic contrast-enhanced MRI as well conventional imaging of the sella postoperatively on the first day (up to 24 h), and on the 3rd and 6th months to examine the remnant or recurrence. Absence of the tumor on postoperative MRI has been evaluated as total resection, while more than 80% resection of the tumor as subtotal resection on the 3rd month postoperatively. Postoperative ophthalmological examination for visual acuity and visual field were performed in the first month.

For secreting adenomas, the results of neuroimaging were correlated by the specific blood assays, to show if a biochemical cure has also been achieved. Furthermore, after incomplete removal, control of the disease was sometimes obtained by using adjuvant pharmacological or other therapies.

Endocrinological remission criteria should be evaluated by comparing preoperative values with early and late (3 months) postoperative values. Tumor remission for endocrine-active pituitary adenomas was defined at prolactin levels below or within the normal range (3–15 ng/ml for men and 3.9–27.7 ng/ml for women), and as freedom from clinical symptoms in patients with prolactin-secreting adenomas (Kristof et al. 2002).

For GH-secreting adenomas, remission was defined as a suppressed serum GH < 1 ng/ml after oral glucose load, and subsequent normal sex- and age-adjusted insulin-like growth factor 1 levels (Kristof et al. 2002). In ACTH-secreting adenomas, remission was defined at a normal circadian rhythm of plasma cortisol levels, normal 24 h urinary cortisol levels, and serum cortisol values of 2 μg/dl after an overnight 2 mg dexamethasone-suppressiontest.Ophthalmological examination can be performed using the Humphrey perimeter for visual field function and the Hess chart evaluation for ocular motility.

Complications

The main complications are related to the hemorrhage control of intracranial vessels and to the closure of the dural and bony defects, with increased risk of postoperative cerebrospinal fluid (CSF) leak, tensive pneumocephalus, and/or meningitis. Postoperative hemorrhage and sphenoid sinusitis are rarely seen complications of endoscopic transphenoidal approach. Postoperative endocrinologic complications included; transient/permanent diabetes insipidus, anterior pituitary insufficiency.

Cranial nerve palsies are one of the complications related to cavernous sinus approaches. Also, ICA injuries for expanded approaches and anterior cerebral artery complex injury for extended approaches are dramatic complications of these approaches. CSF leakage is one of the main problems in extended approaches. Although free grafts or flaps with pedicle have been used for closure, CSF fistula rate is still high in the literature. Usage of free fascia lata graft and mucosal flaps with pedicle for clival lesions and application of lumbar drainage for 5–7 days can be an important advance in decreasing CSF leakage in extended approaches.

Electrophysiological detection of oculomotor, trochlear, and abducent nerves using intraoperative monitoring (Nim-Plus, Xomed) should be performed for cavernous sinus approaches to avoid postoperative cranial nerve palsies.

The CNs stimulation with a monopolar stimulator electrode and eye movements evoked by the stimulation were monitored with an auditory signal. Cavernous sinus bleeding during the procedure can be controlled by using surgical and irrigation. In case of intercavernous venous bleeding, silver surgiclips can be used to control the hemorrhage. Carotid artery injury is the most dramatic complication of endoscopic transphenoidal approach. One case with residual adenoma referred to our department due to massive hemorrhage through the course of the surgical procedure. We defined carotid injury during the surgical procedure. We controlled carotid hemorrhage by two experienced surgeons and removed the residual adenoma. Endovascular intervention was performed following the surgical procedure.

Discussion

Endoscopic Transsphenoidal approach is a newer technique than microscopic transsphenoidal approach. The information in the literature about endoscopic technique is limited in terms of both number of the cases and published series (Cappabianca et al. 2004; Frank et al. 2006b; Kassam et al. 2005).

Endoscopic technique has disadvantages and advantages by comparison with microscopic technique. The main disadvantages of endoscopic pituitary surgery can be brought under four groups; learning curve, requiring two experienced surgeon, bidimensional view and insufficient variety of microinstruments. In endoscopic transsphenoidal surgery, the learning curve and the lack of standard training programs are important disadvantages for the neurosurgeon who is already well trained in microscopic surgery. One surgeon alone can continue the procedure in microsurgical technique, while endoscopic technique requires two surgeons with experience in the use of the endoscope in standard transsphenoidal surgery.

The endoscopic system gives computer-processed bidimensional image on the video monitor, which is a problem for depth perspective; besides, binocular stereoscopic vision of the operating microscopes is superior to endoscopic systems. Endoscope lens produce images with maximal magnification at its center and contraction at its periphery, and the nearest images are disproportionally enlarged, while remote images are falsely miniaturized. This limit can be overcome with continuous in-and-out movements of the endoscope. Handling dedicated endoscopic instruments and the associated different and variably angled tips is challenging when working in the tight corners that the angled endoscopes are designed to expose.

The most important advantage of endoscopic transphenoidal approach is to allow reaching the lesion without brain retraction and with minimal neurovascular manipulation. The majority of microscopic procedures are performed via a sublabial or transseptal route with dissection of the mucosa to expose the anterior face of the sphenoid. If endoscopic endonasal approach is used, nasal packing is not required. This helps to minimize patients' breathing difficulties, headache, and dryness of the oral mucosa and is associated with excellent patient compliance (Dusick et al. 2005).

Endoscopic transsphenoidal approach for anteroinferior portion of the cavernous sinus could be useful for adenoma extending into cavernous sinus, decreasing morbidity and mortality

during surgery in this region. The ideal lesions for endoscopic endonasal approaches are soft tumors such as pituitary adenomas, not suitable for hard tumors such as meningiomas. The advantage of the endoscopic endonasal cavernous sinus approach to the medial wall is that there is no need for dissection of the cranial nerves, which may reduce the incidence of postoperative cranial nerve palsy (Cavallo et al. 2005; Frank et al. 2006b).

Endoscopic extended approaches are mainly for reaching the area from lamina cribrosa to the cranio-cervical junction and require a wider surgical corridor to expose in the different sellar areas (Frank et al. 2006a; Dusick et al. 2005). A binostril approach provides not only a wide surgical area but also for hand maneuverability and allows usage of two or three instruments together through both nostrils. Some pitfalls of extended endoscopic transsphenoidal surgery can be described considering our experience (Ceylan et al. 2009): Wide resection of middle/suprema turbinate and anterior and posterior ethmoid cells can cause lamina cribrosa injury and therefore CSF leakage. Wide medial pterygoid resection extending to vidian canal in lesions with clival extension can cause vidian nerve and anterior genu of petrous carotid artery injury.

The nasal speculum is essential in all types of microscopic approaches, and this causes restriction of the microinstruments usage in the limited area created by the speculum. Sometimes, wide spreading of the transsphenoidal retractor at the anterior wall of the sphenoid sinus can carry some risks of optic nerve damage, facial swelling, and nasal pain (de Divitiis et al. 2007). These risks can be increased in the extended microsurgical approaches, because it requires a wider exposure compared with the standard approach.

The nasal speculum is not required in endoscopic approach. Using Endoscope for transsphenoidal surgery improves postoperative comfort and recovery time as a result of its minimally invasive characteristics. Also endoscopic technique allows better capsular dissection than microscopic technique.

In conclusion, the endoscopic transsphenoidal approach has been reported in the literature as a useful tool to treat sellar and parasellar lesions. Improved visualization with 0° and angled endoscopes allow the surgeon to identify anatomic landmarks, carotid prominence, optic chiasm, etc. The endoscope permits a panoramic view instead of the narrow microscopic view, and it allows the inspection and removal of the lesions of sellar, parasellar, and suprasellar compartments by angled-lens endoscopes.

References

Alfieri A, Jho HD (2001) Endoscopic endonasal approaches to the cavernous sinus: surgical approaches. Neurosurgery 49:354–360

Cappabianca P, Cavallo LM, Colao A, de Divitiis E (2002) Surgical complications associated with the endoscopic endonasal transsphenoidal approach for pituitary adenomas. J Neurosurg 97:293–298

Cappabianca P, Cavallo LM, de Divitiis E (2004) Endoscopic endonasal transsphenoidal surgery. Neurosurgery 55:933–941

Cavallo LM, Cappabianca P, Galzio R, Iaconetta G, de Divitiis E, Tschabitscher M (2005) Endoscopic transnasal approach to the cavernous sinus versus transcranial route: anatomic study. Neurosurgery 56:379–389

Ceylan S, Koc K, Anik I (2009) Extended endoscopic approaches for midline skull-base lesions. Neurosurg Rev 32(3):309–319

Ceylan S, Koc K, Anik I (2010) Endoscopic endonasal transsphenoidal approach for pituitary adenomas invading the cavernous sinus. J Neurosurg 112(1):99–107

Chi JG, Lee MH (1980) Anatomical observations of the development of the pituitary capsule. J Neurosurg 52:667–670

Ciric I, Ragin A, Baumgartner C, Pierce D (1997) Complications of transsphenoidal surgery: results of a national survey, review, of the literature, and personal experience. Neurosurgery 40:225–237

Couldwell WT, Sabit I, Weiss MH, Giannotta SL, Rice D (1997) Transmaxillary approach to the anterior cavernous sinus: a microanatomic study. Neurosurgery 40:1307–1311

Das K, Spencer W, Nwagwu CI, Schaeffer S, Wenk E, Weiss MH, Couldwell WT (2001) Approaches to the sellar and parasellar region: anatomic comparison of endonasal-transsphenoidal, sublabialtranssphenoidal, and transethmoidal approaches. Neurol Res 23:51–54

de Divitiis E, Cappabianca P, Cavallo LM, Esposito F, de Divitiis O, Messina A (2007) Extended endoscopic transsphenoidal approach for extrasellar craniopharyngiomas. Neurosurgery 61:219–227

Dolenc VV (1989) Relation of the cavernous sinus to the sella. In: Dolenc VV (ed) Anatomy and surgery of the cavernous sinus. Springer, Vienna, pp 118–130

Dusick JR, Esposito F, Kelly DF, Cohan P, DeSalles A, Becker DP, Martin NA (2005) The extended direct endonasal transsphenoidal approach for nonadenomatous suprasellar tumors. J Neurosurg 102:832–841

Frank G, Pasquini E (2006) Endoscopic endonasal cavernous sinus surgery, with special reference to pituitary adenomas. Front Horm Res 34:64–82

Frank G, Pasquini E, Doglietto F, Mazzatenta D, Sciarretta V, Farneti G, Calbucci F (2006a) The endoscopic extended transsphenoidal approach for craniopharyngiomas. Neurosurgery 59:ONS75–ONS83

Frank G, Pasquini E, Farneti G, Mazzatenta D, Sciarretta V, Grasso V, Faustini FM (2006b) The endoscopic versus the traditional approach in pituitary surgery. Neuroendocrinology 83:240–248

Guiot G, Rougerie J, Fourestler A, Fournier A, Comoy C, Vulmiere J, Groux R (1963) Une nouvelle technique endoscopique. Exploration endoscopiques intracraniennes. La Presse Medicale 71:1225–1228

Hadad G, Bassagasteguy L, Carrau RL, Mataza JC, Kassam A, Snyderman CH, Mintz A (2006) A novel reconstructive technique after endoscopic expanded endonasal approaches: vascular pedicle nasoseptal flap. Laryngoscope 116:1882–1886

Jankowski R, Auque J, Simon C, Marchal JC, Hepner H, Wayoff M (1992) Endoscopic pituitary tumor surgery. Laryngoscope 102:198–202

Jho HD (2001) Endoscopic transsphenoidal surgery. J Neuro Oncol 54:187–195

Jho HD, Carrau RL (1997) Endoscopic endonasal transsphenoidal surgery: experience with 50 patients. J Neurosurg 87:44–51

Jho HD, Ha HG (2004) Endoscopic endonasal skull base surgery: part 3—the clivus and posterior fossa. Minim Invasive Neurosurg 47:16–23

Kassam A, Snyderman CH, Mintz A, Gardner P, Carrau RL (2005) Expanded endonasal approach: the rostrocaudal axis. Part I. Crista galli to the sella turcica. Neurosurg Focus 19(1):E3

Kitano M, Taneda M, Shimono T, Nakao Y (2008) Extended transsphenoidal approach for surgical management of pituitary adenomas invading the cavernous sinus. J Neurosurg 108:26–36

Knosp E, Steiner E, Kitz K, Matula C (1993) Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery 33:610–618

Koc K, Anik I, Ozdamar D, Cabuk B, Keskin G, Ceylan S (2006) The learning curve in endoscopic pituitary surgery and our experience. Neurosurg Rev 29:298–305

Kristof RA, Schramm J, Redel L, Neuloh G, Wichers M, Klingmuller D (2002) Endocrinological outcome following first time transsphenoidal surgery for GH-, ACTH-, and PRL-secreting pituitary adenomas. Acta Neurochir 144:555–561

Lee EJ, Ahn JY, Noh T, Kim SH, Kim TS, Kim SH (2009) Tumor tissue identification in the pseudocapsule of pituitary adenoma: should the pseudocapsule be removed for total resection of pituitary adenoma? Neurosurgery 64(3 Suppl):62–69

Oldfield EH, Vortmeyer AO (2006) Development of a histological pseudocapsule and its use as a surgical capsule in the excision of pituitary tumors. J Neurosurg 104(1):7–19

Pamir MN, Kiliç T, Ozek MM, Ozduman K, Ture U (2006) Nonmeningeal tumours of the cavernous sinus: a surgical analysis. J Clin Neurosci 13:626–635

Pamir MN, Kiliç T, Belirgen M, Abacioğlu U, Karabekiroğlu N (2007) Pituitary adenomas treated with gamma knife radiosurgery: volumetric analysis of 100 cases with minimum 3 year follow-up. Neurosurgery 61:270–280

Peker S, Kurtkaya-Yapicier O, Kiliç T, Pamir MN (2005) Microsurgical anatomy of the lateral walls of the pituitary fossa. Acta Neurochir (Wien) 147(6):641–648

Snyderman CH, Kassam AB, Carrau R, Mintz A (2007) Endoscopic reconstruction of cranial base defects following endonasal skull base surgery. Skull Base 17:73–78

Songtao Q, Yuntao L, Jun P, Chuanping H, Xiaofeng S (2009) Membranous layers of the pituitary gland: histological anatomic study and related clinical issues. Neurosurgery 64(3 Suppl):1–9

Sonnenburg RE, White D, Ewend MG, Senior B (2004) The learning curve in minimally invasive pituitary surgery. Am J Rhinol 18(4):259–263

Weiss MH (1987) Transnasal transsphenoidal approach. In: Apuzzo MLJ (ed) Surgery of the third ventricle. Williams & Wilkins, Baltimore, pp 476–494

Yasuda A, Campero A, Martins C, Rhoton AL Jr, Ribas GC (2004) The medial wall of the cavernous sinus: microsurgical anatomy. Neurosurgery 55:179–190

Yokoyama S, Hirano H, Moroki K, Goto M, Imamura S, Kuratsu JI (2001) Are nonfunctioning pituitary adenomas extending into the cavernous sinus aggressive and/or invasive? Neurosurgery 49:857–863

Pituitary Adenoma Patients: Hypofractionated Cyberknife Radiosurgery (Method)

24

M. Yashar S. Kalani, Andrew S. Little, David G. Brachman, and William L. White

Contents

Abstract

Pituitary adenomas are common brain tumors, comprising nearly 10% of all intracranial lesions. Although classically treated with transsphenoidal surgery, other methods of treatment that minimize damage to eloquent tissue need to be used as adjuncts to surgery when the tumor encases vital structures, such as the optic nerve and carotid arteries. Over the past 10 years, the literature increasingly has supported the efficacy and safety of fractionated radiosurgery for the treatment of patients with pituitary adenomas. In this chapter we review this literature and describe the method used at our institution for the treatment of pituitary adenomas.

Introduction

Pituitary adenomas are common brain tumors, comprising nearly 10% of intracranial lesions treated at medical centers across the country (Couldwell et al. 2004; Daly et al. 2007; Laws and Jane 2001). Asymptomatic small pituitary tumors are even more prevalent and are estimated to represent 20% of all intracranial lesions (Daly et al. 2007). Patients with pituitary adenomas frequently present with endocrine abnormalities and visual disturbances. Surgery has classically produced excellent results, with long-term control rates of 50–80% while allowing for pathological

M.Y.S. Kalani • A.S. Little • D.G. Brachman • W.L. White (✉)
Division of Neurological Surgery, Barrow Neurological Institute, Saint Joseph's Hospital and Medical Center, Phoenix, AZ, USA
e-mail: neuropub@chw.edu

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_24,

confirmation, rapid reduction of hormone oversecretion and decompression of the optic apparatus (Couldwell et al. 2004). In cases where the adenoma encases critical structures, however, gross total resection of the tumor is not always possible and remnant tumor can serve as a seed for recurrence.

An increasing body of literature now supports the efficacy and safety of fractionated radiosurgery for the treatment of patients with pituitary adenomas (Sheehan et al. 2005). Herein we review the indications and data on the use of different radiation modalities for the treatment of pituitary adenomas and introduce the method used at our institution for the treatment of pituitary adenomas with the CyberKnife radiosurgery suite (Accuray, Inc., Sunnyvale, California).

Radiation Therapy for Pituitary Adenomas

External Beam Radiation. The earliest experience with the use of radiation for treatment of pituitary adenomas dates back a century (Beclere 1909). In the modern era, the use of external beam radiation therapy (EBRT) delivered at doses of 45–54 Gy in 1.8 Gy/d fractions (25–30 treatments over 5–6 weeks) with either a three-dimensional conformal or intensity modulated radiation therapy (IMRT) approach, EBRT provides excellent tumor control and acceptable endocrine results (Breen et al. 1998; Estrada et al. 1997; Grigsby et al. 1989). In the literature tumor control rates with EBRT have ranged from 76 to 97%, and the rate of improvement in endocrine control in secretory lesions has ranged from 38 to 70% at 10 years and 5 years after treatment, respectively (Flickinger et al. 1989; Rush and Cooper 1997; Tsang et al. 1994; Zierhut et al. 1995). Despite its promise for tumor control and improvements in endocrine function, this method has many disadvantages, including panhypopituitarism, the need for multiple daily treatments, slow tumor regression rates for macroadenomas, and a small but non-trivial risk of secondary tumors in patients with long-term follow-up (Breen et al. 1998; Estrada et al. 1997; Grigsby et al. 1989). Visual loss is a rare but reported complication, and is almost always limited to those patients receiving daily doses above 1.8 Gy/day and/or total doses greater than 54 Gy (Parsons et al. 1994, 1996). Another disadvantage is that in patients with functional tumors the median time to hormonal normalization is in excess of 5 years (Landolt and Lomax 2000).

Gamma Knife Radiosurgery. The shortcomings of EBRT and the advent of stereotactic radiosurgery (SRS) paved the way for alternative radiation-based approaches for the treatment of pituitary adenomas. The next modality to find common use was Gamma Knife radiosurgery (GKRS)(Castinetti et al. 2010). The tumor control rate for pituitary adenomas after GKRS has ranged from 90 to 94% with 5 years of follow up. The rate of improvement of endocrinopathies associated with GKRS has ranged from 77.7 to 93%, and the normalization rates have ranged between 21 and 52.4% (Castinetti et al. 2010; Cho et al. 2009). Complication rates for GKRS have ranged from 0 to 12.6%; visual loss is the most common complication (Castinetti et al. 2010; Cho et al. 2009; Landolt et al. 2000). Despite the compelling results reported by retrospective studies, the radiosensitive nature of the optic apparatus provides challenges for the treatment of pituitary adenomas with radiosurgery.

CyberKnife Radiosurgery. In the early 1990s, the introduction of image-guided frameless robotic radiosurgery incorporated in the CyberKnife (Accuray, Inc., Sunnyvale, CA) made it possible to deliver multiple sessions of highly conformal radiation to lesions with an application accuracy that equaled that of conventional stereotactic frames (Romanelli et al. 2006). The combination of relative accuracy, conformality, and homogeneity made the CyberKnife an excellent option for staged radiosurgery for the safe treatment of large lesions and tumors that are adjacent to radiation-sensitive structures, such as the optic apparatus.

Several studies have investigated the use of CyberKnife radiosurgery (CKRS) for the treatment of pituitary adenomas. An early study from Stanford University established the safety and efficacy of using CKRS for the treatment of

pituitary adenomas with 20 Gy of radiation over two to five sessions (Pham et al. 2004). In follow-up studies, the Stanford group reported 19 pituitary adenomas situated within 2 mm of a "short segment" of the optic apparatus and treated with CKRS in two to five sessions. The average tumor volume was 7.7 cm^3. Doses as high as 8 Gy per fraction were administered, and the cumulative average marginal dose was 20.3 Gy. After radiosurgery vision was unchanged in their patients (Adler et al. 2006). The mean follow-up was 49 months (range 6–96 months).

Kajiwara et al. (2005) reported 21 patients with pituitary adenomas treated with CKRS. Fourteen patients had adenomas, three had prolactinomas, two had acromegaly, and two had adrenocorticotrophic hormone-producing tumors. The marginal irradiation dose ranged from 6.4 to 27.7 Gy (mean nonfunctioning adenomas 12.6 Gy, mean functioning adenomas 17.5 Gy), with a single fraction. At a mean follow-up of 35.3 months (range 18–59 months), the tumor control rate was 95.2%. In one patient, visual acuity worsened due to cystic enlargement of the tumor. Hormonal function improved in the seven patients with functioning adenomas.

The Stanford group recently reported their results with the use of CKRS for the treatment of Cushing's disease (Roberts et al. 2007). The treatment protocol in this study was similar to that used in other studies from Stanford. A total dose of 20 Gy of radiation was delivered over two to five sessions. After a mean follow up of 25.4 months (range 6–53 months), CKRS resulted in complete biochemical remission in 4 (44.4%) patients and in biochemical control with the concomitant use of a somatostatin analog in an additional patient. At last follow-up, one new case of panhypopituitarism and two cases of hypogonadism were observed after CKRS in 3 (33%) patients.

A more recent Korean study summarized the results of CKRS treatment in 26 patients (17 with nonfunctioning adenomas, 3 with prolactinomas, and 6 with acromegaly) with pituitary adenomas (Cho et al. 2009). A dose of 14–24 Gy was delivered over one to three sessions. The mean follow-up was 30 months (range 7–47 months). The tumor control rate was 92.3%. Hormonal function improved in all nine (100%) patients with functioning adenomas. Hormonal normalization was observed in four of the nine (44%) patients over a mean duration of 16 months. In two patients (7.6%), visual acuity worsened due to cystic enlargement of their tumor after treatment with CKRS.

We recently reported our results with the use of CKRS for the treatment of pituitary adenomas (Killory et al. 2009). Nineteen patients received a dose of 25 Gy divided over five consecutive fractions. The mean tumor coverage with the full-prescribed dose was 97 ± 2.2% (range 89.8–99.7%), with an average conformity index of 1.3 ± 0.2 (range 1.1–1.9) to an average gross tumor volume of 17.5 ± 12.6 cm^3 (range 2.3–42.3 cm^3). The mean maximal dose to the tumor per fraction was 6.8 Gy (range 5.8–8.3 Gy) and was 4.6 Gy (range 3.7–5.0 Gy) to the optic chiasm, with an average minimal dose of 4.2 Gy per fraction (range 2.6–5.0 Gy per fraction).

At a mean follow-up of 26.6 months (range 10.5–41 months), 14 of 14 patients with intact vision pre-treatment still had intact vision. Among the five patients with impaired vision pre-treatment, all were either stable (two of five patients) or had improved (three of five patients) at followup. Radiographic follow-up with gadolinium-enhanced magnetic resonance imaging (MRI) showed that no tumors increased, 8 had decreased, and 12 had remained unchanged. Among the eight patients who had no pituitary deficits before CKRS, only one developed a new endocrinopathy after a mean follow-up of 25.2 months (range 16.2–37.7 months). In cases of functional tumors (four acromegalic tumors and one prolactinoma), levels of the insulin-like growth factor 1 (IGF-1) in two of the acromegalic tumors normalized at 4 and 23.5 months, respectively. They remained normal at 30.6 and 37.7 months, respectively. The other two acromegalic patients had significantly reduced levels of IGF-1 23.5 and 24.2 months after CKRS, respectively. In our series the single patient with a prolactinoma remains on cabergoline therapy at 41 months. One patient developed transient diplopia 3 months after CKRS, but it resolved after a short course of dexamethasone.

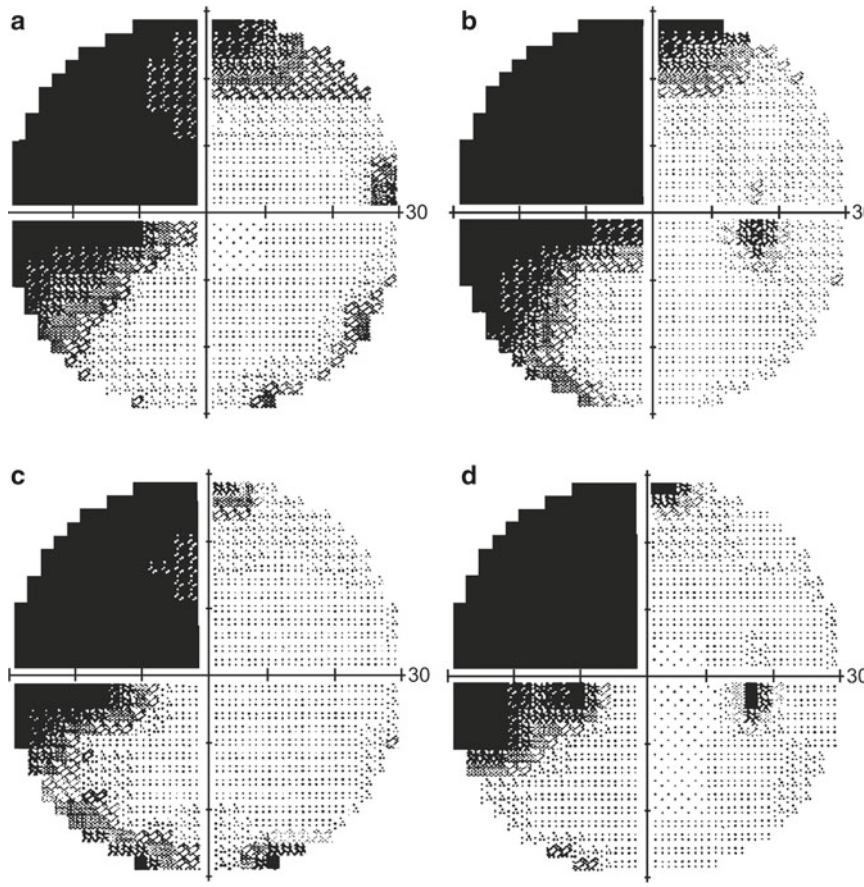

Fig. 24.1 Representative visual field test showing no deterioration of the visual fields with CKRS treatment. (**a**) *Left eye* and (**b**) *right eye* before CKRS treatment. (**c**) *Left eye* and (**d**) *right eye* 10 months after CKRS treatment (*Used with permission from Barrow Neurological Institute*)

Cyberknife Method for Pituitary Adenomas

For the radiosurgical treatment of pituitary adenomas, the current recommendations suggest single-fraction stereotactic radiosurgery at doses of 15–18 Gy for nonfunctioning adenomas and of as much as 30 Gy for functioning adenomas (Pan et al. 2000; Zhang et al. 2000). We prefer CyberKnife treatment for lesions of moderate size or those which are intimately associated with the optic apparatus, as a fractionated (5 Gy × 5 fractions) approach has led to no visual loss and is associated with good endocrine outcomes in our series (Killory et al. 2009). Our current practice is to treat adenomas that lie at least 2–3 mm from the anterior optic pathway with single-fraction stereotactic radiosurgery (usually Gamma Knife) at the recommended single dose of 15–25 Gy, depending on the functional status of the tumor. For these lesions we prefer Gamma Knife treatment because of the convenience of same-day image acquisition, planning, and treatment. The existing literature suggests that lesions within 2 mm of the optic nerves or chiasm are usually treated with a fractionated protocol (CyberKnife) ranging between 15 and 20 Gy of radiation over two to five sessions of radiosurgery (Adler et al. 2006; Kajiwara et al. 2005; Killory et al. 2009; Pham et al. 2004). Lesions described as "very large" or "massive" despite debulking are still treated with fractionated EBXRT to doses of 50.4–54 Gy over 5–6 weeks. Our current protocol for perioptic lesions is described below.

All patients receive a comprehensive pretreatment evaluation, including formal visual field testing (Fig. 24.1a, b) with an ophthalmologist,

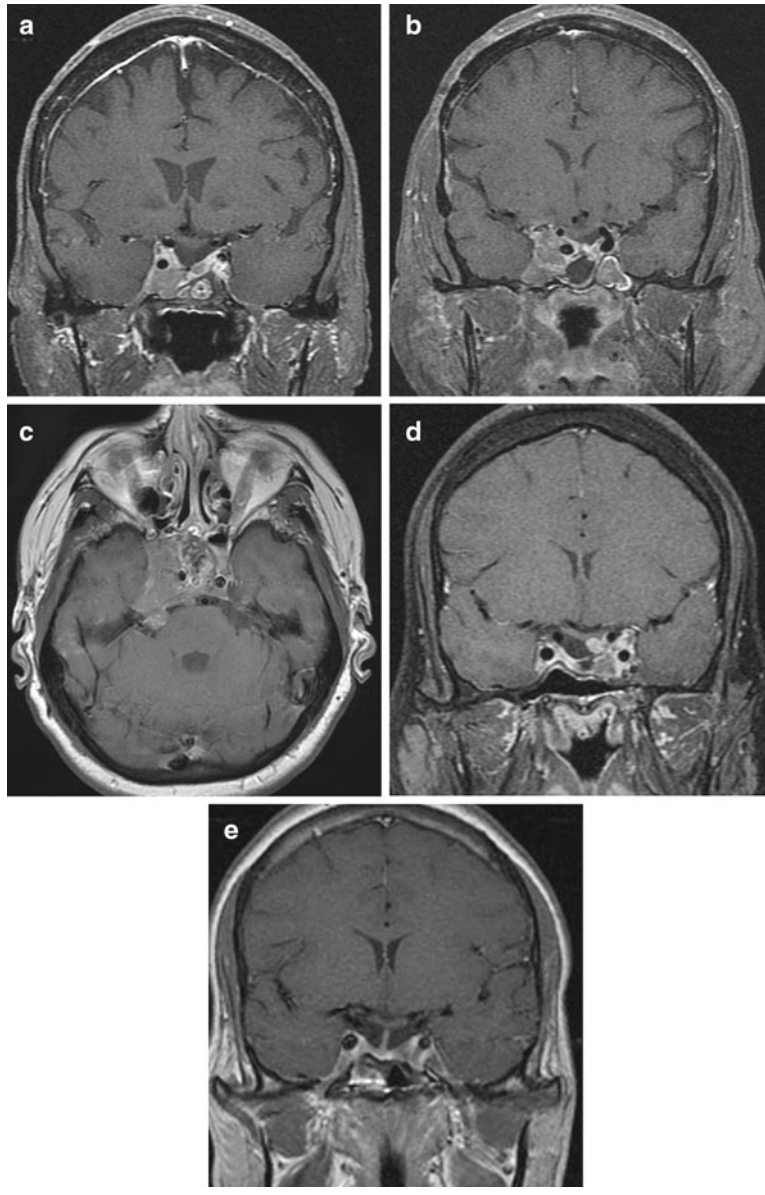

Fig. 24.2 (**a** and **b**) Coronal and (**c**) axial gadolinium-enhanced MRIs showing an adenoma with an extension into the right cavernous sinus abutting Meckel's cave, the optic chiasm, and posterior fossa. Coronal gadolinium-enhanced MRIs obtained (**d**) before and (**e**) after CKRS treatment, showing an overall decrease in the size of an adenoma involving the left cavernous sinus (*Used with permission from Barrow Neurological Institute*)

gadolinium-enhanced MRI (Fig. 24.2a–c), and an evaluation by our team (endocrinologist, neurosurgeon, and radiation oncologist). Patients with secretory lesions who are on suppressive therapies (e.g., bromocriptine, somatostatin, etc.) are always taken off these agents with sufficient time before treatment to allow the tumor to return to an active mitotic state so as not to blunt or negate the effect of the radiation (Landolt et al. 1998). Treatment planning is performed on the Accuray MultiPlan treatment software using thin-slice, high-resolution, computed tomographic (CT) scans with 1.25 mm slice intervals and MRIs with 2- to 3-mm slice intervals obtained after intravenous administration of gadolinium. Next we construct a mask based on the CT scan

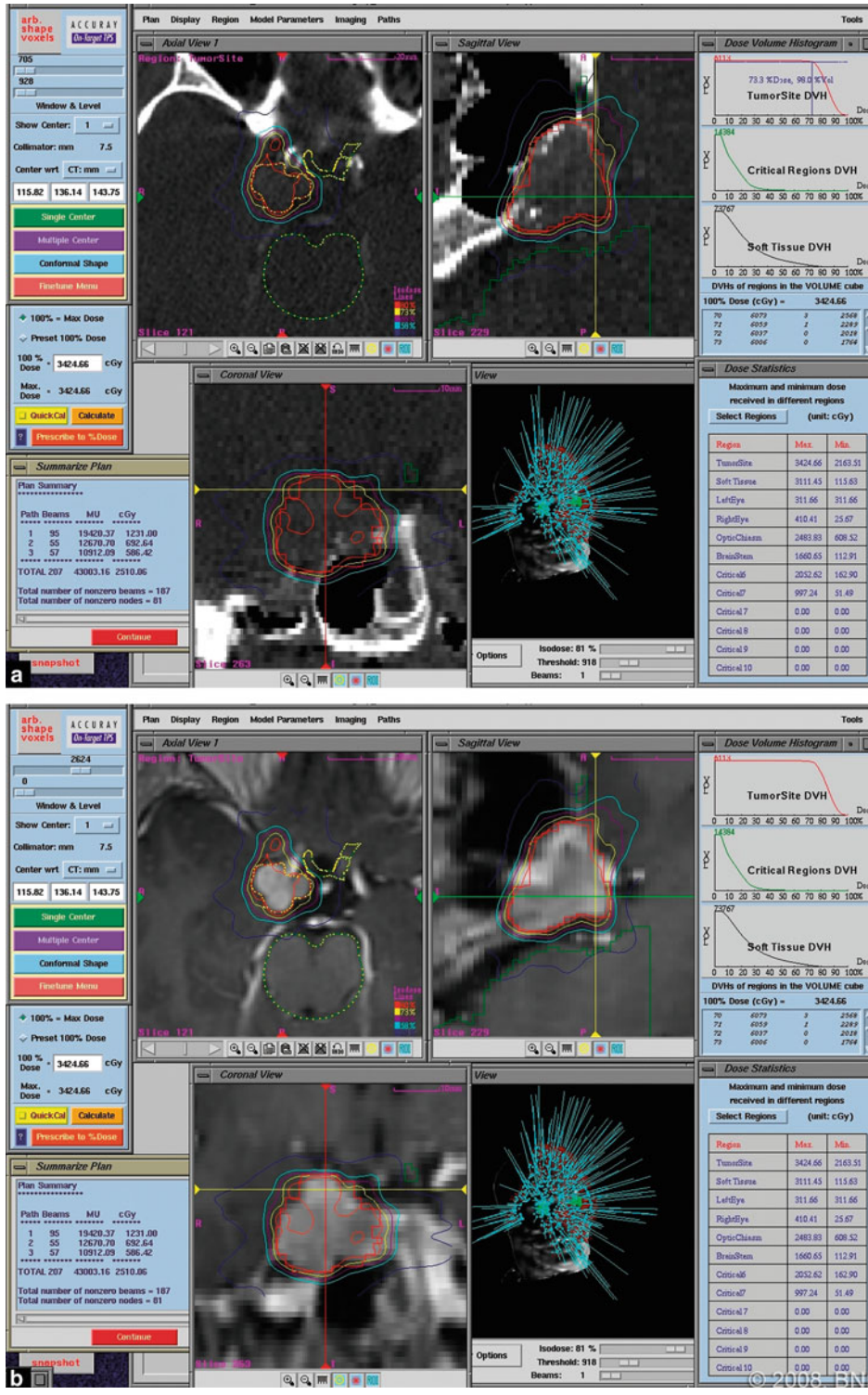

Fig. 24.3 Representative CKRS treatment plans showing solid isodose lines outlining the tumors and dotted outlines of critical structures, based on (**a**) CT scans and (**b**) gadolinium-enhanced MRIs (*Used with permission from Barrow Neurological Institute*)

data to properly position the patient. The Accuray MultiPlan software allows the user to fuse image sets to improve visualization of tumors and neural structures (Fig. 24.3).

Next, the treating surgeon manually outlines the lesion and critical structures, including the tumor, optic nerves, chiasm, brainstem, and eyes. The optic nerves are frequently difficult to identify entirely throughout their course. The position of the optic apparatus is estimated when necessary and drawn in even when not well seen. This structure is also reviewed on the coronal view to confirm that there is an appropriate three-dimensional outline. Our goal has been to limit the maximum optic nerve or chiasm exposure to 5 Gy per session with a total of 25 Gy to the optic apparatus. Tumor margins are treated with a 6 MV X-band photon source to a mean isodose line of 74.5 ± 6.6% (range 60–86%) with an average of 191 ± 57 beams (range 99–283 beams) with one or more collimators. All patients are treated on consecutive weekdays until their radiosurgery is completed. After the completion of radiosurgery treatment, all patients undergo visual field testing (Fig. 24.1c, d) and then gadolinium-enhanced MRI (Fig. 24.2d–e) at 6 months and 1 year after treatment and annually thereafter. Endocrine follow-up is at the discretion of the patient's endocrinologist and can vary on the basis of the patient's pretreatment endocrine status and the functional status of the adenoma.

In conclusion, our experience suggests that this dosing paradigm achieves satisfactory radiographic and endocrinological tumor control for these challenging lesions while remaining below the threshold of radiation damage for the optic apparatus.

References

Adler JR Jr, Gibbs IC, Puataweepong P, Chang SD (2006) Visual field preservation after multisession Cyberknife radiosurgery for perioptic lesions. Neurosurgery 59:244–254

Beclere J (1909) The radiotherapeutic treatment of tumors of the hypophysis, gigantism and acromegaly. Arch Roentgen Radiol III:114

Breen P, Flickinger JC, Kondziolka D, Martinez AJ (1998) Radiotherapy for nonfunctional pituitary adenoma: analysis of long-term tumor control. J Neurosurg 89:933–938

Castinetti F, Regis J, Dufour H, Brue T (2010) Role of stereotactic radiosurgery in the management of pituitary adenomas. Nat Rev Endocrinol 6:214–223

Cho CB, Park HK, Joo WI, Chough CK, Lee KJ, Rha HK (2009) Stereotactic radiosurgery with the CyberKnife for pituitary adenomas. J Korean Neurosurg Soc 45:157–163

Couldwell WT, Weiss MH, Laws ER Jr (2004) Prolactinomas. N Engl J Med 350:1054–1057

Daly AF, Burlacu MC, Livadariu E, Beckers A (2007) The epidemiology and management of pituitary incidentalomas. Horm Res 68(Suppl 5):195–198

Estrada J, Boronat M, Mielgo M, Magallon R, Millan I, Diez S et al (1997) The long-term outcome of pituitary irradiation after unsuccessful transsphenoidal surgery in Cushing's disease. N Engl J Med 336:172–177

Flickinger JC, Nelson PB, Martinez AJ, Deutsch M, Taylor F (1989) Radiotherapy of nonfunctional adenomas of the pituitary gland. Results with long-term follow-up. Cancer 63:2409–2414

Grigsby PW, Simpson JR, Fineberg B (1989) Late regrowth of pituitary adenomas after irradiation and/or surgery. Hazard function analysis. Cancer 63:1308–1312

Kajiwara K, Saito K, Yoshikawa K, Kato S, Akimura T, Nomura S, Ishihara H, Suzuki M (2005) Image-guided stereotactic radiosurgery with the CyberKnife for pituitary adenomas. Minim Invasive Neurosurg 48:91–96

Killory BD, Kresl JJ, Wait SD, Ponce FA, Porter R, White WL (2009) Hypofractionated CyberKnife radiosurgery for perichiasmatic pituitary adenomas: early results. Neurosurgery 64:A19–A25

Landolt AM, Lomax N (2000) Gamma knife radiosurgery for prolactinomas. J Neurosurg 93(suppl 3):14–18

Landolt AM, Haller D, Lomax N, Scheib S, Schubiger O, Siegfried J, Wellis G (1998) Stereotactic radiosurgery for recurrent surgically treated acromegaly: comparison with fractionated radiotherapy. J Neurosurg 88:1002–1008

Landolt AM, Haller D, Lomax N, Scheib S, Schubiger O, Siegfried J, Wellis G (2000) Octreotide may act as a radioprotective agent in acromegaly. J Clin Endocrinol Metab 85:1287–1289

Laws ER, Jane JA Jr (2001) Pituitary tumors–long-term outcomes and expectations. Clin Neurosurg 48:306–319

Pan L, Zhang N, Wang EM, Wang BJ, Dai JZ, Cai PW (2000) Gamma knife radiosurgery as a primary treatment for prolactinomas. J Neurosurg 93(suppl 3):10–13

Parsons JT, Bova FJ, Fitzgerald CR, Mendenhall WM, Million RR (1994) Radiation optic neuropathy after megavoltage external-beam irradiation: analysis of time-dose factors. Int J Radiat Oncol Biol Phys 30:755–763

Parsons JT, Bova FJ, Mendenhall WM, Million RR, Fitzgerald CR (1996) Response of the normal eye to high dose radiotherapy. Oncology (Williston Park) 10:837–847

Pham CJ, Chang SD, Gibbs IC, Jones P, Heilbrun MP, Adler JR Jr (2004) Preliminary visual field preservation

after staged CyberKnife radiosurgery for perioptic lesions. Neurosurgery 54:799–810

Roberts BK, Ouyang DL, Lad SP, Chang SD, Harsh GR, Adler JR Jr, Soltys SG, Gibbs IC, Remedios L, Katznelson L (2007) Efficacy and safety of CyberKnife radiosurgery for acromegaly. Pituitary 10:19–25

Romanelli P, Schaal DW, Adler JR (2006) Image-guided radiosurgical ablation of intra- and extra-cranial lesions. Technol Cancer Res Treat 5:421–428

Rush S, Cooper PR (1997) Symptom resolution, tumor control, and side effects following postoperative radiotherapy for pituitary macroadenomas. Int J Radiat Oncol Biol Phys 37:1031–1034

Sheehan J, Steiner L, Laws ER (2005) Pituitary adenomas: is Gamma Knife radiosurgery safe? Nat Clin Pract Endocrinol Metab 1:2–3

Tsang RW, Brierley JD, Panzarella T, Gospodarowicz MK, Sutcliffe SB, Simpson WJ (1994) Radiation therapy for pituitary adenoma: treatment outcome and prognostic factors. Int J Radiat Oncol Biol Phys 30:557–565

Zhang N, Pan L, Dai J, Wang B, Wang E, Zhang W, Cai P (2000) Gamma Knife radiosurgery as a primary surgical treatment for hypersecreting pituitary adenomas. Stereotact Funct Neurosurg 75:123–128

Zierhut D, Flentje M, Adolph J, Erdmann J, Raue F, Wannenmacher M (1995) External radiotherapy of pituitary adenomas. Int J Radiat Oncol Biol Phys 33:307–314

25 Transsphenoidal/Transcranial Surgery of Pituitary Adenomas: Prognosis-Related Occurence for the Trigemino-Cardiac Reflex

Belachew Arasho, Toma Spiriev, Nora Sandu, Christoph Nöthen, Andreas Filis, Pooyan Sadr-Eshkevari, Hemanshu Prabhakar, Michael Buchfelder, and Bernhard Schaller

Contents

Abstract

This chapter summarizes the well-known trigemino-cardiac reflex (TCR) that was first introduced into skull base surgery by the senior author. We present here the prognostic factors of this brainstem reflex by the example of the transcranial/transsphenoidal operations for pituitary adenomas. A retrospective study of all patients operated on a pituitary adenoma were evaluated between 2000 and 2006. Building two subgroups with and without intraoperative occurrence of the TCR, there was seen no difference in the patients characteristics between these two subgroups. But we found a significant difference for loop diuretics and potassium-sparing diuretic favouring the TCR subgroup. In addition, there is also a significant difference for psychostimulans and morphine analogues. From the present point of knowledge it is not clear whether or not

B. Arasho
Department of Neurology, University of Addis Ababa, Addis Ababa, Ethiopia

T. Spiriev
Department of Neurosurgery, Tokuda Hospital, Sofia, Bulgaria

N. Sandu
Department of Neurosurgery, University of Lausanne, Lausanne, Switzerland

C. Nöthen • A. Filis • M. Buchfelder
Department of Neurosurgery, University of Erlangen, Erlangen, Germany

P. Sadr-Eshkevari
Farzan Clinical Rsearch Institute, Tehran, Iran

H. Prabhakar
Department of Neuroanaesthesiology, All India Institute of Medical Sciences, New Delhi, India

B. Schaller (✉)
Department of Neurosurgery, University of Oradea, Oradea, Romania
e-mail: bernhardjschaller@gmail.com

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_25,

the risk profile is at least dependent of the peripheral or central stimulation of the nerve and may therefore be changed for different skull base operations. So that the present risk factor are only valuable in operations for pituitary adenomas. The knowledge of the TCR is nowadays a MUST for every skull base surgeon and also for every physicians involved in the treatment of patients with skull base pathologies.

Introduction

Since the initial report of the trigemino-cardiac reflex (TCR) during tumor operations in the cerebellopontine angle in 1999 (Schaller et al. 1999), there have been many suggestions as to the exact neural mechanism. The TCR has also been reported to occur during skull base surgery in the region of the petrosal bone, of the falx as well as in microvascular decompression of the trigeminal nerve (Schaller 2005a, b). The physiological function of this phylogenetic old reflex has not yet been fully understood (Schaller 2005a, b). The only electrophysiological studies that exist are those of extramedullary recorded medullary neurons during electrical stimulation of the trigeminal ethmoidal nerve (Golanov and Reis 1996) and are thus of limited value for the TCR initiated by central stimulation. Therefore, there is only a general, somewhat simplified definition for TCR that is the sudden development of cardiac dysrhythmia up to asystole and arterial hypotension after central or peripheral stimulation of any division of the trigeminal nerve (Schaller 2004). Our initially introduced criteria of the occurrence of the TCR namely a simultaneous reduction of mean arterial blood pressure and heart rate of more than 20% of the baseline level after stimulation of any division of the trigeminal nerve is now generally accepted (Schaller et al. 1999). Some authors, however, have discussed that the accepted criteria may exclude cases of a TCR where the drop of blood pressure and heart rate was not as prominent (Bohluli et al. 2011). The anatomical basis of the TCR is the following: sensory nerve endings of the trigeminal nerve send neuronal signals via the Gasserian ganglion to the sensory nucleus of the trigeminal nerve, forming the afferent pathway of the reflex arc. This afferent pathway continues along the short internuncial nerve fibers in the reticular formation to connect with the efferent pathways in the motor nucleus of the vagus nerve. The vagus nerve provides parasympathetic innervations to the heart, vascular smooth muscles, and abdominal viscera. Vagal stimulation via these neural network after trigeminal nerve stimulation is thought to account for the reflexive response (Schaller et al. 2006; Bohluli et al. 2010).

The TCR underlines the essential role of the rostral medulla in the organization and integration of the cerebrovascular adjustment to hypoxia and/or cerebral ischemia (Schaller et al. 2006). It appears that, to a large extent, the cerebrovascular response to hypoxemia is due to this reflex and is generated by an activation of the neurons of the rostral ventrolateral reticular nucleus. This observation is supported by the fact that a stimulation of the rostral ventrolateral reticular nucleus, like by hypoxemia, causes elevated regional cerebral blood flow without changing the cerebral metabolism (Underwood et al. 1992; Golanov and Reis 1994) and that bilateral lesions of the rostral ventrolateral reticular nucleus reduce, by up to 50%, the elevation of the cortical blood flow elicited by hypoxemia, without affecting the cerebrovascular response by hypercapnia or impairing cerebrovascular autoregulation (Underwood et al. 1992; Golanov and Reis 1996). Because the rostral ventrolateral reticular nucleus does not innervate the cerebral cortex (Ruggiero et al. 1989), the intracerebral pathway mediating cortical vasodilation is indirect, and the first synapse resides in the medullary vasodilator area, which is innervated directly from the rostral ventrolateral reticular nucleus (Golanov et al. 2000). An excitation of the medullary vasodilator area elicits changes in the arterial blood pressure, regional cerebral blood flow and EEG that are quantitatively identical to those evoked from the rostral ventrolateral reticular nucleus. Bilateral lesions of the medullar vasodilator area block the cerebrovascular and cortical responses to stimulation of the rostral ventrolateral reticular nucleus, as well as hypoxia-induced cerebral

vasodilatation. The vasodilatation effects of medullary vasodilator area excitation are relayed by the subthalamic nucleus to other areas of the brain resulting in redistribution of blood from the viscera to the brain (Golanov et al. 2001).

It is generally accepted that various noxious stimuli given below the threshold of brain tissue damage are able to induce tolerance of the neurons to deleterious stimuli like hypoxia. This modality is called "cross tolerance" (Golanov et al. 2001) and it probably involves many separate neural networks (Schaller 2004). The one which mediates a reflexive neuroprotection emanates from oxygen sensitive sympatho-excitatory reticulospinal neurons of the rostral ventrolateral medulla oblongata projecting via as yet-undefined pathways to the upper brainstem and/or thalamus and finally ending to a small population of neurons in the cortex which appears to be dedicated to reflexively transducing a neuronal signal into autoregulatory cerebrovascular vasodilatation as well as consecutive synchronization of cortical activity (Golanov et al. 2000).

In the present work we show the occurrence and risk factors for TCR in operations for pituitary adenomas. We have examined potential risk factors that may lead to the intraoperative occurrence of the TCR during transsphenoidal surgery.

Methodology

Patient Population

The trigemino-cardiac reflex was defined as a drop in MABP and HR of more than 20% compared with baseline values before the stimulus and coinciding with the manipulation of the trigeminal nerve (Filis et al. 2008). Cessation of compression or traction of the trigeminal nerve would result in a spontaneous increase in HR and MABP to approximate baseline levels. The phenomenon had to recur when compression or traction was repeated. Only the data of the first occurrence of the reflex in each patient were used in the current study. Repetitive occurrence was only counted as once and the first time occurrence was recorded.

We retrospectively reviewed the case histories of 338 consecutive patients with the histological diagnosis of pituitary adenoma who were operated on by an experienced, world-class neurosurgeon from January 2000 till December 2006 in the Department of Neurosurgery in Göttingen, Germany. These patients were divided into two subgroups on the basis of the occurrence or not of TCR during surgery.

Surgical Technique

All operations were performed in microsurgical technique via a transsphenoidal or transcranial approach as being described above.

Anesthetic Technique

The whole surgical procedure was performed under a standardized anesthetic protocol. Before surgery, patients fasted for atleast 6 h and were premedicated with oral midazolam the evening before surgery. Routine monitoring during surgery included ECG, measurement of blood pressure and end-tidal concentration of carbon dioxide, oxygen saturation in blood and pulse oximetry, and esophageal temperature. An indwelling radial artery catheter was inserted for continous monitoring of MABP and for obtaining intermittend blood gas samples. All hemodynamic variables were monitored continuously and recorded throughout the surgical procedure. General anesthesia was induced with propofol (2 mg kg^{-1}) followed by sufentanyl (1 mg kg^{-1}) and rocuronium (0.6 mg kg^{-1}). Anesthesia was maintained with desflurane; additional boluses of sufentanyl and atracurium were administered if necessary (Schaller et al. 1999).

Statistical Analysis

Data were presented as percentage or mean with standard deviation. For categorical variables, the Fisher-exact test was used while the continuous variables were compared using the two sample

Table 25.1 Patient characteristics of both subgroups

Parameter	TCR (n = 19)	Non-TCR (n = 319)	*p*-value
Gender (male:female ratio)	12:7	178:141	0.53
Mean age (years)	51 (range: 23–79)	48 (range: 14–81)	
Tumor diameter (mm)	19	16	0.13
BMI (kg m^{-2})	27.9	27.5	0.76
Type of surgery			
Trans-sphenoidal	6 (31.6%)	72 (22.6%)	0.4
Transcranial	1 (5.3%)	25 (7.8%)	1
Preoperative administration of drugs			
β-blockers	1 (5.3%)	19 (6%)	0.19
Pre-existing cardiovascular diseases			
Arrhythmia	0	8 (2.5%)	0.48
Ischemic heart disease	4 (21.1%)	37 (11.6%)	0.22

TCR trigeminocardiac reflex

t-test or the Mann–Whitney *U* test. The level of significance was set at $p < 0.05$.

Prognosis-Related Occurence of the Trigemino-Cardiac Reflex

In the present retrospective study, 338 consecutive patients who underwent a transsphenoidal or transcranial operation for pituitary adenoma resection were selected. In 19 patients (6%) TCR was observed. These patients were, therefore, subdivided into the TCR subgroup. The remaining 319 patients (94%), in whom the TCR was not observed during transsphenoidal/ transcranial operation for pituitary adenoma concluded the non-TCR subgroup.

The patient characteristics of the two groups are summarized in Table 25.1. Patients were comparable for gender, age, tumor diameter, BMI and type of surgery in the two study subgroups. In addition, preoperative administration of drugs such as β-blockers that affect the autonomic nervous system, or pre-existing cardiovascular diseases, showed no significant differences between the subgroups.

During the operation, anticholinergic treatment was administered in ten patients (52.6%) of TCR subgroup and in 19 patients (6.0%) of non-TCR subgroup.

In Table 25.2, we have shown the preoperative chronic intake of antihypertensive treatment.

Table 25.2 Antihypertensive treatment as risk factor for the trigemino-cardiac reflex

	TCR (n = 19)	Non-TCR (n = 319)	*p* value
ACE-inhibitor	5 (26%)	39 (12%)	> 0.05
Loop diuretic	4 (21%)	8 (3%)	0.00002
β-blocker	1 (5%)	53 (17%)	> 0.05
Calcium channel blocker	2 (11%)	22 (7%)	> 0.05
Potassium-sparing diuretic	1 (5%)	4 (2%)	0.03633

TCR trigeminocardiac reflex, *ACE* angiotensin-converting enzyme

Table 25.3 Psychostimulans and morphine analogues as risk factor for the trigemino-cardiac reflex

	TCR (n = 19)	Non-TCR (n = 319)	*p* value
Xanthine/caffeine	1 (5%)	0 (0%)	0.00004
Morphine analogue/codeine	1 (5%)	1 (0%)	0.00628

TCR trigeminocardiac reflex

There is a significant difference between the two subgroups for loop diuretic and Potassium-sparing diuretic intake. Regarding the preoperative intake of ACE-Inhibitors, there can be found a statistically non-significant tendency.

Table 25.3 shows the association between the pre-operative intake of (psycho) stimulants and

morphine analogues and the occurrence of TCR. For both classes of substances there is a statistically significant difference.

Discussion

It has been demonstrated that the TCR may occur with manipulation of any of the branches of the trigeminal nerve anywhere along its (intracranial and extracranial) course (Schaller et al. 2009). The oculocardiac reflex (OCR), which is a subvariant of the TCR, and studied far earlier, was said to occur in up to 67% of the patients operated for strabismus (Apt et al. 1973), but a lot of authors only studied the heart rate and a reduction in heart rate by 10% or more was taken as a positive OCR, so that the real incidence may be substantially smaller. The authors have the opinion that the OCR incidence has only a little higher than that one of the TCR. This higher incidence may be only reflected to the fact that there are mainly children that we operated for strabismus and that are more prone to the occurrence of TCR/OCR than adults. In contrast to the studies on OCR, Schaller et al. (1999) took into consideration the heart rate and blood pressure defining TCR as heart rate less than 60 beats per minute and mean arterial blood pressure (MABP) 20% lower than the baseline after the reflex would be elicited.

TCR was also reported during transsphenoidal surgery for pituitary adenoma (Starr et al. 1986; Shende et al. 2000; Schaller et al. 2008a, b). Among 117 patients who underwent transsphenoidal surgery for pituitary adenoma, 10% developed a TCR during the surgical procedure (Shende et al. 2000). Peripheral stimulation of the naso-pharynx may also lead to the occurrence of TCR (Schaller et al. 2008a).

Risk Factors for Occurrence of the Trigeminocardiac Reflex

As there is a lack of detailed knowledge of the physiology of the TCR and since we cannot prevent the occurrence TCR (though) recently some authors have published reports of reducing its occurrence rate by deep anesthesia of the trigeminal nerve branches (Bohluli et al. 2011), it is important to identify possible risk factors. The risk factors already known to increase the incidence of TCR include: (1) hypercapnia; (2) hypoxemia; (3) "superficial" general anesthesia; (4) age (more pronounced in children); (5) the nature of the provoking stimulus (strength and duration of the stimulus); and (6) drugs. Drugs known to increase the TCR include: (1) potent narcotic agents (sufentanil and alfentanil) (Hunsley et al. 1982; Blanc 1991); (2) beta-blockers; and (3) calcium channel blockers (Mirakhur et al. 1982). In addition to beta-blockers and calcium channel blockers in other skull base surgeries for vestibular schwannomas (Schaller et al. 1999), the present study also showed that other anti-hypertensive agents like diuretics (both loop and potassium sparing) and angiotensin converting inhibitors may also be potential risk factors for the occurrence of the TCR. Narcotics may augment vagal tone through their inhibitory action on the sympathetic nervous system (Schaller et al. 1999, 2007a, b, 2008a; Prabhakar et al. 2008). Beta-blockers reduce the sympathetic response of the heart and by so doing, augment the vagal cardiac response resulting in bradycardia. Calcium channel blockers result in peripheral arterial smooth muscle relaxation and vasodilatation causing reduction in blood pressure. This may worsen the vagal output and oppose any reflex-mediated sympathetic stimulation resulting from reducing the blood pressure, also during intraoperative manipulations on the trigeminal nerve (Lang et al. 1991; Schaller and Buchfelder 2006). The individual calcium channel blockers have different relative potencies on various cardiovascular functions what may explain the inconsistency of these findings in different studies. From the present point of knowledge, it is not clear if there exist a specific risk profile for transcranial/transsphenoidal surgery for pituitary adenomas, or the risk profile seems to be the same for all skull base operations. According to the present (preliminary) work, it seems that the risk profile may change for different surgical approaches to the skull base.

Management of the Trigeminocardiac Reflex

There has been a lot of discussion about the best and more effective treatment for TCR. It is beyond the scope of this manuscript to discuss all these issues. Without any doubt, application of atropine is an effective, but only symptomatic treatment. However it is more important for the doctor to recognize and minimize potential predisposing factors of the TCR.

According to the clinical experience on this topic, the management of patients with TCR can be classified into the following categories:

1. Risk factor identification and modification
2. Prophylactic treatment with either vagolytic agents or peripheral nerve blocks in case of peripheral manipulations of the trigeminal nerve (Bohluli et al. 2010).
3. Careful cardiovascular monitoring during anesthesia especially in those with risk factors for TCR.
4. Treatment of the condition when it occurs:
   i. Cessation of the manipulation until normal sinus rhythm returns
   ii. Further administration of vagolytic agents

The risk of TCR should be considered in any neurosurgical intervention, especially at the skull base. If working in the vicinity of the nerve or its branches, the anesthesiologist should be notified by the surgeon. Continuous intraoperative monitoring of hemodynamic parameters has been shown to allow the surgeon to interrupt surgical maneuvers immediately upon the occurrence of TCR. Following this strategy, an uneventful further intraoperative and postoperative course may be achieved.

If there is no contraindication to intravenous anticholinergics, atropine and/or glycopyrrolate IV may be used to partially prevent a TCR (Blanc 1991). Hunsley et al. (1982) evaluated the efficacy of IV atropine and glycopyrrolate in the prevention of the OCR in children operated for strabismus. They tested different doses of the two drugs, glycopyrrolate 5 and 7.5 μg kg^{-1} and atropine 10 and 15 μg kg^{-1}. Overall, there is a reduction in the rate of bradycardia from 23.8 to 33.3%. But, they noticed that even higher doses of the two drugs, atropine 15 mcg kg^{-1} and glycopyrrolate 7.5 μg kg^{-1} IV, given 5 min before induction of anesthesia, are not sufficient to protect completely against the OCR in children. In a control study, Mirakhur et al. (1982), evaluated the efficacy of IV or IM vagolytic agents (atropine and glycopyrrolate) in children undergoing squint surgery, and found out that the administration of the anticholinergic agents in either the IV or the IM form may decrease the occurrence of the OCR. The overall frequency was approximately 40% (62 of 160 patients), but was 90% in those patients who did not receive anticholinergic drugs. The authors concluded that the administration of anticholinergic drugs, even through the IM route, is associated with decreased frequency of the OCR with glycopyrrolate 10 μg kg^{-1} being the most efficacious by this route.

Intramuscular administration of anticholinergics has shown to be ineffective in preventing the TCR (Prabhakar et al. 2006; Schaller et al. 2008b). The use of atropine is, nowadays, questioned because cholinergic blockage reduces but does not totally prevent either bradycardia or hypotension in animals (Schaller et al. 2007b). Another reason is that, a trigeminal depressor response includes both activation of vagal cardioinhibitory fibers and inhibition of adrenergic vasoconstriction as demonstrated after electrical stimulation of the spinal trigeminal tract and trigeminal nuclear complex. In addition, atropine may cause serious cardiac arrhythmias itself, especially when halothane is the primary anesthetic agent and hence the dose must be carefully chosen (Parabhakar et al. 2008). Prabhakar et al. (2008) reported a 48 year-old female who developed severe bradycardia and hypotension during craniotomy for parietal convexity meningioma; she was unresponsive to atropine and successfully managed with epinephrine. Adrenaline causes vasoconstriction via bonding to α_1-adrenergic receptor, thus increasing peripheral resistance. On the other hand, adrenaline mediates through the β_1-adrenergic receptor an increase in heart rate and cardiac output. This important case report underscores the fact that TCR may be refractory to atropine and other vagolytics and may rather need to be managed with epinephrine. This finding is supported by the fact that atropine blocks

only muscarinic acetylcholine receptors being the main but not the only neurotransmitter system used by the parasympathetic nervous system.

References

Apt L, Isenberg S, Gaffney WL (1973) The oculocardiac reflex in strabismus surgery. Am J Ophthalmol 76:533–536

Blanc VF (1991) Trigeminocardiac reflexes. Can J Anesthesia 38:696–699

Bohluli B, Bayat M, Sarkarat F, Moradi B, Tabrizi MH, Sadr-Eshkevari P (2010) Trigeminocardiac reflex during Le fort I osteotomy: a case-crossover study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 110(2):178–181

Bohuli B, Schaller B, Khorshidi-Khiavi R, Dalband M, Sadr-Eshkevari P, Maurer P (2011) Trigeminocardiac reflex, bilateral sagittal split ramus osteotomy, Gow-Gates block: a randomized controlled clinical trial. J Oral Maxillofac Surg 69:2316-20

Filis A, Schaller B, Buchfelder M (2008) Trigeminocardiac reflex in pituitary surgery. A prospective pilot study. Nervenarzt 79:669–675

Golanov EV, Reis DJ (1994) Nitric oxide and prostanoids participate in cerebral vasodilation elicited by electrical stimulation of the rostral ventrolateral medulla. J Cereb Blood Flow Metab 14:492–502

Golanov EV, Reis DJ (1996) Contribution of oxygen-sensitive neurons of the rostral ventrolateral medulla to hypoxic cerebral vasodilatation in the rat. J Physiol 495:201–216

Golanov EV, Ruggiero DA, Reis DJ (2000) A brainstem area mediating cerebrovascular and EEG responses to hypoxic excitation of rostral ventrolateral medulla in rat. J Physiol 529:413–429

Golanov EV, Christensen JR, Reis DJ (2001) Neurons of a limited subthalamic area mediate elevations in cortical cerebral blood flow evoked by hypoxia and excitation of neurons of the rostral ventrolateral medulla. J Neurosci 21:4032–4041

Hunsley JE, Bush GH, Jones CJ (1982) A study of glycopyrrolate and atropine in the suppression of the oculocardiac reflex in strabismus surgery in children. Br J Anaesth 54:459–564

Lang S, Lanigan DT, van der Wal M (1991) Trigeminocardiac reflexes: maxillary and mandibular variants of the oculocardiac reflex. Can J Anaesth 38:757–760

Mirakhur RK, Jones CJ, Dundee JW, Archer DB (1982) I.M. or I.V. Atropine or glycopyrrolate for the prevention of oculocardiac reflex in children undergoing squint surgery. Br J Anaesth 54:1059

Prabhakar H, Anand N, Chouhan RS, Bithal PK (2006) Brief report of special case: sudden asystole during surgery in the cerebellopontine angle. Acta Neurochir (Wien) 148:699–700

Prabhakar H, Ali Z, Rath GP (2008) Trigeminocardiac reflex may be refractory to conventional management in adults. Acta Neurochir (Wien) 150:509–510

Ruggiero DA, Cravo SL, Arango V, Reis DJ (1989) Central control of the circulation by the rostral ventrolateral reticular nucleus: anatomical substrates. Prog Brain Res 81:49–79

Schaller B (2004) Trigemino-cardiac reflex. A clinical phenomenon or a new physiological entity? J Neurol 251:658–665

Schaller B (2005a) Trigemino-cardiac reflex during microvascular decompression in cases of trigeminal neuralgia. J Neurosurg Anesth 17:45–48

Schaller B (2005b) Trigemino-cardiac reflex during transsphenoidal surgery for pituitary adenomas. Clin Neurol Neurosurg 107:468–474

Schaller B, Buchfelder M (2006) Trigemino-cardiac reflex: a recently discovery "oxygen-conserving" response? The potential therapeutic role of a physiological reflex. Arch Med Sci 2:3–5

Schaller B, Probst R, Strebel S, Gratzl O (1999) Trigeminocardiac reflex during surgery in the cerebellopontine angle. J Neurosurg 90:215–220

Schaller B, Knauth M, Buchfelder M (2006) Trigeminocardiac reflex during skull base surgery. A new entity of ischemic preconditioning? The potential role of imaging. Eur J Nucl Med Mol Imaging 33:384–385

Schaller B, Filis A, Buchfelder M (2007a) Cardiac autonomic control in neurosurgery. Arch Med Sci 4:287–292

Schaller BJ, Weigel D, Filis A, Buchfelder M (2007b) Trigemino-cardiac reflex during transsphenoidal surgery for pituitary adenomas: methodological description of a prospective skull base study protocol. Brain Res 1149:1169–1175

Schaller BJ, Filis A, Buchfelder M (2008a) Trigeminocardiac reflex in humans initiated by peripheral stimulation during neurosurgical skull-base operations. Its first description. Acta Neurochir (Wien) 150:715–718

Schaller B, Sandu N, Filis A, Buchfelder M (2008b) Peribulbar block of topical application of local anesthesia combined for paediatric strabismus surgery. Anaesthesia 63:1142–1143

Schaller B, Cornelius JF, Prabhakar H, Koerbel A, Gnanalingham K, Sandu N, Ottaviani G, Filis A, Buchfelder M (2009) The trigemino-cardiac reflex: an update of the current knowledge. J Neurosurg Anesth 21:187–195

Shende D, Sadhasivam S, Madan R (2000) Effects of peribulbar bupivacaine as an adjunct to general anesthesia on peri-operative outcome following retinal detachment surgery. Anaesthesia 55:970–975

Starr NJ, Sethna DH, Estafanos FG (1986) Bradycardia and asystole following rapid administration of sufentanil with vecuronium. Anesthesiology 64:521–523

Underwood MD, Iadecola C, Sved A, Reis DJ (1992) Stimulation of Cl area neurons globally increases regional cerebral blood flow but not metabolism. J Cereb Blood Flow Metab 12:844–855

Part III

Spinal Tumors

Spinal Extradural Meningiomas

26

Amgad Hanna, Phi Nguyen, Harminder Singh, and James Harrop

Contents

Abstract

Spinal extradural meningiomas are rare. They can be mistaken for the more common etiologies in this location like metastases or lymphomas. An initial approach could involve biopsy to obtain a diagnosis. Symptomatic tumors should be operated on. The goal of surgery is to decompress the spinal cord and/or the spinal nerves. Complete resection may not be achievable especially in the cervical spine due to proximity to the vertebral artery.

Introduction

Spinal meningiomas represent 10% of meningiomas and 30% of intraspinal tumors. It has been reported by Vargas et al. (2004) that 90% of cases occur in women older than 40 years. They occur most commonly in the thoracic spine (70%), less frequently in the cervical spine (25%), and more rarely in the lumbar spine (5%). The intradural location is the rule. Pure extradural meningiomas represent ~3.5% of spinal meningiomas, while combined intra- and extradural tumors occur in 4%. Calogero and Moosy (1972) reviewed the literature and found that extradural meningiomas had no significant gender preference, occurred in younger patients, including children. They are typically psammomatous. Nicolas et al. (2007) illustrated that cells may have a plasmacytoid appearance, making the diagnosis difficult especially with a small specimen of fine needle aspiration.

A. Hanna (✉)
Department of Neurological Surgery, University of Wisconsin, 600 Highland Avenue, Madison, WI 53792, USA
e-mail: ah2904@yahoo.com

P. Nguyen • J. Harrop
Department of Neurological Surgery, Thomas Jefferson University, 909 Walnut Street, PA 19107, Philadelphia

H. Singh
Department of Neurological Surgery, Stanford University School of Medicine, 751 S Bascom Ave, San Jose, CA 95128

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_26,

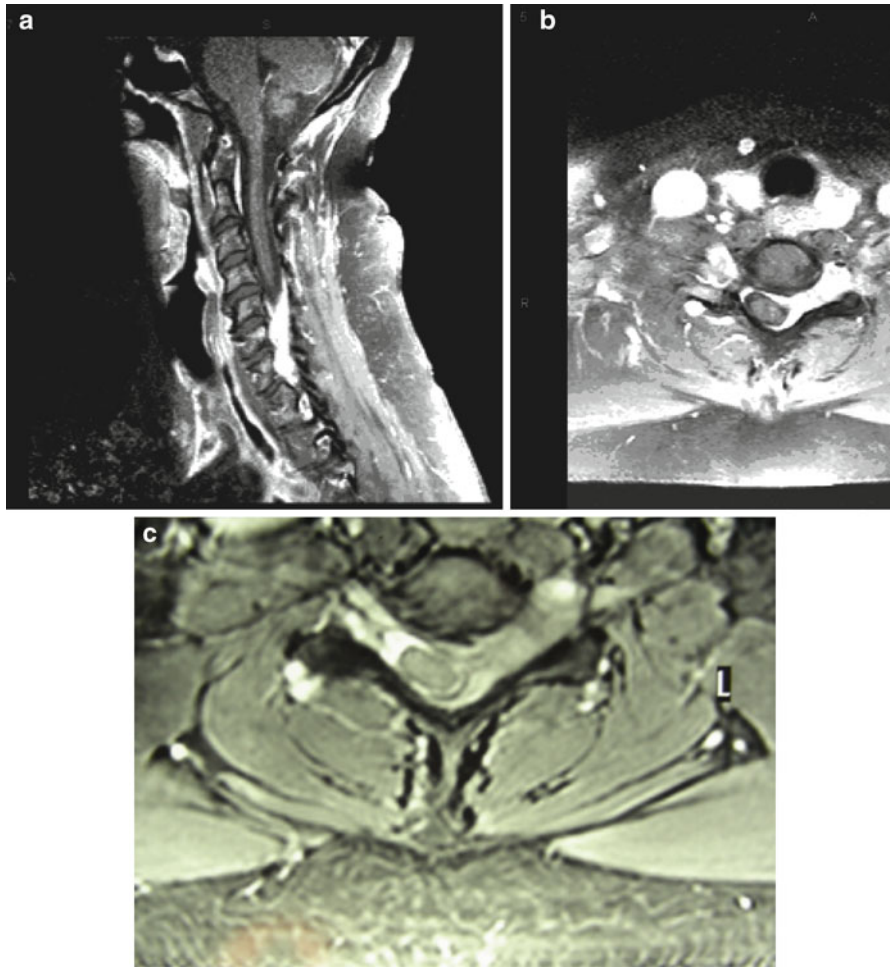

Fig. 26.1 Preoperative MRI, (**a**) Sagittal T1 with contrast, (**b**) Axial T1 with contrast, (**c**) Axial T2, showing extradural mass extending through the left foramina, isointense with the cord, and enhancing with contrast (**a** and **b**) (Reproduced with permission from Frank et al. 2008)

Case Illustration

A 45-year-old female presented with left cervical radiculopathy and neck discomfort persisting for 8 months and progressively worsening. The patient had a remote history of a low-speed motor vehicle collision. On physical exam, the patient had full muscle strength bilaterally except for mild weakness (4+/5) of the intrinsic hand muscles bilaterally and the left triceps. There was no evidence of sensory deficits, abnormal reflexes, or long tract signs. A cervical magnetic resonance image (MRI) revealed an extradural mass at C5-C7. The mass was situated on the left side of the spinal canal and exited through the neural foramina resulting in significant spinal cord compression (Fig. 26.1). The lesion was hyperintense T2-weighted images. MRI of the central nervous system revealed no other lesions. Additionally, computerized tomography of the cervical spine revealed left neuroforaminal widening at C6-C7. A full metastatic evaluation with CT of the chest, abdomen and pelvis revealed no evidence of additional lesions.

Due to the severe cord compression and progressive symptoms a decompression and open biopsy was performed through a left C6-C7 laminectomy and foraminotomy. Pathological examination revealed a psammomatous meningioma.

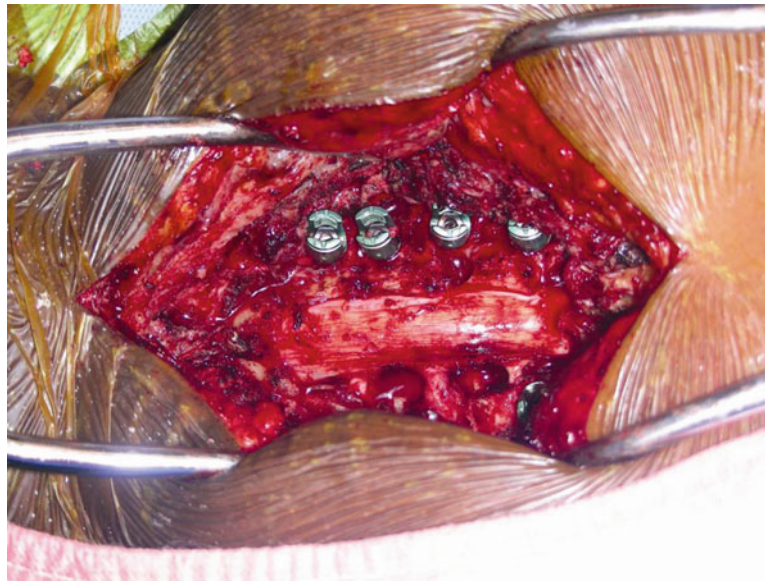

Fig. 26.2 Intraoperative view after tumor resection showing adequate decompression of the spinal cord and nerve roots on the *left side* after wide foraminotomies

A more aggressive surgical resection was performed 2 weeks later via a C5-C7 laminectomy and instrumented fusion with left-sided C5-T1 foraminotomies (Fig. 26.2). The extradural meningioma engulfed the vertebral artery and prohibited gross total resection. The patient's post-operative course was uneventful and her neurologic examination progressively returned to normal. The patient was followed with serial MRI every 3 months. At the most recent follow-up visit, she had no evidence of recurrence and did not report any symptoms.

Embryology

The developing embryo is characterized by three germ layers, which give rise to the various specialized systems of the body. These are the ectoderm, the mesoderm, and the endoderm. The ectoderm forms the nervous system as well as the epidermis with all of its sensory receptors. The mesoderm gives rise to connective tissue as well as the skeletal, muscular, circulatory, and urogenital systems and glands. From the endoderm arise the digestive and respiratory epithelia.

As the embryo develops, coverings called the meninges (of mesodermal origin) develop along with it and completely enclose the brain and spinal cord. The meninges separate the brain and spinal cord from the bony surface lining of the cranium and vertebral canal. In the early embryo, the neural tube is enveloped by a mesenchymal layer. Mesenchyme is a type of undifferentiated loose connective tissue that is derived mostly from mesoderm, but also from other germ layers like the neural crest cells that originate from ectoderm. Most embryologists use the term mesenchyme only for those cells that develop from mesoderm. The mesenchymal layer will differentiate during development to form two different layers: the *pachymeninges*, or dura mater, and the *leptomeninges*, comprising the arachnoid and the pia mater. The leptomeninges may contain both mesodermal and neural crest cells, while the pachymeninges are of mesodermal composition only.

There are anatomic and embryologic differences between the meningeal coverings of the brain and the spinal cord. According to O'Rahilly and Muller (1986), the encephalic meninges originate from both the mesodermal

and the encephalic neural crest, while the meninges of the spine and caudal regions of the head are of mesodermal origin only. This double origin of the meninges is similar to axial skeletal structures in which the spinal column and occipital bone are derived from the paraxial mesoderm, whereas the rest of the cranial vault is developed from neural crest-derived and mesodermal cells (Jiang et al. 2002). In the cranium the dura mater has two layers surrounding the brain: an inner, meningeal layer and the outer, periosteal layer. In the spine there is a single dural layer, the spinal dura mater, which is in fact a continuation of the meningeal layer in the brain; the outer layer is represented by the periosteum lining the vertebral canal.

Buetow et al. (1991) showed that meningiomas are tumors originating from the arachnoid "cap" cells of the arachnoid granulations in the meninges. Arachnoid granulations are small protrusions of the arachnoid into the dura mater. Sometimes, these granulations can traverse the entire width of the dura mater, and allow CSF to exit the brain and spinal cord. Since the location of the arachnoid layer is intradural extramedullary, most meningiomas occur in this location. In the spine, they typically arise in proximity of the dorsal nerve root, which explains their common posterolateral location.

Clinical Presentation

According to Frank et al. (2008), a history of trauma frequently precedes presentation. Depending on their location, spinal extradural meningiomas can present with pain, radiculopathy, myelopathy, or myeloradiculopathy. Local pain could be from direct mass effect of the meningioma and pressure on the dural sac or surrounding bony structures. Pain is more typically nocturnal. Radiculopathy arises from direct compression of the nerve root and could manifest as radicular pain, tingling, numbness, loss of sensation, or less frequently focal weakness. Myelopathy results from mass effect on the spinal cord, and could present with decreased dexterity, stiffness, or loss of balance. Examination can reveal weakness, hyper-reflexia, clonus, Babinski's sign, decreased sensation especially proprioception.

Imaging

The differential diagnosis of spinal extradural masses includes schwannomas, neurofibromas, metastases, lymphomas, meningiomas, chordomas, neuroblastomas, ganglioneuroblastomas, sarcomas, synovial cysts, infections, and hematomas. Wang et al. (1988) demonstrated the appearance of meningiomas on computed tomography (CT) as hyperdense lesions with enlargement of the neuroforamen, and possible calcifications. Santiago et al. (2009) described the magnetic resonance imaging (MRI) findings of meningiomas as isointense to the spinal cord on both T1 and T2-weighted images with intense and homogeneous contrast enhancement. Schwannomas are hypointense on T1, hyperintense on T2, with intense heterogeneous contrast enhancement, sometimes with necrotic or cystic components. Lymphomas are hypointense on T2 in >50% of cases. Myelogram and CT-myelogram are rarely needed if MRI is available.

Treatment

Treatment is primarily surgical. A Simpson grade I resection (Simpson 1957) is usually difficult, since resection of the dura at the nerve root sleeve would make it very difficult to obtain a watertight reconstruction. If possible, gross total resection should be attempted. However, in the cervical spine the tumor can come in close proximity to or even enchase the vertebral artery which renders complete resection a high risk for its injury. Subtotal resection with adequate decompression of the neural elements is a reasonable goal of the surgery. Residual tumor could be observed with serial imaging. Radiation can be used if residual tumor demonstrates regrowth.

The need for spinal instrumentation depends on the amount of facet resection needed, and the potential instability.

In conclusion, spinal extradural meningiomas should be considered in the differential diagnosis of

extradural masses. Symptoms may involve myelopathy and/or radiculopathy. Treatment primarily involves surgical resection with potential need for instrumented fusion. Residual tumor could be observed with serial imaging. Prognosis is generally good.

References

Buetow MP, Buetow PC, Smirniotopoulos JG (1991) Typical, atypical, and misleading features in meningioma. Radiographics 11(6):1087–1106

Calogero JA, Moosy J (1972) Extradural spinal meningiomas: report of four cases. J Neurosurg 37442–447

Frank BL, Harrop JS, Hanna A, Ratliff J (2008) Cervical extradural meningioma: case report and literature review. J Spinal Cord Med 31:302–305. URL: www.maney.co.uk/journals/scm; www.ingentaconnect.com/content/maney/scm

Jiang X, Iseki S, Maxson RE, Sucov HM, Morris-Kay GM (2002) Tissue origins and interactions in the mammalian skull vault. Dev Biol 241(1):106–116

Nicolas MM, Kaakaji R, Russell EJ, De Frias DV, Nayar R (2007) Extradural spinal meningioma as a source of plasmacytoid cells. Acta Cytol 51:68–72

O'Rahilly R, Muller F (1986) The meninges in human development. J Neuropathol Exp Neurol 45(5):588–608

Santiago BM, Rodeia P, Sa.M Cunha e (2009) Extradural thoracic spinal meningioma. Neurol India 57:98

Simpson D (1957) The recurrence of intracranial meningiomas after surgical treatment. J Neurol Neurosurg Psychiatry 20:22–39

Vargas MI, AbuEid M, Bogorin A, Beltechi R, Boyer P, Javier RM, Zollner G, Dietemann JL (2004) Les méningiomes rachidiens extraduraux: données IRM à propos de deux observations. J Neuroradiol 31: 214–219

Wang AM, Gall CM, Shillito J Jr, Schick R, Brooks ML, Haikal N (1988) CT demonstration of extradural thoracic meningioma. J Comput Assist Tomogr 12:536–537

Spinal Cord Ganglioglioma

27

Lee Hwang, Sina Tok, and George Jallo

Contents

Abstract

Ganglioglioma of the spinal cord is a rare tumor that affects predominately the pediatric population. This type of intramedullary spinal cord neoplasm is generally considered benign, corresponding to WHO grade II. Common presenting symptoms include pain, neurologic deficits, and spinal deformity. Because of the indolent clinical course, risk of recurrence, and relative ineffectiveness of additional treatment modalities, early diagnosis with imaging as well as early surgical intervention are highly recommended. Complete, or near complete, resection usually results in long-term progression-free survival, and improvement of neurological status is achievable despite aggressive surgery. Improved microsurgical techniques, preoperative planning with MRI, and intraoperative neurophysiological monitoring have made a significant impact on the management of all spinal cord tumors including gangliogliomas.

Introduction

Although tumors arising from the spinal cord comprise a small subset of central nervous system (CNS) tumors affecting children, they are particularly threatening because of the densely packed neuronal networks and fiber tracts within the spinal cord. Management of these slow growing spinal cord tumors remains controversial. According to the expanding literature, most of

L. Hwang • S. Tok • G. Jallo (✉)
Department of Neurosurgery, Division of Pediatric Neurosurgery, Johns Hopkins University, 600 North Wolfe Street, Harvey 811, Baltimore, MD, USA
e-mail: gjallo1@jhmi.edu

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_27,

these intrinsic tumors can and should be treated with microsurgical resection; whereas, adjunctive therapies are reserved for selected cases of high-grade tumors and recurrences.

The history of intrinsic spinal cord surgery begins in Vienna. von Eiselsberg and Marburg (1917) performed the first successful resection of an intramedullary tumor in 1907. However, the first publication of such a surgery appeared in New York when Elsberg and Beer (1911) described a two-stage operation: a myelotomy followed by a more extensive resection. In subsequent decades, neurosurgeons were reluctant to attempt such radical procedures. The neurological risk of resecting intramedullary neoplasms was considered unacceptably high, which eventually led to the development of more conservative treatment options including biopsy, dural decompression, and radiation therapy regardless of the histological diagnosis (Wood et al. 1954).

The microscope and microsurgery completely revolutionized neurosurgery, allowing surgical resection of intramedullary tumors as a management strategy superior to the biopsy-radiation approach. In addition, magnetic resonance imaging (MRI) dramatically improved visualization of the spinal cord anatomy. Preoperative planning and postoperative follow-up became crucial components of patient care. Gradually the conservative treatment concept evolved into an aggressive surgical strategy. Since most intramedullary tumors are low-grade, complete, or even near complete, surgical resection appears to result in long-term progression-free survival with acceptable neurological morbidity (Constantini et al. 2000). After MRI dramatically improved the anatomical understanding of the surgical pathology, intraoperative neurophysiological monitoring emerged as the supreme method of assessing the functional integrity of the spinal cord pathways during surgery and also during postoperative neurological recovery.

Despite these advances in the surgical management of intramedullary tumors, differences of opinion still exist regarding optimal individualized treatment. The chemotherapeutic agents used for CNS tumors have not been widely used to treat intramedullary neoplasms. Only the higher-grade glial tumors have been treated with combination therapies that include chemotherapy (Allen et al. 1998). For the low-grade tumors, the efficacy of medical therapy remains unknown. Given the recent advances in chemotherapy for malignant gliomas of the brain, development of chemotherapeutic agents for spinal cord neoplasms is highly plausible in the future. Unfortunately, at this time, patients diagnosed with malignant intramedullary tumors are still confronted with a poor prognosis.

Epidemiology and Pathology

Intrinsic tumors of the spinal cord are rare. Gangliogliomas comprise 1.1% of intramedullary spinal cord tumors (Lee and Glasauer 1968). However, some report an incidence as high as 14%, which designates ganglioglioma the second most common intramedullary tumor after astrocytoma under the age of 10 (Constantini et al. 1996). The incidence declines with age, and only a few cases have been reported beyond the fourth decade of life (Hamburger et al. 1997). Males and females appear to be equally affected by intramedullary spinal cord lesions, but male preference has been noted with brain lesions (Lantos et al. 1997). No specific ethnic prevalence has been identified (Lantos et al. 1997). In addition, gangliogliomas are not known to be associated with any specific phakomatoses or inheritable diseases (Lantos et al. 1997); however, there are cases of concomitant type I neurofibromatosis as well as occult spinal dysraphism (Park et al. 2000).

Many studies have evaluated prognostic factors associated with functional outcome after surgical resection of intramedullary spinal cord tumors in adults (Jallo et al. 2001; Sandalcioglu et al. 2005; Shrivastava et al. 2005). On the other hand, studies in the pediatric population are sparse. Intramedullary spinal cord tumors in pediatric patients are histologically different from those affecting adults. The most common intramedullary tumors in children are infiltrating astrocytomas as opposed to adults who more commonly develop intramedullary ependymomas (Shrivastava et al. 2005; Rodewald et al. 1987; Constantini et al. 1996).

Furthermore, most spinal cord tumors in children are benign, either pilocytic or, less frequently, fibrillary astrocytomas (Constantini et al. 1996).

Gangliogliomas are histologically low-grade tumors with well-differentiated neuronal and astrocytic cells (Miller et al. 1990). The neoplastic neurons are large and relatively mature. Characteristic features include population of large ganglion cells with larger but paler nuclei than astrocytes, neurons atypical for perineuronal satellitosis or arranged in normal architecture, as well as fibrosis or desmoplasia in the background (Miller et al. 1990). Although the histopathological diagnosis may be limited by sampling, extensive research has yielded an antibody marker for synaptophysin, a synaptic vesicle membrane glycoprotein, which is sensitive and specific for neoplastic neurons (Miller et al. 1990).

Clinical Presentation

Intramedullary gangliogliomas often grow slowly, following an indolent course; however, some clinicians assert that gangliogliomas can behave aggressively (Lang et al. 1993). The overall potential for malignant transformation of spinal cord gangliogliomas cannot be determined, as the literature only provides five cases of malignant gangliogliomas (Hamburger et al. 1997; Henry et al. 1978). The risk is 10% for intracranial gangliogliomas, mostly within the glial component, but unclear for spinal cord gangliogliomas (Rodewald et al. 1987). In comparison to the mature adult spine, the pediatric spine is more susceptible to postoperative deformity due to the lax ligamentous structures, relatively horizontal orientation of the facet complexes, and dynamic development of the immature spine (Rodewald et al. 1987). Spinal cord gangliogliomas have predilections for a cervical location (Fig. 27.1), although they have been reported in all regions of the spinal cord (Satyarthee et al. 2003). Additionally, they often involve more than eight segments of the spinal cord (Fig. 27.2), typically a greater length than astrocytomas and ependymomas (Lotfinia and Vahedi 2008). There are even reports of gangliogliomas involving the entire length of the spinal cord (Albright and Byrd 1980).

Intramedullary spinal cord tumors may grow considerably before causing clinical symptoms, which are often non-specific. Neck and back pain, a common presenting symptom, is usually

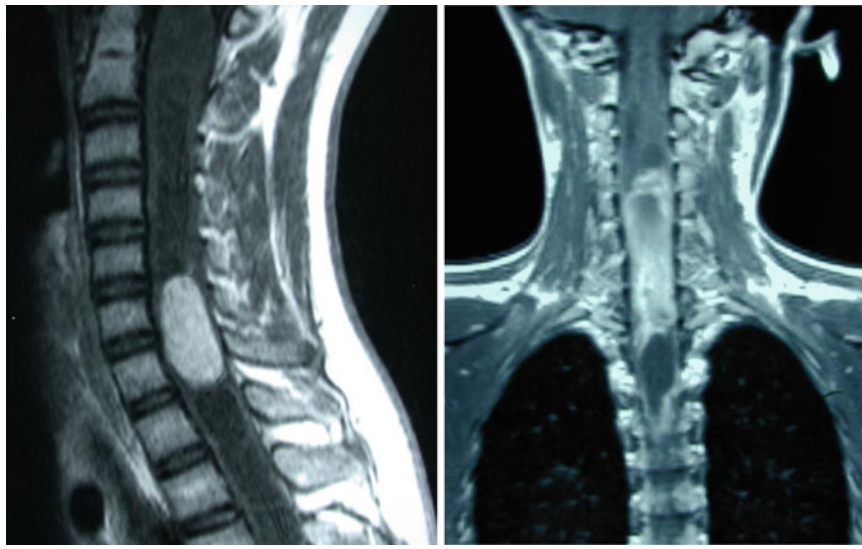

Fig. 27.1 T1-weighted MRI of cervical spinal cord ganglioglioma, sagittal (*left*) and coronal (*right*) views: Although spinal cord gangliogliomas have been reported in all regions of the spinal cord, they frequently involve the cervical location and span multiple segments

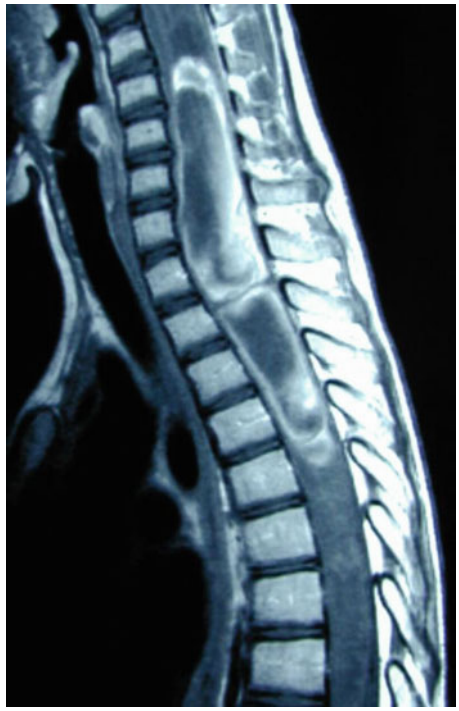

Fig. 27.2 T1-weighted MRI of spinal cord ganglioglioma, sagittal view: More than eight segments of the spinal cord are often involved, sometimes spanning the entire length of the spinal cord. Spinal cord gangliogliomas typically extend a greater length than astrocytomas and ependymomas

diffuse rather than radicular and most prominent in the horizontal position (Constantini et al. 1996). Young children may present with diffuse abdominal pain, motor deficits, motor regression (i.e. regressing from walking to crawling), or gait abnormalities (Constantini et al. 1996). Adolescent patients may present with lower-extremity weakness, clumsiness, or frequent falls (Constantini et al. 2000). Moreover, 20% of children with spinal cord tumors present with torticollis (Kiwak et al. 1983). In contrast, adults typically present with sensory deficits including dysesthesia, numbness in extremity, and myelopathy (Jallo et al. 2001; Shrivastava et al. 2005). Upon examination, most patients exhibit mild-moderate motor deficits as well as upper motor neuron findings, including increased muscle tone, spasticity, hyper-reflexia, and clonus. At least one third of patients have a spinal deformity, most with tumors in the thoracic region (Epstein and Farmer 1990). Furthermore, scoliosis tends to coincide with cystic tumors (Epstein and Farmer 1990). Sphincter dysfunction usually occurs late in the clinical course unless the tumor involves the conus medullaris, in which case such a deficit occurs more frequently and appears earlier (Constantini et al. 2000). In addition, as many as 15% of patients with intramedullary tumors present with hydrocephalus (Rifkinson-Mann et al. 1990). Hydrocephalus is frequently associated with malignant tumors and with cervical tumors (Kothbauer 2007).

The McCormick scale is often used for preoperative and postoperative clinical assessment of neurologic function in patients with intramedullary spinal cord tumors. In addition, the Nurick grade system may be useful for classifying the severity of any associated myelopathy (Park et al. 2000). The American Spinal Injury Association (ASIA) scale may also be used but is limited in younger children.

Diagnosis

Radiological features of intramedullary spinal cord ganglioglioma are usually non-specific, without any characteristic appearance that clearly differentiates them from other intramedullary lesions. MRI is the study of choice to assess the characteristics of spinal cord tumors. On T1-weighted MRI, hyper-intense, hypo-intense, or even irregular signals may be observed (Fig. 27.3). T2-weighted imaging usually demonstrates hyper-intense signals. Following administration of gadolinium (DTPA) contrast, enhancement is frequently observed; however, gangliogliomas enhance less frequently than ependymomas, and the enhancement tends to be heterogeneous (Lang et al. 1993).

If an MRI cannot be obtained (i.e. due to metallic implants) or is difficult to interpret, myelography with water-soluble contrast and computed tomography may be used (Lang et al. 1993). Spinal X-rays should be used for patients with scoliosis, to establish baseline and follow-up management of the spinal deformity (Kothbauer 2007).

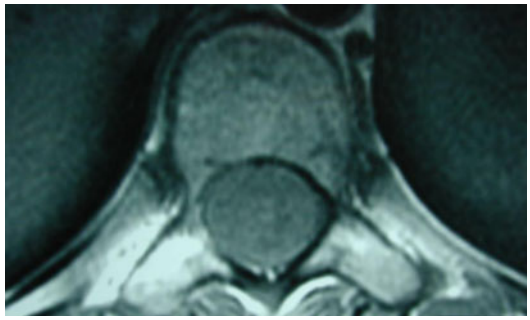

Fig. 27.3 T1-weighted MRI of spinal cord ganglioglioma, axial view: MRI is the study of choice to assess the characteristics of spinal cord tumors, though the radiological features are usually non-specific. T1-weighted MRI may demonstrate hyper-intense, hypo-intense, or even irregular signals

Factors that contribute to a diagnosis include holospinal involvement, scoliosis with bone remodeling, mixed intensity on T1 imagining, associated cysts or syrinx, absence of edema and enhancement within the tumor center, hemosiderin or calcification, as well as young age (Kothbauer 2007).

Surgical Management

As the majority of intramedullary spinal cord tumors are low-grade glial tumors, these tumors can be surgically resected with minimal morbidity and favorable oncologic outcome (Ulutin et al. 2002). There are three indications for surgical treatment of any newly diagnosed intramedullary tumor: to confirm histological diagnosis, to apply the most effective oncologic treatment, and to prevent long-term neurologic dysfunction (Constantini et al. 1996).

Surgery is performed with the patient lying in a prone position. A rigid head-holder (Sugita or Mayfield) is required for cervical and cervicothoracic tumors to secure the head in a neutral position. A set of specialized surgical instruments are used to minimize trauma to normal neural tissue. Microsurgical resection of spinal cord tumors usually incorporates the application of the Cavitron ultrasonic aspirator (CUSA) system. It uses high-frequency sound waves to fragment the tumor tissue, which is then aspirated by the suction apparatus. This device allows a much easier and quicker removal of bulks of tumor with less manipulation of the adjacent neural tissue relative to the suction-cautery technique. In addition, the laser is a useful tool to perform the myelotomy, demarcate the glial-tumor interface, and remove residual tumor fragments.

A laminectomy or osteoplastic laminotomy (Raimondi et al. 1976) is performed with a high-power drill using the craniotome attachment, round burr, and rongeurs. The bone removal must expose the solid tumor (Fig. 27.4) but not necessarily the rostral or caudal cysts. The cysts tend to disappear after tumor resection since the cyst walls usually contain non-neoplastic glial tissue (Kothbauer 2007). In addition, intraoperative sonography allows visualization of the full extent of the spinal cord tumor and its relation to the bone removal, as well as cysts and associated cord displacement (Taniguchi et al. 1993).

The dura is opened in the midline. The spinal cord is frequently expanded and sometimes rotated or distorted, which may make identification of the midline raphe difficult. On the other hand, localization of the midline raphe is a crucial step in entering the spinal cord. Alternatively, for asymmetrical tumors, the spinal cord can be reached through the dorsal root entry zone (Kothbauer 2007).

Intraoperatively, gangliogliomas have a gray-yellow glassy appearance. They are removed in an inside-out fashion until the glial-tumor interface is recognized by the change in color and consistency of the tissue. Because there is no distinct plane between tumor and normal spinal cord, the resection is usually started in the mid-portion rather than at the poles. Following tumor removal, hemostasis is maintained, and the dura is closed primarily in a watertight fashion. If an osteoplastic laminotomy was performed, the bone segments are replaced and secured with non-absorbable sutures bilaterally. The muscle and fascial closures must not be under tension. A subcutaneous drain may be placed in a large incision, a reoperation, or a patient with prior radiation therapy. History of previous surgery or radiation is associated with a higher risk of wound dehiscence and cerebrospinal fluid leakage.

There are only few studies concerning the management of spinal cord gangliogliomas.

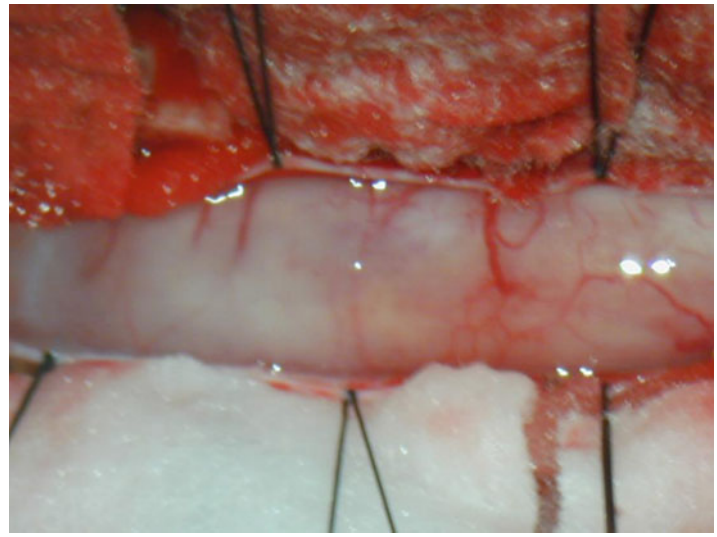

Fig. 27.4 Intraoperative view of spinal cord ganglioglioma: After a laminectomy or osteoplastic laminotomy, the solid tumor is exposed and removed in an inside-out fashion until the glial-tumor interface is recognized by the change in color and consistency of the tissue. Because there is no distinct plane between tumor and normal spinal cord, the resection is usually started in the mid-portion rather than at the poles

Surgical resection has been the recommended treatment for patients with spinal cord gangliogliomas, based on retrospective analysis of gangliogliomas in the cerebral hemispheres (Lang et al. 1993). Many studies advocate gross total resection (GTR) of these tumors. Garrido et al. (1978) treated three patients with spinal cord gangliogliomas and concluded that surgical resection was more effective than biopsy with irradiation. Furthermore, radiotherapy did not prolong the time to recurrence or increase length of survival. Similarly, Lang et al. (1993) described 58 patients with gangliogliomas resected throughout the neuraxis. Jallo et al. (2004) also performed radical surgical resection in 56 patients and reported better survival, low recurrence rate, and good functional outcome. All of these groups recommended surgery followed by observation without adjuvant irradiation. Close monitoring is recommended every 6 months for the first two postoperative years and then annually thereafter (Jallo et al. 2004). Only patients who experience clinical deterioration undergo urgent MRI scans.

Postoperative Complications

The completeness of surgical resection is always weighed against the risk of major neurologic impairment. The incidence of postoperative motor deficits is correlated with preoperative neurologic function: patients with a pre-existing motor deficit prior to surgery are more likely to deteriorate postoperatively (Morota et al. 1997). Short-term motor dysfunction has been reported in up to one third of patients, but the motor deficit usually resolves within hours to days (Kothbauer et al. 1998). Long-term motor outcome tends to be more favorable and directly related to the preoperative functional status (Hamburger et al. 1997). Additionally, symptom duration seems to be an important prognostic factor regardless of the preoperative neurological condition (Park et al. 2000). In general, patients with intramedullary tumors are advised to undergo surgery before developing significant neurologic deficits.

Patients may be affected by postoperative spinal deformities such as scoliosis and kyphosis. However, osteoplastic laminotomy is believed to reduce the incidence of postoperative spinal deformities in children (Abbott et al. 1992). Approximately one third of pediatric patients with a significant deformity eventually require surgical stabilization (Yasuoka et al. 1982). Spinal fusion is more urgent in patients with spinal deformity secondary to tumor relative to patients with idiopathic scoliosis. In patients with a progressive deformity, MRI should be performed to rule out tumor recurrence. Spinal deformity may also result from radiation therapy (Katzman et al. 1969). Higher rates of

post-radiation spinal deformity are associated with younger age at the time of radiation, dose greater than 20 Gy, and asymmetrical radiation fields (Mayfield et al. 1981). As a result, radiation is not a "non-invasive" alternative to surgery.

Other, more rare, complications include cerebrospinal fluid leakage. This risk increases with a history of radiation and/or prior surgery. In addition, impaired proprioception may cause a serious functional disability and requires extensive physical therapy. The postoperative course may also be complicated by pain syndromes, autonomic symptoms, as well as decreased strength secondary to prolonged physical inactivity. Fortunately, paralysis is a rare complication of intramedullary tumor surgery. Generally, close follow-up with plain radiographs is a crucial component of postoperative care.

Adjuvant Therapy

Recurrence due to microscopic tumor remnants is not uncommon regardless of the extent of the surgical resection. For recurrent spinal cord gangliogliomas, a second operation should be considered but radiation therapy may also be an option. Jallo et al. (2004) reported patients with recurrent tumors who underwent a second surgery without increased morbidity.

The role of adjuvant postoperative radiotherapy is not well-established. After GTR, postoperative radiotherapy for benign gangliogliomas is usually unnecessary (Otsubo et al. 1992; Hamburger et al. 1997). Radiation therapy should be reserved for patients with malignant tumors such as anaplastic gangliogliomas, inoperable tumor recurrence, and substantial residual tumor for which additional surgery is too risky. On the other hand, there is some concern for potential malignant transformation after radiation (Ulutin et al. 2002).

The role of chemotherapy is uncertain at this time for intramedullary tumors but seems limited for low-grade tumors (Chamoun et al. 2006). Chemotherapy may be more beneficial in high-grade tumor management but does not appear to significantly prolong survival (Allen et al. 1998).

Outcome

The prognosis of CNS gangliogliomas is not related to the histological appearance but to the extent of tumor as well as its resectability (Lang et al. 1993). Even after a near-complete resection, some residual microscopic fragments always remain. Lang et al. (1993) reported a clinical recurrence rate of 33% with CNS gangliogliomas, and linear regression analysis demonstrated the spinal cord location to have a 3.5-fold increase in the risk of recurrence (47%) relative to gangliogliomas involving the cerebral hemispheres. In addition, Jallo et al. (2004) reported a 30% recurrence rate for spinal gangliogliomas.

A resection that exceeds 80–90% is comparable to an almost-complete resection in terms of long-term progression-free survival (Shrivastava et al. 2005). Operative mortality, death within 1 month of the operation, is extremely low after radical resection regardless of the tumor site (Jallo et al. 2004). In addition, postoperative neurological function is usually improved or maintained during follow-up. Park et al. (1993) conducted a large series of five cases of intramedullary tumors, demonstrating no evidence of tumor recurrence during a follow-up period of 4.1 years.

The 5-year survival rate is 88% for low-grade intramedullary neoplasms and 18% for high-grade intramedullary neoplasms despite surgery and adjuvant therapy (Jallo et al. 2004). Whereas the 10-year survival rate is 84% for CNS gangliogliomas, the 5-year and 10-year survival rates for spinal cord gangliogliomas after radical resection are 89 and 83%, respectively (Lang et al. 1993). The progression-free survival may be up to 65% for spinal cord tumors (Jallo et al. 2004). Henry et al. (1978) reported a mean survival of 7 years for 50 patients with gangliogliomas involving the neuraxis. Moreover, the survival of patients with gangliogliomas is better than that reported for intramedullary astrocytomas (Minehan et al. 1995).

The postoperative functional outcome can be graded using a scale developed by McCormick et al. (1990). According to the literature, the McCormick score is not much higher in patients with recurrent tumors after a

second surgery compared to patients without recurrence. In addition, patients with a worse preoperative functional grade are at risk of developing complete loss of function postoperatively (Jallo et al. 2004). Therefore, early surgical intervention is strongly recommended. Furthermore, the extent of tumor resection, gross total versus subtotal, does not seem to significantly influence the functional prognosis (Jallo et al. 2004).

References

Abbott R, Feldstein N, Wisoff JH, Epstein FJ (1992) Osteoplastic laminotomy in children. Pediatr Neurosurg 18:153–156

Albright L, Byrd RP (1980) Ganglioglioma of entire spinal cord. Childs Brain 6:274–280

Allen JC, Aviner S, Yates AJ, Boyett JM, Cherlow JM, Turski PA, Epstein F, Finlay JL (1998) Treatment of high-grade spinal cord astrocytoma of childhood with '8-in-1' chemotherapy and radiotherapy: a pilot study of CCG-945. Children's Cancer Group. J Neurosurg 88:215–220

Chamoun RB, Alaraj AM, Kutoubi AOA, Abboud RR, Haddad GF (2006) Role of temozolamide in spinal cord low grade astrocytomas: results in two pediatric patients. Acta Neurochir (Wien) 148:175–180

Constantini S, Houten J, Miller DC, Freed D, Ozek MM, Rorke LB, Allen JC, Epstein FJ (1996) Intramedullary spinal cord tumors in children under the age of 3 years. J Neurosurg 85:1036–1043

Constantini S, Miller DC, Allen JC, Rorke LB, Freed D, Epstein FJ (2000) Radical excision of intramedullary spinal cord tumors: surgical morbidity and long-term follow-up evaluation in 164 children and young adults. J Neurosurg 93:183–193

Elsberg CA, Beer E (1911) The operability of intramedullary tumors of the spinal cord. A report of two operations with remarks upon the extrusion of intraspinal tumors. Am J Med Sci 142:636–647

Epstein FJ, Farmer JP (1990) Pediatric spinal cord tumor surgery. Neurosurg Clin N Am 1:569–590

Garrido E, Becker LF, Hoffman HJ, Hendrick EB, Humphreys R (1978) Gangliogliomas in children. A clinicopathological study. Childs Brain 4:339–346

Hamburger C, Buttner A, Weis S (1997) Ganglioglioma of the spinal cord. Report of two rare cases and review of the literature. Neurosurgery 41:1410–1415

Henry JM, Heffner RR, Earle KM (1978) Gangliogliomas of CNS: a clinicopathological study of 50 cases. J Neuropathol Exp Neurol 37:626 (Abstract)

Jallo GI, Danish S, Velasquez L, Epstein F (2001) Intramedullary low-grade astrocytomas: long-term outcome following radical surgery. J Neurooncol 53:61–66

Jallo GI, Freed D, Epstein FJ (2004) Spinal cord gangliogliomas: a review of 56 patients. J Neurooncol 68:71–77

Katzman H, Waug T, Berdon W (1969) Skeletal changes following irradiation of childhood tumors. J Bone Joint Surg Am 51:825–842

Kiwak KJ, Deray MJ, Shields WD (1983) Torticollis in three children with syringomyelia and spinal cord tumor. Neurology 33:946–948

Kothbauer KF (2007) Neurosurgical management of intramedullary spinal cord tumors. Pediatr Neurosurg 43:222–235

Kothbauer KF, Deletis V, Epstein FJ (1998) Motor evoked potential monitoring for intramedullary spinal cord tumor surgery: correlation of clinical and neurophysiological data in a series of 100 consecutive procedures. Neurosurg Focus 4:article 1

Lang FF, Epstein FJ, Ransohoff J, Allen JC, Wisoff J, Abbott IR, Miller DC (1993) Central nervous system gangliogliomas. Part 2. Clinical outcome. J Neurosurg 79:867–873

Lantos PL, Vandenberg SR, Kleihues P (1997) Tumors of the nervous system. Neuronal and neuronal-glial tumors. Greenfield's Neuropathol 2:654–677

Lee JC, Glasauer FE (1968) Ganglioglioma: light and electron microscopic study. Neurochirurgia 11:160–169

Lotfinia I, Vahedi P (2008) Intramedullary cervical spinal cord ganglioglioma, review of the literature and therapeutic controversies. Spinal Cord 47:87–90

Mayfield JK, Riseborough EJ, Jaffe N, Nehme AME (1981) Spinal deformity in children treated for neuroblastoma. J Bone Joint Surg Am 63:183–193

McCormick PC, Torres R, Post KD, Stein B (1990) Intramedullary ependymoma of the spinal cord. J Neurosurg 72:523–532

Miller DC, Koslow M, Budzilovich GN, Zagzag D (1990) Synaptophysin: a sensitive and specific marker for ganglion cells in central nervous system neoplasms. Hum Pathol 21:271–276

Minehan KJ, Shaw EG, Scheithauer BW, Davis DL, Onofrio BM (1995) Spinal cord astrocytoma: pathological and treatment considerations. J Neurosurg 83:590–595

Morota N, Deletis V, Constantini S, Kofler M, Cohen H, Epstein FJ (1997) The role of motor evoked potentials during surgery for intramedullary spinal cord tumors. Neurosurgery 41:1327–1336

Otsubo H, Hoffman HJ, Humphreys R, Hendrick EB, Darak JM, Hwang PA, Beckere LE, Chaung SH (1992) Detection and management of gangliogliomas in children. Surg Neurol 38:371–378

Park SH, Chi JG, Cho BK, Wang KC (1993) Spinal cord ganglioglioma in childhood. Pathol Res Pract 189:189–196

Park CK, Chung CK, Choe GY, Wang KC, Cho BK, Kim HJ (2000) Intramedullary spinal cord ganglioglioma: a report of five cases. Acta Neurochir (Wien) 142:547–552

Raimondi AJ, Gutierrez FA, Di Rocco C (1976) Laminotomy and total reconstruction of the posterior

spinal arch for spinal canal surgery in childhood. J Neurosurg 45:555–560

Rifkinson-Mann S, Wisoff JH, Epstein FJ (1990) The association of hydrocephalus with intra- medullary spinal cord tumors: a series of 25 patients. Neurosurgery 27:749–754

Rodewald L, Miller D, Sciorra L, Barabas G, Lee ML (1987) Central nervous system neoplasm in a young man with martin-bell syndrome: fra (X)-XLMR. Am J Med Genet 26:7–12

Sandalcioglu IE, Gasser T, Asgari S, Lazorisak A, Engelhorn T, Egelhof T, Stolke D, Wiedemayer H (2005) Functional outcome after surgical treatment of intramedullary spinal cord tumors: experience with 78 patients. Spinal Cord 43:34–41

Satyarthee GD, Mehta VS, Vaishya S (2003) Ganglioglioma of the spinal cord: report of two cases and review of literature. J Clin Neurosci 11:199–203

Shrivastava RK, Epstein FJ, Perin NI, Post KD, Jallo GI (2005) Intramedullary spinal cord tumors in patients older than 50 years of age: management and outcome analysis. J Neurosurg Spine 2:249–255

Taniguchi M, Cedzich C, Schramm J (1993) Modification of cortical stimulation for motor evoked potentials under general anesthesia: technical description. Neurosurgery 32:219–226

Ulutin HC, Onguru O, Pak Y (2002) Postoperative radiotherapy for ganglioglioma: report of three cases and review of the literature. Minim Invasive Neurosurg 45:224–227

von Eiselsberg A, Marburg O (1917) Zur Frage der Operabilitat intramedullarer Ruckenmarkstumoren. Arch Psychiatr Nervenkrankh 59:453–461

Wood EH, Berne AS, Taveras JM (1954) The value of radiation therapy in the management of intrinsic tumors of the spinal cord. Radiology 63:11–24

Yasuoka S, Peterson HA, MacCarty CS (1982) Incidence of spinal column deformity after multilevel laminectomy in children and adults. J Neurosurg 57:441–445

Spinal Angiolipoma: Diagnosis and Treatment

28

Miguel Gelabert-González, Ramón Serramito-Garcia, and Eduardo Aran-Echabe

Contents

Abstract

Spinal angiolipomas are benign uncommon neoplasms originating in mesenchymal tissue, and are composed of mature lipocytes admixed with abnormal blood vessels. Angiolipomas frequently develop in the epidural space of the spine and more rarely in the orbit, the cavernous spaces, thalamus, and account for only 0.04–1.2% of all spinal tumors. These neoplasms can be categorized into two subtypes: non-infiltrating and infiltrating. The former is more common and remains confined to the epidural space whereas the latter invades the contiguous bone and adjacent soft tissues.

Spinal angiolipomas are predominantly located in the mid-thoracic region; hence, most patients present slowly progressive signs of spinal cord compression secondary to epidural mass. Angiolipomas show iso- or hyperintensity on T1-weighted images and hyperintensity on T2-weighted images, and most lesions enhance with gadolinium administration. The treatment for spinal extradural angiolipomas is surgical resection. Complete removal of an epidural non-infiltrating angiolipoma is possible in most cases and the prognosis is excellent and no adjuvant therapy should be administered. For infiltrating neo plasms complete removal may entail complications due to heavy bleeding.

M. Gelabert-González (✉) • R. Serramito-Garcia • E. Aran-Echabe
Department of Neurosurgery, University of Santiago de Compostela, Santiago de Compostela, Spain
e-mail: Miguel.gelabert@usc.es

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_28,

Introduction

Spinal angiolipomas are benign tumors composed of both mature fatty tissue and abnormal vascular elements that represent a distinct clinical and pathological entity. These lesions are most commonly found in the subcutaneous tissue of the trunk and extremities, but other sites have been reported as well. They account for 0.04–1.2% of all spinal axis tumors, and are predominantly found in the epidural space, where they represent 2–3% of spinal tumors (Gelabert-González and García-Allut 2009).

The first case of spinal angiolipoma was reported by Berembruch (1890) in a doctoral dissertation in a 16-year-old boy with numerous cutaneous lipomas who developed a progressive paraparesis with hyperreflexia. The tumors were removed, but the boy died in the first postoperative hours. The autopsy revealed a thoracic cutaneous lipoma infiltrating the spinal canal compressing the spinal cord from C6 to T5. The tumor showed fat cells with a predominantly vascular component. At the turn of the twentieth century, Liebscher (1901) was the first to describe a spinal angiolipoma, however, the term angiolipoma was defined by Howard and Helwig (1960) as an anatomopathological entity containing mature fat cells and proliferating vessels. A review of the literature since 1892–2007 revealed 123 cases of spinal angiolipomas (118 extradural and 5 intradural) (Gelabert-Gonzalez and Garcia-Allut 2009), since then, only 14 new cases have been reported.

Epidemiology

According to Gelabert-Gonzalez and Garcia-Allut (2009) Spinal angiolipomas account for 0.14–1.2% of all spinal tumors, of which 2–3% are extradural spinal tumors, and 16–35% spinal lipomas. Although most angiolipomas occur in adults, they have been reported in all age groups with a range of 1.5–85 years (mean 44), and peak incidence in the fourth to fifth decades of life. A female predominance was observed (ratio 1.4:1), but no racial or geographic predominance has been found.

Topography

Angiolipomas are benign tumors which usually appear as painful subcutaneous nodules, particularly in the forearm, trunk, or neck but occur rarely in the spinal canal. Prior to the five reports of intramedullary angiolipomas by Palkovic et al. (1988), Preul et al. (1993), Maggi et al. (1996), Klisch et al. (1999) and Weill et al. (1991), these were thought to occur exclusively in the epidural space. A further six cases of intracranial angiolipomas have been described: two were parasellar i.e., adherent to the dura and in intimate contact with the cavernous sinus, and the others located in the cortico-subcortical area of the left frontal lobe.

Location: As reported by Gelabert-González and García-Allut (2009) about 90% of spinal angiolipomas were in the thoracic region and most extended 2–3 vertebral segments. An explanation for the thoracic predominance of spinal angiolipomas may lie in the spine's regional variation in blood supply, particularly the mid-thoracic spine which is the least perfused. Neovascularization in ischemic tissues is known to occur and is thought to be triggered by an angiogenic factor (Labram et al. 1999).

Histogenesis

The histogenesis of angiolipomas is poorly understood and several theories have been advanced. Ehni and Love (1945) postulate that, following some undefined stimulus, angiolipomas arise from pluripotential mesenchymal stem cells by divergent differentiation along both adipose tissue and angioid lines (smooth muscle and vascular endothelium). Willis (1948) considered these to be a congenital malformation or a benign hamartoma, but most would now agree that angiolipomas and mesenchymal hamartomas arise from primitive mesenchyme. The strong predilection of infiltrating angiolipomas for anterior localization is thought to be due to the early inclusion of the immature pluripotent stem cells in the ventral and lateral spinal canal.

The notion of an interperiosteo-dural space in the development of angiolipomas has been recently proposed by Francois et al. (2010). This space represents an anatomical continuum extending from the coccyx to the orbit, and contains fat tissue that is abundant at the level of the orbit and the epidural spinal space, and sparser at the level of the cavernous spaces. This interperiosteo-dural space is improperly called the epidural space. It contains an important quantity of fat tissue that facilitates the movements of the dural sheath of the spine in the spinal canal, which is covered by the osteoperiosteal layer. Angiolipomas develop from this fat tissue (François et al. 2010).

Hemangiomas and lipomas may represent a spectrum within which angiolipomas constitute an intermediate entity (Fourney et al. 2001). The more invasive infiltrating type of spinal angiolipoma would then represent a shift towards the hemangioma end of the spectrum (Padovani et al. 1982). Pagni and Canavero (1992) support the theory of an abnormal developmental origin, based on their findings of spinal angiolipomas in patients with birth defects outside the central nervous system (CNS). Spinal lipomas differ from angiolipomas in that they are most commonly found in the lumbosacral region, rather than midthoracic region, and may be associated with dysraphic abnormalities. Provenzale and Mclendon (1996) consider spinal angiolipomas to be more aggressive than spinal lipomas.

Pregnancy was an aggravating factor in 15% of diagnosed women and termination of pregnancy, as in the case reported by Preul et al. (1993) may bring about regression of symptoms. Pregnancy may interfere with spinal venous drainage and increase epidural venous pressure from compression of pelvic and abdominal veins; the redistribution of blood, the increase in extracellular fluid, and the forceful Valsalva maneuvers associated with vaginal delivery may acutely exacerbate symptoms (Balado and Morea 1928; Provenzale and McLendon 1996). Others possible factors reported are vascular steal phenomenon as a cause of spinal cord ischemia and pulsatile compressive effect on the adjacent cord because of its vascularity (Turanzas et al. 1994). Hormonal influences and increased adiposity produced by pregnancy may also cause an increase in the size of spinal angiolipomas, as much as obesity may lead to symptoms induced by increasing their fatty component as occurs in patients who receive steroid treatment or are overweight (Gelabert-González and García-Allut 2009). Tumor thrombosis and/or hemorrhaging may cause a sudden deterioration as occurred in two cases reported by Labram et al. (1999) and Anson et al. (1990).

Pathology

Macroscopically, the tumor is an encapsulated or uncapsulated, reddish soft mass extending into the extradural space of the spinal canal. There are subdivided into two subtypes which should be considered and treated differently: non-infiltrating and infiltrating, the later extending into the vertebral body (Lipson et al. 1980). Gonzalez-Crussi et al. (1966) described the first case of vertebral "infiltrating" angiolipoma and stated that this tumor should be distinguished from angiolipomas described by Howard and Hellwig (1960). Lin and Lin (1974) established infiltrating angiolipoma as a separate entity, justifying their decision on differences in clinical course, pathological picture, and prognosis from those of "plain angiolipomas". Histologically, they are composed of mature fat cells and blood vessels, features of which are described as being either normal or mimicking capillary angioma, cavernous angioma or arteriovenous malformations (Fig. 28.1). The fatty tissue is of the adult type and shows no remarkable findings (Pinto-Rafael et al. 2002). The ratio of fat to vessels is variable and ranges from 1:3 to 2:3. When an abundance of smooth muscle fibers are noted on microscopy, the lesion is further sub-classified as angiomyolipomas. A thin capsule, often defective in many areas, may surround the lesion. Secretory activity has been described in one case with lipid-like material in perivascular granules (Bardosi et al. 1985). No atypia, pleomorphism, mitotic figures or karyotypical abnormalities were found (Andaluz et al. 2000), but in the case reported by Fourney et al. (2001) mild atypia was

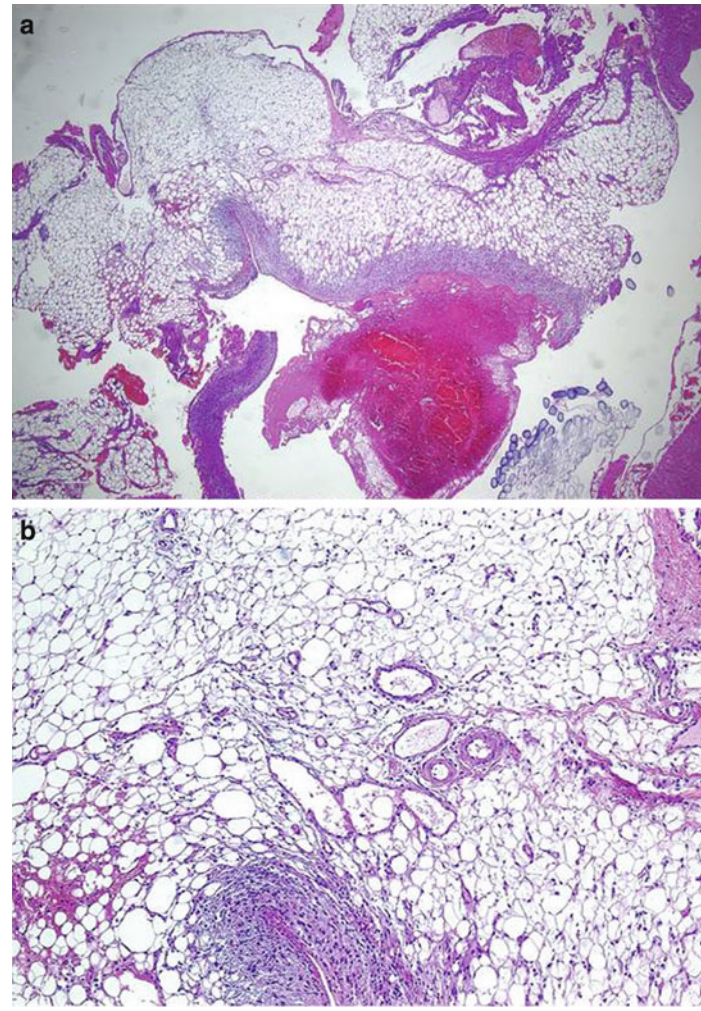

Fig. 28.1 H&E 20× (**a**) and 80× (**b**) contained mature, normal appearing adipose tissue and small-medium caliber blood vessels indicative of angiolipoma

observed in the endothelial cells. Otherwise associations have also been reported with an intramedullary glioblastoma and osteochondroma. In two cases described by Pearson et al. (1970), additional osteoid tissue was observed. Inmunoshistochemical assay was performed in a few cases, and a low proliferation rate was found on Ki-67 (Pinto-Rafael et al. 2002). Samdami et al. (2004) observed a positive stain for CD31, Factor XIIIa, and Factor VIII, and negative for Glio Fibrilar Acid Protein.

Sciot et al. (1997) analysis of the karyotype in 20 angiolipomas found that all were normal; the authors speculate that a normal karyotype supports the theory of angiolipomas as a reactive or hamartomatous lesion. They also suggest that vascular proliferation could be a primary event in the development of these tumors, and the adipose tissue, a secondary, albeit prominent, component.

Clinical Symptoms

From a clinical point of view, spinal angiolipomas do not differ from other benign space-occupying spinal lesions. They generally produce spinal cord compression, and back pain is a frequent initial complaint at presentation (Pagni and Canavero 1992). Subjective complaints are mostly of sensory disorders and motor deficits below the level of the lesion that often progress to weakness in

the lower-limbs for long periods with sphincter dysfunction in the later stages (Gelabert-González and García-Allut 2009).

From the clinical point of view, Gelabert -Gonzalez and García Allut (2009) reports most patients exhibited a progressive clinical course over a period of a few hours to several years. The interval between the initial symptoms and tumor diagnosis ranged from 1 day to 17 years (mean interval 20 months). The duration of symptoms was longer in patients with lumbar pain (1 year), whereas the shortest duration of symptoms occurs in patients involving sudden motor deficit (<1 week) (Gelabert-González and García-Allut 2009). Eight patients with a relapsing clinical course presented paraparesis during pregnancy or puerperyum (Gelabert-González and García-Allut 2009). Other authors consider overweightness and the administration of steroids as symptomatologically aggravating factors (Fourney et al. 2001). Regardless of the duration, rapid deterioration has occurred in a few cases due to factors such as venous thrombosis, tumoral hemorrhage, vascular steal phenomenon, and intratumoral abscess (Anson et al. 1990). In a few cases, a relapsing and remitting course mimicking demyelinating disorder was reported.

Diagnosis

Biochemical studies: Cerebrospinal fluid samples were analyzed in a few cases and slight protein *concentrations were observed* (range 0.70–1.41 mg/dl).

Neurophysiological study: only two reported neurophysiologic cases have been reported i.e., Bailey et al. (2000) patient where the lower limb somatosensory evoked responses were normal, and the case published by Nishiura et al. (1986) where the electromyogram showed a neurogenic pattern in the paravertebral muscles from T8 to T10 bilaterally.

Radiology: X-rays of the spine essentially show no bone abnormalities. The most frequent alterations were erosion of the walls of the vertebras (bodies and pedicles) with enlargement of the interpeduncular distances primarily in infiltrated tumors. Other cases were associated to scoliosis, osteoporosis or Klippel-Feil syndrome (Bardosi et al. 1985; Bucy and Ritchey 1947; Nishiura et al. 1986). In two infiltrating angiolipomas bone erosion had a coarsened trabecular pattern characterisitic of vertebral hemangioma, but no calcifications were found.

Myelography: was the most common radiologic technique for tumor identification prior to 1988; the findings, however, were not specific and showed partial or total blocking of the contrast column that was also characteristic of other extradural lesions compressing the spinal cord (Nishiura et al. 1986; Padovani et al. 1982).

Angiography: Selective spinal angiography was rarely used in the diagnosis of spinal angiolipomas and, of the few cases reported, prominent vascularity in the lateral aspect of the T9 vertebral body was observed in one case (Rabin et al. 2004), and in T6 in the other (Andaluz et al. 2000). In both cases the tumors were supplied by a branch of the intercostals artery.

Computed Tomography CT scan has lost relevance in the diagnosis of spinal tumor; however, CT Scans are useful to visualize lesions affecting the bone such as trabeculation of the vertebral body, erosion of the vertebral body and pedicles, the presence of paravertebral component or/and association with a vertebral tumor such as a hemangioma (Klisch et al. 1999; Weill et al. 1991). In some cases tumor calcifications it can be observed (Rabin et al. 2004). In most of the reported cases, the tumor was hypo dense with the cord (between −20 and −72 HU), and less frequently isodense, slight hyperdensity, or heterogeneous (Weill et al. 1991). After contrast administration tumors generally showed irregular enhancement (Palkovic et al. 1988).

Rubin et al. (1992) report that CT scan revealed dense fatty tissue in most of the extradural angiolipomas. Though CT scan usually demonstrates a hypodense lesion, some tumors are isodense or hyperdense depending on the extent of the vascular

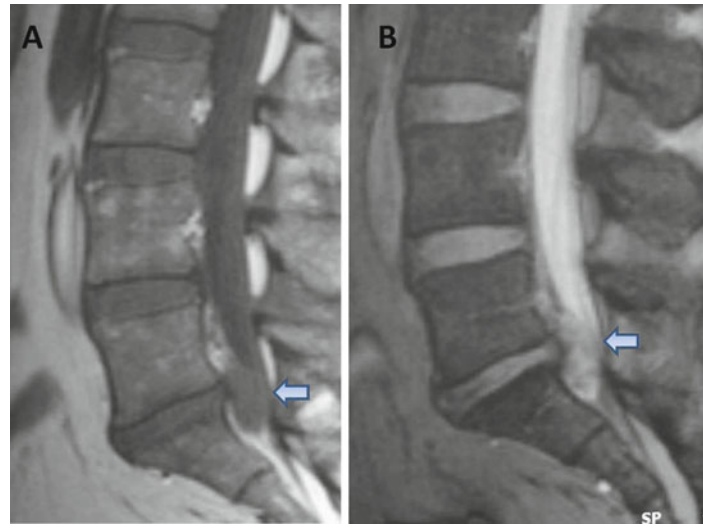

Fig. 28.2 Preoperative sagittal MRI T1-weighted image (TR 560, TE 14) (**a**) T2-weighted image (TR 400, TE 15) (**b**) showing an L5-S1 extradural slightly inhomogeneous epidural mass (*arrows*)

component or the presence of calcification. Pagni and Canavero (1992) suggest that CT is not specific for spinal epidural angiolipomas and can be misleading.

Magnetic resonance imaging (MRI) is the most valuable radiological technique for diagnosing spinal angiolipomas. The variability of the vascular and adipose elements of the tumor causes a significant heterogeneity in imaging studies. On non-contrast T1-weighted images, most of angiolipomas are typically hyperintenses due to their fatty content; however, there are some reports of isointense, hypointense on heterogeneous angiolipomas (Preul et al. 1993; Weill et al. 1991).

In most T2-weigthed sequences, the tumor was hyperintense as compared to cerebrospinal fluid (CSF), but isointense, hypointense or heterogeneous signal have also been reported (Weill et al. 1991) (Fig. 28.2). Following Gd administration, most cases presented remarkably homogeneous enhancement though poor uptake and irregular or heterogeneous enhancements have been described. Gadolinium enhancement may go undetected due to the hyperintensity of angiolipomas on T1-weighted imaging, but gadolinium infusion with fat suppression sequences may enhance areas of abnormal signal intensity within fatty tumors. Moreover, MRI enables the imaging of disc hernia, infiltration of the vertebral body and/or extending to the pedicle, and a paravertebral thoracic component (Andaluz et al. 2000).

Provenzale and Mclendon (1996) showed that large hypointense foci observed within spinal angiolipomas on non-contrast T1-weighted images are correlated with increased vascularity and most lesions enhance with gadolinium administration whereas T2-weighted imaging can be variable but is often hyperintense. Significant heterogeneity in imaging is attributed to the variable vascular and adipose elements of the tumor. The enhancement after Gadolinium administration is due to the vascularity of these tumors; this phenomenon allows differential diagnosis between extradural lipomatosis and spinal angiolipoma, as the former is not enhanced. Non-infiltrating angiolipomas are generally located in the posterior portion of the epidural space, are well delimited from the surrounding tissue, and can usually be removed easily by laminectomy.

Treatment

Spinal angiolipomas are treated exclusively by surgical removal of the lesion. In cases of dorsally

located, non-infiltrating lesions a gross total resection of the tumor and associated capsule is usually achievable. The tumor usually has no adhesions to the dura and can be removed with no difficulty through a laminectomy (Gelabert-González and García-Allut 2009; Pagni and Canavero 1992).

Total removal of infiltrating angiolipomas, that often involve the body of the vertebral than the posterior arch, has been recommended using the anterolateral approach and stabilization of the affected vertebrae is desirable (Gelabert-González and García-Allut 2009). In these lesions, pre-operative angiography and embolization followed by an anterior approach has been described and may provide an opportunity for greater spinal decompression and tumor removal (Rabin et al. 2004). Some authors believe that the tumor-invaded vertebral body should be preserved because, analogous to vertebral hemangiomas, spinal angiolipomas may not enlarge. As these lesions are histopathologically benign and slow growing, no adjuvant therapy in the form of chemotherapy or radiation is required and once adequate surgical decompression of neural elements has been achieved, no further treatment is required. However, in three reported cases, postoperative radiotherapy was administered following a partial excision owing to concerns of potential malignancy (Anson et al. 1990; Bucy and Ritchey 1947; González-Crussi et al. 1966).

Most authors report good outcomes after surgical excision of spinal angiolipomas in spite of severe preoperative neurological deterioration (Andaluz et al. 2000). Only one case of recurrence of an angiolipoma has ever been reporter with successful surgery 12 years after the first intervention (Bender et al. 1974) and none has been reported in all the other cases of infiltrating angiolipomas, even in cases in which a complete removal could not be attained (Rabin et al. 2004). Furthemore, it seems reasonable to suggest that surgical intervention is urgent when there is a sudden clinical deterioration such as occurred in some cases in the literature (Preul et al. 1993; Gonzalez-Crussi et al. 1966).

Prognosis and Outcome

The prognosis of spinal angiolipomas is very favorable and only few cases of death have been reported. Most of this cases being very old references and no clear indication as to the cause of death (Balado et al. 1928; Berenbruch 1890). More recently, one patient who had never undergone surgery died from a cervical glioblastoma (Howard and Helwig 1960) and the other from a respiratory infection.

Tumor recurrence following surgery is rare, and only two cases (1.8%) were reported, one after gross-total removal (Bender et al. 1974) and the other, a infiltrating tumor following subtotal resection. In a review of the literature, outcome was no worse in the infiltrating than in the non-infiltrating lesions and was independent of the completeness of the removal. The postoperative course was diverse ranging 3 months to 10 years. Overall, there were two deaths (1.8%) in patients with non-infiltrating angiolipomas (Bender et al. 1974) and none in the infiltrating subgroup (Gelabert-González and García-Allut 2009). Though the results of surgery are difficult to assess due to the variations in terminology in the literature, most patients were reported to have improved postoperatively.

References

Andaluz N, Balko G, Bui H, Zuccarello M (2000) Angiolipomas of the central nervous system. J Neuroncol 49:219–230

Anson JA, Cybulski GR, Reyes M (1990) Spinal extradural angiolipoma: a report of two cases and review of the literature. Surg Neurol 34:173–178

Bailey D, Andrews J, Mazur E, Cleary J (2000) Dorsal thoracic cord compression from a spinal angiolipoma: case report and brief comment. Conn Med 64:267–269

Balado M, Morea R (1928) Extramedular haemangioma producing paraplegia during pregnancy. Arch Argent Neurol 1:345–351

Bardosi S, Schaale TH, Friede RL, Roessmann U (1985) Extradural spinal angiolipoma with secretory activity. An ultrastructural, clinicopathological study. Virchows Arch 406:253–256

Bender JL, Van Landingham JH, Manno NJ (1974) Epidural lipoma producing spinal cord compression. J Neurosurg 41:100–103

Berenbruch K (1890) Association of multiples angiolipomas and angioma of spinal cord, Tubingen: H Laupp, Jr

Bucy PC, Ritchey H (1947) Klippel-Feil syndrome associated with compression of spinal cord by an extradural hemangiolipoma. J Neurosurg 4:476–481

Ehni G, Love JG (1945) Intraspinal lipomas: report of cases, review of the literature, and clinical and pathological study. Arch Neurol Psychiatry 53:1–28

Fourney DR, Tong KA, Macaulay RJ, Griebel RW (2001) Spinal angiolipoma. Can J Neurol Sci 28:82–88

François P, Zemmoura I, Fouquet AMB, Jan M, Velut S (2010) Lateral sellar angiolipoma: a tumor illustrative of the extradural compartment of the neural axis. J Neurosurg 113:1053–1058

Gelabert-González M, García-Allut A (2009) Spinal extradural angiolipoma: report of two cases and review of the literatura. Eur Spine J 18:324–335

Gonzalez-Crussi F, Enneking WF, Arean VM (1966) Infiltrating angiolipoma. J Bone Joint Surg Am 48A: 1111–1124

Howard WR, Helwig EB (1960) Angiolipoma. Arch Dermatol 82:924–931

Klisch J, Spreer J, Bloss HG, Baborie A, Hubbe U (1999) Radiological and histological findings in spinal intramedullary angiolipoma. Neuroradiology 41:584–587

Labram EK, El-Shunnar K, Hilton DA, Robertson NJ (1999) Revisited: spinal angiolipoma, three additional cases. Br J Neurosurg 13:25–29

Liebscher C (1901) Angiolipoma of the spinal canal with compression of the spinal cord. Pag Med Wochenschr 26:189–191

Lin JJ, Lin F (1974) Two entities in angiolipomas. A study of 459 cases of lipoma with review of literature on infiltrating angiolipoma. Cancer 34:720–727

Lipson SJ, Naheedy MH, Kaplan MM, Bienfang DC (1980) Spinal stenosis caused by epidural lipomatosis in Cushing's syndrome. N Engl J Med 302:336

Maggi G, Aliberti F, Colucci MR, Petrone G, Dorato P, De Giorgi AM (1996) Spinal intramedullary angiolipoma. Childs Nerv Syst 12:346–349

Nishiura I, Kubo Y, Koyama T (1986) Spinal haemangiolipoma: three case reports. Neurochirurgia (Stuttg) 29:63–66

Padovani R, Tognetti F, Speranza S, Pozzati E (1982) Spinal extrathecal hemangiolipomas: report of two cases and review of the literature. Neurosurgery 11:674–677

Pagni CA, Canavero S (1992) Spinal epidural angiolipoma: rare or unreported? Neurosurgery 31:758–764

Palkovic S, Wassmann H, Bonse R, Kashab M (1988) Angiolipoma of the spinal cord. Magnetic resonance imaging and microsurgical management. Surg Neurol 29:243–245

Pearson J, Stellar S, Feigin I (1970) Angiomyolipoma: long term cure following radical approach to malignant-appearing benign intraspinal tumor-report of 3 cases. J Neurosurg 33:466–470

Pinto-Rafael JI, Vázquez- Barquero A, Martín-Laez R, García-Valtuille R, Sanz-Alonso F, Figols-Guevara FJ, Pérez-Castro MC, Abascal F, Cerezal L (2002) Spinal angiolipoma. Report of one case. Neurocirugia 13: 321–325

Preul MC, Leblanc R, Tampieri D, Robitaille Y, Pokrupa R (1993) Spinal angiolipomas. Report of three cases. J Neurosurg 78:280–286

Provenzale JM, McLendon RE (1996) Spinal angiolipomas: MR features. AJNR 17:713–719

Rabin D, Hon BA, Pelz DM, Ang LC, Lee DH, Duggal N (2004) Infiltrating spinal angiolipoma. A case report and review of the literature. J Spinal Disord Tech 17:456–461

Rubin G, Gornish M, Sandbank J, Shevach I, Rappaport ZH (1992) Spinal extradural angiolipoma. Case report and review of the literature. Spine 17:719–724

Samdani AF, Garonzik IM, Jallo G, Eberhart CG, Zahos P (2004) Spinal angiolipoma: case report and review of the literature. Acta Neurochir (Wien) 146: 299–302

Sciot R, Akerman M, Dal Cin P, De Wever I, Fletcher CD, Mandahl N, Mertens F, Mitelman F, Rosai J, Rydholm A, Tallini G, Van den Berghe H, Vanni R, Willen H (1997) Cytogenic analysis of subcutaneous angiolipoma: further evidence supporting its difference from ordinary pure lipomas. A report of the CHAMP Study Group. Am J Surg Pathol 21:441–444

Turanzas FS, Domínguez J, Eyerbe P, Dhimes P (1994) Infiltrating spinal angiolipoma. Case report. Neurocirugia 5:246–249

Weill A, del Carpio-O'Donovan R, Tampieri D, Melanson D, Ethier R (1991) Spinal angiolipomas: CT and MR aspects. J Comput Assist Tomogr 15:83–85

Willis RA (1948) Pathology of tumors. Butterworths, London, p 700

Spinal Cord Injury: Tissue Engineering Using Neural Stem Cells

29

Deniz Yucel, Irem Ayse Kanneci, Damla Arslantunali, Gamze Torun Kose, and Vasif Hasirci

Contents

Abstract

Spinal cord injuries result in catastrophic dysfunctions that impair the quality of a patient's life. Following spinal cord injury (SCI), the cascade of cellular and biochemical reactions during primary and secondary injuries and a dense scar formation leads to a devastating physical and chemical barrier at the lesion site. These effects could not be reversed through conventional treatments. A multidisciplinary approach that includes materials science, engineering, biology, chemistry, and medicine is required to achieve a completely successful treatment for SCI. Tissue engineering, which integrates scaffolds, autologous (preferably) cells, and growth factors, is an encouraging development in the treatment of SCI which aims to replace and restore the anatomical and functional structure of the damaged spinal cord. Neural stem

D. Yucel (✉)
Department of Histology and Embryology, School of Medicine, Acibadem University, 34848 Istanbul, Turkey

BIOMATEN, CoE in Biomaterials and Tissue Engineering, 06800 Ankara, Turkey
e-mail: deniz.yucel@acibadem.edu.tr

I.A. Kanneci • G.T. Kose
Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, 34755 Istanbul, Turkey

BIOMATEN, CoE in Biomaterials and Tissue Engineering, 06800 Ankara, Turkey

D. Arslantunali
Department of Biotechnology, BIOMAT, Middle East Technical University, 06800 Ankara, Turkey

BIOMATEN, CoE in Biomaterials and Tissue Engineering, 06800 Ankara, Turkey

V. Hasirci
Departments of Biotechnology and Biological Sciences, BIOMAT, Middle East Technical University, 06800 Ankara, Turkey

BIOMATEN, CoE in Biomaterials and Tissue Engineering, 06800 Ankara, Turkey

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_29,

cells (NSCs), by differentiating into the cells of the nervous system, are a promising cell source for use in this challenging approach which has a great potential in the therapy of spinal cord injuries.

Introduction

Spinal cord injury (SCI) is among the most devastating disorders that impair the quality of a patient's life. The annual incidence of SCI in the Unites States is estimated to be 40 cases per million population according to the National Spinal Cord Injury Statistical Center (2011). The most common causes of SCI have been reported in 2011 as motor vehicle accidents (40.4%), falls (27.9%), acts of violence especially gunshot wounds (15%), and sports (8%) since 2005.

Spinal cord is an elongated cylindrical part of central nervous system (CNS) and contains bundles of nerves which carry nerve impulses along the spinal tract between the brain and the rest of the body (Snell 1992). It is composed of an inner core of gray matter, contains nerve cell bodies and associated nerve fibers, and an outer covering of white matter with descending and ascending tracts (columns) of myelinated axons. The spinal cord is surrounded by three meninges, the dura mater, the arachnoid mater and the pia mater, and is situated within the rings of bones, called vertebra, which constitute the spinal column. In addition to these protective structures, the cerebrospinal fluid found in the central canal and the subarachnoid space cushions and protects the spinal cord.

The degree of neurological defects varies depending on the extent and level of the SCI (Legos et al. 2002). A complete injury is defined by the total absence of motor and sensory function below the level of the injury which means that the patient has no sensation and voluntary movement. However, in an incomplete injury either the motor or the sensory function is present below the level of injury. Thus, a patient with an incomplete injury may be able to feel but can not move certain parts of the body. The other important criterion is the level of injury which is highly related with the parts of the body that might be affected by paralysis and loss of function. According to their location the vertebra is classified from top to bottom (head-to-toe direction) as cervical vertebra (the eight vertebra in the neck; C-1 to C-8), thoracic vertebra (the twelve vertebra in the chest; T-1 to T-12), lumbar vertebra (in the lower back between the thoracic vertebra and the pelvis), and sacral vertebra (from pelvis down to the end of the spinal cord) (Snell 1992). Generally more dysfunction is expected in patient with SCI in the higher levels of the spinal column. SCI at cervical region usually results in quadriplegia or tetraplegia, and causes loss of function in the arms and legs or complete loss of sensation below the neck (Legos et al. 2002). Moreover, patients having injuries above C-4 level lose spontaneous respiration capability and may require a ventilator to breathe. Thoracic injuries usually affect the chest and the legs, and result in paraplegia, with the hands being not affected. Compared to the other parts, injuries at lumbar and sacral regions result in lesser effects, like decreased control of the legs and hips, and urinary system. Since 2005, the most frequent neurologic categories are reported as follows; incomplete tetraplegia (39.5%), complete paraplegia (22.1%), incomplete paraplegia (21.7%) and complete tetraplegia (16.3%) (National Spinal Cord Injury Statistical Center 2011).

In traumatic SCI, an external physical insult leads to neurological damage because of interruption of communication pathways between the brain and the periphery. The SCI is usually caused by blunt impact, compression, and penetrating trauma, and may result in complete transection. The complex pathophysiology of SCI starts with the mechanical damage to the neurons caused at the time of trauma, called primary injury, which triggers the secondary injury, a cascade of cellular and biochemical reactions (Legos et al. 2002). Formation of free radicals, lipid peroxidation, accumulation of excitatory neurotransmitters, disruption of ionic homeostasis, activation of inflammatory response are among the mechanisms that are parts of the secondary injury that leads to generation of an inhibitory environment for regeneration and repair. The resultant injury leads to necrosis and apoptosis of neurons and glia which in turn results in cystic cavity formation.

The loss of oligodendrocytes results in demyelination of axons, and impairs nerve impulse propagation. Following the tissue loss the cystic cavity is surrounded by reactive astrocytes due to astrogliosis (excessive increase in astrocyte numbers), and ends up with a dense scar tissue upon deposition of collagen. The growth of axons across the cavity is not sufficient, and the injured tissue is not able to achieve self-regeneration due to the presence of the inhibitory environment and the scar tissue which acts as a physical and chemical barrier.

Treatment Strategies in Spinal Cord Injury

Today, there still is no completely successful treatment of spinal cord injury. The routine clinical treatment for SCI is to stabilize the spine and restore its proper alignment along with surgical decompression of the cord. However, the decompressive surgery is a controversial issue regarding the appropriate time for surgical intervention and the ability of surgery to promote neurological recovery. The researchers have been trying to understand the neuronal behavior within the human spinal cord and the studies have been focused on functional training approaches. Neurorehabilitation is a method used for functional recovery of such disorders. For the therapy to be effective it is important to understand the reflexes and motor centers. So, it is necessary to evaluate neuronal function and biomechanical properties. Basically rehabilitation in functional training approaches should aim an improvement of function by taking advantage of the plasticity of the neuronal center. However, a single therapy as neurorehabilitation would not be sufficient to overcome the problems that arise from the secondary injury and scar formation in SCI; therefore, it should be accompanied by other inhibitory and/or regenerative therapies.

The scientific challenges for the treatment of SCI can be summarized under two main topics: neuroprotection and neural regeneration. Neuroprotection aims to prevent the secondary injury mechanisms and/or to minimize the extent of damage caused by autodestruction of neurons and the tissue. Drugs have been used in spinal cord repair as neuroprotective agents to provide functional inhibition of molecules that prevent axonal regeneration. The most promising neuroprotective drug for SCI is methylprednisolone, a synthetic glucocorticoid steroid with anti-inflammatory activity, and is effective in attenuating particular secondary injury processes if administered properly and in time (Anderberg et al. 2007). There are also other drugs, like monosialotetrahexosylganglioside (GM-1), thyrotropin-releasing hormone (TRH), gacyclidine (GK-11), minocycline, and nimodipine, which have neuroprotective effects due to their antioxidant, anti-inflammatory or channel blockage properties. However, the dosage, timing and the way of administration are some technical constraints that should be considered when planning drug administration. Researchers have been investigating epidural drug delivery from injectable hydrogels as a more localized method that prevents the loss of dosage.

The present studies in SCI are particularly focused on neural regeneration. In neuroregeneration, the axonal growth and the neural plasticity are promoted to reorganize the injured spinal cord by forming new neural connections. A milestone in this field was the study carried out by David and Aguayo (1981) which changed the belief that injured axons are not able to regenerate after SCI. A peripheral nerve tissue graft was used to build a bridge as a guide along which axons could form connections and restore the function. It was observed that the axons could be regenerated by providing such a permissive environment, but they failed to elongate when faced with the native CNS tissue. Later, several more regeneration obstacles like nerve cell disability, an insufficient growth response by the injured neurons, and inhibitory environmental factors like NOGO-A and myelin-associated glycoprotein (MAG 13) produced by oligodendrocytes have been identified (Anderberg et al. 2007). In order to solve these problems various regeneration strategies have been developed to modify and/or eliminate the inhibitory properties of the injured spinal cord tissue. Several promising approaches, such as injection of macrophages to enhance the regeneration and removal of chondroitin sulfate proteoglycans to prevent scar

formation, were developed. Neuroregeneration strategies for SCI require the outgrowth of existing and new axons across the lesion site and their remyelination in order to achieve the ultimate goal, the functional recovery of the injured tissue. The potential strategies proposed to provide regeneration and to improve both the pathological and functional outcome in SCI mainly include the use of cell-free biomaterials, the therapy via cell transplants, and a combination of these strategies which is tissue engineering with cell seeded scaffolds.

Tissue Engineering Approaches for Spinal Cord Injury

Recent advances in nerve regeneration show that a combinatorial approach like tissue engineering, which integrates scaffolds, cells, and growth factors, is a creative strategy to achieve a completely successful treatment for SCI. The ultimate goal of tissue engineering in the treatment of SCI is to replace and restore the anatomical and functional structure of the damaged spinal cord. Cell transplantation, involving introduction of cells without scaffolds, encounters some problems like poor cell localization and survival, and uncontrolled differentiation following transplantation. Cell survival is enhanced when the cells are introduced within a cell carrier (scaffold), and thus, their integration and the success rate of their differentiation into appropriate cell types could be improved. This is to be expected because the regenerative process in the injured spinal cord is affected by the absence of extracellular matrix at the lesion site. In the presence of a scaffold, an extracellular matrix analog, the transplanted cells can be directed and organized to promote regeneration in the injured spinal cord. This is because these scaffolds serve as a bridge for the regenerating axons and guide them from one end of the injury site to the other end. In addition, the presence of cellular components in or on the scaffolds increases the healing effects and might shorten the healing time. Consequently, tissue engineering, a new perspective on SCI therapy, is an effective strategy that combines cells, scaffolds and growth factors for proper regeneration of a damaged spinal cord.

Cell Sources Used in the Treatment of Spinal Cord Injury

In SCI, various cell sources are being tested in *in vitro* and *in vivo* studies for their neurogenic and/or neuroregenerative potential. The main goal of the cellular therapy is to fill the cavities (gaps) formed in the injured region by transplantation of cells with or without scaffold. The dead cells that result as a consequence of SCI can be replaced with new neuronal and support cells, such as Schwann cells and/or olfactory ensheathing cells, to restore myelination and/or for the release of neurotrophic factors for axonal regeneration and functional improvements. Another active area in the treatment of SCI is the use of stem cells. These cells are capable of self-renewal and proliferation, and therefore, have a great potential in cellular transplantation for the treatment of degenerative disorders. The stem cells of pluri- and multipotent nature are able to produce specialized, differentiated progeny, and having neurogenic differentiation potential and the plasticity of neurons make them an appropriate cell source for use in the treatment of SCI. These cells can be used to directly replace the damaged cells (neurons, astrocytes, and oligodendrocytes). The stem cells can also be used in undifferentiated state as support cells to release particular growth factors necessary for neural regeneration or are differentiated into neuron or support cells *in vivo* after transplantation. In addition, the stem cells become adapted to the environment, and consequently evolve with the pathology of the spinal cord to provide a sustainable treatment via neuroprotective and neuroregenerative mechanisms.

Embryonic Stem Cells

Embryonic stem cells (ESCs) derived from inner cell mass of the blastocyst are pluripotent cells that are able to differentiate into derivatives of all

the three germ layers, like the neurons and the support cells in the CNS. However, there are some significant issues like teratoma formation that should be considered in cellular transplantation. There are a large number of studies on implantation of ESCs for SCI repair both *in vitro* and *in vivo*. The results have supported the idea that ESCs can survive, differentiate into neurons, and provide axonal regeneration and remyelination, and functional recovery. Since the surrounding of the injury site is not suitable for ESC survival and differentiation, the main strategy is the predifferentiation of cells into neuronal cells and thus improve their regeneration potential before transplantation for treatment of SCI (Cui et al. 2011). The neural cell adhesion molecule L1, a member of the immunoglobulin superfamily, enhances neurite outgrowth and survival. In a recent study, L1 molecule expressing ESCs, which were transfected with a plasmid encoding L1 molecule, were differentiated through SENA (substrate-adherent embryonic stem cell-derived neural aggregates) procedure before implantation (Cui et al. 2011). The results showed a better cell survival and improved locomotor function. Besides, regrowth of catecholaminergic nerve fibers distal to the injury site and recovery of endogenous spinal cord interneurons and motoneurons were achieved by SENAs. In addition to cellular therapy strategies, ESCs can be combined with biomaterials in order to provide regeneration via tissue engineering. It was shown that use of biodegradable polymer scaffolds carrying various growth factors, such as retionic acid, insulin-like growth factor (IGF) and transforming growth factor-β (TGF-β), can induce the differentiation of ESCs and promote cellular survival (Levenberg et al. 2003). Besides, when the cells were transplanted with the poly(lactic acid-co-glycolic acid) (PLGA)-poly(L-lactic acid) (PLLA) polymer blend scaffolds, cellular survival and differentiation continued *in vivo* for up to 2 weeks and a 3D vessel network like structure was observed. Furthermore, when the cell seeded scaffolds were compared with ESC cultures, it was confirmed that 3D cellular organization and a higher expression of differentiation-associated proteins could be supported by the scaffold. Considering these findings, it can be concluded that ESCs with their great differentiation potential is an efficient cell source for cellular replacement in spinal cord injuries. However, it should not be ignored that, there are still ethical concerns and legal issues about the utilization of ESCs because these cells have to be obtained from early embryos.

Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are found in the bone marrow, peripheral blood and umbilical cord blood. These stem cells are responsible for the formation of the blood and immune cells, therefore, they provide a regular renewal of the blood in the body. In addition, it was shown by various studies that HSCs can differentiate into muscle, bone and blood vessel cells, in addition to epithelium, intestine, liver and neural cells. Thus, HSC is a great source for cell therapy applications and there are various studies using engraftment of HSC in the treatment of SCI. It was proven that direct injection of HSCs 1 week after the spinal cord injury in a mouse model promoted functional recovery and cell survival for up to 5 weeks (Koshizuka et al. 2004). Furthermore, expression of astrocyte, oligodendrocyte, and neural precursor markers were detected, but, expression of neural markers could not be observed. In another study, implantation of human CD34+ HSCs isolated from bone marrow into lesions of the developing spinal cord in the chicken embryo resulted in differentiation of HSCs into neuronal cells (Sigurjonsson et al. 2005). Expression of high levels of the neuronal markers, neuronal nuclear antigen (NeuN) and microtubule-associated protein (MAP2), by the transplanted cellular population was observed in addition to axon extension and neuronal cyto-architecture formation.

Mesenchymal Stem Cells

Mesenchymal stem cell (MSC) is the other cell source that can be utilized in the treatment of SCI. Their use in cell therapies does not raise ethical

concerns and debates due to their ease of isolation from various common sources such as bone marrow, umbilical cord blood and matrix, adipose tissues, etc. Each of these cells has different superiorities over the others. Even though the differentiation mechanism of transplanted MSCs in engraftment site is not clear, several *in vivo* studies showed that MSCs can migrate and differentiate into astrocytes and neurons depending on the extracellular matrix and the signal molecules in their environment. Most of the *in vitro* studies indicated that MSCs have a capability to transdifferentiate into neurons or glial cells upon treatment with appropriate combinations of growth factors and chemical stimulants, and several studies showed the functionality of these transdifferentiated neurons. Hu et al. (2010) reported that transplantation of umbilical cord MSCs (UC-MSCs) into rat spinal cord after a traumatic injury improved hind limb locomotor function recovery and achieved lengthening of neurofilament-positive fibers. It was found that the majority of the UC-MSCs were not differentiated into neuronal cells. However, the undifferentiated UC-MSCs released neurotrophin 3 (NT-3) and glial cell-derived neurotrophic factor (GDNF) after implantation. Thus, it was revealed that recovery was maintained through the production of the growth factors and cytokines by the UC-MSCs.

Initially, MSCs were isolated from the bone marrow and to date these cells have been tested in numerous tissue engineering studies. In one such study, the survival, adhesion and proliferation of rat bone marrow-derived MSCs on 3D gelatin sponges coated with a thin layer of PLGA *in vitro* were high, and upon implantation of these MSC seeded scaffolds into rat SCI lesions the inflammatory reactions and the lesion cavity formation were reduced (Zeng et al. 2011).

Neural Stem Cells

During CNS development, after the gastrulation, neural plate is formed from the ectoderm. The proliferation and certain morphological changes of the cells within the neural plate lead to the closure of neural plate, and the neural tube and neural crest are formed. Neural crest gives rise to the peripheral nervous system during embryogenesis while the neural tube develops into the central nervous system. Neural stem cells (NSCs) are derived from the inner epithelial periphery of the neural tube and these neuroepithelial cells are induced by intrinsic factors and the extrinsic soluble signals to differentiate into mature neuronal and glial cells. This whole process is called neurulation. Before the 1990s, it was believed that neuron production in the central nervous system ceased after birth. However, this argument was refuted by numerous studies and the presence of NSCs in the CNS was shown. It was first reported by Reynolds and Weiss (1992) that multipotent stem cells expressing nestin reside in the adult brain and these cells can be isolated through neurosphere formation assay. NSCs, which are self-renewable, are generally cultured as neurospheres. NSCs evolve into precursors committed to specific neural cell lineages, and these cells are capable of differentiation into functional neurons, astrocytes and oligodendrocytes. Therefore, transplantation of these cells into the injured region of the spinal cord can lead to the formation of new neurons and oligodendrocytes, expression of growth factors, and support regeneration and functional recovery. Thus, the role of NSCs in regenerative medicine is to become neural cells to repair communication pathways, or to do remyelination of the growing axons, or to release neurotrophic factors to stimulate regeneration.

NSCs derived from fetal tissue can preserve the full range of pluripotency, while the ones isolated from adult brain or spinal cord are more restricted to a neural phenotype. Fetal stem cells (FSCs) are used for cellular therapies in the treatment of SCI, and result in lower rates of tumor formation after transplantation, and this makes them more advantageous as a cell source compared to the ESCs. However, differentiation capacity of FSCs into motor neurons is lower compared to ESCs. Many studies suggest that induction of FSCs to become a neural cell type before implantation improves the outcomes of treatment with increased differentiation specificity. Tarasenko et al. (2007) reported that grafting of *in vitro* primed human fetal NSCs into the contused spinal cord 9 days after the injury improved the differentiation of cells into cholinergic neurons

with enhanced functional recovery. However, it was revealed that the time of the grafting is also crucial for the success of cell replacement therapy. The neural precursor cells can also be derived from ESCs to be used in the SCI, and result in behavioral improvement after transplantation to the lesion site (Hatami et al. 2009).

The evidence of the presence of NSCs in the adult CNS opens up a new era in the treatment of SCI via cellular therapies. These cells can be isolated and cultured *in vitro*. In the adult brain, NSCs are present in the subgranular zone (SGZ) of hippocampal dentate gyrus and in the subventricular zone (SVZ) of lateral ventricle where the new neurons are generated during adulthood. NSCs are most abundant in SVZ and the newly produced neurons migrate from SVZ to the olfactory bulb through the rostral migratory system. In SGZ, the number of NSCs is lower compared to SVZ. Besides, endogenous NSCs can be isolated from the central canal of the mature spinal cord. However, the NSCs isolated from different sources have different characteristics. They require different trophic factors and have different growth patterns. Thus, the environmental factors are important for specific differentiation of NSCs. Under *in vitro* conditions NSCs isolated from human adult brain were expanded in the presence of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF); however, upon removal of the mitogens from the culture NSCs differentiated into neuronal and glial cells. In an *in vivo* study by Lundberg and Bjorklund (1996) it was reported that the NSCs differentiated primarily into astrocytes after injection into adult CNS. Different studies also support that after transplantation of NSCs to the injury site, the cells differentiate especially into astroglial and oligodendroglial cells. For transplantation of NSCs after spinal cord injury, there is also a controversy about the ideal source of NSCs. One side advocates that all the stem cells are similar and they can be induced to differentiate into any type of cells through the signals of the local environment while others state that the local environment of a stem cell defines its fate and transplantation of these region-specific stem cells improves the outcome of the cell therapy. NSCs isolated from both the spinal cord and the forebrain of the embryonic (day 16) rat were cultured under the same conditions with the medium containing either EGF, bFGF or both (Fu et al. 2005). It was shown that the cells could proliferate and expand in all three mediums, but more rapidly in the presence of both factors. It was also observed that the percentage of the neuronal differentiation of the NSCs isolated from the forebrain was higher compared to those from the spinal cord. This study suggests that use of a NSC source closer to the transplanted region could improve cell survival, engraftment and function. However, NSCs alone may not be sufficient for complete treatment of SCI. Because of the inhibitory environment and the glial scar tissue formation after the injury, neuronal differentiation and axonal extension of NSCs are limited. In the light of these facts, NSCs are also being tested with complementary strategies. Bioengineered scaffolds are utilized as a vehicle for NSC delivery. In addition, by entrapping bioactive agents in these scaffolds, graft performance could be improved. One of the first applications in this direction was in a rat SCI model in which the importance of using NSCs along with the scaffolds for best recovery was shown (Teng et al. 2002). Coordinated, weight-bearing hind limb steps were observed 70 days after the induction of injury and this recovery was stated to be related with the decrease of glial scarring. Besides, elongation of corticospinal tract fibers (from the injury region to the caudal cord) was recorded.

Consequently, each cell type referred to above could be a source for cell therapy and nerve tissue engineering. However, multipotent neural stem cells, as a result of their giving rise to the cells of the nervous system, appear to be the most promising cell source in nerve tissue engineering.

Scaffold Design for the Regeneration of Injured Spinal Cord

A human SCI can be extensive with massive disrupted tissue and scar formation which is represented by a serious lack of cells that would normally contribute to regeneration, and by a

physical barrier across which the axons are not allowed to grow. Therefore, the regeneration of functional axons across this large lesion would be achieved via a bridge like biomaterial scaffold through the injured area. These biomaterials can be used as cell-free structures; however, incorporation of cells and growth factors to such highly porous structures (scaffolds) creates a more permissive environment for healing and offers a longer term solution. In all tissue engineering approaches, the ideal scaffold should be three dimensional (to provide a site for cell attachment and to mechanically support tissue development), biodegradable with non-toxic degradation products and porous (for cell penetration, for nutrient supply and for removal of metabolic waste). Mechanical strength is an important parameter in the design of a nerve guide to prevent the collapse of the tube and the obstruction of regeneration. The chemical composition is critical for the mechanical properties of the material. In addition, crosslinking of the scaffold material or varying the composition of the material are approaches used to improve the mechanical strength of the scaffolds. For many years different biomaterials made of non-degradable and biodegradable polymers have been used in spinal cord repair. Generally, it is the regeneration strategy that determines the material choice.

Non-degradable materials used in tissue engineering are generally of synthetic origin. Their synthesis processes are controllable and generally there is no need for complex protocols. Some non-degradable synthetic materials like silicone, poly(2-hydroxyethyl methacrylate) (PHEMA), polyacrylonitrile-polyvinylchloride (PAN-PVC), and poly(tetrafluoroethylene) (PTFE) are used in the design of nerve guidance tubes. These materials have some advantages over the degradable ones. For example, they do not require a control of the degradation rate or carry the risk of toxicity of the degradation products. However, their permanent presence creates a high risk of chronic inflammation and may result in nerve compression over time. Furthermore, the non-degradable synthetic materials are generally non-cell adhesive.

Biodegradable materials are preferred more for the demanding regeneration environment in SCI. There is no need for their removal since they would degrade as the new tissue regenerates. However, there is a risk of releasing toxic degradation products. Another concern when using biodegradable scaffolds is the degradation rate and time. The degradation time should not be less than the recovery time which is crucial for proper tissue regeneration. The rate of degradation of the construct should be tailored to match the rate of new tissue formation. The choice of polymer type and ratios and the fabrication method can alter the rate of biodegradability of the scaffold as well as its mechanical properties. The degradable materials could be of either natural or synthetic origin. However, materials from natural sources have some problems in uniformity, batch-to-batch variability, purity, and sometimes evoking immune responses. The degradable natural materials like collagen (particularly Type I), chitosan, alginate, fibrin and poly(hydroxyalkanoates) (PHA) and the degradable synthetic polymers like poly(glycolic acid) (PGA), poly(L-lactic acid) (PLLA), poly (lactic acid-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), and some of their copolymers like poly(lactide-co-caprolactone) (PLCL) have been used in various nerve regeneration studies.

Electrically active materials are also used in the design of the nerve guidance channels. The electrical field in the natural extracellular matrix (ECM) is formed by electrically charged materials, like negatively charged proteoglycans that attract sodium ions, acts as a signal to promote axon regeneration. The applied electric field leads to a polarized rearrangement of the cytoskeleton of nerve cells via promotion of the microtubule disassembly locally along the neurite shaft. It is possible to deliver localized electrical stimulus at the injury site. The materials which can achieve these are electrically conducting, generally non-degradable polymers like polypyrrole (PP).

The structural and chemical versatility make biomaterials suitable to replace the scar tissue, fill the gap and serve as a bridge to carry regenerating axons across the gap. Most of these polymeric materials are implanted as solid scaffolds, though some are injected in sol or gel form. Gels,

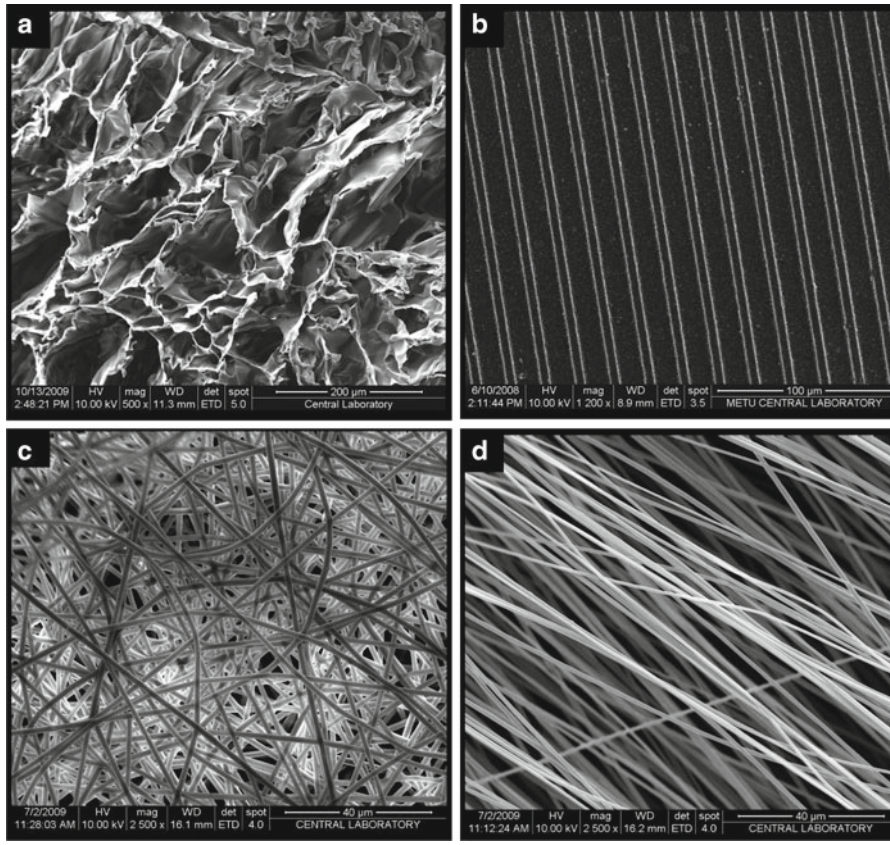

Fig. 29.1 Scanning electron micrograph of scaffolds which were fabricated in different forms (**a**) foam, (**b**) micropatterned film, and (**c**) electrospun random fibers and (**d**) electrospun aligned fibers

highly hydrated, crosslinked polymers, are viscous, and quite suitable to fill a small void preventing astrocytosis from expanding. Gels allow regeneration of axons, and are advantageous for drug delivery to the injured tissue. The property of hydrogels that are used as space filling agents makes these materials ideal for implantation at complex SCI sites. Hydrogels can also mimic the ECM environment with their polymeric network structure and high levels of water. On the other hand, use of a solid scaffold is more advantageous than injection of a hydrogel that sets or solidifies after injection. Polymeric scaffolds can be found in various forms such as sponges, fibers, and full or hollow cylinders that can be used to bridge the gaps. Sponges or foams, are porous and three dimensional, and are generally used for large tissue defects (Fig. 29.1a). They can be conveniently fabricated by freeze drying, solvent casting or particulate leaching, or a combination of these methods and their pore size can be adjusted by various methods such as altering the size of crystals or changing the freezing temperature. However, like in the gels their mechanical strength is not high (mainly due to the high porosity and the inherent properties of polymers) making them unsuitable for long implantation durations. On the other hand, the tubular structures are used in the lesion site to serve as a bridge to cross large gaps. These can be generally porous, hollow tubes or multichannel tubes to provide a pathway for axonal regrowth. The roles of the tubular structures can be listed as to prevent scar formation, to concentrate neurotrophic molecules, and to guide the axon regeneration. Hollow single channel tubes provide limited guidance because of their large size and lack of oriented intraluminal substratum. However, tubular

constructs having anisotropic architecture such as multichannels or aligned textures are promising scaffolds in the unidirectional regeneration of neurons, which is essential for functional recovery after a spinal cord injury.

Upon SCI the damaged axons at the lesion site are misdirected which results in abortive regeneration. The advances in nerve tissue engineering aim to design scaffolds that mimic the architecture and the organization of the uninjured spinal cord. Random structures placed within the lesion site generally lead to a disorganized growth of axons (Stokols et al. 2006). By the incorporation of physical and chemical cues in the design, the oriented scaffold can imitate the anatomical structure of native tissue to provide contact mediated guidance for regeneration. The physical cues such as roughness and topography are important in the cell attachment via nonspecific adsorption. In topographical approaches, inclusion of internal matrices like channels, oriented micro/nanopatterned designs, and micro/nanofibers increase the surface area to promote cell adhesion and enhance the guided tissue regeneration via stereotropism. On the other hand, the surfaces modified with patterned ECM biomolecules, act as chemical guidance cues, facilitate cell attachment and facilitate unidirectional growth of axons, and even cells. In addition to cell attachment and guidance, some physical or chemical cues are used to promote cell survival and differentiation.

The cells adhere to ECM through interactions between ECM proteins and cell adhesion molecules like integrins and membrane receptors. These specific cell-matrix interactions modulate the organization of the actin filaments. The oriented actin cytoskeleton is the evidence of cell alignment via contact guidance. Thus, immobilization of biological molecules as ECM proteins or their constitutional motifs form chemical patterns on scaffolds to guide cells to mimic the oriented structure of the native tissue. The specific peptide sequence Ile-Lys-Val-Ala-Val (IKVAV), poly (D-lysine), and laminin are among the most common chemical cues for nerve guidance. Fibronectin can also serve as a chemical cue since it enhances attachment and proliferation of cells, and induces nerve regeneration.

Scaffold topography, as a non-biological approach to regulate cell behavior, could act as a biomimetic, cell stimulating cue. Even though the whole mechanism of cell response to topography is not clear, it has been proposed that cells adapt to physical, topographic substrates by conditioning growth environments through secretion and modulation of ECM proteins. Surfaces with micro and nano structures could be formed using a variety of techniques. Patterned surfaces could be obtained by lithography and subsequent transfer methods, or by other methods such as molding, surface grafting, ink jet printing, surface etching, and etc. The resultant surfaces could be used as is or as a template to transfer the patterns to films via solvent casting, hot embossing, or microcontact printing. Fibrillar scaffolds, on the other hand, are generated by pressure assisted injection through microsyringes, by self assembling or by electrospinning. Most of the polymers can be easily cast into different patterned films and fibers which are then used separately or in combination.

The most common technique to produce patterns or surface textures with controlled dimensions is microfabrication. Many groups have used the microfabrication techniques like photolithography to create microchannels or microgrooves (Fig. 29.1b). The dimensions of the micropatterned substrates have a strong effect on the behavior of cells that vary with the cell type and cell size. In one study microgrooved substrates of varying channel dimensions (e.g. 8 μm wide groove, 20 μm wide ridge, and 1 or 2 μm depth) were produced to study the effect of these topographical cues on the behavior of neurons derived from chick embryo cerebral hemisphere (Clark et al. 1990). The shallower (1 μm depth) grooves were not effective on the outgrowth of the neurites, with the growth cones crossing over many grooves and ridges. However, on 2 μm deep patterns neurite outgrowth was significantly aligned along the groove axis with little crossing over the edges. It was also reported that the effect of the physical cues (such as micropatterns) on behavior and morphology of cells improved with the contribution of chemical and biological cues.

Recknor et al. (2006) showed that astrocytes and adult rat hippocampal progenitor cells were aligned along the pattern axis of laminin coated, micropatterned films. The co-culture of these progenitor cells on guided astrocytes, which served as the biological cues, enhanced neuronal differentiation and promoted neurite alignment on the patterned textures.

Fiber is one of the most suitable scaffold forms to reestablish the connection between the nerve fibers which is lost upon SCI. The fiber size, material, orientation, and fabrication methods can be altered as needed. Microfibers and nanofibers are the most common scaffold forms in neural tissue engineering. The most preferred techniques to obtain fibers are self assembly and electrospinning. In electrospinning, briefly the polymeric solution is ejected from a needle attached to the tip of a syringe under a high potential created between the needle tip and the collector plate. When the microsyringe is set in motion the solution is ejected through the needle tip in the form of a polymeric jet. The fibers are collected on the grounded collector. Concentration and flow rate of the polymeric solution, diameter of the needle, potential applied and the distance between the needle and the collector directly affect the morphology and diameter of the fibers. In standard electrospinning procedure, the randomly oriented fibers are collected on a metal plate (Fig. 29.1c). A rotating drum, disk, wire drum or a parallel pair of electrodes can be used as a collector to obtain aligned micro/nanofibers (Fig. 29.1d). Since it has been shown that the alignment of the fibers significantly alters the tissue engineered construct's performance, scaffolds with oriented fibers are commonly investigated in neural tissue engineering studies.

Nanoscale surface features can initiate the formation of focal adhesions, and might achieve a precise control of cell directionality and migration in implants. Therefore, cell attachment, proliferation, and differentiation are enhanced on the nanoscale patterned scaffolds. In addition, the nanofibers resemble the native ECM of the spinal cord more than the microfibers. The outcome of many studies involving fibrous scaffolds show that nano or low microscale fibers are quite promising for the treatment of SCI. In addition, the alignment of the fibers is especially important in guided tissue engineering studies. In neural tissue engineering, the cells could be successfully aligned on guided textures like oriented parallel fibers. Moreover, the aligned fibers could direct the neurite growth along the fiber. Therefore, the aligned nanofibrous scaffolds are mostly selected to provide the topography suitable for achieving regeneration of SCI. Effects of both the diameter and the orientation of fibers on NSCs were investigated in *in vitro* studies by Yang et al. (2005). It was observed that NSCs were oriented along the direction of the parallel PLLA fibers, and neurite outgrowth was parallel to these fibers. However, the use of micro or nano scale fibers did not seem to have a significant effect on cell alignment. It was shown that the rate of NSC differentiation was higher on the nanofibers compared to the microfibers. In addition, the neurite extension was faster and longer on the highly aligned scaffold due to better contact guidance effects. The effect of the fiber orientation on NSC behavior was also studied by our group (Yucel et al. 2010). It was observed that mouse NSCs were randomly distributed in all directions on the nonguiding, random fibers. However, on the aligned fibers NSCs responded to the topography and were aligned in clusters. It was shown that the cytoskeleton and nuclei of the cells were also aligned and elongated along the axis of fibers.

In self assembly method, a molecularly designed bioactive matrix is used to provide treatment for SCI and regeneration of axons. A nanofibrous matrix composed of peptide amphiphile (PA) molecules is formed after injection into the lesion site. Thus, the scaffold of cylindrical nanofibers is formed by self assembly from aqueous solution, and bioactive epitopes are found on the surfaces of the nanofibers. In this strategy, neither the cells nor the exogenous proteins are combined with the scaffold, which completely differs from other approaches (Tashiro et al. 1989). The novelty of this study is the incorporation of the neuroactive pentapeptide epitope (IKVAV) of laminin into the negatively charged PA network. Incorporated IKVAV peptide mimicked laminin and supported ECM formation,

which was required for axon outgrowth. When this IKVAV PA solution interacted with the physiological fluid, PA spontaneously formed nanofibers both *in vivo* and *in vitro*. Previous studies on this system showed that the nanofibers including the IKVAV epitope promoted the outgrowth of cultured neurons and suppressed the astrocytic differentiation of neural progenitor cells.

Growth Factor Incorporation into Scaffolds

Growth factors, especially neurotrophic factors, are essential in the development of nervous system, myelination of axons, and supporting survival and differentiation of neurons. In SCI treatment the application of these neurotrophic factors are crucial. These factors are able to stimulate the activation of the regeneration-associated genes to promote the regeneration of injured axon and to enhance the differentiation of NSCs to replace the injured neurons or neuroglia cells. In *in vitro* culture, the differentiation of NSCs is triggered by the withdrawal of mitogens like EGF and bFGF which are essential in NSC expansion. In addition, the potential of NSC differentiation can be enhanced by the use of neurotrophin molecules which improve the survival of postmitotic neurons, and so increase the number of newly committed neurons. The molecules of neurotrophin family promote the maturation process of newly formed neurons, increase the length and branching of neurons, and facilitate formation of functional neurons. Therefore, introducing the main growth factors into the lesion promotes recovery. There are two major approaches for growth factor administration; exogenous delivery and endogenous expression. Different growth factors such as brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5) have been tested. However, the ways these factors influence the neural cells significantly differ. In addition to supporting survival and differentiation of neural cells, these factors play a role on cellular migration and myelination, but with contrasting actions. Unlike endogenous BDNF, NT-3 supports Schwann cell migration but prevents myelination of cells (Yamauchi et al. 2004). As defined in here, the use of these antagonist growth factors after SCI is definitely a proper approach for the regulation of trophic environment (for both migration and myelination of neural cells to support regeneration). In addition to BDNF and NT-3, GDNF and NGF delivery after SCI are appropriate strategies for regeneration. It was reported that injection of genetically modified fibroblasts expressing NGF into the lesion site of rhesus monkeys achieved a greater extension of spinal cord sensory axons and putative coerulospinal axons compared to the control group (Tuszynski et al. 2002). In addition, Schwann cell migration and spontaneous axonal plasticity were observed. It can be concluded that axonal plasticity can be enhanced by expression of trophic factors by the transplanted cells. In addition to NGF, Schwann cell migration to the injured region can be induced by the local expression of GDNF, and as a result, both remyelination and axonal regeneration can be promoted (Blesch and Tuszynski 2003). NT-4/5 is also candidate for growth factor delivery; however, this neurotrophic factor was less investigated compared to the others. NT-4/5 binds to the same receptor tyrosine kinase receptor B (TrkB) with BDNF, but their biological activities significantly differ. It was indicated that NT-4/5 may be more potent than BDNF in SCI treatment (Blesch et al. 2004). Thus, NT-4/5 expressing genetically modified fibroblasts were grafted into the lesion after thoracic spinal cord injury and axonal extension was observed as a result of the effect of NT-4/5. Moreover, remyelination was observed as a result of Scwann cell migration inside the graft, but functional recovery could not be observed.

Tissue engineering approaches with growth factor administration are utilized in order to improve the outcome and overcome the deficiencies in the treatment of SCI. The main strategy is genetic modification of cells in order to express or overexpress growth factors as mentioned above, and performing transplantation

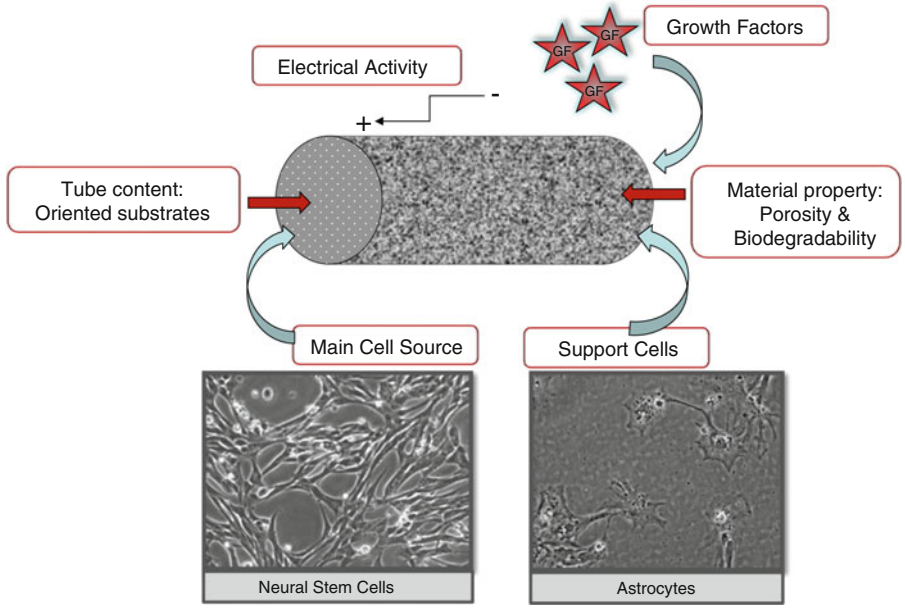

Fig. 29.2 Schematic presentation of an ideal tissue engineered construct seeded with neural stem cells

after seeding and culturing these cells on the scaffolds. On the other hand, growth factors can also be entrapped in the scaffolds (Fig. 29.2). These bioactive molecules can be released from the scaffold via controlled or sustained delivery, and they would support regeneration, cellular proliferation and differentiation after transplantation. Especially growth factor release from the scaffolds can induce the differentiation of the cells seeded on the scaffold, and cellular replacement in the lesion cavity can be provided.

Tissue Engineering Applications Using NSCs for Spinal Cord Injury

There are various encouraging results obtained both in *in vitro* and *in vivo* that combine biomaterial scaffolds with NSCs. These NSCs are restricted to differentiate into neurons, a basic cell type found in the spinal cord, and supportive neuroglial cells, achieving remyelination and release of required neurotrophic factors to promote regeneration in the SCI.

The predetermined differentiation potential of NSCs in three dimensional matrices was demonstrated in several studies. In one such study, NSCs were obtained from embryonic rat cortical or subcortical neuroepithelium, and cultured in collagen type I gels (Ma et al. 2004). It was shown that the differentiated neurons were excitable and ion channels/receptors, neurotransmitters and expression of specific proteins that characterize the polarity of neurons were present. In addition to these results, an active synaptic vesicle recycling among neurons entrapped in collagen presented the formation of functional synapse and neuronal network.

Investigators use different strategies to release the growth factors, especially neurotrophins, into the milieu of the tissue engineered NSC-scaffolds to improve the regeneration process at the site of the lesion of the spinal cord. The permissiveness of the environment can be further enhanced by incorporation of these factors directly into the scaffolds, or use the cells, like NSCs themselves or the support cells, as a source from which to deliver the neurotrophins. In one study, NT-3 and platelet-derived growth factor (PDGF) were incorporated into fibrin scaffolds which also contained mouse embryonic stem cell-derived neural progenitor cells, and these scaffolds were

embedded in a subacute rat model of SCI (Johnson et al. 2010). It was reported that the strategy of using growth factors within the fibrin scaffold enhanced the cell survival and proliferation in the spinal cord lesion 2 weeks after injury. Moreover, the scaffolds including a heparin-binding delivery system for the controlled release of growth factors directed the differentiation of progenitor cells into neurons. Moreover, NSCs by themselves can be used as a growth factor delivery vehicle via incorporation of a therapeutic target gene, particularly a gene that codes for a neurotrophic factor, into these cells via standard genetic material manipulation procedures to achieve the expression or overexpression of specific neurotrophic factors. NT-3 overexpressing NSCs generated via transduction were seeded on PLGA scaffolds and implanted into a canine SCI model (Kim et al. 2010). It was shown that NT-3 overexpression slightly enhanced the survival of transplanted cells. On the other hand, it was observed that it promoted the migration of NT-3 overexpressing NSCs to the spinal cord tissue, and also improved long-term survival of these cells. In the approach of incorporation of the supportive cells like Schwann cells, the cells could be used as a source of neurotrophic substances to improve the survival and axonal regeneration of injured neurons as well as take part in remyelination of axons (Chen et al. 2010). They observed that co-transplantation of NSCs and Schwann cells which were cultured on PLGA scaffolds could promote the functional recovery of the SCI of rats with the higher amplitudes of motor and somatosensory evoked potential of lower limbs compared to the same construct except Schwann cells. Moreover, it was suggested that Schwann cells promoted NSC differentiation into neurons to replace the degenerated counterparts in order to maintain the synaptic connections and to restore the neural pathways.

The other most commonly used strategy in tissue engineered NSC-biomaterial scaffolds for SCI is the use of guided scaffolds to orient axons and newly generated neurons in the same direction. Therefore, the scaffolds were designed by simulating the architecture of the healthy spinal cord. Even in the early studies the guided substrates were constructed to emulate the white matter with longitudinally oriented textures for axonal and neuronal guidance (Teng et al. 2002). For this purpose, the outer part of the scaffold was fabricated via solid–liquid phase separation technique to produce long, axially oriented pores. In a similar approach alginate-based anisotropic capillary hydrogels were used to promote oriented axonal regrowth in the injured spinal cord (Prang et al. 2006). It was observed that this architecture induced guided axon regeneration across the scaffold after implantation into acute cervical spinal cord lesions in adult rats. Moreover, *in vitro* studies showed that neural progenitor cells could be introduced into these oriented constructs to encourage cell contact-mediated axon regeneration in the injured spinal cord. In another study, the multichannel porous PLGA scaffold was combined with a mixture of genetically modified NSCs transfected with either NT-3 or tyrosine receptor kinase C (TrkC), the NT-3 receptors, to obtain neuronal connections *in vitro* (Xiong et al. 2009). In the NT-3/TrkC group, a high percentage of NSCs were differentiated into functional neurons which established connections and exhibited synaptic activities, thus, these neurons could be activated at the molecular level in response to external stimuli. A biomimetic electrospun fibrous PCL/collagen tube was fabricated to be utilized as a delivery vehicle for NGF to facilitate regeneration after SCI by promoting NSC differentiation (Hackett et al. 2010). The gaps formed between nanofibers were small enough to entrap the growth factor inside the scaffold and allowed a slow release of these factors. It was observed that NSCs proliferated and differentiated effectively on nanofibers, even more favorably on the aligned fibers. It was revealed that the electrospun PCL/collagen tubes could have a great potential to serve as a scaffold with the highest proportion of neurons, astrocytes, and Nestin positive cells grown on them. In our study a tissue engineered, guided nerve tube with well defined topographical cues, aligned electrospun fibers and a micropatterned film, was developed by the use of NSCs and NSC derived astrocytes as support cells to repair the transected nerves in

the spinal cord (Yucel et al. 2010). NSCs were cultured both on the aligned, electrospun mat and micropatterned films for a while, then the cells on the micropatterned film were differentiated into astrocytes while the cells on the fibers were kept undifferentiated. The 3D tubular scaffold was formed by rolling the patterned film (with its micropatterns facing inside) over the fibrous mat, and the aligned fibers containing oriented NSCs and the microgrooves containing the aligned astrocytes were parallel to the tube axis. In this design, the aligned astrocytes on the film would serve as a growth and differentiation factor source for the NSCs on the aligned fibers in addition to enhancing their alignment. The success of *in vitro* study in cellular alignment and survival of both NSCs and astrocytes in the tubular scaffold after co-culture demonstrated the potential of the distinct tissue engineered nerve tube design to be used *in vivo* for the structural and functional regeneration of injured spinal cord.

In most of the studies for SCI the NSCs seeded on the biomaterial scaffolds were derived from mouse or rat; however, recently researchers have started using human NSCs. In one such study, human ESC-derived neural precursor cells (NPCs) were cultured in collagen scaffolds to promote recovery in injured rat spinal cord (Hatami et al. 2009). It was observed that human ESC-NPCs are able to differentiate into neurons and glial cells both *in vitro* and *in vivo*. The *in vivo* results showed that the recovery of hind limb locomotor function and sensory responses in an adult rat model of SCI were improved by implantation of collagen scaffolds seeded with human ESC-NPC, and these transplanted cells migrated toward the spinal cord. In another study, engraftment of NT-3 overexpressing human NSC on PCL scaffold, and combining chondroitinase treatment after implantation of cell seeded scaffold significantly promoted behavioral and electrophysiological recovery after SCI in a rat model (Hwang et al. 2011). In this strategy, the scaffold provided the mechanical support for the cells and also acted as a reservoir to provide migratory NSCs to the lesion. On the other hand, NT-3 overexpression of the cells increased the cell survival, differentiation and migration. Results proved that, by combining all these tissue engineering components and performing chondroitinase treatment after implantation, neuroplasticity and axonal remodelling were improved, remyelination of contralateral white matter was promoted and functional recovery was ameliorated.

Through these strategies researchers are seeking to construct the ideal scaffold for SCI treatment (Fig. 29.2). The studies revealed that transplantation of guided, tissue engineered scaffolds seeded with NSCs combining with the developing approaches would be an effective way to achieve the regeneration and functional recovery of SCI.

Future Prospects

The unlimited potential in the scaffold design and the use of NSCs with current approaches open up the way for new treatments for SCI. Researchers are still facing the problem that regenerating axons fail to grow out of the scaffolds. To overcome this obstacle and initiate the entry of regenerated axons into the host environment, highly migratory cells, which replace degenerated cells or facilitate axonal growth, need to be transplanted within the scaffolds designed through advanced engineering approaches. Tissue engineering should be coupled with the manipulation of the milieu for the neutralization of inhibitory signals or the enhancement of effective signals so that significant neural regeneration is achieved in patients with SCI.

References

Anderberg L, Aldskogius H, Holtz A (2007) Spinal cord injury – Scientific challenges for the unknown future. Ups J Med Sci 112:259–288

Blesch A, Tuszynski MH (2003) Cellular GDNF delivery promotes growth of motor and dorsal column sensory axons after partial and complete spinal cord transections and induces remyelination. J Comp Neurol 467:403–417

Blesch A, Yang H, Weidner N, Hoang A, Otero D (2004) Axonal responses to cellularly delivered NT-4/5 after spinal cord injury. Mol Cell Neurosci 27:190–201

Chen G, Hu YR, Wan H, Xia L, Li JH, Yang F, Qu X, Wang SG, Wang ZC (2010) Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells and Schwann cells. Chin Med J (Engl) 123:2424–2431

Clark P, Connolly P, Curtis ASG, Dow JAT, Wilkinson CDW (1990) Topographical control of cell behaviour: II. Multiple grooved substrata. Development 108:635–644

Cui YF, Xu JC, Hargus G, Jakovcevski I, Schachner M (2011) Embryonic stem cell-derived L1 overexpressing neural aggregates enhance recovery after spinal cord injury in mice. PLoS One 6:e17126

David S, Aguayo AJ (1981) Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. Science 214:931–933

Fu SL, Ma ZW, Yin L, Iannotti C, Lu PH, Xu XM (2005) Region-specific growth properties and trophic requirements of brain- and spinal cord-derived rat embryonic neural precursor cells. Neuroscience 135:851–862

Hackett JM, Dang TNT, Tsai EC, Cao X (2010) Electrospun biocomposite polycaprolactone/collagen tubes as scaffolds for neural stem cell differentiation. Materials 3:3714–3728

Hatami M, Mehrjardi NZ, Kiani S, Hemmesi K, Azizi H, Shahverdi A, Baharvand H (2009) Human embryonic stem cell-derived neural precursor transplants in collagen scaffolds promote recovery in injured rat spinal cord. Cytotherapy 11:618–630

Hu SL, Luo HS, Li JT, Xia YZ, Li L, Zhang LJ, Meng H, Cui GY, Chen Z, Wu N, Lin JK, Zhu G, Feng H (2010) Functional recovery in acute traumatic spinal cord injury after transplantation of human umbilical cord mesenchymal stem cells. Crit Care Med 38:2181–2189

Hwang DH, Kim HM, Kang YM, Joo IS, Cho CS, Yoon BW, Kim SU, Kim BG (2011) Combination of multifaceted strategies to maximize the therapeutic benefits of neural stem cell transplantation for spinal cord repair. Cell Transplant 20:1361–1379

Johnson PJ, Tatara A, Shiu A, Sakiyama-Elbert SE (2010) Controlled release of neurotrophin-3 and platelet derived growth factor from fibrin scaffolds containing neural progenitor cells enhances survival and differentiation into neurons in a subacute model of SCI. Cell Transplant 19:89–101

Kim BG, Kang YM, Phi JH, Kim YH, Hwang DH, Choi JY, Ryu S, Elastal AE, Paek SH, Wang KC, Lee SH, Kim SU, Yoon BW (2010) Implantation of polymer scaffolds seeded with neural stem cells in a canine spinal cord injury model. Cytotherapy 12:841–845

Koshizuka S, Okada S, Okawa A, Koda M, Murasawa M, Hashimoto M, Kamada T, Yoshinaga K, Murakami M, Moriya H, Yamazaki M (2004) Transplanted hematopoietic stem cells from bone marrow differentiate into neural lineage cells and promote functional recovery after spinal cord injury in mice. J Neuropathol Exp Neurol 63:64–72

Legos JJ, Gopez JJ, Young WF (2002) Non-surgical management of spinal cord injury. Expert Opin Investig Drugs 11:469–482

Levenberg S, Huang NF, Lavik E, Rogers AB, Itskovitz-Eldor J, Langer R (2003) Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. Proc Natl Acad Sci USA 100: 12741–12746

Lundberg C, Bjorklund A (1996) Host regulation of glial markers in intrastriatal grafts of conditionally immortalized neural stem cell lines. Neuroreport 7:847–852

Ma W, Fitzgerald W, Liu QY, O'Shaughnessy TJ, Maric D, Lin HJ, Alkon DL, Barker JL (2004) CNS stem and progenitor cell differentiation into functional neuronal circuits in three-dimensional collagen gels. Exp Neurol 190:276–288

National Spinal Cord Injury Statistical Center, Birmingham, Alabama, USA (2011) Spinal cord injury: facts and figures at a glance. February 2011. Available at www.spinalcord.uab.edu

Prang P, Muller R, Eljaouhari A, Heckmann K, Kunz W, Weber T, Faber C, Vroemen M, Bogdahn U, Weidner N (2006) The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels. Biomaterials 27:3560–3569

Recknor JB, Sakaguchi DS, Mallapragada SK (2006) Directed growth and selective differentiation of neural progenitor cells on micropatterned polymer substrates. Biomaterials 27:4098–4108

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255:1707–1710

Sigurjonsson OE, Perreault MC, Egeland T, Glover JC (2005) Adult human hematopoietic stem cells produce neurons efficiently in the regenerating chicken embryo spinal cord. Proc Natl Acad Sci USA 102:5227–5232

Snell RS (1992) Neuroanatomy: a review with questions and explanations, 1st edn. Little, Brown and Company, Boston, pp 55–61

Stokols S, Sakamoto J, Breckon C, Holt T, Weiss J, Tuszynski MH (2006) Templated agarose scaffolds support linear axonal regeneration. Tissue Eng 12:2777–2787

Tarasenko YI, Gao J, Nie L, Johnson KM, Grady JJ, Hulsebosch CE, McAdoo DJ, Wu P (2007) Human fetal neural stem cells grafted into contusion-injured rat spinal cords improve behavior. J Neurosci Res 85:47–57

Tashiro K, Sephel GC, Weeks B, Sasaki M, Martin GR, Kleinman HK, Yamada Y (1989) A synthetic peptide containing the IKVAV sequence from the A chain of laminin mediates cell attachment, migration, and neurite outgrowth. J Biol Chem 264:16174–16182

Teng YD, Lavik EB, Qu X, Park KI, Ourednik J, Zurakowski D, Langer R, Snyder EY (2002) Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proc Natl Acad Sci USA 99:3024–3029

Tuszynski MH, Grill R, Jones LL, McKay HM, Blesch A (2002) Spontaneous and augmented growth of axons in the primate spinal cord: effects of local injury and nerve growth factor-secreting cell grafts. J Comp Neurol 449:88–101

Xiong Y, Zeng YS, Zeng CG, Du BL, He LM, Quan DP, Zhang W, Wang JM, Wu JL, Li Y, Li J (2009) Synaptic

transmission of neural stem cells seeded in 3-dimensional PLGA scaffolds. Biomaterials 30:3711–3722

Yamauchi J, Chan JR, Shooter EM (2004) Neurotrophins regulate Schwann cell migration by activating divergent signaling pathways dependent on Rho GTPases. Proc Natl Acad Sci 101:8774–8779

Yang F, Murugan R, Wang S, Ramakrishna S (2005) Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. Biomaterials 26:2603–2610

Yucel D, Kose GT, Hasirci V (2010) Tissue engineered, guided nerve tube consisting of aligned neural stem cells and astrocytes. Biomacromolecules 11: 3584–3591

Zeng X, Zeng YS, Ma YH, Lu LY, Du BL, Zhang W, Li Y, Chan WY (2011) Bone marrow mesenchymal stem cells in a three dimensional gelatin sponge scaffold attenuate inflammation, promote angiogenesis and reduce cavity formation in experimental spinal cord injury. Cell Transplant 20:1881–1899

Pediatric Spinal Tumors: Total Removal Using Laminotomy

30

Yusuf Izci

Contents

Abstract

The management of spinal tumors in children is well established. These tumors are mostly benign and when completely removed, the child is considered cured with an excellent prognosis. Surgical removal of spinal tumors in childhood by laminotomy reduce the incidence of postoperative deformity during the adolescence and adult periods. Laminotomy have some beneficial effects in preventing spinal deformity and epidural scar formation, especially when treating children with remaining growth potential. Patients with good preoperative neurological conditions show excellent postoperative functional outcome and had improved functionally in the long term. This emphasize the importance of early diagnosis and surgical treatment by laminotomy for the majority of children with spinal tumor.

Introduction

Spinal tumors can be divided anatomically by location as intramedullary and extramedullary lesions. Spinal tumors are rare in children with an approximate annual incidence of 1 per 1 million children. In patients under 15 years of age, the ratio of brain to spinal tumors ranges from 8:1 to 22:1 (O'Sullivan et al. 1994). The prevalence of intradural spinal cord tumors is 3–10/100,000 per year and up to 12% of spinal cord tumors arise in the first year of life. They predominantly ocur in the middle third of life (Baysefer et al. 2004). Major improvements

Y. Izci (✉)
Department of Neurosurgery, Gulhane Military Medical Academy, Etlik, Ankara 06018, Turkey
e-mail: yusufizci@yahoo.com

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_30,

have been made in the diagnosis, treatment modalities and outcome of children with spinal tumors over the past several decades. Progress in imaging allows for detailed preoperative evaluation. Although vast majority of the spinal tumors in children are benign, many require surgical intervention to make a definitive diagnosis to determine appropriate treatment.

Pediatric Spinal Tumors

Intradural-extramedullary tumors represent approximately 25% of intraspinal tumors in children (Baysefer et al. 2004). Thirty-five percent of neoplasms in the spinal canals of children are located extradurally (Yamamoto and Raffel 1999). Intradural-intramedullary tumors comprise 35% of all spinal neoplasms in children (Houten and Weiner 2000). Most published studies relating to spinal tumors in children cover the entire spectrum of neoplasms, congenital lesions, developmental anomalies and non-neoplastic masses. Thus these intraspinal tumors account for approximately 20–25% of all pediatric tumors. Since these tumors are relatively infrequent, individual or general neurosurgeons have relatively little experience with this entity. The low prevalence of these lesions and their anatomic complexity have resulted in a very slow evolution of therapeutic principles for the management of spinal tumors in children (Loh et al. 2005).

Cytoreductive (debulking) surgery, radiotherapy and chemotherapy are the treatment strategies for spinal tumors (Schick and Marquardt 2001). Laminotomy and laminectomy are the options to reach the spinal tumor. But the spinal instability is a major problem after surgery in children. Many surgical strategies have been developed to overcome spinal instability in order to achieve the best clinical outcome. Furthermore, the development of effective surgical techniques with reduced instability may lead to patient's satisfaction. Several studies have been performed on enhancing stability using laminotomy. This chapter will focus on the following targets: pediatric spinal tumors, symptomatology and radiology, management strategies and laminotomy technique in spinal tumors.

Symptoms and Radiology

As mentioned above, most of these tumors are benign and slow growing. Pain along the spinal axis is the most common complaint often persisting for several weeks to months before it leads to evaluation and imaging workup. Motor and sensory deficits produced by extramedullary tumors are often segmental or unilateral. In young infants, motor deficits may begin as a regression in motor skills or refusal to walk. Limb weakness is usually spastic, but may be flaccid. Sensory changes are typically radicular.

Urinary disturbances may be present in children with spinal cord tumors. Urinary retention or incontinence may be overlooked by the parents during the first 2 years of the life, but in late childhood these findings are obvious and may be the first signs of a spinal tumor.

Scoliosis and foot deformities are the other signs of a spinal cord tumors. Scoliosis may be found incidentally during the evaluation of a respiratory tract or urinary tract infection. The spinal or foot deformities are usually associated with spinal malformations, which may be also asscociated with spinal inclusion tumors (dermoid or epidermoid tumors) or lipomas. Hypertrichosis, skin color changes or subcutaneous masses are the other signs of spinal malformations. In order to evaluate these malformations, a detailed radiological examination is needed.

Imaging studies for the diagnosis of spinal tumors include plain x-rays, computed tomography (CT) scan, magnetic resonance imaging (MRI), and spinal bone scintigraphy. Whole spine x-rays are the first diagnostic tool of spinal tumors. Anteroposterior, lateral, and oblique views may be necessary to evaluate a spinal bone lesion. This examination shows bony changes in the spine as well as neural foramen and vertebral bodies. The general location of the lesion within the bone, the integrity of the cortex, and the presence of fractures are the important findings in plain x-rays. Whole spine x-rays may also help the surgeon to make a decision regarding overall spinal balance and the necessity of stabilization after tumor removal.

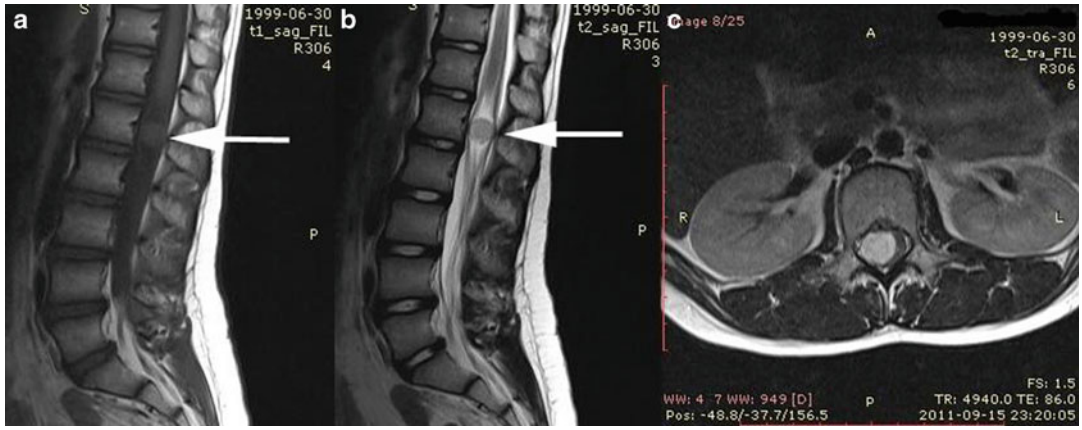

Fig. 30.1 The sagittal T1-weighted (**a**) and T2-weighted (**b**) MRI sections of a 12 years-old female patient showing intradural-extramedullary mass lesion at L1 level. This tumor compress to the rootlets of cauda equina in the axial MRI section (**c**). The *arrows show* the tumor. This tumor removed totally by laminotomy and the diagnosis was epidermoid tumor

CT is the most accurate method for evaluating the extent of osseous involvement and the degree of cancellous and cortical bone loss. Tomography also helps evaluate the risk for vertebral compression. Multidetector CT is performed in most cases with a large field of view and no contrast medium. With an isotropic volume acquisition, it is possible to obtain axial, sagittal, and coronal reformatted images that are of the same quality as the source images. Two-dimensional multiplanar reformatted images are useful in the evaluation of cortical bone destruction and calcified tumor matrix.

MRI is the best imaging modality for the evaluation of spinal canal, the epidural space and neural structures. The MRI protocol should includes sagittal, axial and coronal images in all cases (Fig. 30.1). Coronal images may be helpful for the evaluation of paravertebral soft-tissue extension. Sagittal slices show the stability of the vertebrae. Axial images are needed to show the spinal cord compression and extention of the tumor. T1-weighted images are helpful for delineating normal bone marrow architecture, fat content within masses, subacute hemorrhage and for evaluating tissue enhancement after the administration of contrast material. The administration of gadolinium-based contrast material results in enhancement proportional to soft-tissue vascularity and is helpful for differentiating cystic lesions from solid masses. It is also useful for biopsy of spinal lesions. This allows differentiation of enhanced viable tumor from areas of nonenhanced necrosis. In addition, contrast material is frequently used for better demonstration of extradural extension of the tumor. Contrast material enhancement is best evaluated on fat-saturated T1-weighted MR images. Inhomogeneous suppression of the fat signal can impair the quality of images obtained in the cervical and thoracic spine because of phase-encoded motion artifacts. Thus, contrast material–enhanced nonfat-suppressed T1-weighted images are reliable and remain valuable. Dynamic contrast-enhanced MR imaging may provide information about the rapidity of the enhancement. Most pathologic processes are often highlighted on T2-weighted images due to their increased fluid content. T2-weighted images delineate spinal canal stenosis and high-signal-intensity areas resulting from myelomalacia in spinal cord compression. Short inversion time inversion-recovery imaging is very sensitive for detecting most types of soft-tissue and marrow abnormalities and is recommended if the exploration requires a large field of view, which may result in inhomogeneous suppression of fat signal with T2-weighted sequences. Full-spine and whole-body MR imaging are also very useful in the assessment and diagnosis of multifocal lesions of the skeleton (Rodallec et al. 2008).

Management Strategy

Surgery is the most effective treatment for all spinal neoplasms which located intradurally. Over the last 30 years, primary resection alone has become the treatment of choise for spinal cord tumors in children, since favorable early results following resection have been reported for these generally low-grade tumors, even when microscopic residual tumor is or is assumed to be present (O'Sullivan et al. 1994). Long-term control or cure can be achieved with total removal for the benign extramedullary tumors and lesions. Early diagnosis and aggressive initial surgery provide the best opportunity for long, and progression-free survival. A complete resection of a spinal cord tumor may be treacherous, especially if the mass involves the conus medullaris. Manipulation of the surrounding neural tissue may cause irreversible neurologic injury; therefore adequate exposure and meticulous dissection should be performed during the surgery. The surgical exposure should encompass the tumor with some rostral and caudal margins to allow for adequate visualization. Laminectomy enables a direct approach to spinal cord tumors and wide exposure of the spinal canal. It can be extended intraoperatively either rostrally or caudally. By this technique, the protective role of posterior arch is lost and spinal stability is broken.

Spinal stability depends on balanced growth of bony structures, ligaments and paravertebral musculature (McGirt et al. 2008). The pediatric spine is in a state of dynamic flux, with tensile and compressive forces in exquisite balance during growth and development (Raab et al. 2008). Any disruption of the posterior elements may create unbalanced forces that progress to radiographic, and ultimately clinically significant deformity of the spine (Raab et al. 2008). Disturbance of the posterior structures may result in failure to counterbalance the anterior bending force (Matsumoto et al. 2009). Skeletal, ligamentous or muscular injuries predisposes the patient to spinal-column instability and promotes spinal deformity, often with a rotatory component (Raab et al. 2008). Iatrogenic instability, subluxation, kyphosis may ocur after laminetomy, especially in children or when the facet joints are removed (Matsumoto et al. 2009). Spinal deformity is a well recognized complication of laminectomies in children, certainly in the cervical and thoracic regions (Cochrane and Steinbok 1992). It has been suggested that replacement of the posterior elements may help to prevent spinal deformity in children.

In an effort to reduce potential destabilisation of the spine and the necessity for more extensive surgery, laminotomy was first described by Raimondi et al. (1976) and adopted by Milhorat in 1978 for selected patients (Milhorat 1978). In children or young adults osteoplastic laminoplasty or laminotomy may reduce the incidence of postoperative deformity. Osteoplastic laminotomy preserves the structures that appear to be important for spinal stability. Bony union of the re-attached laminae is critical for the establishing anatomical integrity and stability. Laminotomy has two benefits for the patient: Firstly, it allows the reconstruction of normal anatomy following posterior approaches to the spine at all levels. Secondly, it prevents the development of myodural and epidural scatrices, which can compromise the spinal canal. The possible complications of laminotomy are dural tear and subdural hematoma (Abbott et al. 1992).

Surgical Technique of Laminotomy and Tumor Removal

Following the midline exposure of the supraspinous ligament and lumbodorsal fascia, paramedian incisions are made in the lumbodorsal fascia, preserving the attachment of the supraspinous ligament to the spinous process. The multifidus and interspinalis muscles are reflected from the spinous processes and laminae to expose the medial half of the inferior articular processes. The interspinous ligaments and ligamentum flavum are resected in the midline above and below the planned span of the laminotomy flap. This provide adequate exposure for drilling.

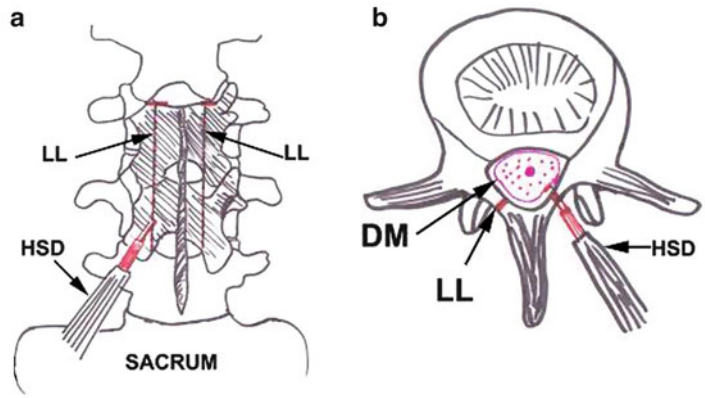

Fig. 30.2 The laminotomy line (*LL*) on the lumbar vertebrae should be linear and symmetrical (**a**). The lamina should be cut by high-speed drill (*HSD*) just above the dura mater (*DM*) (**b**)

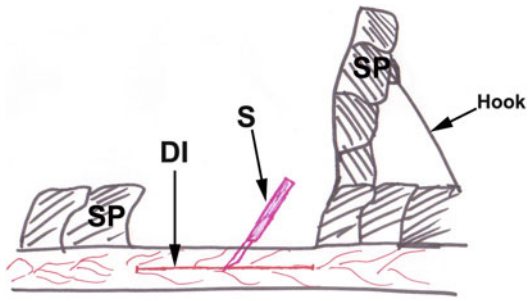

Fig. 30.3 The laminotomy flap should be reflected caudally and fixed with a hook. The spinous processes (*SP*) and interspinous ligaments are protected by laminotomy. The dural incision (*DI*) should be performed by scalpel (*S*) on the midline in order to remove the intradural tumor

The epidural fat is exposed at these levels. The laminae are marked symmetrically at points just medial to the corresponding facet joints. The supraspinous ligament is left intact as it stabilizes the laminae while the laminotomies are performed. The laminotomy should be made as far lateral as possible to provide adequate spinal canal and dural exposure. The high-speed drill is applied at the epidural plane to cut the laminae at a slightly diagonal level (Fig. 30.2). The footplate of the high-speed drill protect the dura while the drill perform the laminotomy. Gentle caudal movement of the handpiece once the laminae is cut allows safe advancement of the footplate under the superior ligamentum flavum. The laminotomy flap is fully released and preserved. Hinging the flap caudally by a hook, rather than removing it, allows the restoration of the integrity of the supraspinous ligament (Fig. 30.3). This technique also preserves partly vascular supply to the flap and ligaments. The epidural veins are carefully coagulated and thin strips of Gelfoam® are applied to the lateral gutters to assist in the tamponate of epidural bleeding.

Following the laminotomy, ultrasound may be used to verify if the bony exposure is adequate to expose the intradural tumor. If it is necessary to improve the exposure, the laminotomy may be extended in the appropriate rostral or caudal directions with high-speed drill. It is unnecessary to extend the laminotomy of the dural opening over rostral or caudal tumor-associated cysts, the latter are typically decompressed after the removal of the solid portion of tumor. For the electrophysiological monitoring, both rostral and caudal electrodes are inserted, secured and then connected to the monitoring unit.

The dura is best opened with a number 11 blade after it has been elevated with a dural hook (Fig. 30.3). A small dissector is used to peel the dura from the adjacent arachnoid mater. Retraction sutures are applied to the dural edges and suspended by hemostat clamps. The operating microscope is brought into the surgical area. After the dural opening, the midline raphe may be identified by the small diagonal vessel typically situated at the medial surface of posterior columns. The extramedullary tumors are easily removed by microinstruments and microdissection. Small cottonoids should be placed between the tumor and neural elements (spinal cord or cauda equina) in order to protect these structures. At the middle thoracic region, the roots can be coagulated and

cut in order to achieve adequate exposure and total removal of the tumor. But it is not suitable for cervical or lumbosacral region tumors. For the intramedullary tumors, the midline dorsal surface of the spinal cord is meticulously coagulated by bipolar cauter. Coagulation of posterior surface veins are usually risk free. The myelotomy incision is best initiated with an arachnoid knife in that region of the cord in which the thickest portion of the tumor exists. Next, the pial traction sutures are applied to open the lips of myelotomy incision. After the intramedullary tumor is identified the posterior columns are gently reflected laterally with the plated bayonet, exposing the lateral aspects of the tumor. The tumor is removed using microtechniques.

After the resection of spinal cord tumor, the laminar flap should be brought into its anatomical position. Symmetrical drill holes made at the both sides of laminotomy incision and suture material passed through these holes tied down in order to achieve the normal anatomical position. In order to minimize the risk of migration of the laminar flap into the canal, the interspinous suture placed at the caudal extent of the flap and those placed through the supraspinous ligament into lumbodorsal fascia are critical. These sutures take advantage of the paravertebral muscle spasm in the early postoperative period and resultant loss of lordosis to support the flap away from the dura. Titanium miniplates may also be used for the reconstruction of laminar roof after the resection of spinal tumor (Wiedemayer et al. 1998). Following the laminar reposition, the muscle retractors are removed and the paravertebral muscles are allowed to reassume their anatomical position. These muscles are sewn into the individual interspinous and supraspinous ligaments, thus facilitating balanced movements and stability in the region of laminar flap after healing (Raimondi et al. 1976).

References

Abbott R, Feldstein N, Wisoff JH, Epstein FJ (1992) Osteoplastic laminotomy in children. Pediatr Neurosurg 18:153–156

Baysefer A, Akay KM, Izci Y, Kayalı H, Timurkaynak E (2004) The clinical and surgical aspects of spinal tumors in children. Pediatr Neurol 31:261–266

Cochrane DD, Steinbok P (1992) Laminotomy: a technical note. Child's Nerv Syst 8:226–228

Houten JK, Weiner HL (2000) Pediatric intramedullary spinal cord tumors: special considerations. J Neurooncol 47:225–230

Loh JK, Lin CK, Hwang YF, Hwang SL, Kwan AL, Howng SL (2005) Primary spinal tumors in children. J Clin Neurosci 12:246–248

Matsumoto Y, Harimaya K, Doi T, Maeda T, Iwamoto Y (2009) Outcome of osteoplastic laminotomy for excision of spinal cord tumours. J Orthop Surg 17:275–279

McGirt MJ, Chaichane KL, Atiba A, Bydon A, Witham TF, Yao KC, Jallo GI (2008) Incidence of spinal deformity after resection of intramedullary spinal cord tumors in children who underwent laminectomy compared with laminoplasty. J Neurosurg Pediatr 1:57–62

Milhorat TH (1978) Pediatric neurosurgery. Contemp Neurol Ser 16:611–623

O'Sullivan C, Jenkin D, Doherty MA, Hoffman HJ, Greenberg ML (1994) Spinal cord tumors in children: long term results of combined surgical and radiation treatment. J Neurosurg 81:507–512

Raab P, Juergen K, Gloger H, Soerensen N, Wild A (2008) Spinal deformity after multilevel osteoplastic laminotomy. Int Orthop 32:355–359

Raimondi AJ, Gutierrez FA, Di Rocco C (1976) Laminotomy and total reconstruction of the posterior spinal arch for spinal canal surgery in childhood. J Neurosurg 45:555–560

Rodallec MH, Feydy A, Larousserie F, Anract P, Campagna R, Babinet A, Zins M, Drapé JL (2008) Diagnostic imaging of solitary tumors of the spine: what to do and say. Radiographics 28:1019–1041

Schick U, Marquardt G (2001) Pediatric spinal tumors. Pediatr Neurosurg 35:120–127

Wiedemayer H, Schoch B, Stolke D (1998) Osteoplastic laminotomy using titanium microplates for reconstruction of the laminar roof: a technical note. Neurosurg Rev 21:93–97

Yamamoto Y, Raffel C (1999) Spinal extradural and intradural-extramedullary neoplasms. In: Albright AL, Pollack IF, Adelson PD (eds) Principles and practice of pediatric neurosurgery. Thieme, New York, pp 685–696

Treatment of Metastatic Spinal Epidural Disease: Surgery Versus Radiotherapy

31

Chester K. Yarbrough, Wilson Z. Ray, and Meic H. Schmidt

Contents

Abstract

Metastatic spinal disease occurs frequently, affecting as many as 40% of cancer patients over time. Approximately 10% of these patients experience malignant epidural spinal cord compression. Metastasis to the spine causes significant morbidity and often leads an inexorable decline into paraplegia and loss of bowel and bladder function. Advances in surgical techniques have allowed a more aggressive approach to treatments for cytoreduction and spinal stabilization. Fractionated radiotherapy has proved effective in treatment of these patients, but inability to design and implement highly conformal plans initially limited the use of radiation delivery until the development of stereotactic and highly conformal radiation delivery techniques. The current data supporting surgery and radiation for treatment of metastatic spinal disease will be reviewed in this chapter.

Introduction

Malignant epidural spinal cord compression (MESCC) is defined as radiographic displacement of the spinal cord by a neoplastic lesion (Loblaw et al. 2003). The natural history of untreated MESCC is grim, with the expected outcome being stepwise motor and sensory decline, pain, and loss of bowel and bladder control. Between 20,000 and 30,000 new cases of MESCC are diagnosed yearly in the United States, with

W.Z. Ray • M.H. Schmidt (✉)
Department of Neurosurgery, University of Utah, Clinical Neurosciences Center, 175 N. Medical Drive East, Salt Lake City, UT 84132-2303, USA
e-mail: meic.schmidt@hsc.utah.edu

C.K. Yarbrough
Department of Neurosurgery, Washington University, 660 S. Euclid Avenue, 63110, St. Louis, MO, USA

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_31,

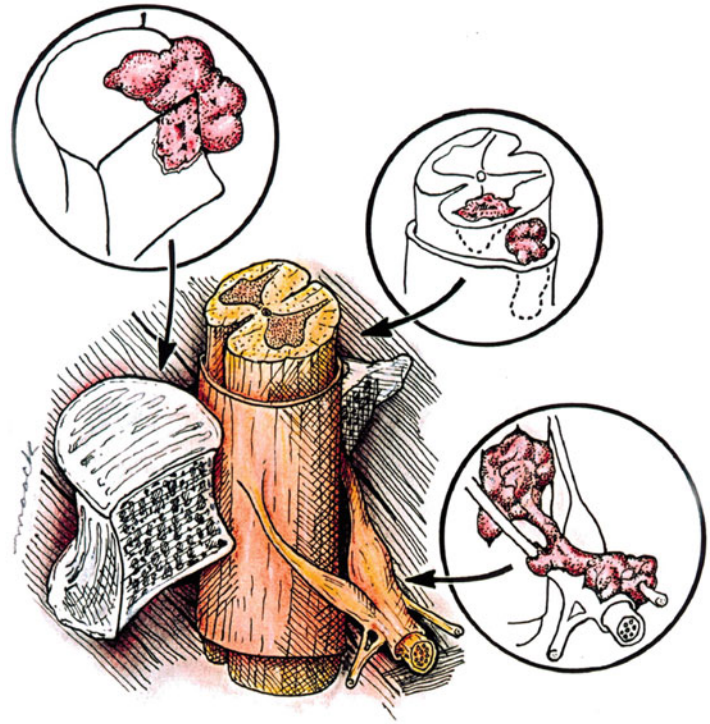

Fig. 31.1 Locations of metastases to the spine. Most tumor emboli are found in the vertebral column surrounding the spinal cord, with the posterior half of the vertebral body being the most common initial focus (*upper left inset*). Tumor can also originate in a paravertebral location and track along the spinal nerves to enter the spinal column by way of the neural foramina (*lower right inset*). Both of these mechanisms can lead to epidural spinal cord compression. Intramedullary and subdural/leptomeningeal metastatic deposits are rarely encountered (*middle inset*) (Reprinted with permission from Klimo and Schmidt 2004. Copyright reserved by AlphaMed Press)

peak incidence among adults aged 40–65 years (Perrin and Laxton 2004; Kwok et al. 2006). Men are affected more often than women, likely because of the propensity of prostate cancer to metastasize to the spine (Perrin and Laxton 2004; Zaikova et al. 2011). MESCC occurs in the setting of many primary malignancies, although breast, lung, and prostate primary disease account for approximately 50% of cases (Byrne 1992). Breast and lung cancer appear more likely to cause spinal metastases than prostate cancer (Gerszten and Welch 2000). Some studies have estimated that 5–10% of cancer patients will suffer from spinal metastases during the disease course (Barron et al. 1959; Schaberg and Gainor 1985; Bach et al. 1990). In patients with spinal metastases, MESCC was the initial presenting event in 20% of patients in one series (Schiff et al. 1997), and only 16% of patients in another population-based study presented with a solitary lesion (Zaikova et al. 2011). Approximately 2.5% of cancer patients in one study were admitted to the hospital because of MESCC during the last 5 years of life (Loblaw et al. 2003). In patients requiring treatment for MESCC, one study showed median survival of approximately 3 months (Loblaw et al. 2003), while another showed median survival of 5 months, with 26% of patients dying within 2 months of treatment (Zaikova et al. 2011). Approximately 30% of patients presenting with MESCC will survive 1 year or longer (Rades et al. 2011; Zaikova et al. 2011). Survival after treatment has been associated with primary site, with patients with melanoma, renal cell carcinoma, gastrointestinal cancer, and unknown primary disease showing shorter survival after treatment (Zaikova et al. 2011).

The majority of metastases occur in the thoracic spine, although metastasis is possible anywhere in the spine (Constans et al. 1983; Bach et al. 1990; Helweg-Larsen 1996). Anatomically, MESCC may involve neoplasia in any portion of the spinal canal, with or without involvement of the vertebral body (Fig. 31.1). The metastasis arises in the vertebral

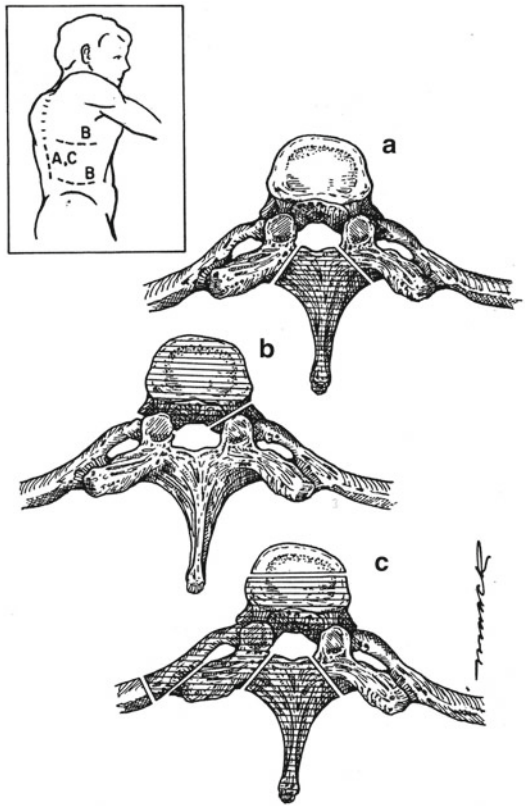

Fig. 31.2 Surgical approaches to the spine. The *shaded areas* indicate the bone removed in each of the approaches. (**a**) Laminectomy. The spinous process and the adjacent lamina are removed up to the junction of the pedicles. This was the standard surgical procedure for many years regardless of where the tumor was actually located within the vertebra. It can still be used for disease isolated to the posterior elements. (**b**) Transthoracic or retroperitoneal. These anterior approaches provide direct access to the vertebral body in the thoracic (transthoracic) and thoracolumbar/lumbar regions (retroperitoneal). (**c**) Posterolateral. For patients who cannot tolerate an anterior approach or who have significant posterior extension of their disease, a posterolateral approach provides excellent access to both the anterior and posterior elements. The *inset* shows the skin incisions for each of the approaches. The laminectomy (**a**) and posterolateral (**b**) approaches can be performed through a midline incision. The transthoracic (*upper 'B' line*) and retroperitoneal approaches (*lower 'B' line*) require flank incisions (Reprinted with permission from Klimo and Schmidt 2004. Copyright reserved by AlphaMed Press)

body in 85% of cases, the paravertebral space in 10–15%, and the subarachnoid, subdural, or epidural space in under 5% of cases (Byrne 1992). Approximately 30% of patients with epidural metastases have multiple lesions (Schiff et al. 1998). Imaging of the entire spine is paramount, as failure to do so may miss as many as 20% of additional lesions (Schiff et al. 1998).

Management of metastatic cancer is complex and requires a multidisciplinary approach involving surgical and medical specialists. In this chapter, we seek to provide a broad but concise discussion of management options of MESCC. Treatment of metastatic spinal disease that does not cause cord compression will be included as part of this discussion. We believe this is appropriate, because the natural history of spinal metastases frequently leads to MESCC and the treatment options for spinal metastases not causing MESCC are the same as those for MESCC. Whether surgery or radiotherapy (XRT) or both is performed, the goals of treatment—local control for neurological preservation and mechanical stability—are the same. Evaluation and treatment of suspected MESCC should be urgent, and intervention should occur within 24 h (National Institute for Health and Clinical Excellence 2008).

Surgery for Epidural Metastases

Initially, surgery for MESCC involved laminectomy alone, with or without XRT (Fig. 31.2). The results were generally disappointing, as posterior decompression alone often did not address the location of the neoplastic process (Young et al. 1980). As discussed above, most spinal metastases occur in the vertebral body and cause neurologic decline with secondary extension into the spinal canal or neural foramen. In 1984, Sundaresan et al. (1984) reported a large group of patients with MESCC in whom ambulation was preserved with vertebral column resection from an anterior approach. The ambulation rate improved from 48% preoperatively to 78% postoperatively, and pain decreased in 84% of patients undergoing surgery (Sundaresan et al. 1984). Coincident with this report, others authors reported case series with similar outcomes using more complete resection of metastatic lesions (Siegal et al. 1982;

Harrington 1984; Overby and Rothman 1985). As data supporting more extensive surgical approaches to metastatic spinal lesions accrued, concurrent improvements in surgical techniques provided better strategies for stabilization after resection.

A landmark randomized control trial supported use of surgical decompression in patients with a single lesion causing MESCC when the likely primary site was not known to be exquisitely sensitive to XRT (Patchell et al. 2005). This study included 101 patients who were randomized either to surgery with adjuvant XRT or to XRT alone. Exclusion criteria included patients with more than one site of MESCC and those with known primary types of small cell lung carcinoma, myeloma, and lymphoma. After treatment, 84% of patients in the surgical group were able to walk, whereas 57% of patients in the radiotherapy-alone group were walking. Additionally, patients who underwent surgery plus XRT remained ambulatory for a median of 122 days, where the XRT alone group did so for a median of 13 days (Patchell et al. 2005). This study supported the use of decompressive surgery in addition to XRT for a specific and well-defined group of patients—those suffering acute (<48 h) neurological symptoms from a single lesion with a known primary site, excluding those with exquisitely radiosensitive primary disease. Interestingly, patients who underwent surgery in this landmark study also showed a survival benefit of 26 days (median 126 versus 100 days) (Patchell et al. 2005).

After Patchell et al. (2005) definitively supported the use of surgery for the preservation and improvement of neurologic function in the setting of MESCC, consideration of surgery became more important. The many technical advancements in spinal instrumentation that have occurred in the past several decades will not be reviewed here; however, we note that improvements in surgical fixation have increased the ability of surgeons to offer successful cytoreductive and stabilizing surgeries. The particular techniques used are well reviewed elsewhere (Klimo and Schmidt 2004; Quraishi et al. 2010).

Radiotherapy for Epidural Metastases

Although early surgical strategies sometimes involved adjuvant XRT for the treatment of MESCC, the use of XRT for metastases has evolved over time in concert with evolving technology in the field of radiation oncology. Most recently, development of stereotactic radiosurgery (SRS) has broadened the armamentarium with which neurosurgeons and radiation oncologists may treat MESCC. SRS allows for higher treatment doses without a coincident increase in irradiation of unaffected, adjacent structures by utilizing image guidance and stereotaxis to target the affected area (Fig. 31.3).

XRT is a noninvasive treatment, but side effects and toxicity can limit the ability to tolerate a treatment course. Nevertheless, in one large series, 94% of patients completed the recommended fractionated XRT (Zaikova et al. 2011). Although a detailed discussion of radiation biology and the molecular effects of XRT on normal spinal cord is outside the scope of this chapter, a short discussion of dose toxicity is warranted. Conventional external beam XRT has limited ability to contour radiation treatment to exclude normal anatomy. Inability to contour the treatment plan increases the chances of dose toxicity to surrounding structures. In treatment of spinal metastases, the spinal cord typically causes the greatest concern for treatment toxicity. Radiation of the spinal cord may lead to myelopathy, although the risk of symptomatic myelopathy after conventional XRT appears to be low (Kirkpatrick et al. 2010). A review of studies of XRT to cervical spine metastases revealed 14 of 335 (4.2%) patients demonstrated symptoms of myelopathy after treatment using a variety of treatment plans (Kirkpatrick et al. 2010). Conventional XRT to the thoracic spine exhibits a lower incidence of post-treatment myelopathy at 2.8% (55 of 1946 patients) (Kirkpatrick et al. 2010). These rates are quite low, but treatment dosing has been guided by preclinical studies showing myelopathy as severe as paralysis in animal models. Of note, preclinical studies have shown variation in radiosensitivity of white matter and gray matter

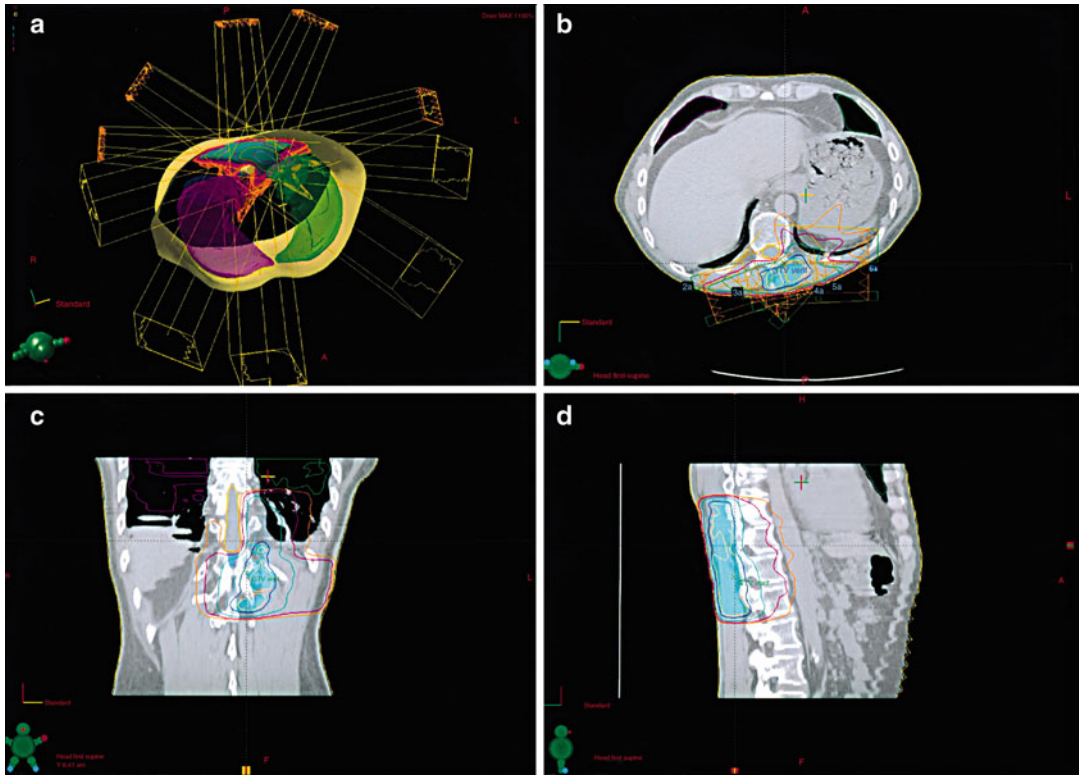

Fig. 31.3 IMRT. This 56-year-old man has metastatic lung cancer to the left paraspinal region in the midthoracic area. (**a**) Axial model of the patient showing the location of his tumor with respect to the spinal cord and lungs and the radial arrangement of the beams. (**b**) Axial, (**c**) coronal, and (**d**) sagittal images of the planned treatment showing the isodose lines: 100% (*yellow*), 90% (*dark blue*), 80% (*light blue*), 50% (*green*), 30% (*purple*), and 20% (*orange*). The spinal cord is outlined in *yellow*. Note how the beams are contoured to avoid the spinal cord (Reprinted with permission from Klimo and Schmidt 2004. Copyright reserved by AlphaMed Press)

in the cervical spinal cord (Bijl et al. 2005). The central gray matter appears to be more resistant to the effects of radiation, while the white matter exhibits changes at much lower dosages, and white matter necrosis is present in rats suffering from radiation-related paralysis (Bijl et al. 2005).

Traditionally, fractionated external beam radiation was used for treatment, with a total of 30 Gy given to the field over a period of ten sessions (Kwok et al. 2006). This treatment paradigm has significant limitations, as the initial technology did not allow accurate and conformal treatment planning. In a recent nonrandomized study, patients were treated with either hypofractionated radiotherapy (8 Gy × 1 session or 5 Gy × 4 sessions) or with varying schedules of standard fractionated radiotherapy (3 Gy × 10 sessions, 2.5 Gy × 15 sessions, or 2 Gy × 20 sessions) (Rades et al. 2011). The results of this study, which involved patients with a wide variety of primary lesions, showed similar motor outcomes between the two treatments. A trend towards improved survival was shown in the standard groups, with 30% of patients alive at 1 year in the standard group versus 23% of patients in the hypofractionated group (Rades et al. 2011). The authors found a significant difference in local recurrence rates at 1 year (61% in the hypofractionated group versus 81% in the standard group) (Rades et al. 2011). This study suggests that short-term (i.e., weeks to months) outcomes concerning motor function and pain assessments are similar between hypofractionated and standard XRT algorithms, thus supporting the use of less demanding treatments in

patients with significant impairment but short expected survival. This study confirmed the findings of an earlier randomized-controlled trial that showed equivalent pain relief between single fraction (8 Gy × 1 session) and a longer treatment paradigm (5 Gy × 4 sessions) (Nielsen et al. 1998).

The advent of SRS for treatment of extracranial neoplasms has allowed for improvements in dosimetry planning for spinal metastases and shorter, more tolerable treatment periods (Gerszten et al. 1995; Chang et al. 2007; Sheehan et al. 2009). Additionally, more than one lesion may be safely targeted without necessarily increasing the radiation dose to critical unaffected structures. Sheehan et al. (2009) reported excellent local control in patients by using SRS (mean tumoral dose 17.3 Gy; range 10–24 Gy) as a primary or adjunctive treatment for MESCC. In 40 patients with 110 masses and a median follow-up of 12.7 months, 72% of patients with lung cancer and 64% of patients with prostate cancer metastases showed radiographic response to treatment. Yamada et al. (2008) reported excellent control in a wide variety of primary disease types using intensity-modulated radiotherapy and tumoral doses of 18–24 Gy and showed convincingly the improvement in local control with increasing dose given to a lesion. Local control in this study was 90%, with a median follow-up of 15 months (Yamada et al. 2008). Similarly, Gerszten et al. (2007) presented 500 patients treated with SRS (median tumoral dose 20 Gy; range 12.5–25 Gy) for a wide variety of primary disease, showing 88% radiographic control across all patients at last follow-up. Radiographically, SRS has been shown to decrease tumor volume by up to two thirds within 2 months, with durable improvement in thecal sac compression (Ryu et al. 2010). Advances in three-dimensional planning and technology have allowed treatment of larger masses with increasing doses of radiation without increasing risk of toxicity to surrounding structures (Bilsky et al. 2004; Chang et al. 2007; Gerszten et al. 2007; Yamada et al. 2008). An increasing amount of data has demonstrated the safety and utility of conformal XRT when used as primary or as adjunctive treatment in the management of metastatic spinal disease.

Discussion

Metastatic spinal disease occurs in up to 40% of cancer patients, and MESCC occurs in up to 10% (Barron et al. 1959; Schaberg and Gainor 1985; Bach et al. 1990). As the population ages and mortality decreases from non-neoplastic chronic medical illnesses, MESCC may increase in incidence. Management of MESCC will continue to be a challenging issue, as there are multiple factors to consider when deciding on treatment. Rarely will surgery alone be considered adequate treatment. XRT either via conventional fractionated XRT or via SRS provides approximately 90% local control of a variety of primary cancers (Gerszten et al. 2007; Yamada et al. 2008; Sheehan et al. 2009; Zaikova et al. 2011). However, XRT by any method cannot provide re-stabilization of a spine with destabilizing neoplastic disease.

Different methods for quantifying spinal stability in the context of neoplastic illness have been proposed (Kostuik and Weinstein 1991; Fourney et al. 2011). Regardless of method, it is clear that some patients will require surgical stabilization in the course of treatment. Given the improved methods of addressing metastases regardless of which portion of the vertebral column is involved (Quraishi et al. 2010), surgical decompression must be considered should the patient be healthy enough to undergo surgery. Given the specific situations in which surgical intervention is indicated (Patchell et al. 2005), multidisciplinary evaluation of each patient with MESCC is warranted to ensure that treatment is enacted urgently, whether surgery or XRT is determined to be the primary treatment. Technological advancements in XRT allow much higher doses of radiation to the tumoral bed while preserving surrounding structures, so cancers once perceived to be radioresistant may now show improved outcomes when treated with highly conformal XRT.

Direct comparison of surgery and XRT for treatment of metastatic spinal disease is fraught with confounding factors such as patient age, health, presence of additional metastatic disease, involvement of multiple spinal levels, and radiosensitivity of the neoplasm. Whether or not surgery is performed, nearly all patients healthy enough to undergo XRT will be offered it. Despite the difficulty directly comparing surgery and XRT, comprehensive care of cancer patients requires the availability of both.

In conclusion, metastatic spinal disease affects a large portion of cancer patients. MESCC causes significant disability in cancer patients, and requires urgent treatment. Advances in surgical and radiation treatments have broadened the options available to the cancer patient and improved quality of life and local control possible with treatment. A multidisciplinary approach to the cancer patient with MESCC is required for optimal patient-centered care.

References

Bach F, Larsen BH, Rohde K, Borgesen SE, Gjerris F, Boge-Rasmussen T, Agerlin N, Rasmusson B, Stjernholm P, Sorensen PS (1990) Metastatic spinal cord compression. Occurrence, symptoms, clinical presentations and prognosis in 398 patients with spinal cord compression. Acta Neurochir (Wien) 107:37–43

Barron KD, Hirano A, Araki S, Terry RD (1959) Experiences with metastatic neoplasms involving the spinal cord. Neurology 9:91–106

Bijl HP, van Luijk P, Coppes RP, Schippers JM, Konings AW, van Der Kogel AJ (2005) Regional differences in radiosensitivity across the rat cervical spinal cord. Int J Radiat Oncol Biol Phys 61:543–551

Bilsky MH, Yamada Y, Yenice KM, Lovelock M, Hunt M, Gutin PH, Leibel SA (2004) Intensity-modulated stereotactic radiotherapy of paraspinal tumors: a preliminary report. Neurosurgery 54:823–830, discussion 830–831

Byrne TN (1992) Spinal cord compression from epidural metastases. N Engl J Med 327:614–619

Chang EL, Shiu AS, Mendel E, Mathews LA, Mahajan A, Allen PK, Weinberg JS, Brown BW, Wang XS, Woo SY, Cleeland C, Maor MH, Rhines LD (2007) Phase I/II study of stereotactic body radiotherapy for spinal metastasis and its pattern of failure. J Neurosurg Spine 7:151–160

Constans JP, de Divitiis E, Donzelli R, Spaziante R, Meder JF, Haye C (1983) Spinal metastases with neurological manifestations. Review of 600 cases. J Neurosurg 59:111–118

Fourney DR, Frangou EM, Ryken TC, Dipaola CP, Shaffrey CI, Berven SH, Bilsky MH, Harrop JS, Fehlings MG, Boriani S, Chou D, Schmidt MH, Polly DW, Biagini R, Burch S, Dekutoski MB, Ganju A, Gerszten PC, Gokaslan ZL, Groff MW, Liebsch NJ, Mendel E, Okuno SH, Patel S, Rhines LD, Rose PS, Sciubba DM, Sundaresan N, Tomita K, Varga PP, Vialle LR, Vrionis FD, Yamada Y, Fisher CG (2011) Spinal instability neoplastic score: an analysis of reliability and validity from the spine oncology study group. J Clin Oncol 29:3072–3077

Gerszten PC, Welch WC (2000) Current surgical management of metastatic spinal disease. Oncology (Williston Park) 14:1013–1024, discussion 1024, 1029–1030

Gerszten PC, Lunsford LD, Rutigliano MJ, Kondziolka D, Flickinger JC, Martinez AJ (1995) Single-stage stereotactic diagnosis and radiosurgery: feasibility and cost implications. J Image Guid Surg 1:141–150

Gerszten PC, Burton SA, Ozhasoglu C, Welch WC (2007) Radiosurgery for spinal metastases: clinical experience in 500 cases from a single institution. Spine (Phila Pa 1976) 32:193–199

Harrington KD (1984) Anterior cord decompression and spinal stabilization for patients with metastatic lesions of the spine. J Neurosurg 61:107–117

Helweg-Larsen S (1996) Clinical outcome in metastatic spinal cord compression. A prospective study of 153 patients. Acta Neurol Scand 94:269–275

Kirkpatrick JP, van der Kogel AJ, Schultheiss TE (2010) Radiation dose-volume effects in the spinal cord. Int J Radiat Oncol Biol Phys 76:S42–S49

Klimo P Jr, Schmidt MH (2004) Surgical management of spinal metastases. Oncologist 9:188–196

Kostuik JP, Weinstein JN (1991) Differential diagnosis and surgical treatment of metastatic spine tumors. In: Frymoyer JW (ed) The adult spine: principles and practice. Raven, Baltimore, pp 861–888

Kwok Y, Tibbs PA, Patchell RA (2006) Clinical approach to metastatic epidural spinal cord compression. Hematol Oncol Clin North Am 20:1297–1305

Loblaw DA, Laperriere NJ, Mackillop WJ (2003) A population-based study of malignant spinal cord compression in Ontario. Clin Oncol (R Coll Radiol) 15:211–217

National Institute for Health and Clinical Excellence (2008) Metastatic spinal cord compression. Retrieved 15 Sep, 2011, from http://guidance.nice.org.uk/CG75

Nielsen OS, Bentzen SM, Sandberg E, Gadeberg CC, Timothy AR (1998) Randomized trial of single dose versus fractionated palliative radiotherapy of bone metastases. Radiother Oncol 47:233–240

Overby MC, Rothman AS (1985) Anterolateral decompression for metastatic epidural spinal cord tumors. Results of a modified costotransversectomy approach. J Neurosurg 62:344–348

Patchell RA, Tibbs PA, Regine WF, Payne R, Saris S, Kryscio RJ, Mohiuddin M, Young B (2005) Direct decompressive surgical resection in the treatment of spinal cord compression caused by metastatic cancer: a randomised trial. Lancet 366:643–648

Perrin RG, Laxton AW (2004) Metastatic spine disease: epidemiology, pathophysiology, and evaluation of patients. Neurosurg Clin N Am 15:365–373

Quraishi NA, Gokaslan ZL, Boriani S (2010) The surgical management of metastatic epidural compression of the spinal cord. J Bone Joint Surg Br 92:1054–1060

Rades D, Lange M, Veninga T, Stalpers LJ, Bajrovic A, Adamietz IA, Rudat V, Schild SE (2011) Final results of a prospective study comparing the local control of short-course and long-course radiotherapy for metastatic spinal cord compression. Int J Radiat Oncol Biol Phys 79:524–530

Ryu S, Rock J, Jain R, Lu M, Anderson J, Jin JY, Rosenblum M, Movsas B, Kim JH (2010) Radiosurgical decompression of metastatic epidural compression. Cancer 116:2250–2257

Schaberg J, Gainor BJ (1985) A profile of metastatic carcinoma of the spine. Spine (Phila Pa 1976) 10:19–20

Schiff D, O'Neill BP, Suman VJ (1997) Spinal epidural metastasis as the initial manifestation of malignancy: clinical features and diagnostic approach. Neurology 49:452–456

Schiff D, O'Neill BP, Wang CH, O'Fallon JR (1998) Neuroimaging and treatment implications of patients with multiple epidural spinal metastases. Cancer 83:1593–1601

Sheehan JP, Shaffrey CI, Schlesinger D, Williams BJ, Arlet V, Larner J (2009) Radiosurgery in the treatment of spinal metastases: tumor control, survival, and quality of life after helical tomotherapy. Neurosurgery 65:1052–1061, discussion 1061–1062

Siegal T, Robin G, Lubetzki-Korn I, Fuks Z (1982) Anterior decompression of the spine for metastatic epidural cord compression: a promising avenue of therapy? Ann Neurol 11:28–34

Sundaresan N, Galicich JH, Bains MS, Martini N, Beattie EJ Jr (1984) Vertebral body resection in the treatment of cancer involving the spine. Cancer 53:1393–1396

Yamada Y, Bilsky MH, Lovelock DM, Venkatraman ES, Toner S, Johnson J, Zatcky J, Zelefsky MJ, Fuks Z (2008) High-dose, single-fraction image-guided intensity-modulated radiotherapy for metastatic spinal lesions. Int J Radiat Oncol Biol Phys 71:484–490

Young RF, Post EM, King GA (1980) Treatment of spinal epidural metastases. Randomized prospective comparison of laminectomy and radiotherapy. J Neurosurg 53:741–748

Zaikova O, Fossa SD, Bruland OS, Giercksky KE, Sandstad B, Skjeldal S (2011) Radiotherapy or surgery for spine metastases? Acta Orthop 82:365–371

Metastatic Spinal Cord Compression from Synovial Sarcoma: Surgical Resection

32

Karen K. Anderson, Paul M. Arnold
and Maura F. O'Neil

Contents

Abstract

Synovial sarcoma is rare, and metastasis of synovial sarcoma to the spine is even rarer. Surgeons should have a high index of suspicion when evaluating patients who present with a history of synovial sarcoma, a spine lesion, and concomitant back pain and neurologic symptoms. Thorough radiological work-up should include CT and MRI in order to confirm the diagnosis and ensure appropriate management. Although not curative, surgical resection followed by spinal decompression, fusion, and fixation can lead to neurologic improvement in patients who present with spinal cord compression.

In this chapter, two illustrative cases are presented describing the surgical treatment of spinal cord compression caused by metastatic synovial sarcoma. The clinical presentation and prognostic factors of synovial sarcoma are discussed, as well as its cellular and histopathologic classification. Methods of diagnosis are described, which include histopathology, immunohistochemistry, and molecular studies,

K.K. Anderson • P.M. Arnold (✉)
Department of Neurosurgery, University of Kansas Medical Center, 3901 Rainbow Boulevard, Mail Stop 3021, Kansas City, KS 66160, USA
e-mail: parnold@kumc.edu

M.F. O'Neil
Departments of Pathology and Laboratory Medicine, University of Kansas Medical Center, 3901 Rainbow Boulevard, Mail Stop 3021, Kansas City, KS 66160, USA

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_32, © Springer Science+Business Media Dordrecht 2013

as well as the use of radiographs, CT, and MRI. Lastly, the treatment of both primary and metastatic synovial sarcoma is discussed, with sections on surgery, radiation, and chemotherapy.

Introduction: Spinal Metastases

Metastases will develop in approximately two-thirds of patients with cancer (Ratliff and Cooper 2004). The skeletal system is the third most common site of metastasis after lungs and liver. Within the skeletal system, the spine is the most common location for metastatic deposits (Ratliff and Cooper 2004; Holman et al. 2005; Quraishi et al. 2010).

The most common cancers involving the spine are prostate (90%), breast (75%), melanoma (55%), lung (45%), and renal cell carcinoma (30%) (Ratliff and Cooper 2004). Symptomatic metastatic spinal cord compression is seen most frequently in cancers of the breast (22%), lung (15%), and prostate (10%). Clinical evidence of spinal cord, cauda equina, or nerve root compromise is seen in 5% of all cancer cases (Ratliff and Cooper 2004).

Emergent evaluation is required when a patient presents with metastatic disease and concomitant neurologic deficit due to spinal cord or nerve root compromise (Ratliff and Cooper 2004). Metastatic disease involving the spine can cause a variety of neurologic deficits from mild radicular weakness to paraparesis. An initial mild weakness may be followed by gradual progression due to increasing compression of the spinal cord, cauda equina, and nerve roots. Vertebral body metastases may cause local, radicular, or axial pain (Ratliff and Cooper 2004; Holman et al. 2005). A pathologic fracture or vertebral body fracture/dislocation may cause an acute onset of severe back pain (Ratliff and Cooper 2004).

The incidence of metastatic epidural spinal cord compression (MESCC) in patients with extraspinal soft tissue sarcomas is ~3% (Merimsky et al. 2004). Approximately 90% of patients present with local and/or radicular pain, and up to 50% of patients may have sensory and/or bladder/bowel dysfunction and be non-ambulatory (Loblaw and Mitera 2011). Spinal cord compression is an emergent event and may lead to long-lasting disability in patients who are misdiagnosed or treated unsuccessfully (Merimsky et al. 2004). If untreated, the patient may develop intractable and progressive pain, paralysis, sensory loss, and sphincter dysfunction. Spinal metastases can have a significant impact on the amount of time a patient spends in the hospital, and median survival after diagnosis of MESCC is only 3–5 months (Merimsky et al. 2004; Loblaw and Mitera 2011).

Nearly all major types of systemic cancers can spread to the spine, and spinal metastases can occur at any time during the patient's primary disease course (Ratliff and Cooper 2004). In postmortem studies of patients with cancer, metastases in the spine have been seen in ~90% of patients (Holman et al. 2005; Quraishi et al. 2010), and symptomatic metastatic spinal disease has been seen in ~30% of patients (Ratliff and Cooper 2004; Holman et al. 2005).

The thoracic region is the site of 70% of spinal metastases, compared to only 20% in the lumbar region and 10% in the cervical region (Holman et al. 2005). Metastatic spinal lesions may be asymptomatic, though at least 90% of patients present with back pain (Ratliff and Cooper 2004). The pain may gradually increase as the vertebral body is progressively destroyed, and movement may worsen the pain.

Background: Synovial Sarcoma

Synovial sarcoma is a rare malignant soft tissue neoplasm of uncertain differentiation (Fletcher et al. 2002), which comprises <1% of all malignancies and only 5–10% of all soft tissue sarcomas in adults (Baptista et al. 2006; Eilber and Dry 2008; Sultan et al. 2009; Koehler et al. 2009; Puffer et al. 2011). It is a high-grade tumor and is considered to have a poor prognosis compared with other soft tissue sarcomas (Eilber et al. 2007). The annual incidence is ~2–3 cases per 100,000 people (Sakellaridis et al. 2006; Sultan et al. 2009), with <5,000 cases each year in the United States (Fedors et al. 2010).

Synovial sarcoma is the fourth most common adult soft tissue sarcoma (Baptista et al. 2006; Fedors et al. 2010), and the third most common adult extremity soft tissue sarcoma (Eilber and

Dry 2008; Koehler et al. 2009). Unlike some other soft tissue histologies, synovial sarcoma has no specific predisposing etiological agent or genetic condition (Eilber and Dry 2008; Fedors et al. 2010). Synovial sarcoma has no predilection for ethnicity or gender.

Synovial sarcoma occurs primarily in the extremities of adolescents and young adults between the ages of 15–40, with median age of 35 years (Eilber and Dry 2008; Koehler et al. 2009; Sultan et al. 2009; Fedors et al. 2010; Puffer et al. 2011). Most other soft tissue sarcomas in adults occur in the 50s (Eilber and Dry 2008; Koehler et al. 2009; Fedors et al. 2010). Approximately 30% of cases occur before the age of 20 (Laor 2004; Sultan et al. 2009; Puffer et al. 2011) and 90% occur before the age of 50 (Sakellaridis et al. 2006).

These tumors are commonly found adjacent to joints in the soft tissue of the extremities and bear microscopic resemblance to developing synovium (Eilber and Dry 2008); thus, they commonly have been designated tumors of the synovium. The name is a misnomer (Ravnik et al. 2009) because no evidence indicates that synovial sarcoma derives from or differentiates toward synovial tissue (Fletcher et al. 2002; Eilber and Dry 2008; Koehler et al. 2009; Fedors et al. 2010; Puffer et al. 2011). Ultrastructural studies and immunochemistry have identified the cells of origin as epithelial (Eilber and Dry 2008; Koehler et al. 2009; Puffer et al. 2011), arising from undifferentiated mesenchymal tissues (Laor 2004; Suh et al. 2005; Davicioni et al. 2008). Despite the advances in understanding of its molecular biology, its cellular genesis remains unclear (Ladanyi et al. 2002; Greene et al. 2006; Davicioni et al. 2008).

Synovial sarcoma may occur at any anatomical site (Fletcher et al. 2002; Baptista et al. 2006; Eilber and Dry 2008), though >80% arise in deep soft tissues adjacent to joints or tendon sheaths of an extremity, especially the lower extremity (Laor 2004; Baptista et al. 2006; Eilber and Dry 2008; Ravnik et al. 2009; Fedors et al. 2010; Puffer et al. 2011), and particularly the knee (Fletcher et al. 2002; Suh et al. 2005; Fedors et al. 2010; Puffer et al. 2011). The majority of tumors (40–50%) are found within 5–7 cm of a joint (Fedors et al. 2010), and the most common location is the knee. Only ~5% of cases arise within a joint or bursa (Fletcher et al. 2002; Puffer et al. 2011), and these intra-articular lesions are often more aggressive (Fedors et al. 2010).

Only ~20% of all primary synovial sarcomas arise in nonextremity sites (Guillou et al. 2004; Ferrari et al. 2004; Suh et al. 2005; Eilber and Dry 2008). Up to 15% are found in the body axis (Koehler et al. 2009; Puffer et al. 2011), including the trunk (~8%) (Ferrari et al. 2004; Guillou et al. 2004; Eilber and Dry 2008); the head and neck (~5%) (Ferrari et al. 2004; Guillou et al. 2004; Murphey et al. 2006; Eilber and Dry 2008; Koehler et al. 2009; Fedors et al. 2010); the pelvis (~8%) (Murphey et al. 2006; Fedors et al. 2010); the thorax/chest wall (~7%) (Murphey et al. 2006; Fedors et al. 2010); and the retroperitoneum/abdomen (~7%) (Ferrari et al. 2004; Guillou et al. 2004; Murphey et al. 2006; Eilber and Dry 2008; Koehler et al. 2009; Fedors et al. 2010).

Illustrative Case 1: Metastatic Synovial Sarcoma with Cervical Spinal Cord Compression Treated with Posterior Ventral Resection

A 26-year-old female presented in May 2007 with neck pain and left arm weakness. The patient's history was significant for metastatic synovial sarcoma. At ~age 10, while living in Mexico, the patient was diagnosed with synovial sarcoma in the right knee, which was resected. Three additional resections were performed for three subsequent recurrences. The fourth recurrence, at ~18 years of age, was resected and followed by radiation. Shortly after this, the patient immigrated to the United States. Five years later, in the fall of 2004, the tumor recurred in the right knee and right thigh. The patient underwent seven cycles of chemotherapy, finishing in May 2005. For the next 20 months, she did well without any symptoms. In January 2007, a mass was found in the lung. Pathological evaluation of that resected mass was consistent with monophasic synovial sarcoma.

Ten weeks after resection of the lung mass, the patient was informed by the cancer service at our

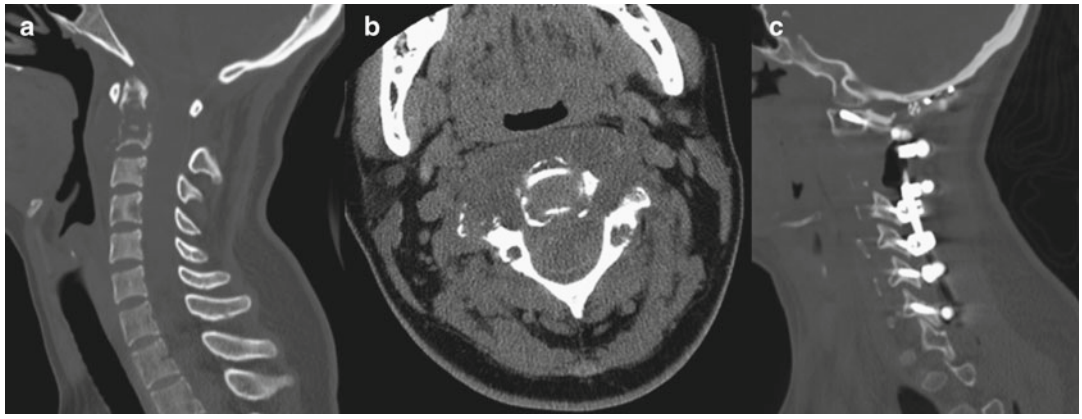

Fig. 32.1 Sagittal CT (**a**) shows tumor destroying C2. Axial CT (**b**) shows tumor involvement at C2. Post-operative sagittal CT (**c**) study shows resection of the C2 body and placement of hardware

medical center that she had widely extensive metastatic disease. Four weeks later, she presented to our service with neck pain and left arm weakness. Neurological examination revealed motor strength to be 5/5 in all muscle groups of the upper and lower extremities, with the exception of the left upper extremity. She was found to have 3/5 left deltoid strength, 3/5 left biceps, 4+/5 left triceps, and 5/5 left hand grip strength. The rest of the neurological exam was normal.

Computed tomography (CT) of the cervical spine showed lytic destruction of the dens and body of C2 with narrowing of the spinal canal and compression of the cord at that level. There was also an adjacent soft tissue component extending into the prevertebral soft tissues, and a pathologic fracture with complete disassociation of the dens from the body of C2 without significant displacement (Fig. 32.1). CT of the thoracic spine revealed lytic lesions involving the T4 vertebral body centrally as well as the right aspect of the T6 and T7 vertebral bodies and the posterior T8 and T11 vertebral bodies.

MRI of the cervical spine revealed a mass replacing most of the C2 vertebral body. The enhancing soft tissue extended into the prevertebral space anterior to C2 and C3. There was an additional bilateral paravertebral component extending throughout C2 and C3. There was also extension on the posterior aspects of the C2-C4 vertebral bodies with an epidural component identified (Fig. 32.2). The epidural tumor caused marked narrowing of the spinal canal to ~5 mm and mass effect on the cord. The left vertebral artery was not well visualized at the level of the mass between the C2 and C4 levels, which was suspicious for vertebral artery occlusion.

Preoperative staging of the neoplasm was not necessary because widely metastatic disease was confirmed only 1 month prior to consultation. Because of the extent of the ventral tumor, the patient underwent a C2-C3 laminectomy, posterior C2 corpectomy with occipital-C7 fixation, and allograft fusion. After the laminectomy was performed, the pars and pedicles were resected bilaterally, allowing access to the ventral epidural space. The tumor was then debulked and the spinal cord decompressed. Post-surgical radiographs of the cervical spine revealed the resection of the C2 vertebral body. Post-operatively the patient's left upper extremity strength became normal. Due to the patient's poor prognosis and known metastatic disease, complete resection by an anterior approach was not attempted.

Pathological evaluation of both the soft tissue and bone revealed malignant spindle cell tumor consistent with metastatic sarcoma, primarily synovial with some areas associated with a slight myxoid to lipoblastic appearance. The histological appearance of the tumor was essentially the same as that seen in previous sections from the knee and lung; the tumor was composed of mitotically active

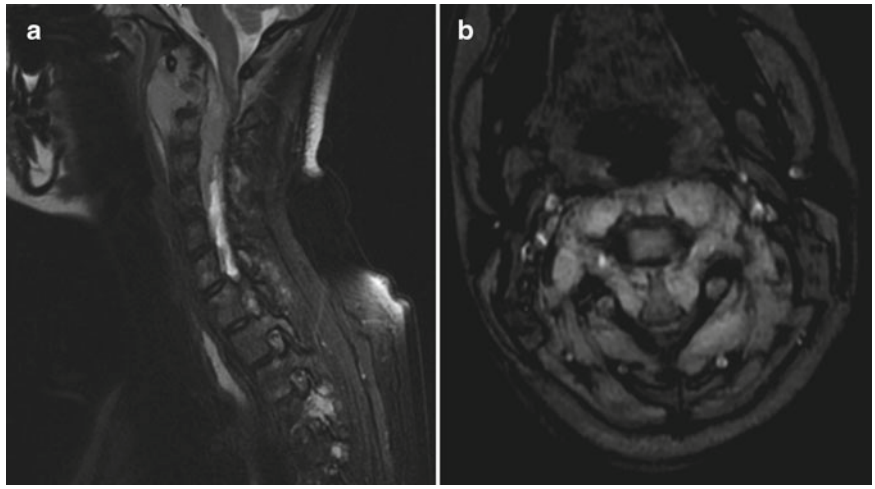

Fig. 32.2 Sagittal T2 MRI (**a**) of the cervical spine shows tumor in the cervical ventral epidural space from C2-C5, and anterior to the body of C2. Pathologic analysis revealed synovial sarcoma. Axial T2 MRI (**b**) shows tumor compressing the spinal cord as well as in front of the C2 body

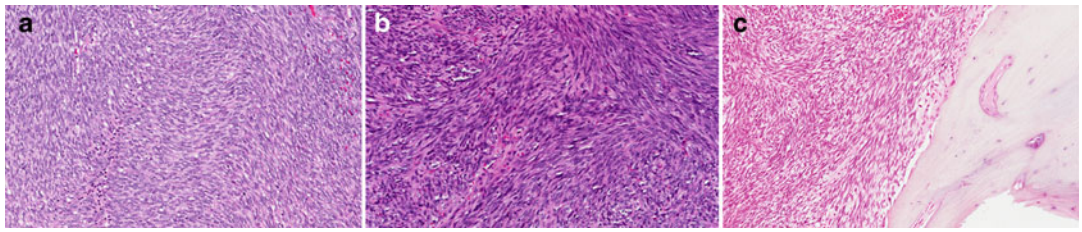

Fig. 32.3 (**a**) (2004 recurrent tumor in the right knee) This monophasic synovial sarcoma is characterized by a monotonous population of spindle cells arranged in sheets and fascicles in a collagenous stroma. Mitotic figures are present, however the mitotic index is low (typically <10/50 hpf). The nuclei are elongated with pointed ends and the cytoplasm lacks conspicuous cell borders. (**b**) (2007 lung tumor) This lung metastasis is histologically identical to the previously resected knee tumor. Again the tumor is characterized by fascicles and sheets of uniform spindle cells. This hypercellular tumor is admixed with eosinophilic fibrous stroma. Neither of these tumors contained unfavorable histologic features such as high mitotic count (>15/10 hpf), the presence of rhabdoid features, tumor necrosis, or poorly differentiated histology. (**c**) (2007 C2 vertebral body) The bone metastasis shown here is identical to those from the knee and lung. The section is pale due to decalcification, but the hypercellular sheets of spindle cells are still appreciated. The metastatic tumor completely replaces the normal marrow cavity

spindle cells arranged in a fascicular pattern, with no epithelial component identified, typical of monophasic synovial sarcoma (Fig. 32.3).

The patient did well post-operatively and was transferred to the medicine service several days postoperatively, where she continued to improve. Physical therapy was continued as tolerated. One month after surgery, the patient was to undergo palliative chemoradiation but developed fever, leukocytosis and acidosis, and then ultimately became septic. CT scans showed multiple metastases to the liver and abdominal parenchyma. She was no longer eligible for systemic chemotherapy and radiation therapy due to the heavy tumor burden. She died 6 months post-operatively of disease progression.

Illustrative Case 2: Thoracic Spinal Cord Compression Secondary to Metastatic Synovial Sarcoma

A 47-year-old man presented with a 3-month history of low back pain radiating to the right side of the midline. He had a history of synovial sarcoma of the right lower back, originally

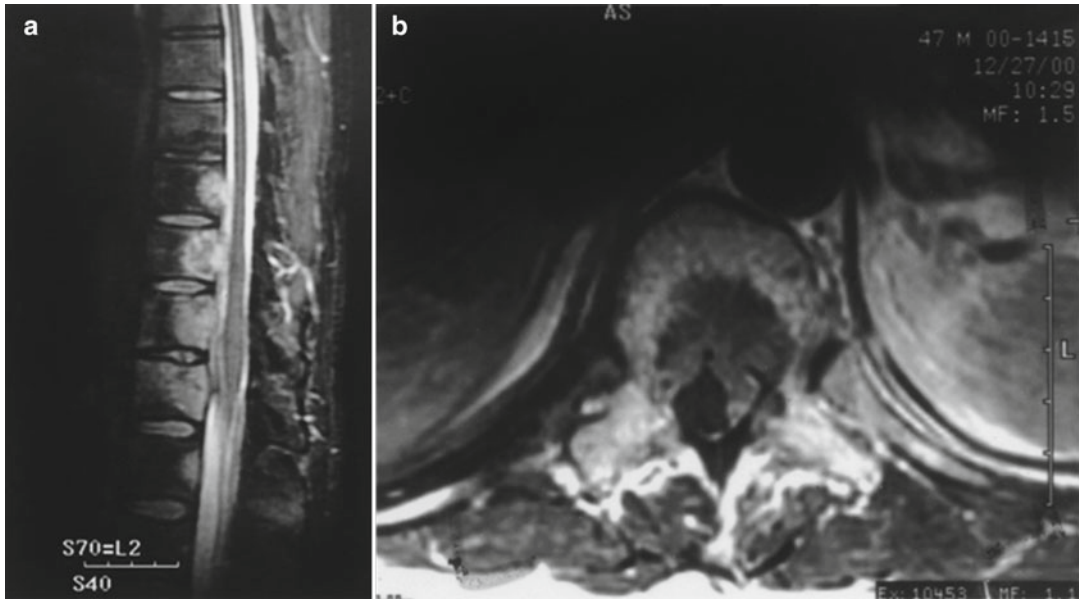

Fig. 32.4 Sagittal (**a**) and axial (**b**) T2-weighted MRI images show multiple spine metastases. There is spinal cord compression at T12

diagnosed in 1992 at age 38, and staged as III a,b (T1, N0, M0, GIII) when initially resected. This was followed by external beam radiation treatment for 7 weeks. The patient presented in 1997 with metastasis to the left upper lobe of the lung and subsequently underwent thoracotomy with lobectomy, followed by a second surgery for recurrence 4 months later. In 1998, the patient was found to have a penile lesion, which was biopsied and found to be consistent with synovial sarcoma. He subsequently received a 70 Gy radiation treatment. In 2000, he underwent total penectomy and perineal urethrostomy for recurrent synovial sarcoma, with an uneventful postoperative course.

The patient began complaining of low back pain 7 months later, which was controlled initially by anti-inflammatory medication and morphine. The patient also complained of lower extremity paresthesias but did not complain of any bowel or bladder incontinence. Neurological examination was normal. MRI of the thoracolumbar spine demonstrated lesions in five contiguous segments with circumferential compression at T11-T12, with the mass at T12 compressing the spinal cord (Fig. 32.4). The patient underwent T11-T12 laminectomy, transpedicular decompression, tumor debulking, T7-L3 transverse process fusion with iliac crest bone graft, and pedicle screw fixation. The tumor, which appeared to be encapsulated, could be seen ventral and lateral to the spinal cord. Much of the tumor had a liquid consistency and was suctioned out easily.

Pathological evaluation revealed synovial sarcoma consistent with previously recovered tissue, representing a highly cellular small cell malignant neoplasm with tumor cells having scant cytoplasm, occasionally demonstrating eccentric small hyperchromatic nuclei, seemingly pushed aside by eosinophilic cytoplasm lending some of the tumor cells a somewhat "rhabdoid" shape (Fig. 32.5). The tumor cells also could be seen as compressing capillary blood vessels to a narrow slit; however, the endothelial lining cells of those vessels appeared normal, though slightly elongated under the pressure. The close apposition of tumor cells to the small vessel walls extending to the immediate subependymal layer of the vessels is highly reminiscent of the pattern seen in hemangiopericytomas. This subgroup of synovial

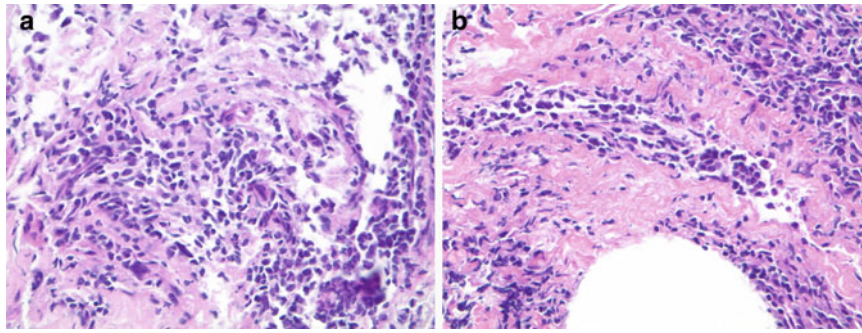

Fig. 32.5 (**a**) (2001 "spinal epidural and vertebral tissue") In contrast to the first case, this tumor is composed predominantly of small blue cells with very little cytoplasm admixed with hyalinized pink stroma. Histology alone would not be enough to diagnosis synovial sarcoma. In these tumors with less differentiation, cytogenetic and molecular ancillary tests are required. (**b**) (2001 "spinal epidural and vertebral tissue") This metastasis was characterized predominantly by intravascular tumor, which is shown in the center of the picture

sarcomas has (at least focally) a pattern very similar to that of hemangiopericytomas. In addition, in many areas of the excised tumor, numerous intraluminal tumor cells could also be observed in the blood vessels (mostly capillaries and venules). The patient did well postoperatively and was discharged to home on postoperative day 4. Subsequent post-operative X-rays showed the hardware in place. He remained neurologically normal. The patient died 6 months after surgery due to progression of disease.

Clinical Presentation of Synovial Sarcoma

The presentation of synovial sarcoma is similar to that of other sarcomas, with an initially painless, palpable, well-circumscribed multinodular mass that enlarges slowly (Laor 2004; Koehler et al. 2009; Ravnik et al. 2009; Fedors et al. 2010; Puffer et al. 2011). The average duration of symptoms prior to diagnosis is 2–4 years (Murphey et al. 2006), and 20-year histories have been reported (Fletcher et al. 2002). Diagnosis may be delayed or incorrect because of the prolonged signs and symptoms, deceptive indolent imaging findings, and long periods of dormancy preceding a period of aggressive, rapid growth (Fedors et al. 2010). Most lesions are 3–5 cm, but they may grow to 15 cm or larger (Sakellaridis et al. 2006); 85% of synovial sarcomas are >5 cm (Koehler et al. 2009).

The size and location of the tumor affect the presenting clinical symptoms. Tumors originating in an extremity may present with swelling or pain, and if the tumor is located near a joint, range of motion may be limited (Foreman and Stahl 2011). Pain and tenderness at the site may occur if the tumor begins to press on nerves or muscles, but pain without a corresponding palpable lesion has also been reported (Fedors et al. 2010), as has the presence of a lesion without pain (Fletcher et al. 2002). Tumors arising at non-extremity sites can present with symptoms related to mass effect on adjacent structures (Fletcher et al. 2002; Ravnik et al. 2009). Compression of nerves by the growing tumor may cause the gradual onset of neurological deficits (Foreman and Stahl 2011).

Cellular and Histopathologic Classification of Synovial Sarcoma

Synovial sarcoma continues to be one of the most aggressive soft-tissue sarcomas, despite improvements in staging, surgical methods, and adjuvant treatments (Baptista et al. 2006). Synovial sarcomas are considered primarily high-grade soft-tissue sarcomas (Eilber and Dry 2008), although in some cases the biologic behavior can be more benign (Baptista et al. 2006). While the tumor

grade can be estimated by mitotic index, percentage of necrosis, and tumor differentiation, this tumor should always be considered a high-grade sarcoma as it is characterized by local invasiveness and a propensity to metastasize (Fletcher et al. 2002; Guillou et al. 2004; Ferrari et al. 2004; Eilber and Dry 2008; Sultan et al. 2009).

Synovial sarcoma is characterized by varying proportions of epithelioid and spindle cells arranged in a classic biphasic or monophasic pattern (Fletcher et al. 2002; Ferrari et al. 2004; Suh et al. 2005; Baptista et al. 2006; Davicioni et al. 2008; Eilber and Dry 2008; Fedors et al. 2010; Puffer et al. 2011), or the newly identified poorly differentiated pattern (Greene et al. 2006; Eilber and Dry 2008; Sultan et al. 2009; Koehler et al. 2009; Puffer et al. 2011). Other authors consider the three histologic subtypes to be biphasic, monophasic fibrous, and monophasic epithelial (Sakellaridis et al. 2006; Barus et al. 2009; Fedors et al. 2010). The histological features are identical in children and adults (Fletcher et al. 2002; Guillou et al. 2004; Ferrari et al. 2004; Sultan et al. 2009).

The monophasic histologic subtype is composed entirely of spindle cells (Fletcher et al. 2002; Laor 2004; Davicioni et al. 2008; Eilber and Dry 2008; Koehler et al. 2009). The monophasic subtype is further subdivided into monophasic fibrous (presence of only the spindle cell component) and monophasic epithelial (presence of only the epithelial component only) (Ferrari et al. 2004). The biphasic subtype is composed of both epithelial and spindle cells in varying proportions (Fletcher et al. 2002; Laor 2004; Greene et al. 2006; Eilber and Dry 2008; Davicioni et al. 2008; Koehler et al. 2009; Fedors et al. 2010).

The recently described poorly-differentiated subtype of synovial sarcoma is aggressive and metastasizes in a high number of cases. PDSS has the same immunophenotype and genetic abnormalities as regular synovial sarcoma (Fletcher et al. 2002). Less than 5% of synovial sarcoma are the pure PD subtype (Fedors et al. 2010), though up to 20% of synovial sarcomas may contain PD areas (Fletcher et al. 2002; Eilber and Dry 2008). The classification "poorly differentiated" may be used as a histologic modifier for any of the other SS types (Barus et al. 2009). PDSS are composed of densely packed, uniform, small ovoid blue cells; consequently, this subtype poses the greatest diagnostic challenge because it can mimic so many other tumors (Eilber and Dry 2008). PDSS is characterized by the presence of three histologic patterns: PD large cell type, PD small cell type, and PD high-grade spindle cell type (Ferrari et al. 2004). The PDSS subtype frequently shows necrosis, hemorrhage, high mitotic activity, the presence of a prominent hemangiopericytomatous pattern (Ferrari et al. 2004), as well as more marked nuclear atypia and pleomorphism (Koehler et al. 2009).

Synovial sarcoma has a specific and consistent cytogenetic chromosomal translocation involving chromosomes X and 18 (Fletcher et al. 2002; Laor 2004; Suh et al. 2005). This translocation fuses two normal genes, SYT and SSX1 (or the related SSX2) to create SYT/SSX, an abnormal fusion protein (Suh et al. 2005). This fusion protein is present in both the spindled and epithelial elements (Eilber and Dry 2008), and the fusion partners (SYT-SSX1 or SYT-SSX2) remain consistent between the primary and metastatic tumors and is constant during the course of the disease (Ladanyi et al. 2002; Guillou et al. 2004; Eilber and Dry 2008).

This unique genetic reciprocal translocation t(X:18) is found in >90–95% of the 150 cases of synovial sarcoma that have been reported (Fletcher et al. 2002; Ferrari et al. 2004). The genes affected by the t(X;18) translocation are SS18 (a.k.a. SYT or SSXT), from chromosome 18, and SSX1, SSX2 and SSX4 from the X chromosome (Fletcher et al. 2002; Sakellaridis et al. 2006). The translocation t(X;18) (p11.2;q11.2) represents the fusion of the SYT gene on chromosome 18 with one of three homologous SSX genes on the X chromosome (SSX1, SSX2, and SSX4) (Ferrari et al. 2004; Greene et al. 2006; Eilber and Dry 2008; Fedors et al. 2010). Approximately two-thirds of reported cases have an SYT/SSX1 fusion and one-third have an SYT/SSX2 fusion, with only a few cases having a fusion between SYT and SSX4 (Fletcher et al. 2002; Eilber and Dry 2008; Sultan et al. 2009).

Because the t(X;18) translocation arises exclusively in synovial sarcomas and is considered the

cytogenetic hallmark (Fletcher et al. 2002; Sakellaridis et al. 2006), its detection provides a definitive diagnosis (Greene et al. 2006; Eilber and Dry 2008). The biphasic subtype usually has the SYT/SSX1 translocation, and the monophasic and PDSS subtypes may have either the SSX1 or SSX2 partner (Guillou et al. 2004; Eilber and Dry 2008). It is thought that SSX2 may repress epithelial differentiation, or SSX1 may promote it (Eilber and Dry 2008).

Diagnosis of Synovial Sarcoma: Histopathology, Immunohistochemistry, and Molecular Studies

In the last 20 years, there has been much progress in the diagnosis of synovial sarcoma, including identification of translocations, new imaging modalities, continued use of immunohistochemistry, and refinements in prognosis (Borden et al. 2003). Because sarcomas are not defined by a specific organ system but can arise virtually anywhere in the body, diagnosis is based on the characteristic histopathology of the tumor and the clinical presentation. Differentiating SS from other soft-tissue sarcomas is especially challenging when SYT-SSX expression is undetected or when only poorly differentiated cells are seen (Davicioni et al. 2008). Because of difficulties in definitive histologic diagnosis, ancillary techniques including immunohistochemical analysis for cytokeratins, vimentin, Bcl- 2, S100 protein, CD34, smooth muscle actin (SMA), and desmin are used to narrow the differential diagnosis (Eilber and Dry 2008; Fedors et al. 2010). The actins SMA and MSA, as well as desmin, CD34, and CD31 typically are negative in synovial sarcomas (Eilber and Dry 2008). Because ~90% of all synovial sarcomas express cytokeratins (CK) (Fletcher et al. 2002; Sakellaridis et al. 2006; Fedors et al. 2010), a definitive diagnosis is possible upon positive staining for CK or a focal biphasic pattern (Sakellaridis et al. 2006). Epithelial markers are the most helpful, and they include pankeratin, CAM 5.2, and EMA. The epithelial component of synovial sarcoma stains strongly for at least one of these markers in nearly all cases (Eilber and Dry 2008). Small biopsy specimens can be problematic, as the cells present may or may not stain with the chosen markers (Eilber and Dry 2008).

Among soft-tissue sarcomas, there are multiple variants in morphological appearance, age at diagnosis, anatomical distribution, and propensity for metastasis, as well as histopathologic features that overlap; thus, microarray-based gene expression signatures are being used more frequently to aid in definitive diagnosis of synovial sarcoma and to aid in prognosis assessment for newly diagnosed patients (Davicioni et al. 2008). Synovial sarcomas demonstrate strong and homogeneous gene expression profiles, and recurrent biological pathways that are observed across the expression signatures, which may be useful in differential diagnosis (Davicioni et al. 2008).

Definitive diagnosis may not be possible by histology and immunochemistry alone. Molecular testing should be performed when there is a high clinical suspicion for synovial sarcoma that cannot be confirmed by histologic and immunohistochemical analysis (Eilber and Dry 2008; Fedors et al. 2010), and when there is low-to-moderate clinical suspicion for synovial sarcoma and the results of histologic and immunochemical analysis are ambiguous.

Molecular tests that have been used to diagnose synovial sarcoma include cytogenetics, interphase FISH and conventional and real-time RT-PCR (Eilber and Dry 2008). To address the disadvantages of cytogenetic analysis, a lengthy process that requires fresh tissue that must propagate in culture, highly specific interphase FISH and (real-time) RT-PCR tests for synovial sarcoma were developed which can be performed on routinely processed, formalin-fixed, paraffin embedded tissues in only a few days (Eilber and Dry 2008). These highly sensitive tests are now the preferable clinical testing methods for rapid diagnosis of synovial sarcoma (Fletcher et al. 2002; Eilber and Dry 2008; Fedors et al. 2010). A histological diagnosis usually can be confirmed using RT-PCR or FISH (Davicioni et al. 2008).

Diagnosis of Synovial Sarcoma: Imaging

Imaging studies can be instrumental in earlier diagnosis of synovial sarcoma, because the initial slow growth and long duration of symptoms can mimic benign processes and cause a delay in diagnosis (Fedors et al. 2010). Imaging studies used in patient evaluation include MRI, CT scanning, and plain films. Imaging results provide information critical to diagnosis, staging, treatment planning, treatment evaluation, and post-treatment assessment (Gomez and Morcuende 2004). The goals of imaging in suspected spinal metastases are to identify and localize the lesion(s), assess calcification, neural element compromise, periosteal new bone formation, extent of bony destruction, presence or absence of systemic dissemination, and presence or absence of instability (Ratliff and Cooper 2004). By combining the clinical presentation with the nonspecific imaging features of synovial sarcoma, the differential diagnosis can be narrowed (Fedors et al. 2010). Despite the nonspecific CT and MR imaging findings, an understanding of the morphologic appearance may help distinguish low- and high-grade tumors, allow some degree of prognosis prediction, and aid in predicting survival (Tateishi et al. 2004). Ultimately, however, accurate diagnosis will require biopsy and thorough pathologic investigation (Fedors et al. 2010).

Radiographs/Plain Films

Plain films are often the initial imaging obtained in patients with a non-emergent presentation (Fedors et al. 2010), and they are often used as a screening tool when spinal metastases are suspected (Ratliff and Cooper 2004). There are no definitive characteristics of synovial sarcoma on radiologic examination (Puffer et al. 2011). The slow growth of the tumor allows radiographic identification of periosteal new bone formation, bony erosion, osteoporosis, and soft tissue calcification (Koehler et al. 2009; Fedors et al. 2010). Plain films may be normal in up to 50% of patients with synovial sarcoma (Suh et al. 2005; Puffer et al. 2011). Plain films can reveal compression fractures, and, when supplemented with dynamic views, can reveal vertebral body alignment and stability. Furthermore, plain films may be normal even in patients with clinical evidence of metastatic spinal cord compression; thus, plain films cannot rule out vertebral body metastases, especially early in their development (Ratliff and Cooper 2004). Bony abnormalities cannot be seen on plain films until significant vertebral body destruction has occurred or the tumor has eroded adjacent bony structures. Technetium-99 bone scintigraphy is more sensitive than plain films in detecting bony metastases, and the entire body may be viewed in a single study (Ratliff and Cooper 2004).

On plain films, synovial sarcoma usually appears as well-circumscribed or lobulated, homogeneous, round-to-ovoid juxta-articular soft tissue mass (Tateishi et al. 2004; Eilber and Dry 2008; Koehler et al. 2009; Fedors et al. 2010). Approximately 30% of cases demonstrate tumor calcification, with or without ossification (Fletcher et al. 2002; Laor 2004; Suh et al. 2005; Eilber and Dry 2008; Koehler et al. 2009), which is a higher percentage than in other soft tissue sarcomas and may help in differential diagnosis (Tateishi et al. 2004; Fedors et al. 2010).

Computed Tomography (CT)

CT imaging is very useful in identifying subtle soft-tissue calcifications and local bony changes (Koehler et al. 2009), as well as areas of internal necrosis or hemorrhage (Fedors et al. 2010). The effect of a tumor on adjacent bone can range from the more common slowly-developing pressure erosions to aggressive bone destruction (Fedors et al. 2010). CT imaging provides an accurate assessment of the relationship of synovial sarcomas to adjacent bones, especially in complex areas such as the spine (Koehler et al. 2009). CT with sagittal reformatting is better than plain films in showing spinal alignment at the cervicothoracic and thoracolumbar junctions (Ratliff and Cooper 2004). CT is excellent in revealing neural

element compromise from retropulsed bony fragments in compression fractures, as well as identifying the extent of bony destruction and lesion distribution (Ratliff and Cooper 2004). CT should be used to evaluate calcifications, because absence of calcification is associated with high-grade tumor and poor prognosis (Fletcher et al. 2002; Tateishi et al. 2004). Small lesions may be imperceptible on CT, but when they are visible, they often appear as well-demarcated, hypodense, heterogeneous, multinodular soft tissue masses with a density similar to skeletal muscle (Fedors et al. 2010), and thus this tumor is easily confused with other tumors (Puffer et al. 2011).

For visualizing the bony anatomy of a vertebral body metastasis, CT remains the best imaging modality (Ratliff and Cooper 2004). CT with myelography will clearly show the level and extent of metastatic spinal cord compression in patients with suspected compressive lesions. However, due to the invasiveness of CT myelography, most facilities now use MRI (Ratliff and Cooper 2004).

Magnetic Resonance Imaging (MRI)

For the evaluation of most soft-tissue abnormalities that require cross-sectional imaging, MRI is now used instead of CT (Laor 2004). MR imaging is not used to make a specific diagnosis, but rather to delineate the extent of a lesion and the involvement of adjacent structures, to evaluate therapy response, and to assess postoperative complications (Laor 2004). On MRI, synovial sarcomas usually appear as well-defined, nonspecific, heterogenous soft-tissue masses and may have a multilocular configuration with varying degrees of septation with or without fluid-fluid levels (Tateishi et al. 2004; Koehler et al. 2009).

Although the appearance of synovial sarcoma on MRI remains nonspecific, MR imaging is the modality of choice for detecting and characterizing synovial sarcoma (Koehler et al. 2009; Fedors et al. 2010; Puffer et al. 2011). The high-contrast tissue resolution and multi-planar capability (Gomez and Morcuende 2004) provide superior tissue characterization, demonstrate involvement of neurovascular structures or bone marrow, aid in preoperative planning, and assist in tumor grading and assessing clinical prognosis (Suh et al. 2005). Small lesions often have predominantly homogeneous signal intensity similar to adjacent muscle (Suh et al. 2005; Fedors et al. 2010); larger lesions are usually lobular, well-defined masses that have T1 signal intensity slightly lower than adjacent muscle (Fedors et al. 2010).

Most tumors display heterogeneous intermediate signal intensity on T1-weighted images, occasionally with fluid-fluid levels (Laor 2004; Suh et al. 2005). The presence of fluid-fluid levels is described in 10–25% of cases (Fedors et al. 2010), but is nonspecific and cannot be considered diagnostic (Tateishi et al. 2006; Fedors et al. 2010). On T2-weighted images, most tumors display heterogeneous, predominantly high T2-signal intensity (Fedors et al. 2010). Intratumoral hemorrhage is common in synovial sarcoma, in contrast to other soft tissue sarcomas (Tateishi et al. 2006; Fedors et al. 2010), with an incidence of ~40–73% of cases demonstrating high signal on both T1- and T2-weighted images (Koehler et al. 2009; Fedors et al. 2010). The "triple signal pattern," one of the patterns on T2-weighted images, is useful in indicating the presence of both cystic and solid elements, hemorrhage/proteinaceous products, and fibrous tissue (Fedors et al. 2010), and is considered to be suggestive of the diagnosis (Tateishi et al. 2006). Approximately 35% of synovial sarcomas demonstrate the "triple signal" of hyperintensity, isointensity, and hypointensity relative to fat within a single lesion (Koehler et al. 2009; Fedors et al. 2010).

MRI cannot differentiate postoperative edema, inflammation, and hemorrhage from residual or recurrent disease (Laor 2004), and neither can it differentiate monophasic from biphasic subtypes (Gomez and Morcuende 2004; Koehler et al. 2009; Fedors et al. 2010). In addition, patient movement or spinal instrumentation may impede visualization, and may be contraindicated in patients with certain implants (Ratliff and Cooper 2004). Because most soft tissue masses have no specific MR features characteristic of malignancy,

the sensitivity and usefulness of MRI as a predictor of malignancy is unclear (Gomez and Morcuende 2004; Laor 2004). In addition, there is significant overlap in the findings commonly associated with both benign and malignant lesions (Gomez and Morcuende 2004; Laor 2004).

Gomez and Morcuende (2004) described three enhancement patterns that may predict malignancy on MRI: (1) different pulse sequences showing intensity and homogeneity of the MR signal; (2) T2-weighted images showing high-signal intensity; and (3) T1-weighted images showing homogeneity. However, these have shown unacceptably low specificity. Other signs that may indicate malignancy, including the presence of tumor necrosis, bone or neurovascular involvement, irregular or partially irregular margins, and a mean diameter of >66 mm, have not shown enough reliability to accurately assess the malignancy of a lesion on MRI. MR signal characteristics assist in the management of synovial sarcoma, but the results are far more useful when considered in the context of the clinical history as well as the morphology, internal architecture, growth pattern, and other anatomic relationships (Gomez and Morcuende 2004).

For evaluating spinal metastases, MRI is the modality of choice. MRI can detect changes in bone marrow, and thus can provide earlier evidence of vertebral body involvement; in addition, MRI may be more sensitive in detecting epidural metastases (Ratliff and Cooper 2004). The entire spine can be imaged with just multiple planes of view, which can reveal multiple sites of bony involvement. Other features that may be revealed on MRI are paravertebral tumor extension, the delineation of tumor extent, and neural compression from epidural tumor or bony destruction (Ratliff and Cooper 2004).

Treatment of Primary Synovial Sarcoma

Treatment of synovial sarcoma is often multimodal (Fedors et al. 2010). Significant progress has been made in the last 40 years in the treatment of soft tissue sarcomas in adults, including: improved specificity in pathological definition and staging; improvements in the use of radiotherapy and, in some cases chemotherapy, as adjunctive therapies; and advances in surgical treatment for preservation of function (Borden et al. 2003).

Surgery for Synovial Sarcoma

The surgical management of synovial sarcoma is generally the same as for all other soft tissue sarcomas (Fedors et al. 2010). Complete surgical resection of the primary lesion with wide negative margins is essential for optimal outcomes and is considered the cornerstone of treatment (Suh et al. 2005; Eilber and Dry 2008; Fedors et al. 2010; Puffer et al. 2011). An incisional biopsy site should be resected completely along with the specimen (Eilber and Dry 2008). For some patients, surgery alone can be curative (Davicioni et al. 2008). Intralesional resections can be performed as palliative procedures, though with poor local control rates. When curettage resection techniques are used (for both initial surgery and recurrence), neurological and functional outcomes are excellent (Bilsky et al. 2001). In contrast, Eilber and Dry (2008) state there is no role for incomplete gross resection or intralesional excisions.

Surgery is considered the most effective treatment for spinal instability causing pain and paralysis because the patient can experience immediate relief (Tokuhashi et al. 2009). Synovial sarcomas are often located very close to neurovascular structures, or found invading joints or bones (Eilber and Dry 2008); these tumors are more challenging and may even be inoperable (Laor 2004). However, sacrifice of these neurovascular structures during dissection is usually not necessary (Eilber and Dry 2008).

Radiation for Synovial Sarcoma

Because synovial sarcomas are considered high-grade tumors, complete surgical resection is followed by adjuvant radiotherapy and/or chemotherapy to

treat any residual disease and improve local control (Bilsky et al. 2001; Ferrari et al. 2004; Eilber and Dry 2008; Ravnik et al. 2009; Fedors et al. 2010; Puffer et al. 2011), and prevent amputation for most patients with extremity tumors (Suh et al. 2005). Radiotherapy should not begin until at least 2 weeks after surgery in order to allow for sufficient wound healing (Ratliff and Cooper 2004).

Surgery followed by radiation yields better neurologic outcomes than patients treated with radiation alone (Patchell et al. 2005). Radiation has been proven to be superior to chemotherapy alone as adjuvant therapy following primary surgical excision (Puffer et al. 2011). Preoperative radiotherapy combined with chemotherapy has a reported 5-year overall survival rate of 75% (Fedors et al. 2010).

The doses of radiation necessary to control tumor growth are greater than the spinal cord can tolerate (Bilsky et al. 2001). In patients with microscopic residual tumors, radiation doses considered effective for local tumor control are from 6,000 to 7,000 cGy, while maximal spinal cord tolerance is only 4,500 cGy (Bilsky et al. 2001). Postoperative radiotherapy has been shown to delay recurrence despite the suboptimal doses, and thus is recommended after surgical resection in eligible patients (Bilsky et al. 2001).

Preoperative radiotherapy has been correlated with an increased risk of perioperative complications. Studies comparing pre- and postoperative radiation have found the rate of wound complications with preoperative radiotherapy to be 35% compared to a rate of 17% with postoperative radiotherapy (Fedors et al. 2010). Ghogawala et al. (2001) found that the rate of wound infection was increased ~threefold (from 12 to 32%) after preoperative radiotherapy.

Various utilities and modalities are used for adjuvant radiation, including external beam therapy (neoadjuvant or adjuvant), brachytherapy, and intensity modulated radiation therapy (IMRT) (Eilber and Dry 2008; Fedors et al. 2010). Each modality has been proven to decrease the local recurrence rate, thus extending event-free intervals (Bilsky et al. 2001; Eilber and Dry 2008; Fedors et al. 2010; Puffer et al. 2011). Each modality has its own advantages and disadvantages, and no single modality has been proven more effective for the adjuvant treatment of synovial sarcoma (Eilber and Dry 2008).

Ratliff and Cooper (2004) proposed indications for choosing surgery or radiotherapy as the primary treatment modality. Indications for the use of surgery as the primary treatment intervention include: retropulsed bone producing neural compression; spinal deformity producing pain or neural compression; axial pain; spinal instability from metastatic bony or ligamentous destruction; progressive neurological deficit; failure of radiation (progression of deficit/pain during radiation, previous radiation with recurrence/progression after treatment); and an unknown primary. Indications for the use of radiation as the primary treatment intervention include: radioresponsive tumor; moderately radioresponsive tumor in a patient with minimal deficit/ limited pain; isolated epidural neural compression; isolated local pain; expected survival of <3 months; poor operative candidate; and complete neurological deficit.

Chemotherapy for Synovial Sarcoma

The diversity and rarity of soft tissue sarcomas, especially synovial sarcomas, has prevented the accrual of an adequate number of high-risk patients for randomized trials evaluating the use of chemotherapy (Ferrari et al. 2004; Eilber and Dry 2008); thus, formal proof of the efficacy of adjuvant chemotherapy in the treatment of adult soft tissue sarcoma remains unavailable (Ferrari et al. 2004). Chemotherapy may be useful for extremity soft tissue sarcoma lesions (Laor 2004), adjuvant chemotherapy can offer a limited survival benefit for high-risk patients (Ferrari et al. 2004), and, in some cases, neoadjuvant chemotherapy may be beneficial (Fedors et al. 2010).

The chemotherapy agents generally used for soft tissue sarcomas are doxorubicin and ifosfamide (Fedors et al. 2010; Puffer et al. 2011). Synovial sarcoma is considered a particularly chemosensitive soft tissue sarcoma (Eilber and Dry 2008), although no one single chemotherapy protocol has been proven most effective in treating

synovial sarcoma (Puffer et al. 2011). The results of several recent studies suggest that high-dose ifosfamide-based chemotherapy regimens should be considered in adult patients with primary high-risk extremity synovial sarcomas greater than or equal to 5 cm (Eilber and Dry 2008), as these regimens have shown improved disease-specific survival (Eilber et al. 2007; Puffer et al. 2011).

Both adjuvant and neoadjuvant chemotherapy have been used in the past 30 years for patients with high-risk primary extremity soft tissue sarcomas in order to improve survival. However, due to the toxicity of chemotherapy and its limited impact on survival, the use of chemotherapy in patients with primary soft tissue sarcomas has been controversial (Suh et al. 2005; Greene et al. 2006; Eilber and Dry 2008; Barus et al. 2009), and thus the use of adjuvant chemotherapy remains a case-by-case decision (Fedors et al. 2010).

Prognostic Factors for, and Sites of, Synovial Sarcoma Metastasis

Metastatic synovial sarcoma portends a poor prognosis. Once a patient develops metastases, then mortality is high (Ferrari et al. 2004; Spurrell et al. 2005; Eilber and Dry 2008). Death from synovial sarcoma is almost always due to distant metastases (Ladanyi et al. 2002; Eilber and Dry 2008). In patients with Grade IV disease, there is a 75% mortality rate within 2 years of diagnosis (Ladanyi et al. 2002; Greene et al. 2006); most patients presenting with metastatic disease die within 3 years (Ladanyi et al. 2002). The average duration of disease, from symptom onset to death, is ~6.5 years (Barus et al. 2009). In patients with metastatic disease, the median time to disease-specific death is 10–22 months (Ferrari et al. 2004; Spurrell et al. 2005; Eilber and Dry 2008).

Only a small number of patients with synovial sarcoma have detectable metastatic disease at the time of diagnosis (Davicioni et al. 2008). Some patients experience distant metastasis relatively soon after surgery and die due to disease progression, whereas other patients have a prolonged disease course without experiencing any recurrence or metastasis (Tateishi et al. 2004). Local recurrence is associated with an increased risk for metastases and tumor-related death, and it is reported that ~50% of patients with synovial sarcoma experience recurrence and/or metastasis after their initial treatment (Sakellaridis et al. 2006; Davicioni et al. 2008). Lewis et al. (2000) examined the clinical and pathologic prognostic factors in adult localized primary extremity synovial sarcoma. The 5-year local recurrence rate was 12%, a rate lower than that in other studies (Koehler et al. 2009), presumably due to improved surgical methods and the use of adjuvant radiation therapy (Eilber and Dry 2008). Local recurrence can be controlled by adequate surgical resection with negative margins, as well as the use of adjuvant radiotherapy (Fletcher et al. 2002; Barus et al. 2009). In the same study, Lewis et al. (2000) reported a 5-year distant recurrence rate of 39%.

Eilber and Dry (2008) reported that distant metastasis occurs in up to 53% of patients with primary extremity synovial sarcomas that are greater than or equal to 5 cm, with a resultant 5-year disease-specific mortality rate of 37%. Conversely, the 5-year disease-specific survival rate for primary synovial sarcoma at any site is reported to range from 60 to 75% (Fletcher et al. 2002; Ferrari et al. 2004; Guillou et al. 2004; Eilber and Dry 2008; Koehler et al. 2009). The 10-year survival rate is reported to range from 11 to 63% (Fletcher et al. 2002; Tateishi et al. 2004; Sakellaridis et al. 2006; Koehler et al. 2009; Puffer et al. 2011). Tumor recurrence usually occurs within 2 years after initial therapy (Fletcher et al. 2002; Sakellaridis et al. 2006; Barus et al. 2009); there are reports of recurrence >30 years after diagnosis, which emphasizes the need for long-term follow-up (Eilber and Dry 2008; Koehler et al. 2009).

The metastatic pattern of synovial sarcoma is based on the location of the primary tumor (Eilber and Dry 2008). Because most synovial sarcomas arise in the extremities, the vast majority then metastasize to the lung (Ferrari et al. 2004; Laor 2004; Guillou et al. 2004; Baptista et al. 2006; Eilber and Dry 2008), which is the first site of distant recurrence in nearly 80% of patients (Bedre et al. 2007). Synovial sarcoma also metastasizes

to regional lymph nodes and bone (bone marrow) (Fletcher et al. 2002; Sakellaridis et al. 2006; Barus et al. 2009; Koehler et al. 2009; Puffer et al. 2011). Eilber and Dry (2008) report that synovial sarcomas develop lymph node metastases more commonly than most other soft tissue sarcomas, at an incidence of ~10–12% compared to ~3–5% for other soft tissue sarcomas. Similar to the SYT fusion partners remaining consistent between the primary and metastatic tumors, regional lymph node and distant metastases typically appear similar to the primary tumor (Fletcher et al. 2002; Eilber and Dry 2008).

Patient outcome is correlated with the stage of the initial disease, tumor size, and the extent of surgical resection (Greene et al. 2006). Determining a prognosis before treatment helps determine the treatment modalities, particularly surgical procedures (Tokuhashi et al. 2009). Prognosis does not differ between monophasic and biphasic histology (Fletcher et al. 2002; Ladanyi et al. 2002), and histologic grade is also not a factor, because synovial sarcomas are considered primarily high-grade tumors (Ladanyi et al. 2002). Patient age has been found to be an inconsistent variable affecting survival (Ladanyi et al. 2002; Ferrari et al. 2004; Guillou et al. 2004; Eilber and Dry 2008).

Many studies that have examined the prognostic factors for synovial sarcoma have been inconsistent in their inclusion criteria, making definitive conclusions difficult (Ferrari et al. 2004; Guillou et al. 2004; Eilber and Dry 2008). However, large tumor size (>5 cm) has shown a consistent association with the development of distant metastasis, as well as an association with decreased disease-specific survival (Ladanyi et al. 2002; Ferrari et al. 2004; Guillou et al. 2004; Laor 2004; Eilber and Dry 2008; Fedors et al. 2010). Guillou et al. (2004) found that strong prognostic factors for death from malignancy were age >35 years; large tumor size (>7 cm); poorly differentiated histology; high mitotic rate; tumor necrosis; high histologic grade (grade 3); and advanced stage at diagnosis. Sakabe et al. (2008) reported that the most significant prognostic factor related to disease-specific mortality in their study was inadequate surgical margins at the time of initial surgical resection. Two years earlier, Baptista et al. (2006) evaluated 20 cases of nonmetastatic synovial sarcoma of the extremities to identify prognostic factors for survival and local recurrence. They found the prognostic factors that influenced survival were spontaneous necrosis >25%, tumors proximal to the knee or elbow, and a high histologic grade (according to their criteria). The only prognostic factor that influenced local recurrence was a positive microscopic surgical margin; interestingly, however, neither local recurrence nor large tumor size (>10 cm) showed statistical significance in association with worse survival prognosis. Guillou et al. (2004) considered the most important factors involved in sarcoma recurrences to be microscopically positive surgical margins, lack of adjuvant radiotherapy, large tumor size, high histologic grade, and axial tumor location. The factors reported by Zagars et al. (2003) to be predictive of local recurrence included positive or uncertain surgical margins, tumors in the head, neck, and deep trunk, age >64 years, tumor size >10 cm, and high pathologic grade.

Tateishi et al. (2004) reported five factors that had a significant association with disease-free survival: proximal distribution, large tumor size, the absence of calcification, the presence of hemorrhage, and the presence of the triple signal intensity pattern. The statistically significant imaging findings associated with a diagnosis of high-grade synovial sarcoma included those five factors plus the presence of cystic components. The risk factors for metastasis or death reported by Barus et al. (2009) included age >25 years; tumor size >5 cm; tumor with >20% poor differentiation; and primary tumor in the lower extremities. In patients with negative surgical margins and no risk factors, the 5-year risk of local recurrence was 9%, but in patients with two risk factors, the 5-year risk of local recurrence increased to 31% (Fedors et al. 2010).

Much has been written regarding the prognostic significance of the molecular genetics of synovial sarcoma (Eilber and Dry 2008). Until recently, all studies indicated a worse prognosis for patients with localized tumors with the SYT/SSX1 translocation, with a higher proliferative rate, shorter metastasis-free survival, and overall survival times compared to those with the SYT/SSX2 translocations (Ladanyi et al. 2002; Suh

et al. 2005; Eilber and Dry 2008; Puffer et al. 2011). Prior to the work by Guillou et al. (2004), SYT-SSX fusion type was cited as the single most significant prognostic factor in multivariate analysis for patients with localized disease at diagnosis (Ladanyi et al. 2002). After subdividing synovial sarcomas into intermediate and high-grade tumors, Guillou et al. (2004) showed that for patients with localized synovial sarcoma, it is histologic grade, not SYT-SSX fusion type, which is a strong independent prognostic factor for disease-specific survival and metastasis-free survival.

Detection of the SYT-SSX fusion genes by molecular tests is very useful in making a definitive diagnosis, but it remains unclear whether expression of these fusion genes conveys significant prognostic information (Davicioni et al. 2008). There are at least seven SYT-SSX variants, and both the SYT-SSX1 and SYT-SSX2 translocation partners have been seen in the same primary tumor in up to 10% of synovial sarcomas (Eilber and Dry 2008). Thus, it is unclear whether the different fusion gene variants expressed in synovial sarcoma will be prognostic on their own (Davicioni et al. 2008).

Synovial Sarcoma Metastatic to the Spine

Synovial sarcomas arising from, near, or metastatic to the spine are rare (Bilsky et al. 2001; Puffer et al. 2011). Data regarding treatment consist primarily of case reports (Otsuka et al. 1985; Signorini et al. 1986; Yoshikawa et al. 1997; Hanada et al. 1999; Bilsky et al. 2001; Merimsky et al. 2004; Reichel et al. 2004; Sakellaridis et al. 2006; Bedre et al. 2007; Scollato et al. 2008; Arnold et al. 2009, 2010; Zairi et al. 2011), making optimal treatment strategies as well as functional, neurological, and oncological outcomes unclear (Bilsky et al. 2001). Approximately 10–20% of synovial sarcomas invade adjacent bone (Sakellaridis et al. 2006; Barus et al. 2009).

Intradural spinal metastases are a rare condition, most often with a fatal outcome (Schick et al. 2001). Of symptomatic metastases affecting the spinal cord, intradural metastases comprise only 0.8–3.9%, and are found in only ~2% of patients with end-stage disease (Schick et al. 2001). Intradural metastases are often seen with rapidly progressing neurologic deficit, widely metastatic disease, and very limited life expectancy (Schick et al. 2001). Characteristic findings of intradural extramedullary tumors are outward displacement of epidural fat or compression of the spinal cord with widening of the subarachnoid space above and below the mass (Schick et al. 2001). There are only a few reported cases of metastatic intradural synovial sarcoma in the literature. Sakellaridis et al. (2006) reported a primary tumor at L2, with a first metastasis to L1-L3 lumbar dura mater, and a second metastasis to intradural extramedullary C7 (C6-T1) and the mediastinum in front of the T5-10 vertebral bodies. Scollato et al. (2008) reported an intramedullary metastasis at C3-5. Surgeons must maintain a high index of suspicion during surgery to detect the presence of intradural tumors (Bilsky et al. 2001). In patients with intradural tumors, aggressive tumor resection has improved neurological symptoms and provided significant neurological palliation (Bilsky et al. 2001). The treatment of choice is subtotal resection with preservation of neurological function (Schick et al. 2001).

In a patient with known metastatic disease, back pain must be considered a spinal metastasis until proven otherwise (Ratliff and Cooper 2004). Pain caused by a metastasis to the vertebral body may be local, radicular, or axial. Local pain, which is constant and not worsened by movement, results from tumor mass effect or distortion of the periosteum from tumor destruction. Radicular pain is caused by epidural tumor extension causing nerve root compression. Axial pain, which worsens with movement and is alleviated with rest, is caused by a structural abnormality of the spinal column and may indicate instability (Ratliff and Cooper 2004) Table 32.1.

Treatment of Synovial Sarcoma Metastatic to the Spine

Treatment options for tumors metastatic to the spine include systemic therapies such as hormonal therapy or chemotherapy, as well as local therapies such as radiotherapy, bracing, or surgery

Table 32.1 A summary of the 17 cases of spinal metastases from synovial sarcoma found in the literature from 1985 to 2011

Author	Year reported	Primary (if spinal)	Location of spinal primary	Metastasis	Location of spinal metastasis	# of cases
Otsuka et al.	1985	–	–	Metastasis to L4-L5	Lumbar	1
Signorini et al.	1986	T2	Thoracic	T2, vertebral canal, posterior mediastinum	Thoracic	1
Yoshikawa et al.	1997	–	–	Metastasis to C5-C6	Cervical	1
Hanada et al.	1999	–	–	Metastasis to lumbar vertebra (level not specified)	Lumbar	1
Bilsky et al.	2001	–	–	Metastatic "spindle cell sarcoma" to spine; spinal levels not specified in any patient	–	3
Merimsky et al.	2004	–	–	1. Metastasis to T7-T8; 2. Metastasis to C2-C3; 3. Metastasis to S1-S3	Cervical, thoracic, sacral	3
Reichel et al.	2004	–	–	Metastasis to T8	Thoracic	1
Sakellaridis et al.	2006	L2	Lumbar	First metastasis to L1-L3 lumbar dura mater, then second metastasis to intradural extramedullary C7 (C6-T1) and at mediastinum in front of T5-T10 vertebral bodies	Cervical, lumbar, thoracic	1
Bedre et al.	2007	–	–	Metastasis to T12-L4, eroding the transverse process, body, and pedicle of L2	Lumbar	1
Scollato et al.	2008	–	–	Intramedullary metastasis at C3-C5	Cervical	1
Arnold et al.	2009	–	–	Fifth metastasis to T11-T12, with mass at T12 compressing the spinal cord	Thoracic	1
Arnold et al.	2010	–	–	Seventh metastasis to C2-C3 with spinal cord compression; also metastases to T4, T6-T8, and T11	Cervical, thoracic	1
Zairi et al.	2011	C1-C2 and posterior soft tissue of C4	Cervical	Third metastasis to superior cervical region with extension into the occipital region and involvement of occipital bone and underlying dura; then fourth metastasis to skull base with extension into the left jugular foramen and left cervical foramina (C1-C4)	Cervical	1

Note that two reports described three cases each, and one of those reports did not specify spinal levels in any of the three cases. The report by Suh et al. (2005) is not a report of a metastasis as reported in previous literature

(Tokuhashi et al. 2009). The choice of treatment is based on tumor pathology and its sensitivity to adjuvant treatments, as well as the general condition of the patient and expected survival (Gasbarrini et al. 2004; Tokuhashi et al. 2009). Treatment of spinal column metastases is seldom curative; thus, the goal of treatment in most cases is palliative relief of pain (Ratliff and Cooper

2004) and preservation or restoration of neurological function (Schick et al. 2001).

Advances in MRI, sophisticated instrumentation for spinal stabilization, and an increased understanding of the biomechanics of the spine have led to increased treatment options for spine metastases. The advent of spinal instrumentation as well as transpedicular or ventral decompression has allowed favorable outcomes in treating and possibly reversing significant neurological deficit, compared to previous treatment methods of radiation, decompressive laminectomy without stabilization, or combined radiation and laminectomy.

Several authors have reported postoperative neurologic improvement in ~70% of patients (Ratliff and Cooper 2004; Holman et al. 2005), a significant increase compared to traditional therapies; this increase is attributed to newer approaches emphasizing anterior decompression and vertebral body reconstruction, and the introduction of more reliable anterior and posterior segmental spinal stabilization systems (Holman et al. 2005). These authors also demonstrated that superior rates of pain relief could be achieved when anterior or posterior stabilization is combined with neural decompression to eliminate tumor-related axial spine instability. Approximately 50% of patients who are nonambulatory before surgery will regain ambulation (Ratliff and Cooper 2004). Poor postoperative results are an indication of poor preoperative (baseline) neurologic function. Patients who are ambulatory at the time of initial treatment are more likely to remain ambulatory after intervention (Ratliff and Cooper 2004).

Complete surgical resection with wide negative margins is best for local tumor control and long-term survival (Bilsky et al. 2001). However, concern for preservation of neural tissue as well as the amount of osseous destruction and involvement of the spine at presentation may preclude *en bloc* resection for wide or marginal tumor removal (Bilsky et al. 2001; Barus et al. 2009). Many physicians still believe that radiotherapy should be the initial treatment for all spinal metastases (Holman et al. 2005), and thus surgery is considered only for patients who require a tissue diagnosis or for patients who fail to improve or deteriorate during radiation therapy (Ratliff and Cooper 2004; Holman et al. 2005). The majority of patients referred for surgical consultation for vertebral body metastases will already have received neo-adjuvant chemotherapy, radiotherapy, or both. These therapies may increase the rate of perioperative complications, impaired local wound healing, or immunosuppression (Ratliff and Cooper 2004).

Surgery for Metastatic Synovial Sarcoma

Tumors metastatic to the spine cause severe pain, paralysis, and/or significant impairment of activities of daily living (Tokuhashi et al. 2009). The finding of spinal metastases suggests limited life expectancy and limited treatment options (Tokuhashi et al. 2009). Many studies report median survivals of at least 1 year after surgery (Ratliff and Cooper 2004).

Surgery has a critical role in the treatment of patients with metastatic spinal disease (Holman et al. 2005). Patient survival depends on the primary tumor, degree of spread, and tumor biology (Ratliff and Cooper 2004). Because eradication of metastases is seldom possible, the goals of surgery for patients with sarcoma metastatic to the spine are to relieve pain, prevent or reverse neurologic compromise, improve functional status (ADLs), and possibly achieve long-term local control (Bilsky et al. 2001; Ratliff and Cooper 2004; Holman et al. 2005; Tokuhashi et al. 2009). In properly selected patients, surgery may also provide prevention of late neurologic deterioration as well as excellent acceptable perioperative morbidity and mortality (Ratliff and Cooper 2004). Surgery should be considered as initial therapy in many patients presenting with metastatic spinal disease because improved surgical techniques have resulted in improved outcomes (Ratliff and Cooper 2004; Tokuhashi et al. 2009).

A negative margin in sarcoma surgery is traditionally defined as 5 cm (Puffer et al. 2011). However, attempts at wide or marginal resections of epidural, bilateral pedicle, multilevel vertebral body, and/or large paraspinal tumors may necessitate resection of neural tissue, resulting in a

functional deficit (Bilsky et al. 2001; Barus et al. 2009; Ravnik et al. 2009). To avoid injuring critical structures, gross total resection with only marginal negative margins is recommended (Barus et al. 2009; Puffer et al. 2011). A marginal resection in the spine dissects the pseudocapsule of the tumor, and wide resection provides >2 mm of normal tissue (healthy bone, reactive periosteum, or pleura) (Bilsky et al. 2001).

Surgery: Patient Selection

Careful patient selection is required for surgical treatment of metastatic synovial sarcoma (Eilber and Dry 2008), and is an important determinant of surgical outcome (Ratliff and Cooper 2004). Patients should be selected by extent of disease, longer disease-free interval, and favorable response to systemic chemotherapy (Eilber and Dry 2008). Minimum requirements are a will to live, immunocompetence, a reasonably healthy condition, and a predicted survival of 3–6 months or longer (Ratliff and Cooper 2004; Tokuhashi et al. 2009). Functional recovery is significantly affected by the expected survival period (Tokuhashi et al. 2009). Preoperative neurologic status determines postoperative neurologic function. In patients with neurologic deficits, early intervention is required in order to maximize recovery (Ratliff and Cooper 2004). In patients having rapidly progressing paralysis, surgery may provide transient recovery but deterioration often resumes within 1–2 weeks after surgery (Tokuhashi et al. 2009).

Surgical treatment of spinal metastases may be extensive and is not without risk of complications (Ratliff and Cooper 2004; Holman et al. 2005; Tokuhashi et al. 2009). Thorough preoperative evaluation and optimization of the patient's current medical condition will reduce the incidence of perioperative complications (Ratliff and Cooper 2004). Common complications include cerebrospinal fluid leakage and wound breakdown, as well as pulmonary, cardiac, and gastrointestinal complications (Ratliff and Cooper 2004). Severe complications include perioperative neurologic deterioration, wound breakdown, significant blood loss, and mortality, although the rate of perioperative mortality is <5% (Ratliff and Cooper 2004), and many cases of neurologic deficits are transient (Ratliff and Cooper 2004).

Complication rates are increased by the use of high-dose steroids, radiation, and chemotherapy. An increased risk of postoperative wound infection or breakdown is associated with long-term steroid use, preoperative radiation or chemotherapy, immunosuppression, poor nutritional status, and prior surgery (Ratliff and Cooper 2004). Ghogawala et al. (2001) found a nearly 30% increase in the incidence of wound breakdown in patients treated with preoperative radiation, and a 46% wound complication rate in patients who had surgery within 7 days of beginning radiotherapy.

Indications for Surgery

Currently, the common indications for surgery for metastatic spine tumors are:

- Spinal instability and intractable axial spine pain caused by metastatic bony destruction (Bilsky et al. 2001; Ratliff and Cooper 2004; Holman et al. 2005; Tokuhashi et al. 2009);
- Progressive neurologic deficit due to spinal cord compression (including retropulsed bone and disc fragments) despite radiotherapy, steroids, or chemotherapy (Bilsky et al. 2001; Ratliff and Cooper 2004; Holman et al. 2005; Tokuhashi et al. 2009);
- Radioresistant tumors, tumors that have progressed or recurred after radiation, (Bilsky et al. 2001; Ratliff and Cooper 2004; Holman et al. 2005), or pain caused by radioresistant tumors (Gasbarrini et al. 2004; Tokuhashi et al. 2009);
- Unknown primary for which CT-guided biopsy was not possible or successful (Ratliff and Cooper 2004; Holman et al. 2005);
- Long-term local control in patients who have localized lesions and a life expectancy of at least 1 year (Tokuhashi et al. 2009).

Discussion of Surgical Interventions

The optimal operative strategy for metastatic spinal disease continues to be debated. Improved surgical techniques including anterior, posterior, or combined decompressions with stabilization have been shown to provide better decompression

of the spinal cord and better gross total resection when compared to the use of radiation alone, decompressive laminectomy alone without stabilization, or combined radiation and laminectomy (Bilsky et al. 2001; Ratliff and Cooper 2004). In addition, these improved surgical techniques have resulted in dramatic improvements in the neurological and functional outcomes of patients treated for sarcomas metastatic to the spine (Bilsky et al. 2001). When compared with laminectomy alone, studies of laminectomy combined with posterior stabilization report neurologic improvement in ~72% of patients (Ratliff and Cooper 2004). Most patients with locally recurrent disease can be treated with limb-sparing surgery (Eilber and Dry 2008), with no difference in survival compared to amputation (Fedors et al. 2010). Pain control may be achieved with surgical stabilization regardless of tumor type or spinal level (Ratliff and Cooper 2004).

Surgical procedures for metastatic spine tumors can be classified as excisional or palliative procedures (Tokuhashi et al. 2009). Excisional procedures, such as complete resection of the involved vertebrae or the tumor followed by reconstruction of the vertebrae using spinal instruments or implants, include those aimed at piecemeal resection of the tumor (such as intralesional excision or debulking), and those aimed at *en bloc* resection of the involved vertebrae (Gasbarrini et al. 2004; Tokuhashi et al. 2009). *En bloc* resection, which includes both marginal and wide resection, should be considered in patients with a good prognosis and involvement of only a single vertebra, or patients who have hypervascularized lesions (Gasbarrini et al. 2004; Tokuhashi et al. 2009). A palliative procedures consists of posterior laminectomy, decompression, and stabilization with instrumentation for relief of pain or paralysis, and, if possible, excision of as much of the tumor as possible (Gasbarrini et al. 2004; Tokuhashi et al. 2009). Palliative procedures are indicated for multivertebral metastases involving two or more vertebrae, or single vertebral metastases in a patient who has a predicted survival period of <1 year, and are frequently performed as emergency procedures (Tokuhashi et al. 2009).

Adjunctive Therapy for Metastatic Synovial Sarcoma

Neither preoperative nor postoperative chemotherapy for primary or metastatic sarcoma seem to have a major impact on outcome (Bilsky et al. 2001). In addition, chemotherapy has little effect in reversing spinal cord compression and improving neurologic deficit (Ratliff and Cooper 2004). Complications of chemotherapy may include immunosuppression and delayed wound healing, as well as an increased risk of wound dehiscence or perioperative wound infections (Ratliff and Cooper 2004).

A large local recurrence that develops shortly after initial treatment may indicate the presence of a biologically aggressive tumor with a high disease-specific mortality (Eilber and Dry 2008). These patients should be treated with neoadjuvant systemic therapy prior to re-operation (Eilber and Dry 2008). The first-line treatment for patients with metastatic synovial sarcoma is ifosfamide-based chemotherapy (with or without doxorubicin) because of the significant responses seen on this regimen (Ferrari et al. 2004; Spurrell et al. 2005; Eilber and Dry 2008). However, the effect of this same regimen on the survival of adults with primary disease has not been clear (Eilber and Dry 2008).

Radiation may alleviate local pain from metastasis and radicular pain from epidural compression (Ratliff and Cooper 2004). Relief of axial or mechanical pain requires surgical stabilization. The best predictor of neurologic outcome after radiotherapy is baseline neurologic function. Patients with severe neurologic deficit before radiotherapy are not likely to improve (Ratliff and Cooper 2004).

In patients with high-grade soft tissue sarcomas, brachytherapy has not been associated with a reduction in distant metastasis or improvement in disease-specific survival (Bilsky et al. 2001). In addition, to prevent radiation myelopathy, the spinal cord must be shielded during treatment; however, this shielding limits its use, especially

in patients being treated for epidural tumors (Bilsky et al. 2001).

In most patients with vertebral body metastases, radiotherapy is the initial therapeutic modality used, whereas in patients with widespread metastatic disease and poor prognosis, radiotherapy may be the only modality used (Ratliff and Cooper 2004). The dose of radiation depends on the type of tumor and the length of treatment; to prevent spinal cord damage, the dose is usually limited to 4,500 cGy (Ratliff and Cooper 2004). Radiation is appropriate for patients with radioresponsive tumors, minimal or no neurologic findings, no evidence of instability, and no neural compression by bone (Ratliff and Cooper 2004).

In conclusion, the need for effective palliative and potentially curative treatment for metastatic spinal disease is greater than ever because of earlier detection of metastases and increased patient survival (Holman et al. 2005). Borden et al. (2003) lists three factors critical to the reduction of sarcoma morbidity and mortality: (*a*) molecular and pathological redefinitions of sarcomas; (*b*) improvement in primary management, including imaging modalities; and (*c*) identification and development of targeted systemic therapies. It is clear that more effective, less toxic therapies for synovial sarcoma are needed (Eilber and Dry 2008). The SYT-SSX fusion protein may be a useful molecular target that will lead researchers to a histology-specific therapy (Eilber and Dry 2008).

Synovial sarcoma is rare, and metastasis of this tumor to the spine is even rarer. Metastases may occur in isolated or contiguous regions; the resultant loss of stability or recurrent neural compression may necessitate reoperation for neurologic recovery or preservation (Bilsky et al. 2001; Ratliff and Cooper 2004). Surgeons should have a high index of suspicion when evaluating patients who present with a history of synovial sarcoma, a spine lesion, and concomitant back pain and neurologic symptoms. Thorough radiological work-up should include CT and MRI in order to confirm the diagnosis and ensure appropriate management. Although not curative, surgical resection followed by spinal decompression, fusion and fixation can lead to neurologic improvement in patients who present with spinal cord compression.

References

Arnold PM, Park MC, Newell K, Kepes JJ, Thrasher JB (2009) Thoracic spinal cord compression secondary to metastatic synovial sarcoma: case report. Coluna/Columna 8:206–211

Arnold PM, Roh S, Ha TM, Anderson KK (2010) Metastatic synovial sarcoma with cervical spinal cord compression treated with posterior ventral resection: case report. J Spinal Cord Med 33:80–84

Baptista AM, Camargo OP, Croci AT, Oliveira CR, Azevedo Neto RS, Giannotti MA, Caiero MT, Santos TM, Abadi MD (2006) Synovial sarcoma of the extremities: prognostic factors for 20 nonmetastatic cases and a new histologic grading system with prognostic significance. Clinics (Sao Paulo) 61:381–386

Barus CE, Monsey RD, Kalof AN (2009) Poorly differentiated synovial sarcoma of the lumbar spine in a fourteen-year-old girl. A case report. J Bone Joint Surg Am 91:1471–1476

Bedre G, Gupta T, Rajasekharan P, Munshi A, Jalali R (2007) Cerebellar, pancreatic, and paraspinal metastases in soft tissue sarcomas: unusual sites or changing patterns? JOP 8:444–449

Bilsky MH, Boland PJ, Panageas KS, Woodruff JM, Brennan MF, Healey JH (2001) Intralesional resection of primary and metastatic sarcoma involving the spine: outcome analysis of 59 patients. Neurosurgery 49:1277–1286

Borden EC, Baker LH, Bell RS, Bramwell V, Demetri GD, Eisenberg BL, Fletcher CD, Fletcher JA, Ladanyi M, Meltzer P, O'Sullivan B, Parkinson DR, Pisters PW, Saxman S, Singer S, Sundaram M, van Oosterom AT, Verweij J, Waalen J, Weiss SW, Brennan MF (2003) Soft tissue sarcomas of adults: state of the translational science. Clin Cancer Res 9:1941–1956

Davicioni E, Wai DH, Anderson MJ (2008) Diagnostic and prognostic sarcoma signatures. Mol Diagn Ther 12:359–374

Eilber FC, Dry SM (2008) Diagnosis and management of synovial sarcoma. J Surg Oncol 97:314–320

Eilber FC, Brennan MF, Eilber FR, Eckardt JJ, Grobmyer SR, Riedel E, Forscher C, Maki RG, Singer S (2007) Chemotherapy is associated with improved survival in adult patients with primary extremity synovial sarcoma. Ann Surg 246:105–113

Fedors NH, Demos TC, Lomasney LM, Mehta V, Horvath LE (2010) Radiologic case study: your diagnosis? Synovial sarcoma. Orthopedics 33:861

Ferrari A, Gronchi A, Casanova M, Meazza C, Gandola L, Collini P, Lozza L, Bertulli R, Olmi P, Casali PG (2004) Synovial sarcoma: a retrospective analysis of 271 patients of all ages treated at a single institution. Cancer 101:627–634

Fletcher CDM, Unni KK, Mertens F (eds) (2002) World Health Organisation classification of tumours. Pathology and genetics of tumours of soft tissue and bone. IARC Press, Lyon

Foreman SM, Stahl MJ (2011) Biphasic synovial sarcoma in the cervical spine: case report. Chiropr Man Therap 19:12

Gasbarrini A, Cappuccio M, Mirabile L, Bandiera S, Terzi S, Barbanti Bròdano G, Boriani S (2004) Spinal metastases: treatment evaluation algorithm. Eur Rev Med Pharmacol Sci 8:265–274

Ghogawala Z, Mansfield FL, Borges LF (2001) Spinal radiation before surgical decompression adversely affects outcomes of surgery for symptomatic metastatic spinal cord compression. Spine 26:818–824

Gomez P, Morcuende J (2004) High-grade sarcomas mimicking traumatic intramuscular hematomas: a report of three cases. Iowa Orthop J 24:106–110

Greene S, Hawkins DS, Rutledge JC, Tsuchiya KD, Douglas J, Ellenbogen RG, Avellino AM (2006) Pediatric intradural extramedullary synovial sarcoma: case report. Neurosurgery 59:E1339

Guillou L, Benhattar J, Bonichon F, Gallagher G, Terrier P, Stauffer E, Somerhausen Nde S, Michels JJ, Jundt G, Vince DR, Taylor S, Genevay M, Collin F, Trassard M, Coindre JM (2004) Histologic grade, but not SYT-SSX fusion type, is an important prognostic factor in patients with synovial sarcoma: a multicenter, retrospective analysis. J Clin Oncol 22:4040–4050

Hanada T, Iwashita M, Matsuzaki T, Hanamure Y, Fukuda K, Furuta S (1999) Synovial sarcoma in the parapharyngeal space: case report and review of the literature. Auris Nasus Larynx 26:91–94

Holman PJ, Suki D, McCutcheon I, Wolinsky JP, Rhines LD, Gokaslan ZL (2005) Surgical management of metastatic disease of the lumbar spine: experience with 139 patients. J Neurosurg Spine 2:550–563

Koehler SM, Beasley MB, Chin CS, Wittig JC, Hecht AC, Qureshi SA (2009) Synovial sarcoma of the thoracic spine. Spine J 9:e1–e6

Ladanyi M, Antonescu CR, Leung DH, Woodruff JM, Kawai A, Healey JH, Brennan MF, Bridge JA, Neff JR, Barr FG, Goldsmith JD, Brooks JS, Goldblum JR, Ali SZ, Shipley J, Cooper CS, Fisher C, Skytting B, Larsson O (2002) Impact of SYT-SSX fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. Cancer Res 62:135–140

Laor T (2004) MR imaging of soft tissue tumors and tumor-like lesions. Pediatr Radiol 34:24–37

Lewis JJ, Antonescu CR, Leung DH, Blumberg D, Healey JH, Woodruff JM, Brennan MF (2000) Synovial sarcoma: a multivariate analysis of prognostic factors in 112 patients with primary localized tumors of the extremity. J Clin Oncol 18:2087–2094

Loblaw DA, Mitera G (2011) The optimal dose fractionation schema for malignant extradural spinal cord compression. J Support Oncol 9:121–124

Merimsky O, Kollender Y, Bokstein F, Issakov J, Flusser G, Inbar MJ, Meller I, Bickels J (2004) Radiotherapy for spinal cord compression in patients with soft-tissue sarcoma. Int J Radiat Oncol Biol Phys 58:1468–1473

Murphey MD, Gibson MS, Jennings BT, Crespo-Rodríguez AM, Fanburg-Smith J, Gajewski DA (2006) From the archives of the AFIP: imaging of synovial sarcoma with radiologic-pathologic correlation. Radiographics 26:1543–1565

Otsuka N, Morita R, Yamamoto T, Muranaka A, Tomomitsu T, Yanagimoto S, Sone T, Fukunaga M (1985) A case of synovial sarcoma with bone metastasis identified by bone marrow scintigraphy. Clin Nucl Med 10:260–263

Patchell RA, Tibbs PA, Regine WF, Payne R, Saris S, Kryscio RJ, Mohiuddin M, Young B (2005) Direct decompressive surgical resection in the treatment of spinal cord compression caused by metastatic cancer: a randomised trial. Lancet 366:643–648

Puffer RC, Daniels DJ, Giannini C, Pichelmann MA, Rose PS, Clarke MJ (2011) Synovial sarcoma of the spine: a report of three cases and review of the literature. Surg Neurol Int 2:18

Quraishi NA, Gokaslan ZL, Boriani S (2010) The surgical management of metastatic epidural compression of the spinal cord. J Bone Joint Surg Br 92:1054–1060

Ratliff JK, Cooper PR (2004) Metastatic spine tumors. South Med J 97:246–253

Ravnik J, Potrc S, Kavalar R, Ravnik M, Zakotnik B, Bunc G (2009) Dumbbell synovial sarcoma of the thoracolumbar spine: a case report. Spine 34:E363–E366

Reichel O, Blasenbreu-Vogt S, Suckfüll M, Naumann A, Leunig A (2004) Synovial sarcoma of the hypopharynx – two case reports. Laryngorhinootologie 83:96–101

Sakabe T, Murata H, Konishi E, Takeshita H, Ueda H, Matsui T, Horie N, Yanagisawa A, Kubo T (2008) Evaluation of clinical outcomes and prognostic factors for synovial sarcoma arising from the extremities. Med Sci Monit 14:CR305–CR310

Sakellaridis N, Mahera H, Pomonis S (2006) Hemangiopericytoma-like synovial sarcoma of the lumbar spine. Case report. J Neurosurg Spine 4:179–182

Schick U, Marquardt G, Lorenz R (2001) Intradural and extradural spinal metastases. Neurosurg Rev 24:1–5

Scollato A, Buccoliero AM, Di Rita A, Gallina P, Di Lorenzo N (2008) Intramedullary spinal cord metastasis from synovial sarcoma. Case illustration. J Neurosurg Spine 8:400

Signorini GC, Pinna G, Freschini A, Bontempini L, Dalle Ore G (1986) Synovial sarcoma of the thoracic spine. A case report. Spine 11:629–631

Spurrell EL, Fisher C, Thomas JM, Judson IR (2005) Prognostic factors in advanced synovial sarcoma: an analysis of 104 patients treated at the Royal Marsden Hospital. Ann Oncol 16:437–444

Suh SI, Seol HY, Hong SJ, Kim JH, Kim JH, Lee JH, Kim MG (2005) Spinal epidural synovial sarcoma: a case of homogeneous enhancing large paravertebral mass on MR imaging. AJNR Am J Neuroradiol 26:2402–2405

Sultan I, Rodriguez-Galindo C, Saab R, Yasir S, Casanova M, Ferrari A (2009) Comparing children and adults with synovial sarcoma in the Surveillance, Epidemiology, and End Results program, 1983 to 2005: an analysis of 1268 patients. Cancer 115:3537–3547

Tateishi U, Hasegawa T, Beppu Y, Satake M, Moriyama N (2004) Synovial sarcoma of the soft tissues: prognostic

significance of imaging features. J Comput Assist Tomogr 28:140–148
Tateishi U, Hasegawa T, Nojima T, Takegami T, Arai Y (2006) MRI features of extraskeletal myxoid chondrosarcoma. Skeletal Radiol 35:27–33
Tokuhashi Y, Ajiro Y, Oshima M (2009) Algorithms and planning in metastatic spine tumors. Orthop Clin North Am 40:37–46, v-vi
Yoshikawa H, Ueda T, Mori S, Araki N, Kuratsu S, Uchida A, Ochi T (1997) Skeletal metastases from soft-tissue sarcomas. Incidence, patterns, and radiological features. J Bone Joint Surg Br 79:548–552
Zagars GK, Ballo MT, Pisters PW, Pollock RE, Patel SR, Benjamin RS, Evans HL (2003) Prognostic factors for patients with localized soft-tissue sarcoma reated with conservation surgery and radiation therapy: an analysis of 1225 patients. Cancer 97:2530–2543
Zairi F, Assaker R, Bouras T, Chastanet P, Reyns N (2011) Cervical synovial sarcoma necessitating multiple neurosurgical procedures. Br J Neurosurg 25:769–771

Adult Spinal Intramedullary Ependymomas: Complete Resection

33

Hyun-Jib Kim, Seung-Jae Hyun, Sang Hoon Yoon, and Ki-Jeong Kim

Contents

Abstract

Achieving gross total resection of the tumor is one of the most important factors in promoting long-term recurrence-free survival in the management of spinal intramedullary ependymomas. Therefore, total resection is primary goal of treatment in the modern era. In this perspective, presence of natural dissection plane between the tumor and the spinal cord is crucial to achieve gross total resection. Some large tumors, often devoid of dissection plane, may preclude gross total resection and increase the risk of postoperative neurological compromise. Moreover, modalities of intraoperative neurophysiological monitoring have evolved recently to avoid additional neurological deficits in resection of these tumors. In this chapter, we describe up-to-date knowledge about spinal intramedullary ependymomas including refined operative techniques for complete resection and surgical outcome.

Introduction

Ependymoma arises from ependymal cells that form the lining of the ventricles of the brain and central canal of the spinal cord. Ependymoma of the spinal cord is a rare tumor, accounting for less than 2% of all primary central nervous system tumors. However, it is the most common intraspinal tumor. It comprises 15% of spinal cord tumors and up to 60% of spinal cord gliomas (Peschel et al. 1983). The WHO classified ependymomas into four

H.-J. Kim (✉) • S.-J. Hyun • S.H. Yoon • K.-J. Kim
Department of Neurosurgery, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 300 Gumi-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-707, South Korea
e-mail: jibkim@snu.ac.kr

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_33,

distinct subtypes: subependymomas, myxopapillary ependymomas, classic ependymomas, and anaplastic ependymomas (Nagasawa et al. 2011).

Spinal cord ependymomas are usually benign and curable with surgical resection or in combination with radiotherapy. However, anaplastic ependymomas are less common and have worse prognosis. The primary sites of spinal cord ependymomas are the cervical and cervico-thoracic segments. Surgery is essential for the establishment of a histologic diagnosis and the primary removal of the ependymoma. More importantly, patients with complete resection may not need postoperative radiotherapy. However, complete resection is not always possible because of its location. In such cases, postoperative radiotherapy is often necessary. Standard treatment usually includes radical resection followed by radiotherapy for known or suspected residual tumors. Compared with intracranial ependymomas, spinal ependymomas are less prevalent, occur in a younger population and exhibit a better prognosis. Therefore, they establish a characteristic clinical entity and require their own management flow.

Diagnosis

The median age at diagnosis with ependymoma is 44 years. The incidence of ependymoma is 77% of all primary spinal cord tumors in childhood (0–19 years) but is 23% in all tumors (Schellinger et al. 2008). In the SEER (Surveillance, Epidemiology, and End Results Program) data (malignant tumors only) used for these analyses, the most common histologic diagnosis by age group was astrocytoma (total) for the 0–19 year group, ependymoma for the 20–44 and 45–64 year groups, and lymphoma for the 65+ year group (Schellinger et al. 2008).

Ependymomas are typically non-infiltrative lesions with a distinct tumor–spinal cord plane and are considered resectable, whereas fibrillary astrocytomas, due to their infiltrative characteristic, are considered non-resectable (Nakamura et al. 2008). The advent of magnetic resonance (MR) imaging has improved the capability of visualizing the extent of intramedullary spinal cord tumors and differentiating between cystic and solid components. While MR imaging has been shown to provide earlier detection of spinal cord tumors, prediction of histological grade on the basis of MR imaging is often inaccurate. The typical MR imaging protocol for evaluation of the spinal cord lesions includes unenhanced sagittal and axial T1-weighted and T2-weighted images, as well as post-gadolinium-enhanced sagittal and axial T1-weighted images. Post-gadolinium-enhanced images are advantageous to determine the solid portion of an intramedullary neoplasm, cysts, other enhancing pathologic entities, or other features that may modify the differential diagnosis.

Ependymomas cause expansion of the spinal cord typically over three to four segments (Fig. 33.1a). The lesions are hypointense or isointense relative to normal spinal cord on T1-weighted images (Fig. 33.1b) and are typically heterogeneous on T2-weighted images (Fig. 33.1c). Areas of hypercellularity appear as hypointense within the bulk of a relatively hyperintense lesion on T2-weighted images. Hemorrhage can exhibit variable signal on both sequences. Ependymomas tend to enhance intensely, but heterogeneously, and often have well-defined margins. In addition, intratumoral and peritumoral cysts can be often identified (Fig. 33.1c).

The many efforts using MR imaging for prevention neurological deterioration after surgery are introduced. Diffusion tensor imaging of the spinal cord is one of them but is limited by bony structures surrounding the spinal cord and the low signal-to-noise ratio. These difficulties were overcome by the advent of 3-T technologies and the development of new diffusion tensor imaging protocols. Diffusion tensor imaging and tractography have been used successfully in the intramedullary tumors (Vargas et al. 2008). Until now, however, there have been no large-scale studies comparing the results of diffusion tensor imaging tractography and microsurgical treatment of intramedullary tumors.

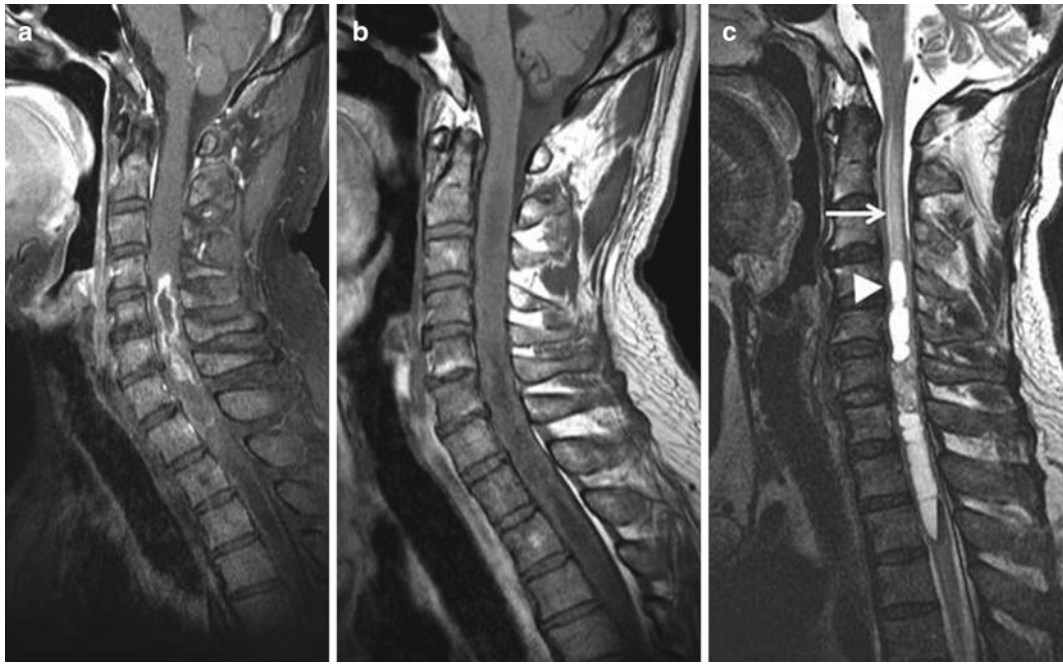

Fig. 33.1 T1 weighted magnetic resonance (*MR*) image with gadolinium enhancement showed intramedullary peripheral enhancement around low signal intensity area from C4 to T1 level. (**a**) Histopathologic examination confirmed cellular ependymoma (WHO grade II). T1 weighted sagittal MR image showed hypo- or iso- signal intensity in the tumor. (**b**) In the other case of ependymoma, typical heterogenous high signal intensity in T2 weighted image was noticed in the whole cervical spinal cord. (**c**) *Arrow* indicated peritumoral edema and *arrow head* indicated syrinx cavity each other

Intraoperative Neurophysiologic Monitoring

Recent advances in technology and neurophysiological methodologies have significantly altered intraoperative neurophysiological monitoring (INM) during spinal and spinal cord surgery. Somatosensory evoked potentials (SSEPs) were first used about 30 years ago to monitor the spinal cord during surgical correction for scoliosis (Nash et al. 1977). Despite preservation of SSEPs, serious motor deficits were observed, thus bringing into question their capacity to monitor spinal cord motor tracts.

More recently, transcranial, electrically-elicited, motor evoked potentials (Tc-MEPs) have been used to assess the integrity of motor tracts during removal of spinal cord tumors, correction of scoliosis and cervical spine surgery (Deletis and Sala 2008). Over individual monitoring alone, combined SSEP and MEP monitoring has several theoretical advantages, including the ability to monitor a larger number of patients, the increased accuracy provided by complementary information from two independent systems with a reduced risk of false negatives, and perhaps an increased sensitivity in detecting early spinal cord dysfunction (Hyun et al. 2009).

Anesthetic Considerations

Anesthesia is maintained with continuous infusion of propofol (10 mg/kg/h) and remifentanil (0.25 μg/kg/min). A single bolus of non-depolarizing short acting muscle relaxant (rocuronium) is given at induction to facilitate endotracheal intubation and ventilation. No paralytic agents are used after induction and intubation. The level of neuromuscular block is monitored by recording the compound muscle action potentials (CMAPs) to a train of four stimuli. Invasive blood

pressure, electrocardiogram (ECG), end-tidal carbon dioxide concentration ($ETCO_2$), pulse oximetry and temperature are monitored. Patients are actively warmed throughout the procedure.

Intraoperative SSEPs

Stimulation of SSEP is accomplished with square-wave electrical pulses of 0.3 ms duration and a maximum intensity of 25 mA at a frequency of 5 Hz. Surface stimulating electrodes are placed over each median nerve at the wrist and over each posterior tibial nerve at the ankle. Evoked potentials are recorded in a referential fashion from the C3 (right median nerve stimulation), C4 (left median nerve stimulation) and Cz (right and left tibial nerve stimulation) positions, and from a reference electrode at FPZ (International 10–20 system). The filter bandwidth is 20–1,500 Hz, and the SSEP amplitude is measured peak-to-peak.

Intraoperative MEPs

Multi-pulse transcranial electrical stimulation is performed using a commercially available IOM electrical stimulator. Nine-millimeter disc or subdermal needle electrodes are attached to the scalp with collodion 6 cm anterior to Cz and at C3 and C4 (International 10–20 system). Bipolar stimulation is used. Trains of either four or five pulses (individual stimulus duration of 50 ms) with interstimulus intervals of 2, 3, or 4 ms are used, depending on which provided the best recording, with a period of at least 30 s between two successive trains. Stimulus intensity is gradually increased (50 V increments from 100 V to a maximum of 600 V) until MEP amplitudes are maximized above a minimum of 20 mV. If response amplitudes of at least 20 mV cannot be obtained from either leg, MEP monitoring is abandoned. MEPs are recorded simultaneously from the tibialis anterior and abductor halluces muscles of both legs and from the abductor pollicis muscles of both arms using a pair of non-insulated subcutaneous needle electrodes inserted 3 cm apart in each muscle. The time base is 100 ms, and the filter bandpass was 20–5,000 Hz, using restricted high-pass filters.

Electrophysiological Monitoring

Neurophysiologic monitoring during surgery was performed. Baseline readings are obtained prior to skin incision and after opening of the dura mater. Waveforms are analyzed for latency and peak-to-peak amplitude. Stimulation is alternated between SSEP and MEP in continuous order. Reduced SSEP amplitude of >50% and increased latency of >10% of baseline values are regarded as significant (Sala et al. 2006). When using propofol for maintenance anesthesia, decrements in MEP amplitude >50% of baseline values, providing the levels of neuromuscular blockade and general anesthesia remain unchanged, are indicative of a significant change. The level of neuromuscular block is monitored by recording the CMAPs from either the abductor hallucis or tibialis anterior muscle to a train of four supramaximal stimuli (2 Hz, 0.2 ms duration) delivered to the tibial nerve at the ankle or the peroneal nerve at the fibula head. CMAPs are recorded either before or after each MEP trial. The surgical team is immediately informed of any significant IOM changes.

Surgical Impact of Combined INM

In our experiences, combined INM resulted in alteration of surgical strategy in all cases with significant potential changes. MEP deterioration prompted an immediate pause of the operation and a thorough inspection of the operating field after considering external factors (i.e. mean arterial pressure, hemoglobin, hematocrit, technical problems, etc.) as a cause of amplitude reduction. Depending on the procedure and the cause of positive recordings, specific measures, such as temporarily ceasing the retraction of the dorsal columns, rinsing of the operative site with nimodipine and longer pausing, were undertaken. Corrective measures for the management of MEP deterioration typically include temporary or definite halt of resection, temporary cessation in

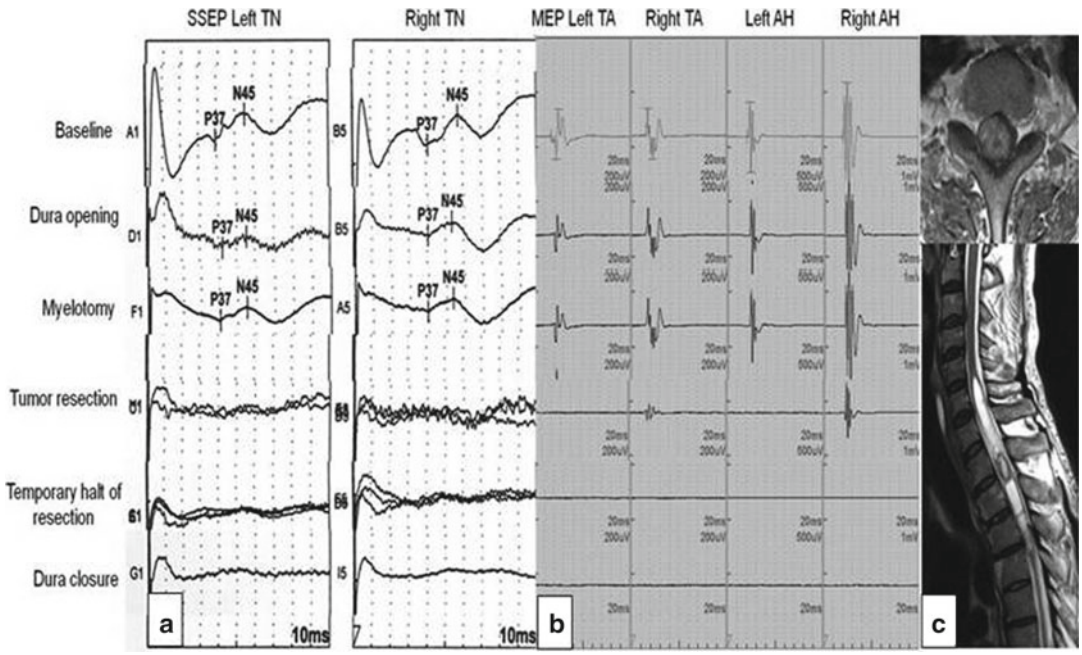

Fig. 33.2 Tracing of SSEP (**a**) and MEP (**b**) during the critical stage of the operation in patient having intramedullary ependymoma in the upper thoracic spinal cord. Bilateral loss of MEP was accompanied by SSEP loss and no recovery. A permanenet motor deficit of both legs occured post-operatively. (**c**) T1-weighted gadolinium-enhanced MRI demonstrating an intramedullary spinal cord tumor with syrinx formation. (histology: ependymoma) *TN* tibial nerve, *TA* tibialis anterior, *AH* abductor halluces muscle

the retraction of the dorsal columns, and rinse of the operative site with nimodipine (Fig. 33.2).

Quinones-Hinojosa et al. (2002) eloquently demonstrate the use of spinal cord mapping for the tumor resections. They used intraoperative spinal stimulation for the elicitation of SSEPs. The antidromically elicited SSEPs allowed the accurate definition and determination of the location of the midline in the spinal cord distorted by tumor. This provides for a more confident and accurate placement of the myelotomy incision. They directly stimulated the spinal cord and found that stimulation of the abnormal tissue did not elicit electromyographic activity. However, stimulation of the periphery of the tumor elicited an electromyographic response before normal spinal cord was visualized, suggesting that traction on the tumor margin could endanger the corticospinal tracts. It is unclear whether the mapping facilitated tumor resection, because the authors did not specify whether there was an obvious cleavage plane between the tumor and the spinal cord.

Corrective Measures in MEP Deterioration

Three useful factors are known to promote the recovery of lost myogenic MEPs or deteriorated SSEPs during spinal cord surgery (Sala et al. 2006).

Time is the first factor. INM can provide real-time neurophysiological data that can accurately assess the well-being of the spinal cord and suggest whether or not it is ready to sustain further manipulation (Deletis and Sala 2008). From this perspective, surgery timing is probably one of the most critical factors affecting the surgical outcome. The second factor is irrigation. Irrigation of the surgical field with warm saline dilutes potassium, which accumulates in the extracellular space and may block conduction. In addition, irrigation generally removes irritating blood products and metabolites. The third factor is a combination of local application of papaverine and increasing mean arterial pressure. These steps

have been found to improve local tissue perfusion to counteract an incipient ischemia. In the current study, correction of systemic hypotension was correlated with the rate of recovery from intraoperative EP deterioration.

Does the use of monitoring improve the functional outcome? The real impact of neurophysiological monitoring on the neurological outcome after intramedullary ependymoma surgery remains controversial and very difficult to prove based on control studies (Hyun and Rhim 2009). In fact, many neurosurgeons who operate with the assistance of IOM and believe in its efficacy to prevent neurological deficit, would not accept a prospective randomized study given the ethical and medico-legal concerns of designating a "control group". However, a study by Sala et al. (2006) suggests an advantage in neurological outcome in the monitored group over the control group. Since the extent of resection did not differ in both groups, the use of monitoring does not make the surgeon too "timid" to proceed until the tumor resection is, indeed, complete. Also, in our experience, the use of monitoring does not influence or hinder surgeon's performance in the tumor resection.

Operative Procedures

Preparation of Patients

Under general anesthesia, the patient is placed on the operating table in the prone position with the chest well padded. A Mayfield skull clamp may be useful for cervical tumors. Neurophysiologist sets up intraoperative neuromonitoring and checks baseline waves of somatosensory evoked potentials (SSEP) and motor evoked potentials (MEP). Some authors prefer perioperative corticosteroids (Hanbali et al. 2002).

Surgical Approach

After standard antiseptic painting and draping, midline skin incision is made. The paraspinal muscles are elevated in subperiosteal fashion, and all the soft tissues are cleared from the spinous processes and laminae. A laminoplasty is carried out with high-speed drill at the appropriate level, and the laminae and spinous processes are elevated and removed in one piece after incising the interspinous ligaments and removing ligamentum flavum with a small Kerrison rongeur. Bleeding from epidural venous plexus and free margins of laminae is controlled appropriately. Spinal intramedullary ependymomas are usually echogenic; intraoperative ultrasonography may be used before dural opening to determine that the entire tumor is sufficiently exposed (Hanbali et al. 2002).

Under operative microscope, a midline dural incision and tack-up sutures with 4–0 silk or Nurolon are made. Arachnoid membrane is opened in the midline and anchored to the dura with small size hemoclips. Arachnoid trabeculations can be released by the use of microscissors, and tortuous posterior spinal veins in the midline can be cauterized and divided. Some authors prefer dissection and preservation of large pial vessels (Iwasaki et al. 2000).

Myelotomy

Recognition of the accurate midline prior to myelotomy is critical to minimizing neurological defects. The midline in a normal cord is identified by the dorsal median sulcus between the bilateral posterior columns (Nagasawa et al. 2011). Dorsal median sulcus can be confirmed by several methods. First is to note the penetrating pial vessels into the dorsal median sulcus (Fig. 33.3a) (Hoshimaru et al. 1999). Second is to inspect dorsal root entry zones bilaterally, especially when the spinal cord is distorted by tumor growth and swelling. Third is to map the dorsal column using a grid electrode (Yanni et al. 2010).

Once identified, pia mater along the dorsal median sulcus is cut by sharp dissection with either No. 11 blade or surgical laser (Hoshimaru et al. 1999; Hsu et al. 2009). Myelotomy can be deepen until identifying the tumor by gently spreading posterior columns with microforceps or fine microdissectors and should be extended to expose rostral and caudal poles of the tumor. The use of pial traction

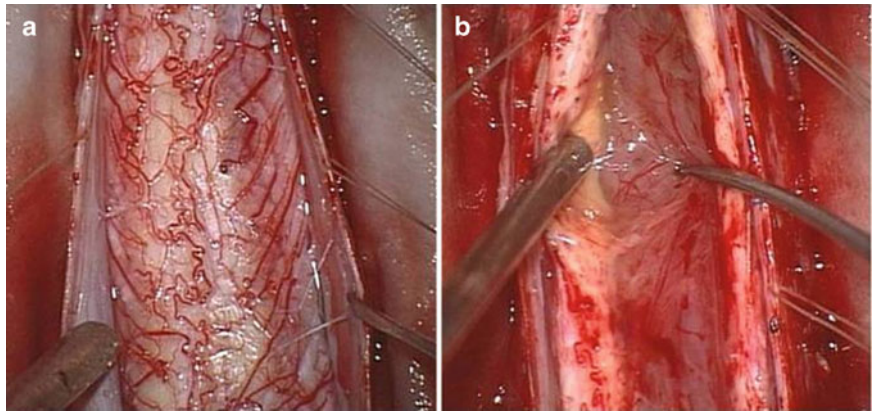

Fig. 33.3 An intraoperative photograph shows dorsal aspect of spinal cord at cervical spine. (**a**) It denotes the dorsal median sulcus at the mid portion between the bilateral dorsal rootlets. During dissection between the normal spinal cord and the tumor, clear natural dissection plane is visible (**b**)

suture remains controversial. Pial traction suture can provide a clear surgical field and steady tension in posterior columns. However, some argue that pial sutures can potentially increase the incidence and severity of postoperative dorsal column dysfunction (DSD) (Hanbali et al. 2002; Kucia et al. 2011).

Tumor Dissection

The tumor is usually soft and friable in consistency and reddish to gray in color. The intraoperative specimen should be sent to confirm the diagnosis of ependymoma. Most of the intramedullary ependymomas have clear natural dissection plane between the tumor and normal spinal cord (Fig. 33.3b), and relatively small tumors can be resected in en-bloc. For large tumors, debulking of the tumor using an ultrasonic aspirator facilitates the mobilization of the tumor. When the cavity allows the lateral edges of the tumor to be easily infolded, the surgeon should try to identify a natural dissection plane. Dissection of tumor can proceed with holding the tumor edge using a small tumor forceps, and gentle retraction should be applied to dissect the tumor away from the spinal cord. When mobilizing the tumor, all small transversing arteries that are still attached to the tumor should be cauterized and cut (Hsu et al. 2009). However, some tumors are poorly encapsulated, and total resection may be impossible without jeopardizing normal structures. A surgeon should pay more careful attention to the changes of intraoperative neuromonitoring in these circumstances, and subtotal resection of the tumors may be inevitable. In most cases, the rostral pole is often associated with a tumor-related syrinx. When the rostral pole is freed, the tumor is lifted and gently dissected out of the spinal cord. The caudal pole is more tapered and usually is connected to the central canal by a dense fibrous band that is also tapered caudally. This fibrous band is cut away. Ventral dissection along the anterior median raphe is usually the most difficult part of the surgery, because the tumor tends to adhere to this particularly thinned-out portion of the cord, and feeders branched from anterior spinal artery emerges (Hanbali et al. 2002). These small feeders must be cauterized with great care to avoid injury to the anterior spinal artery. The tumor bed should be inspected to confirm the absence of residual tumors, and meticulous hemostasis is needed. Intraoperative ultrasonography may also be used to confirm the completeness of the resection (Hanbali et al. 2002).

Closure

There are con and pro whether approximating both ends of posterior columns with pial sutures is necessary or not because it may lead to dorsal

column dysfunction. The authors preferred pial approximation because it may prevent tethering from neural structure to dura mater. Arachnoid membrane can be repaired with 8–0 Prolene. Some surgeons prefer a thin Gore-Tex sheet to be placed over the spinal cord and fixed at the rostral and caudal ends of dural incision in order to prevent postoperative adhesion between the spinal cord and the dura mater (Iwasaki et al. 2000). The dura mater is closed in water-tight fashion with a running 6–0 prolene suture. Fibrin glue or other adhesive agent may be used to prevent CSF leakage, and some authors use prophylactic lumbar drainage (Manzano et al. 2008). After copious irrigation and meticulous hemostasis, lamina is reconstructed by miniplates, and the muscle and skin is closed layer by layer.

Oncologic Outcome

Gross total resection of the tumor is one of the most important factors to promote long-term recurrence-free survival in the management of intramedullary ependymomas (Chang et al. 2002; Gomez et al. 2005; Hsu et al. 2009). Therefore, gross total resection is primary goal of treatment in the modern era (Hsu et al. 2009; Kucia et al. 2011; Nagasawa et al. 2011). In this perspective, presence of natural dissection plane between the tumor and the spinal cord is crucial to achieve gross total resection. Some large tumors often devoid of dissection plane and may preclude gross total resection or increase the risk of postoperative neurological compromise.

Since the incidence of intramedullary ependymomas is very low, and many studies are based on the data collected over several decades in the midst of rapid advancement in surgical and imaging technologies, the rates for total resection and progression free survival (PFS) have a wide variance. In a study of 126 spinal ependymomas from 1953 to 2000, the rate of gross total resection was 50% (Abdel-Wahab et al. 2006). In another study of 37 spinal ependymoma patients, who underwent surgery as early as 1955, the rate of gross total resection was only 11% (Gomez et al. 2005). However, more recent studies indicate much higher rate of total resection. Hoshimaru et al. (1999) reported total resection of 34 among 36 patients (94%) with intramedullary ependymomas. Other studies published after 2000 indicate the rate of total resection was 73–90% (Iwasaki et al. 2000; Hanbali et al. 2002; Nakamura et al. 2008; Bostrom et al. 2011; Karikari et al. 2011; Kucia et al. 2011). However, these more recent data lack sufficient sample size and long-term follow up. Nevertheless, these results reflect changing trend of the role of microsurgery and treatment scheme for intramedullary ependymomas over the past several decades.

Recurrence rates of intramedullary ependymomas have ranged from 3.8 to 9% according to recent studies (Hanbali et al. 2002; Bostrom et al. 2011; Karikari et al. 2011; Kucia et al. 2011). And Boström et al. (2011) reported that the PFS rate of all 57 patients with spinal cord ependymomas was 89% at 5 years and 84% at 10 years. Abdel-Wahab et al. (2006) reported a PFS rate of 74, 60, and 35% at 5, 10, and 15 years, respectively, in 126 patients undergoing operations dating back as early as 1953. Gomez et al. (2005) reported a PFS rate of 75, 50, and 46% at 5, 10, and 15 years, respectively. Their patients underwent surgery as early as 1955, with an overall recurrence rate of 57% in 37 patients. Recurrence rates by ependymoma subtype have ranged from 25 to 32% for myxopapillary tumors and from 4 to 43% for classic/anaplastic ependymomas. However, Boström et al. (2011) reported results for 57 patients undergoing surgery for spinal ependymomas.

Radiation Therapy

The role of postoperative radiation therapy continues to be controversial. In general, the utilization of postoperative radiation following gross total resection of a spinal ependymoma is not necessary (Nagasawa et al. 2011). Furthermore, classic ependymomas have less incidence of recurrence or dissemination in comparison to myxopapillary type. A gross total resection of classic ependymoma can be assumed curative and postoperative radiation is not

required. Postoperative radiation following incomplete resections is generally recommended by most investigators (Gavin Quigley et al. 2007; Hsu et al. 2009; Kucia et al. 2011). It is known that the utilization of postoperative radiotherapy following incomplete resections provide better survival and decreased and delayed recurrence (Schwartz and McCormick 2000; Gilbert et al. 2010). In addition, patients with anaplastic ependymomas or severe metastatic disease may also be good candidates for adjuvant radiotherapy (Bostrom et al. 2011; Nagasawa et al. 2011).

As recurrence is almost always a local phenomenon, prophylactic radiation of the entire cranio-radiation axis is not recommended (Hsu et al. 2009). Moreover, radiation exposure may incur tissue edema, radiation myelopathy, or radiation necrosis, spinal deformity, especially in pediatric population, and wound healing problem (Hanbali et al. 2002; Hsu et al. 2009; Nagasawa et al. 2011). Moreover, radiotherapy may result in reactive gliosis and fibrosis, hardening of the ependymoma, and disruption of the natural dissection planes (Nagasawa et al. 2011).

Prognosis

Neurological Outcome

Few studies have assessed neurological function after treatment. In a recent study, 28% of patients experienced a deterioration of neurological function in the immediate postoperative period (Halvorsen et al. 2010). They found no correlation between neurological deterioration in the postoperative period and tumor subtype (intramedullary vs. extramedullary, myxopapillary ependymoma vs. ependymoma). Sixty-seven percent of the patients with deterioration of neurological function during the immediate postoperative period had postoperative deficits that were related to midline myelotomy. Dorsal column dysfunction, an expected complication after midline myelotomy, accounted for 75% of morbidity, whereas paresis/paralysis/bladder dysfunction/bowel dysfunction and unexpected complications of midline myelotomy accounted for 25% of morbidity. These findings suggested that it is not uncommon for the presence of tumor—which frequently causes the midline of the medulla to be indiscernible due to edema, fibrosis and/or neovascularization—to precipitate unintentional dissection through the dorsal columns, resulting in postoperative sensory deficits, including the loss of proprioception (Manzano et al. 2008). Deterioration of neurological function during the immediate postoperative period was permanent in 67% and transient in 33% (Halvorsen et al. 2010).

Some authors state that for the most part, severe neurological deficit at the onset does not improve after treatment, and that only minor symptoms, such as pain and mild weakness, improve after treatment (Garcia 1985). Kopelson et al. (1980) report that most neurological deficits that are present before surgery either do not resolve or deteriorate. In this study, duration of symptoms relative to postoperative neurological deficit was not statistically significant; however, good preoperative neurological function was a positive prognostic factor for long-term neurological function. As do others, the authors recommend immediate removal of the ependymoma after diagnosis instead of delaying surgery until neurological deterioration occurs. At follow-up, most of our patients had neurological function compatible with an independent life.

Prognostic Factors for Functional Outcome

The prognostic importance of the preoperative functional status has also been stressed by others; Jenkinson et al. (2006) revealed the role of preoperative status as the only predicting factor in multivariate analysis. However, extent of tumor was not tested in their model, and only one postoperative examination was done at variable time intervals. The preoperative neurological status is prognostically important for functional outcome. Woodworth et al. (2007) identified the ability to walk preoperatively to be a significant factor for the postoperative ability to walk. They also revealed preoperative radiation therapy (conducted for unknown reasons) and preoperative serum

glucose level greater than 170 mg/dl to be predicting factors for poor outcome. However, both authors did not differentiate between early and late outcomes.

Acknowledgement The authors give special thanks to Judy (Eun-hui) Park, BS in Neuroscience, Wellesley College, Boston, USA for invaluable assistance and kind support.

References

Abdel-Wahab M, Etuk B, Palermo J, Shirato H, Kresl J, Yapicier O, Walker G, Scheithauer BW, Shaw E, Lee C, Curran W, Thomas T, Markoe A (2006) Spinal cord gliomas: a multi-institutional retrospective analysis. Int J Radiat Oncol Biol Phys 64:1060–1071

Bostrom A, von Lehe M, Hartmann W, Pietsch T, Feuss M, Bostrom JP, Schramm J, Simon M (2011) Surgery for spinal cord ependymomas: outcome and prognostic factors. Neurosurgery 68:302–308, discussion 309

Chang UK, Choe WJ, Chung SK, Chung CK, Kim HJ (2002) Surgical outcome and prognostic factors of spinal intramedullary ependymomas in adults. J Neurooncol 57:133–139

Deletis V, Sala F (2008) Intraoperative neurophysiological monitoring of the spinal cord during spinal cord and spine surgery: a review focus on the corticospinal tracts. Clin Neurophysiol 119:248–264

Garcia DM (1985) Primary spinal cord tumors treated with surgery and postoperative irradiation. Int J Radiat Oncol Biol Phys 11:1933–1939

Gavin Quigley D, Farooqi N, Pigott TJ, Findlay GF, Pillay R, Buxton N, Jenkinson MD (2007) Outcome predictors in the management of spinal cord ependymoma. Eur Spine J 16:399–404

Gilbert MR, Ruda R, Soffietti R (2010) Ependymomas in adults. Curr Neurol Neurosci Rep 10:240–247

Gomez DR, Missett BT, Wara WM, Lamborn KR, Prados MD, Chang S, Berger MS, Haas-Kogan DA (2005) High failure rate in spinal ependymomas with long-term follow-up. Neuro Oncol 7:254–259

Halvorsen CM, Kolstad F, Hald J, Johannesen TB, Krossnes BK, Langmoen IA, Lied B, Ronning P, Skaar S, Spetalen S, Helseth E (2010) Long-term outcome after resection of intraspinal ependymomas: report of 86 consecutive cases. Neurosurgery 67:1622–1631, discussion 1631

Hanbali F, Fourney DR, Marmor E, Suki D, Rhines LD, Weinberg JS, McCutcheon IE, Suk I, Gokaslan ZL (2002) Spinal cord ependymoma: radical surgical resection and outcome. Neurosurgery 51:1162–1172, discussion 1172–1164

Hoshimaru M, Koyama T, Hashimoto N, Kikuchi H (1999) Results of microsurgical treatment for intramedullary spinal cord ependymomas: analysis of 36 cases. Neurosurgery 44:264–269

Hsu W, Pradilla G, Constantini S, Jallo GI (2009) Surgical considerations of spinal ependymomas in the pediatric population. Childs Nerv Syst 25:1253–1259

Hyun SJ, Rhim SC (2009) Combined motor and somatosensory evoked potential monitoring for intramedullary spinal cord tumor surgery: correlation of clinical and neurophysiological data in 17 consecutive procedures. Br J Neurosurg 23:393–400

Hyun SJ, Rhim SC, Kang JK, Hong SH, Park BR (2009) Combined motor- and somatosensory-evoked potential monitoring for spine and spinal cord surgery: correlation of clinical and neurophysiological data in 85 consecutive procedures. Spinal Cord 47:616–622

Iwasaki Y, Hida K, Sawamura Y, Abe H (2000) Spinal intramedullary ependymomas: surgical results and immunohistochemical analysis of tumour proliferation activity. Br J Neurosurg 14:331–336

Jenkinson MD, Simpson C, Nicholas RS, Miles J, Findlay GF, Pigott TJ (2006) Outcome predictors and complications in the management of intradural spinal tumours. Eur Spine J 15:203–210

Karikari IO, Nimjee SM, Hodges TR, Cutrell E, Hughes BD, Powers CJ, Mehta AI, Hardin C, Bagley CA, Isaacs RE, Haglund MM, Friedman AH (2011) Impact of tumor histology on resectability and neurological outcome in primary intramedullary spinal cord tumors: a single-center experience with 102 patients. Neurosurgery 68:188–197, discussion 197

Kopelson G, Linggood RM, Kleinman GM, Doucette J, Wang CC (1980) Management of intramedullary spinal cord tumors. Radiology 135:473–479

Kucia EJ, Maughan PH, Kakarla UK, Bambakidis NC, Spetzler RF (2011) Surgical technique and outcomes in the treatment of spinal cord ependymomas: part II: myxopapillary ependymoma. Neurosurgery 68:90–94, discussion 94

Manzano G, Green BA, Vanni S, Levi AD (2008) Contemporary management of adult intramedullary spinal tumors-pathology and neurological outcomes related to surgical resection. Spinal Cord 46:540–546

Nagasawa DT, Smith ZA, Cremer N, Fong C, Lu DC, Yang I (2011) Complications associated with the treatment for spinal ependymomas. Neurosurg Focus 31:E13

Nakamura M, Ishii K, Watanabe K, Tsuji T, Takaishi H, Matsumoto M, Toyama Y, Chiba K (2008) Surgical treatment of intramedullary spinal cord tumors: prognosis and complications. Spinal Cord 46:282–286

Nash CL Jr, Lorig RA, Schatzinger LA, Brown RH (1977) Spinal cord monitoring during operative treatment of the spine. Clin Orthop Relat Res 126:100–105

Peschel RE, Kapp DS, Cardinale F, Manuelidis EE (1983) Ependymomas of the spinal cord. Int J Radiat Oncol Biol Phys 9:1093–1096

Quinones-Hinojosa A, Gulati M, Lyon R, Gupta N, Yingling C (2002) Spinal cord mapping as an adjunct for resection of intramedullary tumors: surgical technique with case illustrations. Neurosurgery 51:1199–1206, discussion 1206–1197

Sala F, Palandri G, Basso E, Lanteri P, Deletis V, Faccioli F, Bricolo A (2006) Motor evoked potential

monitoring improves outcome after surgery for intramedullary spinal cord tumors: a historical control study. Neurosurgery 58:1129–1143, discussion 1129–1143

Schellinger KA, Propp JM, Villano JL, McCarthy BJ (2008) Descriptive epidemiology of primary spinal cord tumors. J Neurooncol 87:173–179

Schwartz TH, McCormick PC (2000) Intramedullary ependymomas: clinical presentation, surgical treatment strategies and prognosis. J Neurooncol 47:211–218

Vargas MI, Delavelle J, Jlassi H, Rilliet B, Viallon M, Becker CD, Lovblad KO (2008) Clinical applications of diffusion tensor tractography of the spinal cord. Neuroradiology 50:25–29

Woodworth GF, Chaichana KL, McGirt MJ, Sciubba DM, Jallo GI, Gokaslan Z, Wolinsky JP, Witham TF (2007) Predictors of ambulatory function after surgical resection of intramedullary spinal cord tumors. Neurosurgery 61:99–105, discussion 105–106

Yanni DS, Ulkatan S, Deletis V, Barrenechea IJ, Sen C, Perin NI (2010) Utility of neurophysiological monitoring using dorsal column mapping in intramedullary spinal cord surgery. J Neurosurg Spine 12:623–628

Spinal Intramedullary Astrocytomas: Prognostic Factors

34

Vladimír Beneš III, Pavel Buchvald, and Petr Suchomel

Contents

Abstract

Intramedullary astrocytoma is one of the less common tumors of the central nervous system. The impact of prognostic factors on survival can play a major role in treatment planning and help in patient counseling; however conflicting conclusions are reported in the literature. We try to assess the importance of various patient-, tumor- and treatment-related factors on disease prognosis and patient survival. Particular attention is turned to the following: patient age, gender, history length, functional status, tumor location and extent, tumor associated cyst or syrinx presence, as well as tumor grade. Treatment strategies (resection extent and radiotherapy) are also evaluated.

Introduction

Intramedullary spinal cord tumors (IMSCT) account for approximately 2–4% of all central nervous system tumors and for 20–25% of spinal tumors (Helseth and Mork 1989; Slooff et al. 1964). The most common tumors encountered are ependymomas and astrocytomas. Ependymoma diagnosis is rare among children; it is usually encountered in middle-aged patients. Contrary, intramedullary astrocytomas constitute the majority of IMSCTs found in children and young adults (Constantini et al. 2000; Helseth and Mork 1989; Slooff et al. 1964). Whereas ependymoma can be considered a surgical disease with a clear plane of

V. Beneš III (✉) • P. Buchvald • P. Suchomel
Department of Neurosurgery, Regional Hospital Liberec, Husova 10, 46063 Liberec, Czech Republic
e-mail: vladimir.benes@nemlib.cz

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6_34,

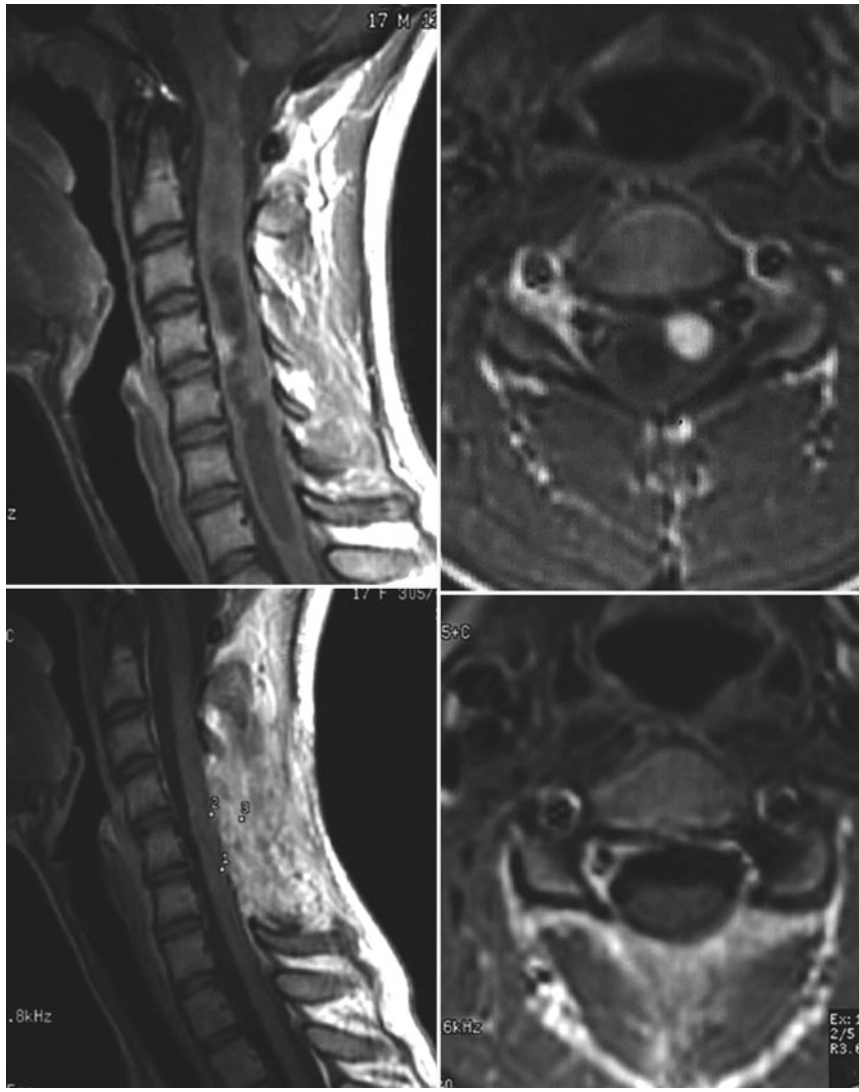

Fig. 34.1 Grade II intramedullary astrocytoma. Contrast enhanced MRI showing an intramedullary cystic lesion with an enhancing nodule. Preoperative (*upper row*) and postoperative scan confirming gross total resection (*lower row*)

dissection (Brotchi et al. 1991), infiltratively growing astrocytoma can seldom be resected radically (Fig. 34.1) (Innocenzi et al. 1997; Jallo et al. 2001; Kim et al. 2001; Minehan et al. 2009; Przybylski et al. 1997). Therefore, less radical surgery or biopsy is recommended to prevent neurological decline in cases where clear spinal cord-tumor interface cannot be identified (Garces-Ambrossi et al. 2009).

The majority of intramedullary astrocytomas are low grade lesions; grade III and IV tumors represent together approximately 8–13% of intramedullary tumors of astrocytic origin (Santi et al. 2003; Slooff et al. 1964). These are usually associated with rapid progression. The addition of radiotherapy to the treatment regimen can be considered a necessity in the setting of high grade tumor (Stupp et al. 2005), however its use is controversial in low grade lesions. Thus, it varies considerably among centers: patients are irradiated according to extent of resection (Kim et al. 2001; Sandler et al. 1992), rapidity of preoperative disease progression (Rossitch et al. 1990) or it is a standard part of treatment protocol (Huddart

et al. 1993; Jyothirmayi et al. 1997; Kopelson et al. 1980).

Despite advances in surgical techniques and adjuncts, diagnostic imaging and adjuvant oncological therapy observed during the last decades, the diagnosis of intramedullary astrocytoma remains a therapeutical challenge. The knowledge of impact of various factors on prognosis can play a major role in treatment planning and may help in patient counseling. Prognostic factors affecting survival are reported in the literature with sometimes conflicting results. We try to summarize these findings and point out the controversies. The obvious goal would be definition of an optimal treatment strategy, however due to the rarity of the tumor and resulting lack of randomized trials this task is impossible. Regarding the discussion and conclusions drawn, the reader is asked to keep certain reservations in mind. We were unable to find a single intramedullary astrocytoma study with prospective data collection (Benes et al. 2009). Studies usually span decades and only few patients are treated each year. The largest study to date includes 136 patients and spans 43 years (Minehan et al. 2009). Reports are therefore abundant with many methodological errors: small number of patients precluding conclusions, patient exclusion and selection bias, incomplete follow-up, changing surgical philosophy and treatment strategy or lack of proper statistical analysis, to name just a few. This is not to criticize the authors, our own study suffers from these shortcomings as well (Benes et al. 2010). Rather, it is the reflection of rarity of intramedullary astrocytoma contrary to its intracranial counterpart. Additionally, intramedullary astrocytoma patients sometimes constitute only a part of an IMSCT study, and results are not infrequently reported together with other histologies, further limiting possible conclusions.

Patient Related Factors

Age

Prognostic significance of age is reported in many studies and key findings are shortly summarized. In accordance several authors found increased age to negatively affect survival or recurrence rates (Benes et al. 2010; Lee et al. 2003; Minehan et al. 2009; Rodrigues et al. 2000; Sandler et al. 1992). Furthermore, Santi et al. (2003) in a study of malignant astrocytoma also reported shorter median survival for patients older than 40 years in comparison to younger patients (Santi et al. 2003). A large multicenter review from France dealing exclusively with pediatric patients found patients younger than 7 years to have better 10-year overall survival (OS) than patients older than 7 years: 76 vs. 38%, $p=0.04$ (Bouffet et al. 1998). Unfortunately, this study did not specify histological or therapeutically aspects within these age groups, inadvertently bias could be introduced. Other authors failed to find significant relationship between age and prognosis (Benes et al. 2009). This can be attributed for example to comparison across age groups within the study or histological composition (Minehan et al. 2009). Of note, several studies report long term survivors (exceeding 10 years) among children with high grade tumors (Bouffet et al. 1998; Constantini et al. 2000; McGirt et al. 2008; O'Sullivan et al. 1994; Przybylski et al. 1997), which is a rather unusual finding in adult population. Comparing pediatric patients to adults within one study cannot detect difference in survival among children, particularly when the analysis is limited by small number of patients. Possibly, pediatric intramedullary astrocytoma is a biologically diverse disease and this was reflected in the large French study. With reservations mentioned in the Introduction, we can conclude that prognosis is more favorable in younger patients.

Gender

Gender as a prognostic factor is reported in numerous studies, although the majority of these fail to find an association (Benes et al. 2009). Female patients were found to have better 10-year OS (100%) compared to male patients (34%, $p<0.01$) in the study of (Huddart et al. 1993). This significance was kept after stratification by grade. Similarly, Jyothirmayi et al. (1997) reported

increased progression-free survival (PFS) for female patients (90 vs. 65%, p = 0.03) (Jyothirmayi et al. 1997). On the other hand, the allready mentioned French study found increased 10-year OS in boys (79 vs. 39%, p = 0.04) (Bouffet et al. 1998), which further supports the hypothesis about tumor diversity in children. However, based on the available literature, no conclusion regarding influence of gender on survival can be drawn.

History Length

Impact of history length on survival is reported in several studies. Whereas some fail to identify a relationship (Benes et al. 2009), other in accord report shorter history length to be associated with worse prognosis. Symptom duration shorter than 2 months (Bouffet et al. 1998), 6 months (Minehan et al. 2009; Rodrigues et al. 2000) and 1 year (Innocenzi et al. 1997) were all reported to be associated with decreased survival rates. Perhaps not surprisingly, patients with grade III tumors had significantly longer history length (12 ± 18 months) when compared to grade IV patients (2 ± 1 months, p = 0.07) in a study dealing with malignant astrocytomas (McGirt et al. 2008). Minehan et al.(2009) likewise found patients harboring pilocytic astrocytoma to have less frequently symptom duration shorter than 6 months (32%) contrary to patients with infiltrative tumors (51%, p = 0.03) (Minehan et al. 2009). Availability of MRI could have played an important role in shortening time to diagnosis. Raco et al. (2005) reported average time of symptom duration to be 3.7 years in the first 10 years of study and 2.1 years in the last 10 years of study when MRI was routinely used (Raco et al. 2005). With the expansion and accessibity of MRI in recent years, this is no longer an issue. Keeping these reservations in mind, shorter preoperative disease duration can be considered a negative prognostic factor.

Functional Status

Favorable preoperative neurological condition is the main predictor for satisfactory postoperative neurological recovery and function (Brotchi et al. 1991; Constantini et al. 2000; Cristante and Herrman 1994; Epstein et al. 1992; Innocenzi et al. 1996; Lee et al. 2003; Nakamura et al. 2006; Raco et al. 2005; Rossitch et al. 1990). Severe and long-lasting deficits rarely, if ever, resolve. Thus, not surprisingly, three studies in accord reported better survival rates and longer survival periods (Innocenzi et al. 1997; Kim et al. 2001; Lee et al. 2003). In fact functional status was the only important prognostic factor among low grade astrocytomas (Kim et al. 2001). In other studies the difference did not reach significance (Benes et al. 2009; Huddart et al. 1993; Jyothirmayi et al. 1997). Regarding preoperative neurological condition, we can safely conclude, that the best chance for satisfactory postoperative functional recovery and survival is in patients operated early in the course of disease before functional decline occurs.

Tumor Related Factors

Tumor Location

Tumor location in thoracic spinal cord was associated with better survival rates in both low and high grade tumors (Nakamura et al. 2006). Similarly, Minehan et al. (2009) reported improved median survival for tumors located outside the cervical region when compared to those located in the cervical spinal cord (306 vs. 63 months, p = 0.048) as well as for tumors located in the thoracic spinal cord when compared to those with no thoracic involvement (254 vs. 49 months, p = 0.02). This distinction was kept when analysed within pilocytic and infiltrative astrocytoma subgroups (Minehan et al. 2009). Respiratory paralysis as a consequence of medulla involvement is believed to be the cause of death (Nakamura et al. 2006; Raco et al. 2010) and tumors located in the cervical spinal cord need shorter time to reach it. However, several other studies did not identify a significant relationship between survival and tumor location (Benes et al. 2009), thus these conclusions cannot be accepted without reservations.

Tumor Extent

Although several studies report tumor extent as a prognostic factor (Benes et al. 2009), only two identified a significant relationship. Kim et al. (2001) found tumors spanning four or more segments to be associated with shorter mean survival than less extensive tumors (46.1 vs. 119.6 months, $p<0.05$). In the largest intramedullary astrocytoma study performed to date, patients with holocord involvement had shorter median survival than those without (7 vs. 186 months, $p=0.048$) (Minehan et al. 2009). In contrast, analysis of pilocytic and infiltrative astrocytoma subgroups found only nonsignificantly improved survival among pilocytic tumors spanning five or more levels (Minehan et al. 2009). Thus, no conclusions regarding the impact of tumor extent on prognosis can be drawn.

Syrinx/Cyst Presence

The presence of tumor associated cysts or intramedullary peritumoral syrinx was associated with better prognosis in two studies in terms of improved 5-year OS (Huddart et al. 1993; Jyothirmayi et al. 1997) or 5-year PFS (Jyothirmayi et al. 1997). Contrary, other studies found no such influence (Benes et al. 2009) precluding any meaningful conclusions.

Tumor Grade

Unsurprisingly, studies in accord report better survival rates for low grade tumors (grade I and II) when compared to high grade tumors (grade III and IV) (Benes et al. 2009). For low grade astrocytoma, 5-year OS ranged between 58% (Kopelson et al. 1980) and 100% (Robinson et al. 2005) and 10-year OS between 43% (Hulshof et al. 1993) and 83% in children (Przybylski et al. 1997). Majority of studies reported 5-year OS to range between 60 and 80% (Benes et al. 2009). In addition, Minehan et al. (2009) found significantly greater 10-year OS (78%) as well as median survival (39.9 years) for grade I tumors when compared to grades II-IV (17% and 1.85 years, $p<0.001$). Similarly, survival worsened with each histological grade (Minehan et al. 2009). Significant difference between grade III and IV tumors in median survival (72 vs. 9 months, $p=0.0001$) as well as 5-year OS (59 vs. 0%, $p=0.0001$) was reported in a study of malignant astrocytoma (McGirt et al. 2008), although other studies of high grade tumors did not confirm this difference (Raco et al. 2010; Santi et al. 2003). Similarly to intracranial glioblastoma, median survival in grade IV tumors is generally less than a year (Benes et al. 2009), with the allready mentioned exception of a subset of pediatric patients (Bouffet et al. 1998; Constantini et al. 2000; McGirt et al. 2008; O'Sullivan et al. 1994; Przybylski et al. 1997). Survival analyses in studies reporting 25 or more patients are presented in Table 34.1. Regarding tumor grade, we can safely conclude that it is the most decisive factor influencing survival.

Treatment Related Factors

Resection Extent

Resection radicality needs to be considered in the context of histology. Regardless of tumor grade, gross total resection (GTR) was reported to be superior to less extensive resection in two studies. Przybylski et al. (1997) found reduced risk of recurrence in pediatric patients (0 vs. 69%, $p=0.029$) (Przybylski et al. 1997). Similarly, Abdel-Wahab in a multicenter review reported significant risk reduction for 15-year PFS for GTR, however, its impact on 15-year OS was non-significant (Abdel-Wahab et al. 2006). Additionally, another pediatric series compared subtotal resection (STR) to biopsy and reported increased 7-year OS (100 vs. 42%, $p=0.02$) (Reimer and Onofrio 1985). On the other hand, several other studies did not report significantly improved survival rates for more extensive resection (Benes et al. 2009).

Resection extent specified for low grade tumors was found to bear no influence on survival in numerous reports (Benes et al. 2009).

Table 34.1 Survival according to tumor grade in intramedullary astrocytoma studies reporting 25 and more patients

Author, year	Patients	Grade	Patients	Outcome	Result	Remark
Reimer and Onofrio (1985)[a]	32	LG	27	5-year/10-year OS (%)	80/55	**p<0.001**
		HG	5		0	
Epstein et al. (1992)	25	LG	19	Recurrence (mean FU, months)	0 (50.2)	Significance NR
		HG	6	Death, progression (years)	6 (2)	
Huddart et al. (1993)	27	LG	19	5-year OS (%)	69	**p<0.05**
		HG	6		33	
		Unknown	2			
Innocenzi et al. (1996)[a]	65	Gr. I	29	5-year OS (%)	76	p NR, "significant role"
		Gr. II	26		68	
		Gr. III	10	5-year OS (%)/median survival (months)	0/15	
Bouffet et al. (1998)[a]	73	LG	49	10-year OS (%)	76	**p=0.00008**
		HG	24		32	
Rodrigues et al. (2000)	52	LG	37	5-year PFS/CSS (%)	64/73	**p=0.01**
		IMG or HG	15		20/30	**p=0.004**
Constantini et al. (2000)[a]	76	LG	58	Estimate 5-year PFS (%)	80	p NR
		Gr. III	14		35	
		Gr. IV	4		0	
Kim et al. (2001)	28	LG	18	Median survival (months)	184	**p<0.05**
		HG	10		8	
Santi et al. (2003)[b]	36	Gr. II→IV	2	Median survival (months)	33	p=0.482
		Gr. III	13		10	
		Gr. IV	21		10	
Lee et al. (2003)	25	LG	15	5-year LC/PFS/OS (%)	48/43/78	**p=0.001**
		Gr. III	4		0/0/67	**p<0.001**
		Gr. IV	6		0/0/17	**p<0.001**
Raco et al. (2005)	86	Gr. I	27	5-year PFS (%)	91	p NR
		Gr. II	41		63	
		HG	18	Mean survival (months)	15.5	
Nakamura et al. (2006)	30	LG	18	Estimate 5-year OS (%)	88	**p=0.0011**
		HG	12		32	

Abdel-Wahab et al. (2006)	57	LG	40	15-year PFS; HR HG vs. LG	2.67	**p = 0.02**
		HG	10	15-year OS; HR HG vs. LG	Univariate: 4.06	**p < 0.01**
		unknown	7	15-year OS; aHR HG vs. LG	Multivariate: 4.86	**p < 0.01**
McGirt et al. (2008)[b]	35	Gr. III	27	1-year/5-year OS (%)	85/59	**p = 0.0001**
				Median survival (months)	72	
		Gr. IV	8		31/0	
					9	
Garces-Ambrossi et al. (2009)	35	Gr.I	16	Estimate 2-year PFS (%)	75	**p < 0.0001**
		Gr. II	10		52	
		Gr. III-IV	9		0	
Minehan et al. (2009)	136	Gr. I	69	10-year OS (%)/median survival (years)	78/39.9	**p < 0.001**
		Gr. II	39	5-year OS (%)/median survival (years)	50/49	
		Gr. III-IV	28		0/10	

Modified from Benes et al. (2009)

LG low grade, *HG* high grade, *IMG* intermediate grade, *gr.* grade, *NR* not reported, *OS* overall survival, *PFS* progression free survival, *CSS* cause specific survival, *LC* local control, *FU* follow up, *HR* hazard ratio, *aHR* adjusted hazard ratio

[a]denotes pediatric astrocytoma series

[b]denotes malignant astrocytoma series

Constantini et al. (2000) in a pediatric series reported significantly better 10-year PFS for patients undergoing GTR or STR in comparison to less than 80% resection, however the difference between GTR and STR was insignificant (Constantini et al. 2000). Another study reported decreased 10-year OS for biopsied patients (Nakamura et al. 2006), contrary to Minehan et al. (2009) who found a trend for improved survival for biopsied patients with pilocytic tumors (p = 0.07) (Minehan et al. 2009). Of note, Epstein et al. (1992) observed no recurrence among 17 patients receiving GTR during a mean follow-up of 50 months (Epstein et al. 1992). On the other hand, Jallo et al. (2001) found "GTR and STR equally efficacious for long-term survival" (Jallo et al. 2001).

Among patients with grade III tumors, GTR was associated with better 4-year OS in comparison to STR (78 vs. 38%, p = 0.028) (McGirt et al. 2008). This study also did not observe any dissemination among GTR patients, whereas 60% of STR patients developed disseminated disease (p = 0.01). However, the difference only trended toward significance on multivariate analysis. An additional study found decreased 5-year OS for biopsied patients when compared to those undergoing GTR or less extensive resection (Nakamura et al. 2006). Contrary, several other studies did not report any survival advantage for more extensive resection (Benes et al. 2009). Based on the available evidence, no sound conclusions regarding the influence of resection extent on survival can be made.

Radiotherapy

Similarly to resection extent, the addition of radiotherapy has to be considered in the context of histology. No significant difference between irradiated and non-irradiated patients was reported in studies which did not analyze patients according to tumor grade (Benes et al. 2009). The addition of radiotherapy in low grade tumors was not found to significantly prolong survival in the majority of reports (Benes et al. 2009). Only Abdel-Wahab et al. (2006) reported significantly reduced adjusted hazard ratio (0.24, p = 0.02) for 15-year PFS in patients treated with radiotherapy following surgery in comparison to surgery alone. However, 15-year OS was not affected (Abdel-Wahab et al. 2006). In the largest study published to date, radiotherapy among patients with pilocytic tumors resulted in nonsignificantly improved survival (80 vs. 73%, p = 0.33) (Minehan et al. 2009). The role of radiotherapy in the treatment of low grade intramedullary astrocytoma needs to be clarified in the future.

Patients with high grade tumors are likely to undergo radiotherapy as a part of the treatment regimen (McGirt et al. 2008; Santi et al. 2003), thus only few reports comparing treated and untreated patients are available. Minehan et al. (2009) found prolonged median survival after radiotherapy among patients with infiltrative (grade II-IV) astrocytomas (Minehan et al. 2009). Similarly, a recent study of malignant astrocytomas reported increased mean survival for patients treated with radio- and chemotherapy in comparison to patients undergoing surgery only. Mean survival increased from 10.6 to 22 months (p = 0.05) (Raco et al. 2010). The rather poor prognosis of adult high grade intramedullary astrocytoma should prompt the treating physician to administer oncological therapy known to prolong life-expectancy, such as radiotherapy plus concomitant and adjuvant chemotherapy with temozolomide as has been shown in intracranial glioblastoma (Stupp et al. 2005).

Discussion

Although no studies on natural history of intramedullary astrocytoma have been performed to date, it is believed to be slowly progressive. Patients and treating physicians are then faced with inconspicuously worsening neurological status which warrants treatment. Once a neurological deficit is present, its regression after surgery cannot be guaranteed. Best functional results in intramedullary surgery are achieved among patients operated in good preoperative function (Brotchi et al. 1991; Constantini et al. 2000; Cristante and Herrmann 1994; Epstein

et al. 1992; Innocenzi et al. 1996; Lee et al. 2003; Nakamura et al. 2006; Raco et al. 2005; Rossitch et al. 1990). A policy of watchful waiting has to be carefully considered and does not play a major role in IMSCT management. Surgical treatment allows for obtaining a representative histological sample. Furthermore, possible mass effect on surrounding tissue is relieved by tumor debulking. Duraplasty and laminoplasty can provide additional space for future tumor growth. Smaller residual tumor burden is advantageous for adjuvant oncological therapy. As has been shown in intracranial gliomas, prolonging survival with more radical resection is an achievable aim (Sanai and Berger 2008). However, intracranial tumors are not always located in eloquent and functionally important areas, contrary to intramedullary astrocytoma which are always surrounded by motor and sensory pathways and neurons. In fact, studies have demonstrated the presence of normal neurons within intramedullary astrocytoma itself (Epstein et al. 1992), so the notion of radical resection of infiltratively growing intramedullary astrocytoma is at best questionable. Postoperative function takes precedence over resection radicality. Patients with worsened postoperative functional status have decreased survival as well (Innocenzi et al. 1997; Kim et al. 2001; Lee et al. 2003), so the advantage of more extensive resection is lost. In cases, where clear spinal cord-tumor plane of dissection can be identified, more radical resection can be safely pursued. Actually, Garcés-Ambrossi et al. (2009) found increased PFS for astrocytoma patients where such a plane of resection was identified (Garces-Ambrossi et al. 2009). Further assurance is brought by the addition of intraoperative electrophysiological monitoring, which can nowadays be considered a standard part of surgical armamentarium. Postoperative MRI should be another standard part of evaluation and is considered superior to intraoperative assessment of resection extent, as it is in intracranial glioma surgery. Not many studies reported superior survival results for more extensive resection (Benes et al. 2009). As only few patients undergo GTR, these are sometimes analyzed together with those undergoing STR in order to increase statistical power. These patients are then compared to less extensive resection or biopsy (Benes et al. 2009). Obviously, superiority of GTR over STR cannot be detected this way, however inferior results of less extensive resection or biopsy can be found (Nakamura et al. 2006). Less extensive resection can contain a wide spectrum of surgical results and when compared to GTR may overestimate importance of GTR (Abdel-Wahab et al. 2006; Sanai and Berger 2008).

Radiotherapy in the treatment of low grade intramedullary astrocytoma is controversial. Studies of low grade intracranial astrocytomas have demonstrated increased PFS after less than radical resection, however, OS was not improved (Karim et al. 2002), which is similar to Abdel-Wahab's conclusions in intramedullary astrocytoma (Abdel-Wahab et al. 2006). Similarly to intracranial gliomas, disease progression is usually manifested locally (Rodrigues et al. 2000). Furthermore, Minehan et al. (2009) and Garcia (1985) found a dose–response relationship for patients receiving more than 35 and 40 Gy, respectively (Garcia 1985; Minehan et al. 2009), however doses exceeding 50 Gy were not associated with increased survival (Benes et al. 2009). These findings speak for the addition of radiotherapy with the goal of slowing disease progression and preventing associated neurological decline. Contrary, radiotherapy may not be as effective in slow growing tumors where cells are not undergoing mitosis (Innocenzi et al. 1996). Limited tolerance of the spinal cord to radiation is further impaired in the presence of an expansive lesion (Marcus and Million 1990). Biopsy and external decompression alone may lead to long-term survival (Innocenzi et al. 1997; Przybylski et al. 1997). Also, keeping radiotherapy in reserve for disease progression following radical surgery is an acceptable treatment option (Epstein et al. 1992; Jallo et al. 2001). Avoidance of radiotherapy in children and infants where skeleton and spinal cord is still developing is also essential (Constantini et al. 2000). In addition, radiotherapy can increase the risk of development of second malignancy: a 13% risk at 20 years has been reported (O'Sullivan et al. 1994). Referral of patients in poorer condition to radiotherapy may be the reason for inferior survival results in

some studies (Przybylski et al. 1997; Sandler et al. 1992).

In conclusion, the diagnosis of low grade intramedullary astrocytoma continues to pose a significant treatment dilemma. Wait and see policy does not seem to be a therapeutical option in newly diagnosed tumors. Although disease progression can be delayed by maximal safe resection controlled by intraoperative neurophysiological monitoring, worsened functional status must not be the cost for radicality. The addition of radiotherapy in the setting of low grade intramedullary astrocytoma is at best controversial and needs further study.

Treatment of high grade intramedullary astrocytoma continues to be defeated by the aggressive nature of this tumor with the possible exception of some pediatric patients. Biological relation to its intracranial counterpart is suggestive of therapy with best achievable results: maximal safe resection followed by radiotherapy plus concomitant and adjuvant chemotherapy with temozolomide, among many prognostic factors reported in the literature, tumor grade can be regarded as the one influencing prognosis the most.

References

Abdel-Wahab M, Etuk B, Palermo J, Shirato H, Kresl J, Yapicier O, Walker G, Scheithauer BW, Shaw E, Lee C, Curran W, Thomas T, Markoe A (2006) Spinal cord gliomas: a multi-institutional retrospective analysis. Int J Radiat Oncol Biol Phys 64:1060–1071

Benes V 3rd, Barsa P, Benes V Jr, Suchomel P (2009) Prognostic factors in intramedullary astrocytomas: a literature review. Eur Spine J 18:1397–1422

Benes V 3rd, Bradac O, Ostry S, Buchvald P, Kramar F, Kaiser M, Suchomel P, Benes V Jr (2010) Intramedullary astrocytoma – A series of 15 patients and literature overview. Ceska Slov Neurol Neurochir 73:169–177

Bouffet E, Pierre-Kahn A, Marchal JC, Jouvet A, Kalifa C, Choux M, Dhellemmes P, Guerin J, Tremoulet M, Mottolese C (1998) Prognostic factors in pediatric spinal cord astrocytoma. Cancer 83:2391–2399

Brotchi J, Dewitte O, Levivier M, Baleriaux D, Vandesteene A, Raftopoulos C, Flament-Durand J, Noterman J (1991) A survey of 65 tumors within the spinal cord: surgical results and the importance of preoperative magnetic resonance imaging. Neurosurgery 29:651–656

Constantini S, Miller DC, Allen JC, Rorke LB, Freed D, Epstein FJ (2000) Radical excision of intramedullary spinal cord tumors: surgical morbidity and long-term follow-up evaluation in 164 children and young adults. J Neurosurg 93:183–193

Cristante L, Herrmann HD (1994) Surgical management of intramedullary spinal cord tumors: functional outcome and sources of morbidity. Neurosurgery 35:69–74

Epstein FJ, Farmer JP, Freed D (1992) Adult intramedullary astrocytomas of the spinal cord. J Neurosurg 77:355–359

Garces-Ambrossi GL, McGirt MJ, Mehta VA, Sciubba DM, Witham TF, Bydon A, Wolinksy JP, Jallo GI, Gokaslan ZL (2009) Factors associated with progression-free survival and long-term neurological outcome after resection of intramedullary spinal cord tumors: analysis of 101 consecutive cases. J Neurosurg Spine 11:591–599

Garcia DM (1985) Primary spinal cord tumors treated with surgery and postoperative irradiation. Int J Radiat Oncol Biol Phys 11:1933–1939

Helseth A, Mork SJ (1989) Primary intraspinal neoplasms in Norway, 1955 to 1986. A population-based survey of 467 patients. J Neurosurg 71:842–845

Huddart R, Traish D, Ashley S, Moore A, Brada M (1993) Management of spinal astrocytoma with conservative surgery and radiotherapy. Br J Neurosurg 7:473–481

Hulshof MC, Menten J, Dito JJ, Dreissen JJ, van den Bergh R, Gonzalez GD (1993) Treatment results in primary intraspinal gliomas. Radiother Oncol 29:294–300

Innocenzi G, Raco A, Cantore G, Raimondi AJ (1996) Intramedullary astrocytomas and ependymomas in the pediatric age group: a retrospective study. Childs Nerv Syst 12:776–780

Innocenzi G, Salvati M, Cervoni L, Delfini R, Cantore G (1997) Prognostic factors in intramedullary astrocytomas. Clin Neurol Neurosurg 99:1–5

Jallo GI, Danish S, Velasquez L, Epstein F (2001) Intramedullary low-grade astrocytomas: long-term outcome following radical surgery. J Neurooncol 53:61–66

Jyothirmayi R, Madhavan J, Nair MK, Rajan B (1997) Conservative surgery and radiotherapy in the treatment of spinal cord astrocytoma. J Neurooncol 33:205–211

Karim AB, Afra D, Cornu P, Bleehan N, Schraub S, De Witte O, Darcel F, Stenning S, Pierart M, Van Glabbeke M (2002) Randomized trial on the efficacy of radiotherapy for cerebral low-grade glioma in the adult: European organization for research and treatment of cancer study 22845 with the medical research council study bro4: an interim analysis. Int J Radiat Oncol Biol Phys 52:316–324

Kim MS, Chung CK, Choe G, Kim IH, Kim HJ (2001) Intramedullary spinal cord astrocytoma in adults: postoperative outcome. J Neurooncol 52:85–94

Kopelson G, Linggood RM, Kleinman GM, Doucette J, Wang CC (1980) Management of intramedullary spinal cord tumors. Radiology 135:473–479

Lee HK, Chang EL, Fuller GN, Aldape KD, Atkinson GJ, Levy LB, McCutcheon IE, Maor MH (2003) The prognostic value of neurologic function in astrocytic spinal cord glioma. Neuro Oncol 5:208–213

Marcus RB, Jr Million RR (1990) The incidence of myelitis after irradiation of the cervical spinal cord. Int J Radiat Oncol Biol Phys 19:3–8

McGirt MJ, Goldstein IM, Chaichana KL, Tobias ME, Kothbauer KF, Jallo GI (2008) Extent of surgical resection of malignant astrocytomas of the spinal cord: outcome analysis of 35 patients. Neurosurgery 63:55–61

Minehan KJ, Brown PD, Scheithauer BW, Krauss WE, Wright MP (2009) Prognosis and treatment of spinal cord astrocytoma. Int J Radiat Oncol Biol Phys 73:727–733

Nakamura M, Chiba K, Ishii K, Ogawa Y, Takaishi H, Matsumoto M, Toyama Y (2006) Surgical outcomes of spinal cord astrocytomas. Spinal Cord 44:740–745

O'Sullivan C, Jenkin RD, Doherty MA, Hoffman HJ, Greenberg ML (1994) Spinal cord tumors in children: long-term results of combined surgical and radiation treatment. J Neurosurg 81:507–512

Przybylski GJ, Albright AL, Martinez AJ (1997) Spinal cord astrocytomas: long-term results comparing treatments in children. Childs Nerv Syst 13:375–382

Raco A, Esposito V, Lenzi J, Piccirilli M, Delfini R, Cantore G (2005) Long-term follow-up of intramedullary spinal cord tumors: a series of 202 cases. Neurosurgery 56:972–981

Raco A, Piccirilli M, Landi A, Lenzi J, Delfini R, Cantore G (2010) High-grade intramedullary astrocytomas: 30 years' experience at the Neurosurgery Department of the University of Rome "Sapienza". J Neurosurg Spine 12:144–153

Reimer R, Onofrio BM (1985) Astrocytomas of the spinal cord in children and adolescents. J Neurosurg 63:669–675

Robinson CG, Prayson RA, Hahn JF, Kalfas IH, Whitfield MD, Lee SY, Suh JH (2005) Long-term survival and functional status of patients with low-grade astrocytoma of spinal cord. Int J Radiat Oncol Biol Phys 63:91–100

Rodrigues GB, Waldron JN, Wong CS, Laperriere NJ (2000) A retrospective analysis of 52 cases of spinal cord glioma managed with radiation therapy. Int J Radiat Oncol Biol Phys 48:837–842

Rossitch E Jr, Zeidman SM, Burger PC, Curnes JT, Harsh C, Anscher M, Oakes WJ (1990) Clinical and pathological analysis of spinal cord astrocytomas in children. Neurosurgery 27:193–196

Sanai N, Berger MS (2008) Glioma extent of resection and its impact on patient outcome. Neurosurgery 62:753–766

Sandler HM, Papadopoulos SM, Thornton AF Jr, Ross DA (1992) Spinal cord astrocytomas: results of therapy. Neurosurgery 30:490–493

Santi M, Mena H, Wong K, Koeller K, Olsen C, Rushing EJ (2003) Spinal cord malignant astrocytomas. Clinicopathologic features in 36 cases. Cancer 98:554–561

Slooff JL, Kernohan JW, MacCarty CS (1964) Primary intramedullary tumors of the spinal cord and filum terminale. WB Saunders Company, Philadelphia/London

Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996

Index

M.A. Hayat (ed.), *Tumors of the Central Nervous System, Volume 10*,
DOI 10.1007/978-94-007-5681-6, © Springer Science+Business Media Dordrecht 2013