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.

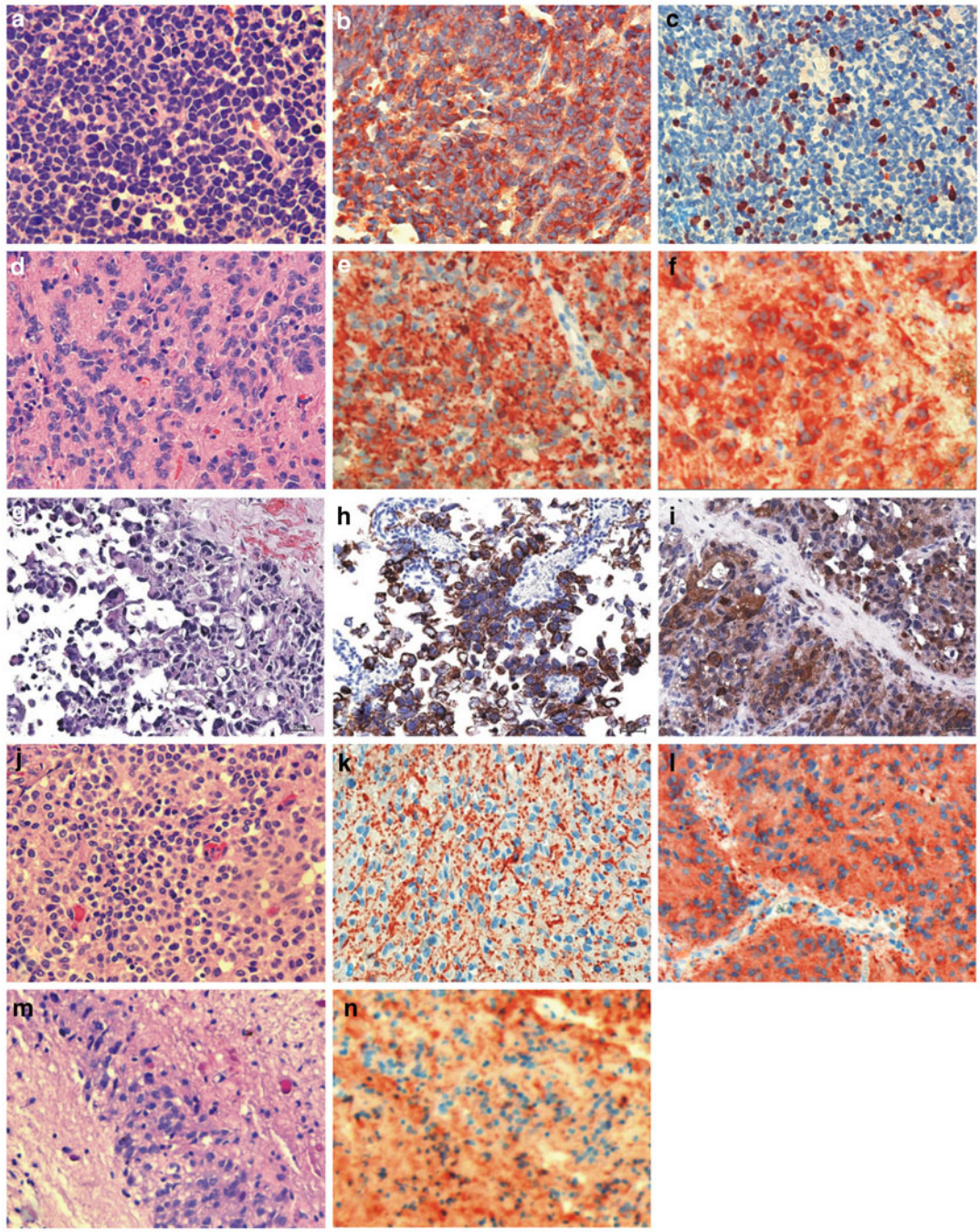

Fig. 4.1 (**a**) Pineoblastoma: diffuse sheets of small hyperchromatic cells. HE staining; (**b**) Pineoblastoma: strong expression of synaptophysin; (**c**) Pineoblastoma: high Ki-67 labeling index; (**d**) Pineocytoma: irregulary placed tumor cells around islands of neuropil. HE staining; (**e**) Pineocytoma: 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×

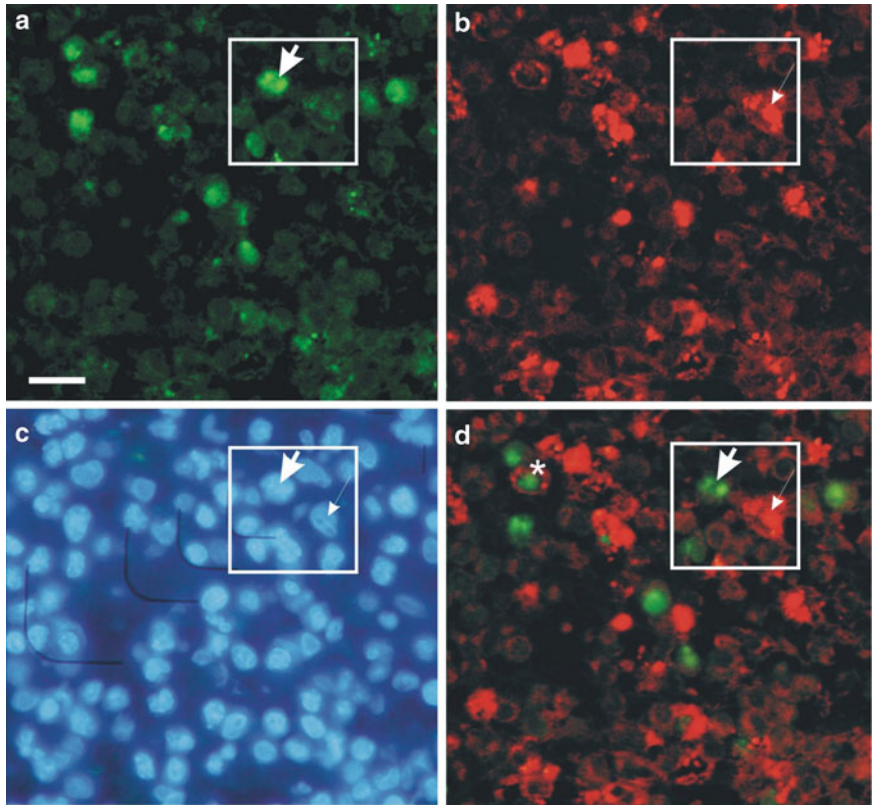

Fig. 4.2 Microphotograph of pineoblastoma specimen analyzed with the immunohistochemical double labeling demonstrating clearly nuclear positive reaction for Ki-67 (*green*, **a**), positive reaction for Bcl-2 antigen (*red*, **b**; this protein is present only in the cells' cytoplasm), DAPI stained cells 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