The Spectrum of Neuroendocrine Neoplasia

A Practical Approach to Diagnosis, Classification and Therapy

Sylvia L. Asa
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Preface

Neuroendocrine neoplasms comprise a large family of proliferative lesions that involve almost every part of the body. Our understanding of their cells of origin as well as the pathology, pathophysiology, and genetics of these neoplasms has made tremendous advances in the last few decades. While they are often discussed as separate entities in textbooks of gastroenteropancreatic pathology and pulmonary pathology, their scope is much broader. In this book, we hope to convey the similarities and differences of these fascinating tumors that may be found from the hypothalamus and pituitary to the rectum, and in soft tissue as well as in many organs. We emphasize their structural, functional, predictive, and prognostic features and attempt to provide the clinical context that allows improved diagnosis and therapy, while building on the genetics that clarifies patterns of inheritance and predisposition to tumor development through precursor lesions.

We thank our colleagues who contributed to the chapters in this text, the many researchers who did the work that we cite, and, most importantly, the patients who have taught us so many invaluable lessons about neuroendocrine neoplasia.

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Neuroendocrine Neoplasms: Historical Background and Terminologies

1

Sylvia L. Asa, Ricardo V. Lloyd, and Arthur S. Tischler

Introduction

The spectrum of neuroendocrine neoplasms encompasses lesions of classical endocrine organs including the pituitary and parathyroid; tumors of the dispersed neuroendocrine cells, including the thyroid, lungs, gastrointestinal tract, thymus, breast, and prostate; as well as paraganglia throughout the body including the adrenal medulla. Many of these lesions are increasing in incidence, and their pathology is becoming more complex with increased understanding of molecular pathology and a high incidence of familial disease.

It is important to define these lesions within the scope of endocrine pathology. Endocrine tissues are of three main types [1]. Steroid hormones are produced by the mesodermal-derived steroidogenic tissues that include the adrenal cortex and gonads. Thyroid follicular cells are epithelial cells of endodermal origin that synthesize thyroid hormones which are lipophilic and iodinated. The largest component of the endocrine system is the neuroendocrine system that is found throughout the body in almost every organ.

The history of neuroendocrine neoplasia is filled with fascinating anecdotes and curious discoveries. The path to our current understanding of the functions of

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neuroendocrine cells, the mechanisms of tumorigenesis, the complexity of their pathology, and the genetics underlying the development of these tumors has been long and convoluted. In this brief chapter, we summarize the important historical events, the controversies, and recent progress in achieving a holistic view of this field of science.

Neuroendocrine Tumors in the Ancient World

The earliest documentation of the manifestations of a neuroendocrine tumor is probably the biblical story of David and Goliath [2]. Goliath was a giant who likely had a pituitary neuroendocrine tumor causing growth hormone excess and visual field loss. It has been speculated that he died of pituitary apoplexy caused by the trauma of being hit in the forehead by a stone. It may be that he also had multiple endocrine neoplasia (MEN) type 1 with hyperparathyroidism or McCune-Albright syndrome with fibrous dysplasia, either of which can be associated with pituitary tumors causing acromegaly, and which would have compounded the impact of the local trauma.

The Egyptian Pharaoh Akhenaton was portrayed in many statues and carvings as a rather hypogonadal man with a sagging stomach, thick thighs, large breasts, and long, thin face. There has been speculation about genetic disorders, but these have been excluded by genetic testing, and given his documented fertility, his phenotype must have been delayed in onset. The features can be explained by a pituitary tumor with hypogonadal manifestations; this might have been a nonfunctional lesion, but prolactin excess can account for the large breasts, and his prominent jaw may have resulted from acromegaly.

Several Egyptian mummies have had findings of pituitary disease, including radiologic evidence of sellar enlargement and erosion [3], but sadly the pituitary was not included in otherwise well-preserved mummies because the embalming process involved removal of the organs, and the brain was removed through the nose, pulling the sellar contents out with it. Interestingly, the ancient Egyptians thought of the heart as the center of thoughts and emotions, not the brain, and they considered that the deceased would not need the brain in the afterlife.

The Scientific Revolution

Endocrine disorders were a curiosity for many centuries before they began to be understood from a scientific perspective. Many examples were illustrated in art; for example, the painting *Magdalena Ventura with Her Husband and Son* (also known as *The Bearded Lady*) by the Spanish artist Jusepe de Ribera is one example from a gallery of portraits of people with various endocrinopathies, including dwarfism and gigantism, that were often featured in circuses (Fig. 1.1).

The importance of neuroendocrinology as the mechanism of integrating mind and body was born in the pioneering work of Descartes, the father of the Scientific

Fig. 1.1 (**a**) *Magdalena Ventura with Her Husband and Son* (also known as *The Bearded Lady*) by the Spanish artist Jusepe de Ribera (1631) illustrates hirsutism in a lactating and fertile female (**b**) Fountain of Neptune (1564), Giambologna, Piazza Maggiore, Bologna, Italy. One of four mermaids at base of the fountain has the artistic rendering of florid galactorrhea. (Photograph courtesy of Dr. Ronald Lechan)

Revolution, who in 1649 proposed that the brain controlled the functions of the mind and body [4]. At the time, he identified the control center as the pineal, but the functions he proposed ultimately came to be recognized as residing more properly in the hypothalamus and pituitary. It is possible that Descartes' thinking was anticipated more than a hundred years earlier by Michelangelo, who was evidently familiar with hypothalamic-pituitary anatomy if not its function (Fig. 1.2).

The first description of the pituitary was by Galen (129–201AD) who considered it a gland that produced nasal mucus. It was not until the seventeenth century that Vesalius (1514–1564) would describe it as the "glans, in quam pituita destillat," translated as the "gland in which slime (*pituita*) drips," which gave rise to the terminology *glandula pituitaria*. The eighteenth century saw progress in recognizing the relationship between the brain and endocrine organs. Morgagni (1733), Soemmering (1792), Meckel (1802), and Zander (1890) described the absence of adrenal glands in anencephalic fetuses, pointing to a direct connection between these anatomically distant organs [5]. In 1849, Claude Bernard described "le piqûre diabetique" as he showed that injury to the floor of the fourth ventricle caused excessive urination [6]. The role of the pituitary in disease was proven after the description of the clinical syndrome of acromegaly by Pierre Marie in 1886 [7] when 1 year later, Minkowski documented the association of acromegaly with a pituitary tumor [8].

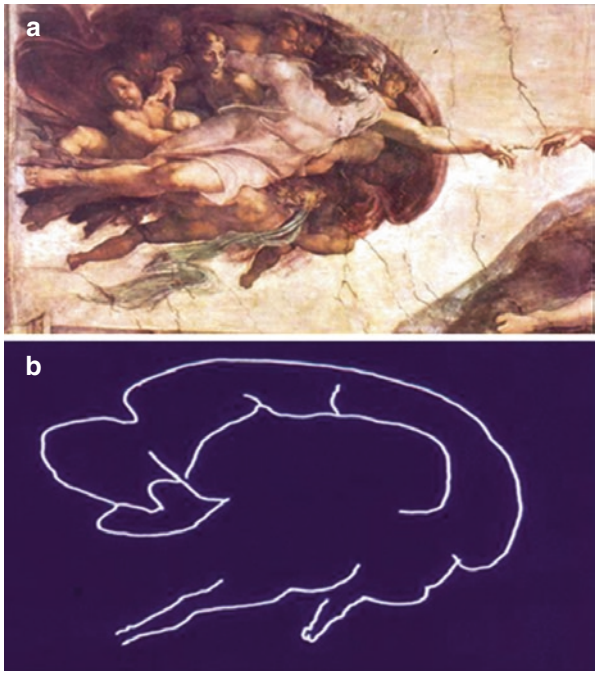

Fig. 1.2 Detail from the fresco, "Creation of Adam" by Michelangelo Buonarroti, visible on the ceiling of the Sistine Chapel in the Vatican at Rome, Italy, painted between 1508 and 1512. (**a**) Photograph of the fresco showing God giving spiritual life and intellect to Adam through his touch; (**b**) the contour of the same image is reminiscent of a midline sagittal section of the brain and includes the hypothalamus, pituitary, and brainstem. (From Toni et al. [84])

Langerhans, working in the laboratory of Virchow in 1867, discovered clusters of distinct cells within the acinar parenchyma of the pancreas [9]. After von Mering and Minkowski found that removal of the pancreas caused an increase in blood sugar and diabetes mellitus in 1889 [10], Laguesse postulated that the "islets of Langerhans" produce an internal secretion that was responsible for diabetes [11], and ultimately in 1922, Banting and Best would use the terminology "insulin" for this substance [12].

Parathyroid glands were first recognized by Owen in the Indian rhinoceros in 1850; he reported his findings of "a small, compact yellow glandular body attached to the thyroid at the point where the veins emerge" to the Royal College in London, but the paper was only published 12 years later [13]. In the interim, Remak described parathyroids in the cat in 1855 [14] and Virchow identified the human counterparts in 1863 [15]. Ivar Sandström studied the parathyroids in several species and attributed to them a functional, structural, and embryological relationship to the thyroid, giving them their name "glandulae parathyroidae" in 1880 [16]. However in 1895, Kohn identified their independent origin and proposed the name "Epithelkörperchen" [17, 18]. Studies by Gley and Erdheim showed that lack of these glands caused death by tetany [19–21]; the role of parathyroid hormone in calcium regulation was proven by Hanson in 1924 [22] and by Collip in 1925 [23], but the hormone was not isolated and characterized until 1959 when Auerback was able to accomplish this [24].

The adrenal medulla was recognized to be chemically different from the adrenal cortex, particularly because of its ability to turn brown in the presence of chromate salts, as shown by Werner in 1857 [25, 26]. However its function was not known until much later. The concept of a system of paraganglia was proposed by Alfred Kohn in 1903 [27] when he identified multiple extra-adrenal ganglia with the same chemical qualities in the retroperitoneum and in the carotid body that had been previously studied by Stilling [25, 26]. Recognizing these ganglion-like structures which he thought derived from ganglion anlagen and believing them to be part of the sympathetic nervous system but not genuine ganglia, he called them paraganglia [27]. Kohn was also responsible for the terms "chromaffin reaction" for the color change and "chromaffin cells" for the reactive cells.

The Progress of Modern Science

In 1902, Bayliss and Starling discovered a "secretin" produced in the duodenum and jejunum that stimulated pancreatic secretion [28]. They coined the term "hormone" to describe a chemical produced by one organ and secreted into the blood for circulation to a distant organ where it would exert its function. This concept was fundamental to the understanding of neuroendocrine cells, their importance in physiology, and their impact in patients with neuroendocrine tumors.

The landmark report of "karzinoide" ("carcinoma-like") tumors of the ileum by Siegfried Oberndorfer in 1907 [29] was the beginning of a new, albeit erroneous, terminology. Oberndorfer initially mistakenly considered these to be benign; however in 1929 he recognized their metastatic potential; the terminology "carcinoid" has plagued the field ever since. Oberndorfer did not recognize the endocrine nature of these tumors. It was not until 1953 that Lembeck identified serotonin as a product of these neoplasms [30] and made the connection between their cells of origin and the enteroendocrine cells that had been described by Kulchitsky in 1897 [31].

The plethora of diseases associated with neuroendocrine organs expanded in the first half of the twentieth century. Simmonds described hypopituitarism as "pituitary cachexia" in 1914 [32], and Cushing identified pituitary-dependent adrenal cortical hyperfunction in 1932 [33]. Banting and Best purified insulin and successfully treated diabetes mellitus with it in 1922 [12]. In 1937, Sheehan described the variant of hypopituitarism associated with postpartum hemorrhage [34]. In 1948, Harris identified several anterior pituitary hormones and clarified their relationship to the hypothalamus [35]. In 1953, Sanger sequenced the gene encoding insulin [36], a finding that led to the first of his two Nobel prizes. In 1954, du Vignaud was awarded the Nobel Prize in Chemistry for having accomplished the synthesis of a polypeptide hormone. The Nobel Prize in Physiology or Medicine was dedicated to endocrinology in 1977; half was bestowed on Rosalyn Yalow for her success in developing radioimmunoassays of peptide hormones, and the other half was shared by Roger Guillemin and Andrew V. Schally for the isolation and characterization of hypothalamic-pituitary hormones.

The Evolution of Definitions and Terminologies

The neuroendocrine system is composed of cells that produce amine and peptide hormones. Many of these same secretory products are also produced in neurons, where they function in neuronal signaling as neurotransmitters. The difference between neurotransmission and classic endocrine transmission is geographical; neurons discharge their product at a synapse where it targets a receptor on an adjacent cell, whereas neuroendocrine cells release their products into the bloodstream to affect receptors in target cells in other parts of the body. Some products produced by neuroendocrine cells may also act in a paracrine mode on nearby cells or in an autocrine mode on the same cells that produce them.

A unifying feature of neuroendocrine cells is their ability to take up and decarboxylate the amines required for hormone synthesis; this characteristic led Pearse to propose the terminology "amine precursor uptake and decarboxylation (APUD)" for this system of widely dispersed endocrine cells [37]. Because many neuroendocrine cells were known to originate in neuroectoderm, that too became a feature that was thought to be consistent, and the neural crest was proposed to be the origin of most APUD cells [38–41]. Although it became clear in the 1980s that neuroendocrine cells of the pituitary, lung, pancreas, and gastrointestinal tract are of endodermal origin, belief in the neural crest origin of the paraganglia and thyroid C-cells has persisted almost to the present. Modern embryologic lineage-tracing studies do in fact confirm that paraganglia are derived from neural crest precursors [42], although not as straightforwardly as previously believed. The majority of paraganglionic chief cells now appear to originate from progenitors termed "Schwann cell precursors" (SCPs) that first migrate from the neural crest to dorsal root ganglia and then to preganglionic sympathetic nerves which provide guidance for migration to the adrenal medulla and extra-adrenal paraganglia [43, 44]. It has been suggested that SCPs constitute a pool of neural crest-like cells employed for expansion and diversification of neural crest-derived tissues after the neural crest itself ceases to exist [45]. In contrast to paraganglia, thyroid C-cells and the parathyroid have recently been shown to be derived from endoderm [46]. These findings emphasize the lack of relevance of embryologic derivation and point instead to the importance of cellular differentiation that is dependent on common functional and structural characteristics. The unifying features include well-developed rough endoplasmic reticulum that is required for peptide synthesis, large Golgi complexes where hormone products are packaged for secretion, and numerous membrane-bound secretory granules that store and transport hormones to the cell surface for release by exocytosis. Starting with chromogranin A [47, 48], neuroendocrine cells were found to express numerous shared functional markers involved in the secretory apparatus, as well as numerous enzymes involved in hormone synthesis and processing, transcription factors, and other structural features (Table 1.1). Today, the definition of these cells relies on the expression of biomarkers expressed by virtually all neuroendocrine cells and their tumors [49, 50]. Subtyping of the various cells is performed using a second level of biomarkers, including transcription factors and peptide hormones (Table 1.1), and can also be performed in some cases by

Table 1.1 Biomarkers of neuroendocrine cells and tumors

Location	Cell type	Transcription factor(s)	Hormones	Others
Hypothalamus	Neurons	NeuN, TTF1	GRH, TRH, CRH, GnRH, Dopamine, Somatostatin Vasopressin, Oxytocin	Neurofilaments
Pituitary	Corticotroph	TPIT, NeuroD1	ACTH, other POMC derivatives	Keratins (+++)
	Somatotroph	PIT1	GH, αSU	Keratins[a]
	Lactotroph	PIT1, ER	PRL	(Keratins)
	Mammosomatotroph	PIT1, ER	GH, PRL, αSU	Keratins
	Thyrotroph	PIT1, GATA2/3	TSH	(Keratins)
	Gonadotroph	SF1, ER, GATA 2/3	FSH, LH	(Keratins)
Thyroid	C-cell	PAX8, TTF1	Calcitonin, CGRP	Keratins, CEA[b]
Parathyroid	Chief cell + variants	GATA3, GCM2	PTH	Keratins
Thymus	Unclassified NE cells	(TTF1, PAX8[c])	Calcitonin, CGRP	
Lung	P1, P2, P3	TTF1	Bombesin, Serotonin, Calcitonin, CGRP	Keratins (CEA[b])
Stomach	ECL	(CDX2)	Histamine	VMAT2, Keratins
	EC		Serotonin	Keratins
	D		Somatostatin	Keratins
	G		Gastrin	Keratins
	XP, D		Xenin, Ghrelin	Keratins
Pancreas	A	PDX1, ISL1, CDX2	Glucagon	Keratins
	B		Insulin	Keratins
	D		Somatostatin	Keratins
	PP		Pancreatic polypeptide	Keratins
Bowel	G	*Duodenum:* ISL1, PDX1, CDX2 *Jejunum and ileum:* CDX2 *Colon:* (CDX2), SATB2	Gastrin	Keratins *Colon:* PSAP
	D		Somatostatin	
	I		CCK	
	K		GIP	
	S		Secretin	
	MO		Motilin	
	N		Neurotensin	
	L		GLI, PYY, PP	
	EC		Serotonin	

(continued)

Table 1.1 (continued)

Location	Cell type	Transcription factor(s)	Hormones	Others
Prostate, kidney, bladder, gonads, breast	Unclassified NE cells	Variable	Variable	Keratins (PSAP)
Skin	Merkel cell	PAX5, SATB2	Unknown	Dot-like CK20, TdT, Merkel polyomavirus
Paragangliomas	Neuroendocrine or chief cells	GATA3	Dopamine, Adrenaline, Noradrenaline	Tyrosine hydroxylase, L-Dopa-decarboxylase, phenylethanolamine N-methyltransferase (PNMT)

Table reproduced with permission from Asa [85] with modifications
[a]Pattern of keratin positivity distinguishes tumor types; see Chap. 4
[b]Using monoclonal antibody
[c]PAX8 in thymic lesions is controversial and likely due to cross-reactivity of polyclonal antisera
()Items in brackets are not consistent findings in normal cells; tumors may have significant variability

characterizing the morphology of secretory granules that are distinct in the various cell types [51–55].

Although the current terminology has settled on the use of "neuroendocrine" to collectively describe endocrine cells with neuron-like features, several other terms are used for subsets of these cells or have been used and are now vestigial. The chromaffin reaction is now obsolete, but the term "chromaffin cell" is still used for the neuroendocrine cells of the adrenal medulla and sometimes for their extra-adrenal counterparts. Interestingly, "pheochromocytoma" (from the Greek *phaios*, dusky + *chroma*, color), which was applied to adrenal medullary tumors because of the color change imparted by the chromaffin reaction, remains the term of choice for adrenal medullary paragangliomas; outside the adrenal, the term paraganglioma is used [56, 57]. In view of the chemoreceptor function of the carotid bodies, Kjaergaard proposed that paraganglia in the head and neck be classified as chemodecta (singular chemodecton) (from the Greek *dechesthai*, to receive) [58], a name reflected in the now-discouraged "chemodectoma," for head and neck paragangliomas. A vestigial synonym for head and neck paraganglia is "glomus" (from the Latin *glomus*, ball), based on a nineteenth-century hypothesis that the carotid body is a vascular structure [4]; this term has caused confusion because it is also used to describe arteriovenous anastomoses that function as thermoregulatory structures in skin and other locations (such as the glomus coccygeum) and their corresponding tumors (glomus tumors or glomangiomas) that are completely unrelated to paraganglia [7].

The terminology applied to tumors of epithelial neuroendocrine cells has been equally complex and confusing. "APUDoma" was only transiently successful. "Carcinoid tumor" is outdated in that it implies a benign "carcinoma-like" entity yet

the tumors are not benign. It also causes confusion with the carcinoid syndrome and has resulted in clinical problems because many oncologists reflexly measure the metabolite of serotonin, urinary 5′5-hydroxyindoleacetic acid (5HIAA), in patients with these tumors [59], yet this test is only valuable for the subset of tumors that actually make serotonin.

Neuroendocrine tumors of the pancreas were called "islet cell tumors," but this implied origin within the islets, a concept that has been controversial. While there is some evidence of precursor lesions in the islets of patients with hereditary predisposition to the development of pancreatic endocrine neoplasia [60, 61], there is also evidence that these tumors frequently arise from ductal elements as defined by Laidlaw with the term nesidioblastosis [62]. The term "endocrine tumors" has also been used; this may be appropriate in the pancreas, gut, or lung to distinguish these neoplasms from the non-endocrine tumors that occur in those sites but would be inappropriate in tissues where multiple endocrine tumor types can occur, such as the thyroid, where follicular cell-derived tumors are also endocrine but not neuroendocrine like C-cell tumors.

The last two decades have seen progress in applying the term "neuroendocrine tumor" to epithelial neuroendocrine tumors. The concept of low malignant potential and the relevance of the unifying features of these lesions in various body sites has been supported by molecular data that also provide valuable information about genetic predisposition syndromes. However, there have been two important challenges. One difficulty has been the distinction between "neuroendocrine tumor" and "neuroendocrine carcinoma"; on the one hand, there are more aggressive and clearly distinct tumors that are carcinomas with neuroendocrine features, but on the other hand, it seemed odd to classify a metastatic lesion as a neuroendocrine tumor when it clearly represented a carcinoma. The other issue with the terminology is that paragangliomas, even when malignant, cannot be classified as carcinomas since they are not epithelial.

This problem was recently addressed by the World Health Organization in an attempt to develop a unifying classification scheme [63]. This proposal provides a clear definition of the criteria for the application of terminologies. In this proposal the term "neuroendocrine neoplasms (NENs)" is used to describe tumors that can arise at almost any anatomical site, including in organs of all types as well as in soft tissues, recognizing that NENs at various sites can be of epithelial or neuronal/neuroectodermal origin. The proposal further specifies that NENs share major morphological and protein expression signatures depending on differentiation, including markers of general neuroendocrine differentiation (such as chromogranins and synaptophysin) as well as site-specific markers such as hormones and transcription factors. The concept indicates that "neuroendocrine neoplasm (NEN)" is a term encompassing all tumor classes with predominant neuroendocrine differentiation, including both well- and poorly differentiated forms; that "neuroendocrine tumor (NET)" be applied to tumors that retain a well-differentiated phenotype, irrespective of their grade (conventionally G1 low grade, G2 intermediate grade, and G3 high grade, based on proliferation rates); whereas the term "neuroendocrine carcinoma (NEC)" should be applied to the poorly differentiated malignancies of this

family. In this classification, paragangliomas, which are NENs of non-epithelial origin, are regarded as a third family of NENs.

The justification for this proposal lies in the molecular changes that underlie the development of these lesions and the genetic predisposition syndromes with which they are associated. NECs are associated with mutations that are known to underlie other cancers [64], and the mutation profiles of the large cell and small cell carcinomas show additional alterations, sometimes associated with known pathogenetic mechanisms, for example, smoking and small cell lung carcinomas. In contrast, NETs tend to have lower mutation burdens, often involving epigenetic regulators, the hallmark example being menin, the protein that is altered in MEN1 [65–69]. Pheochromocytomas and paragangliomas have a low mutation burden and a largely distinct set of driver mutations not usually shared with common cancers [70].

The Genetics of Neuroendocrine Neoplasia

Erdheim described multiple endocrine neoplasia (MEN) type 1 in 1903 [71], but the genetics of this autosomal dominant disorder was recognized by Wermer in 1954 [72]. MEN type 2 was described in 1966 [73]. Since then, multiple, additional familial endocrine syndromes have been identified such that almost every neuroendocrine tumor may be part of a genetic syndrome [74], including MEN4, Carney complex, Carney triad, hyperparathyroidism-jaw tumor (HPT-JT) syndrome, von Hippel-Lindau disease, the multitude of familial paraganglioma syndromes [75], and even Lynch syndrome. This aspect of neuroendocrine neoplasia will be discussed in detail in a dedicated chapter of this book.

The genetic alterations underlying sporadic NENs are more complex and often elusive. As will become evident from the information in the following chapters, these tumors tend to have a low mutational burden, and it is becoming increasingly apparent that epigenetics is as important as genetics in endocrine neoplasia.

The Modern Approach to Neuroendocrine Tumors

While other cancers attributed to environmental factors are decreasing due to prevention and screening, the incidence of neuroendocrine neoplasms is increasing [76, 77]; parathyroid tumors are common, especially in aging women [78], and pituitary tumors are no longer the rare esoteric disease they were once considered, as we have identified a prevalence of approximately 1 per 1000 population [79, 80].

The challenge in this field is to advance the detection and diagnosis of these tumors to an earlier phase, to ensure a more accurate approach to tumor classification and subclassification, and to predict better therapeutic approaches. The use of immunohistochemistry allows pathologists to apply more sophisticated biomarkers to facilitate the molecular histopathologic classification of endocrine tumors. The application of biomarkers within the context of thorough morphological evaluation of tumors and their associated non-tumorous parenchyma can identify underlying

genetic predisposition, even in seemingly sporadic cases. The ability to identify tumors that will respond to targeted therapies, including but not limited to the ubiquitous somatostatin receptors that are the hallmark of these tumors [81], will lead to better outcomes for patients.

The complexity of these tumors requires a thorough understanding, not just of structure and morphological alterations, but also of functional activities of these tumors, including the phenomenon of ectopic hormone production, and the promiscuity of hormone receptor expression. It is no longer sufficient to identify a lesion as a NET or even to simply give it a grade; the sophistication of what can be done requires access to the proper tools that allow complete analysis of transcription factor and hormone profiles, as well as accurate image analysis of proliferation markers [82, 83] and molecular testing to achieve genotype-phenotype correlations.

We hope that the following chapters of this book will prepare pathologists to successfully overcome this challenge with deeper understanding of this fascinating field of endocrine oncologic pathology.

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The Diagnosis of Neuroendocrine Neoplasms

2

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Introduction

The diagnosis of neuroendocrine tumors is based on the recognition of signs and symptoms of the structural and functional effects of these tumors. The structural impact depends on the location of the tumor; in some, such as the pituitary, the structural impact can be significant because of mass effects in a small enclosed and critical area, whereas in others, such as distal pancreas or retroperitoneal sites like adrenal, tumors can grow to be very large without major mass effects. The functional aspects of these tumors involve biochemical confirmation that can be very complex. Radiologic diagnosis and confirmation of a NET is discussed in a separate chapter (Chap. 3).

In this chapter, the various hormone excess syndromes will be discussed, along with the specific structural considerations at the common sites of neuroendocrine tumors. The major syndromes are listed in Table 2.1.

Gastroenteropancreatic and Lung Neuroendocrine Neoplasms

Neuroendocrine tumors (NETs) are a heterogeneous group of tumors that can originate from various sites and arise from endocrine glands or dispersed neuroendocrine cells. Their classification is complex, based among other features on cell and tissue of origin. They can produce and secrete hormones and be functional or nonfunctional.

Pancreatic NETs arise from the endocrine cells of the pancreas and/or their precursors. They are estimated to occur in approximately 25–30 per 100,000

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Table 2.1 Endocrine syndromes associated with neuroendocrine neoplasms

Syndrome	Hormone	Clinical features	Site(s)	Notes
Carcinoid	Serotonin	Flushing Diarrhea	Small bowel Lung Pancreas Gonads Other NENs	Requires metastasis outside portal circulation
Carcinoid-like	Calcitonin	Flushing Diarrhea	Medullary thyroid carcinoma	
Hypoglycemia	Insulin	Shakiness, dizziness, blurred vision, sweating, hunger, anxiety, syncope	Pancreas	
	IGF-2		Soft tissue tumors	
Zollinger-Ellison	Gastrin	Recurrent peptide ulcerations	Pancreas Duodenum	
Glucagonoma	Glucagon	Diabetes mellitus, migratory rash	Pancreas Duodenum	
Verner-Morrison	VIP Rarely PP	Severe watery diarrhea	Pancreas Adrenal medulla	
Somatostatinoma	Somatostatin	Diabetes mellitus, gallstones	Pancreas Duodenum	
Adrenergic	Adrenaline, noradrenaline	Hypertension, palpitations	Adrenal Other (usually abdominal) paragangliomas	
Hyperprolactinemia	Prolactin	Gonadal dysfunction	Pituitary	Many other causes
Cushing syndrome	ACTH	Centripetal obesity, diabetes, hypertension, osteoporosis	Pituitary Lung Other NETs with ectopic sources	Also: primary adrenal tumors, hyperplasias, and exogenous steroids
	CRH	As above	Pancreas Adrenal Other NETs	
Acromegaly	GH	Acral enlargement, soft tissue swelling, diabetes, hypertension	Pituitary Rarely pancreas	
	GHRH	As above	Pancreas Adrenal medulla Lung Other NETs	
Hyperthyroidism	TSH	Palpitations, heat intolerance, weight loss	Pituitary	Also: primary thyroid causes
Hyperparathyroidism	PTH	Kidney stones, osteoporosis	Parathyroid	
	PTHrP	As above	Multiple epithelial tumors	

population in the United States but with rising frequency likely due to refinements in diagnostic approaches. Nearly 10% can be familial in the form of one of the heritable conditions including multiple endocrine neoplasia type 1 (MEN-1), von Hippel-Lindau (VHL), neurofibromatosis type 1 (NF-1), and other newly identified syndromes [1–4]. Gastrointestinal NETs arise from enterochromaffin cells of the digestive tract and are further divided based on the embryonic divisions of the gut [5].

While some gastroenteropancreatic NETs are slow-growing, some can behave aggressively and lead to metastatic disease. The most common site of metastasis regardless of the site of the primary is the liver, followed by the mesentery and the retroperitoneum. Gastroenteropancreatic NETs can be completely asymptomatic and found incidentally, but most patients will have symptoms related to either hormonal secretion of these tumors, as described below, or symptoms related to the mass effect of these masses, causing bowel obstruction, pancreatitis, biliary obstruction, or bowel ischemia [6]. With regard to laboratory testing, chromogranin A is an excellent marker to follow tumor progression and recurrence but is less useful for the diagnosis as it can be elevated for multiple reasons and is therefore nonspecific. However, specific hormonal testing at diagnosis should be included depending on patient symptoms. These tests will be detailed below [7].

Lung NETs account for 1–2% of all lung malignancies in adults. They arise from neuroendocrine cells and are the second most common location for NETs after the gastrointestinal tract. Patients can present with cough, wheezing, dyspnea, and hemoptysis in the presence of an obstructive mass and/or with symptoms of hormonal hypersecretion. The most common hormonal syndrome associated with lung NETs is carcinoid syndrome, followed by Cushing syndrome and acromegaly [8–10]. Importantly, the endocrine manifestations may occur in the absence of metastatic liver disease.

Carcinoid Syndrome

The carcinoid syndrome represents a constellation of symptoms associated with certain NETs. Most of the NETs that present with carcinoid syndrome arise from the midgut (jejunum, ileum, appendix, and ascending colon), as the syndrome is a result of the overproduction of serotonin which is the main secretory product of NETS at those sites. However, due to breakdown of serotonin by the liver, which is the site of venous drainage of these tumor locations, the syndrome almost invariably reflects metastasis to the liver or other extrahepatic sites, including lung NETs, that do not drain into the portal vasculature The manifestations of carcinoid syndrome are episodic cutaneous flushing of the face and thorax, secretory diarrhea, wheezing and bronchospasm, facial telangiectasia, and right-sided valvular disease [1, 11]. The main biochemical test used to confirm the presence of the carcinoid syndrome is the measurement of 24-hour urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA), which is the end product of serotonin metabolism and is elevated in patients with carcinoid syndrome [7].

Hypoglycemia

Insulinoma is a type of functional NET characterized by fasting hypoglycemia caused by an inappropriately high secretion of insulin. Although this type of tumor is very rare, with an incidence of 1–32 new cases per million per year, it is one of the most common functional pancreatic NETs. Most insulinomas present as a single, nonmetastatic lesion, but nearly 7% of patients will have multiple primary tumors and 6% will have metastatic insulinomas. Virtually all insulinomas arise in the pancreas [12].

The main symptoms of insulinoma occur as a result of fasting hypoglycemia, although some patients report both fasting and postprandial symptoms. Clinical manifestations of hypoglycemia include autonomic symptoms (tremor, palpitations, anxiety, sweating) and neuroglycopenic symptoms (dizziness, drowsiness, confusion, and altered mental status) [12, 13]. Only patients in whom hypoglycemia is documented at the time these symptoms occur and whose symptoms are relieved by correcting the hypoglycemia can be diagnosed with a hypoglycemic disorder and further evaluated for insulinoma. This is referred to as Whipple's triad [14]. The diagnosis is made during a proven symptomatic hypoglycemia, spontaneous or during a 72-hour fasting test, in the presence of elevated or inappropriate insulin, proinsulin, and c-peptide in the absence of detectable hypoglycemic agents in the blood as well as negative insulin antibodies [14, 15].

The same syndrome can be mimicked by soft tissue tumors which ectopically produce an excess of IGF-2. This growth factor excess can interact with or facilitate access to the insulin receptor resulting in insulin-like effects.

Zollinger-Ellison Syndrome

This syndrome is characterized by an excess of gastrin production by a "gastrinoma." Along with insulinomas, these represent the most common functional pancreatic NET syndromes. Nearly 70% of gastrinomas are located in the duodenum and 25% are located in the pancreas. The majority of these tumors are metastatic, and approximately 30% of them are associated with the syndrome of multiple endocrine neoplasia type 1 (MEN-1) [8]. These patients often present with peptic ulcer disease, abdominal pain, acid reflux, diarrhea, and/or weight loss [16]. The diagnosis is made in the presence of a very low gastric pH in association with very high gastrin levels, either in a fasting state or while performing a secretin stimulation test [7].

Glucagonoma Syndrome

This rare syndrome is associated with functioning NETs that secrete inappropriately excessive amounts of glucagon. Virtually all glucagonomas arise from the pancreas, and more than half of them are metastatic. Although the vast majority of cases are sporadic, there is an association with MEN-1 in 10% of cases [8]. Most patients will complain of weight loss and will have glucose intolerance or diabetes mellitus, and

70% of patients will present with necrolytic migratory erythema, a paraneoplastic erythematous, pruritic, and painful rash involving the face, trunk, perineum, and extremities. The diagnosis is made by documenting a fasting serum glucagon that is markedly and inappropriately elevated [17, 18].

Verner-Morrison Syndrome

This syndrome is characterized by severe secretory watery diarrhea, as well as symptoms related to hypokalemia and dehydration, which include lethargy, nausea, vomiting, and muscular cramps. These are rare functioning NETs that secrete vasoactive intestinal polypeptide or pancreatic polypeptide.

Tumors that secrete VIP are called VIPomas. The majority or nearly 90% of these tumors arise in the pancreas, but they can also be neural, adrenal, or paraganglionic in origin. These usually occur as solitary, isolated tumors but in 5% of patients, they are part of the MEN-1 syndrome. More than half of these patients will have metastases at the time of diagnosis [8]. The diagnosis of VIPomas is made in the presence of unexplained secretory diarrhea and elevated serum VIP levels.

Tumors that secrete pancreatic polypeptide (PP) are called PPomas [19, 20]. They are very rare tumors that often arise from the head of the pancreas, where 99% of PP-producing cells are located. More than 90% of cases are metastatic at diagnosis. Given that PP is thought to be a biologically inactive hormone, the majority of these tumors reach large dimensions at the time of diagnosis and cause symptoms related to mass effect rather than hormone excess. However, occasional patients develop watery diarrhea, gastrointestinal bleeding, or diabetes mellitus [21–23].

Somatostatinoma Syndrome

Most patients do not exhibit obvious symptoms related to the excess of somatostatin, but in those who do, abdominal pain and weight loss are the most common. A small number of pancreatic somatostatinomas present with the somatostatinoma syndrome, characterized by diabetes/glucose intolerance, diarrhea/steatorrhea, and cholelithiasis. These tumors tend to originate in the pancreas, duodenum, or jejunum, and more than 70% present with metastases at the time of diagnosis. Nearly 40% of these tumors occur in patients with MEN-1, and they are also seen in 10% of patients with NF-1. In these patients, the diagnosis is established by the presence of elevated serum somatostatin.

Pheochromocytomas and Paragangliomas

Pheochromocytomas and paragangliomas (PPGLs) are NETs that arise from chromaffin cells of the adrenal medulla or from the sympathetic or parasympathetic ganglia; by convention, adrenal lesions are classified as pheochromocytomas, whereas extra-adrenal lesions are classified as paragangliomas. PPGLs are

estimated to occur in about 2–8 per 1 million persons per year and about 0.1% of hypertensive patients harbor a PPGL. About 10% of patients with PPGL present with adrenal incidentaloma. The most common paragangliomas are found as masses in the neck or base of the skull; these are known as carotid body tumors and jugulotympanic paragangliomas that used to be confused with glomus tumors. PPGLs may be sporadic but they are strongly hereditary; more than 35% of PPGLs are associated with familial genetic alterations with nearly 20 known susceptibility genes. The most common mutations involve the genes encoding the SDH complex (*SDHB*, *SDHD*, *SDHC*, *SDHA*, and *SDHAF2*), *VHL*, *RET* (the gene responsible for MEN-2), and *NF1*; other less frequently affected genes include *TMEM127*, *MAX*, *EPAS1*, *KIF1Bβ*, *PHD2*, *FH*, *MDH2*, and *MEN1* [24].

PPGLs can be functional in which case they secrete catecholamines, but as with other endocrine malignancies, they can also be nonfunctional. Symptoms associated with catecholamine excess include the classic triad of headache, sweating, and paroxysmal or sustained hypertension as well as chest pain, palpitations, tremors, dizziness, and less frequently postural hypotension. These symptoms can be continuous or episodic spells occurring several times daily or as infrequently as once every few months [25]. An interesting clinical scenario is micturition-induced symptomatology due to bladder paragangliomas [26].

Some very rare cases of PPGLs exclusively produce dopamine. They usually present in extra-adrenal sites and present differently than classical PPGLs, which secrete catecholamines. Patients with dopamine-secreting PPGLs can be asymptomatic or have vague symptoms attributed to high circulating dopamine levels, like fever, malaise, weight loss, or diarrhea, making the detection of these PPGLs more difficult. The diagnosis is made in the presence of an elevated dopamine concentration on a 24-hour urine collection or an elevated serum level of 3-methoxytyramine, a metabolite of dopamine [27, 28].

Nonfunctional PPGLs may be diagnosed after surgical resection and pathology review [29].

It is important to recognize that paragangliomas can occur almost anywhere in the body; while the most well recognized are adrenal pheochromocytomas and those arising in the organ of Zuckerkandl, they can present as soft tissue masses in the head and neck, as para-aortic masses that can mimic metastatic disease in lymph nodes, or as primary tumors of vital organs such as the heart, liver, or lung [30]. It is important to distinguish these from other neuroendocrine tumors for both functional and genetic testing purposes.

The clinical diagnosis is performed by confirming catecholamine excess biochemically by measuring either serum metanephrines or normetanephrines which are the active metabolites of catecholamines or by obtaining a 24-hour urine collection for metanephrines, normetanephrines, and their catecholamine (dopamine, norepinephrine, epinephrine) precursors, followed by localizing imaging studies. As with gastroenteropancreatic NETs, CT, MRI, and 68-Gallium DOTATATE PET are useful imaging modalities [7, 31].

Medullary Thyroid Cancer

Medullary thyroid cancer (MTC) accounts for 1–2% of thyroid cancers in the United States.

These tumors are derived from C-cells in the thyroid gland that normally produce calcitonin and carcinoembryonic antigen (CEA) which serve as excellent biomarkers for the disease. Medullary thyroid cancer is often hereditary; 25% of cases form a heritable syndrome of multiple endocrine neoplasia type 2A or B. Each of these syndromes is associated with germline mutations in the RET protooncogene. Sporadic forms of the disease, in contrast, have been linked to somatic mutations of the same gene rendering them targetable for medical therapy when surgery is not curative. MTCs can be suspected as a thyroid nodule on neck ultrasound, and diagnosis is usually confirmed upon surgical resection of the thyroid tumor. They usually present with asymptomatic goiter, multinodular goiter, or even thyroid nodules that can be found incidentally on imaging with no clinical signs on examinations. Calcitonin and CEA are both used as biochemical markers for diagnosis and follow-up.

Pituitary Neuroendocrine Tumors

Pituitary neuroendocrine tumors (PitNETs) are the most common type of sellar masses found in adults. The differential diagnosis includes craniopharyngiomas, metastasis from other cancers, and Rathke's cleft cysts, to name a few [32]. PitNETs can be sporadic or can be associated with genetic mutations, such as MEN-1 and MEN-4, McCune-Albright syndrome, Carney complex, and AIP mutations that are the underlying cause of the "familial isolated pituitary adenoma" syndrome [10]. PitNETs are often classified based on their size, with microtumors measuring less than 10 mm and macrotumors measuring 10 mm and more. Given the confined space in the sella turcica, the fragility of the pituitary gland, and the close proximity to important structures such as the optic chiasm, these tumors can be symptomatic even when very small [33]. While some patients with PitNET are asymptomatic, others can have neurological symptoms due to mass effects such as headaches as well as visual field defects or diplopia caused by compression of the optic chiasm. PitNETs are further clinically classified into nonfunctioning or functional, the latter describing masses that secrete one or more hormones in excess. These will be reviewed in detail below. Patients can have symptoms associated with the excess of hormones and/or symptoms of hypopituitarism, secondary to deficiency in one or more of the pituitary hormones. The latter is a result of compression of the normal pituitary gland by the tumor causing it to malfunction. Hypopituitarism is more common in larger lesions, usually above 6 mm [34].

When a pituitary mass is found fortuitously in patients undergoing head imaging for another reason, it is referred to as an "incidentaloma." In these patients as well

as in any patient presenting with symptoms suggestive of a pituitary tumor, the evaluation should include a thorough history and physical examination, focusing on compressive symptoms and evaluation for hormone excess and hormone deficiencies. The next step is to obtain an MRI of the sellar region, as this is the primary imaging modality for the pituitary gland. Furthermore, evaluation for hypopituitarism and for excess hormonal secretion should be done in all patients, including asymptomatic patients. This includes measuring morning cortisol, TSH, free thyroxine (T4), triiodothyronine (T3), growth hormone (GH), insulin-like growth factor 1 (IGF-1), prolactin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), as well as one of the first-line tests for Cushing syndrome described below [34].

Hyperprolactinemia

Prolactinomas or lactotroph tumors account for 40% of all PitNETs and are the most common type of functional tumors. Other causes of elevated prolactin include compression of the pituitary stalk by other sellar masses or infiltrative diseases, medication such as antipsychotics and SSRIs, hypothyroidism, pregnancy, breastfeeding, and chronic renal failure [35, 36].

Hyperprolactinemia often leads to hypogonadotropic hypogonadism in both men and women [37]. The signs and symptoms in premenopausal women include galactorrhea, amenorrhea, or oligomenorrhea and infertility. Postmenopausal women have fewer symptoms as they are already in a hypogonadal state, and galactorrhea is infrequent in these women. Hyperprolactinemia in men is often manifested by decreased libido, erectile dysfunction, and infertility, as well as gynecomastia and, very rarely, galactorrhea. Elevated prolactin can also cause headaches in both men and women [38]. The presence of a pituitary tumor and a markedly elevated serum prolactin is highly suggestive of a prolactinoma [37]. However, in the presence of a macrotumor, caution should be exercised when the prolactin levels are only mildly elevated. This can be due to what is called the hook effect; this occurs when the level of prolactin is extremely high, saturating the assay and causing a falsely or inappropriately lower prolactin level. If the hook effect is suspected, this artifact can be avoided by repeating a prolactin level on a diluted serum sample [39]. However if the prolactin is truly only mildly elevated with a large tumor, it is likely due to compression of the pituitary stalk by a tumor that is not of lactotroph differentiation.

Cushing Syndrome

Cushing syndrome (CS) represents the constellation of symptoms that occur in the presence of chronic hypercortisolism. The signs and symptoms of CS are multisystemic and include proximal muscle weakness, muscle wasting in the extremities, redistribution of fat toward the abdomen and the face, obesity, facial plethora, mood disturbances, bone mass loss, and cardiovascular and metabolic disturbances [10].

The diagnosis of hypercortisolism is established in the presence of suggestive clinical manifestations as well as at least two abnormal first-line tests. The four first-line tests are the 24-hour urinary free cortisol, late-night salivary cortisol, overnight 1 mg dexamethasone suppression test (DST), and the longer low-dose DST. Once the diagnosis is made, the etiology has to be established. It is generally divided into adrenocorticotropin (ACTH)-dependent and ACTH-independent CS. Seventy percent of cases are ACTH-dependent and are caused by a pituitary tumor [40]. This is referred to as Cushing disease. Other rare causes of ACTH-dependent CS include ectopic ACTH-producing tumors that are usually NETs at other sites, most commonly the lung, and the rare CRH-producing tumors that are also usually NETs. Adrenal tumors, both benign and malignant, account for almost all cases of ACTH-independent CS. Measurement of plasma ACTH is, therefore, the first step in determining the etiology, with elevated or inappropriately normal levels with elevated cortisol, suggestive of pituitary disease or ectopic CS, while suppressed ACTH points toward an adrenal etiology [7]. This will help orient the clinician toward the appropriate dynamic testing if needed, as well as optimal imaging to further establish the etiology [41]. In the case of ACTH-dependent CS, the first test used to help distinguish between pituitary disease and ectopic CS is a high-dose dexamethasone suppression test, the rationale being that ACTH secretion by pituitary tumors is only partially resistant to the negative feedback of glucocorticoids and, therefore, introducing an even higher dose of dexamethasone will eventually suppress ACTH/cortisol production, whereas ectopic CS are typically the least sensitive to negative feedback where the elevated ACTH/cortisol secretion persists despite high-dose glucocorticoids [42].

Acromegaly

Acromegaly is a disorder characterized by an excess of growth hormone (GH). The vast majority of cases are due to a GH-secreting PitNET but can very rarely be associated with other causes such as an ectopic production of growth hormone-releasing hormone (GHRH) or GH [43]. Pituitary tumors secreting GH can also co-secrete other pituitary hormones, most often PRL but occasionally TSH [10]. Patients with acromegaly have signs and symptoms related to the excess GH and insulin-like growth factor 1 (IGF-1) such as coarse facial features, spacing of the teeth, macroglossia, increased size of the hand and feet, arthralgias, thyroid goiter and nodules, obstructive sleep apnea, hypertension, and cardiomyopathy [44]. Acromegaly has an insidious onset and is slowly progressive, leading to an average delay between the beginning of symptoms and the time of diagnosis of 12 years [45]. If GH excess begins in childhood or adolescence before fusion of the epithelial growth plates, it leads to gigantism as well, which is characterized by extremely tall stature. The diagnosis of acromegaly is confirmed by an elevated IGF-1 level in the presence of typical manifestations of the disease [46]. An oral glucose tolerance test can be used to further confirm the diagnosis, demonstrating a lack of GH suppression following glucose administration in patients with acromegaly [42].

Hyperthyroidism

TSH-secreting PitNETs are very rare and account for less than 1% of cases of hyperthyroidism. These tumors secrete TSH in an autonomous fashion and do not respond appropriately to TRH stimulation or thyroid hormone inhibition. The majority of these tumors only secrete TSH, but almost 25% will co-secrete other pituitary hormones, mainly prolactin and GH [10]. These patients can present with typical symptoms of hyperthyroidism, such as weight loss, palpitations, heat intolerance, and tremors, but can also present with a diffuse goiter as well as symptoms specific to these types of pituitary tumors, such as visual field defects, headache, and galactorrhea. On thyroid function tests, patients will have elevated free T3 and T4 concentrations, in the presence of elevated or inappropriately normal TSH. Most patients will also have an elevated serum alpha subunit of the glycoprotein hormones [34].

Parathyroid Adenoma and Carcinoma

Hyperparathyroidism is one of the more common neuroendocrine tumor manifestations, occurring in 20 to 90 per 100,000 people with a prevalence of up to 3%. This disorder is most common in females in their 40s and 50s. Parathyroid adenoma is the cause of approximately 90% of cases of sporadic primary hyperparathyroidism [47]. In contrast, parathyroid carcinoma is a rare neuroendocrine malignancy, responsible for <1% of cases of sporadic primary hyperparathyroidism. This syndrome can be mimicked by epithelial tumors ectopically elaborating the closely related PTH peptide PTHrp.

The classical manifestations of hyperparathyroidism are symptoms of hypercalcemia including renal involvement with nephrocalcinosis, nephrolithiasis, impaired renal function, and impaired bone metabolism resulting in osteitis fibrosa cystica, subperiosteal resorption, “salt and pepper” skull, and diffuse osteopenia. However, in much of the world, screening identifies the biochemical abnormality prior to the onset of clinical symptoms in most patients.

Parathyroid tumors are also part of heritable multiple endocrine neoplasia syndromes including MEN-1 and MEN-2 [48]. They can also be the sole manifestation in familial isolated hyperparathyroidism. Patients with the hyperparathyroidism-jaw tumor (HPTJT) syndrome due to germline mutation of *CDC73* have a high incidence of parathyroid neoplasia that is more often malignant [10].

The diagnosis of parathyroid adenoma is not a difficult one clinically and after appropriate imaging, surgical resection results in cure. However parathyroid carcinoma, while rare, is an indolent but lethal disease. In many instances, the diagnosis of parathyroid carcinoma is made only in retrospect when hypercalcemia recurs because of local spread of tumor or distant metastases [49] . Only a minority of patients with parathyroid carcinoma achieve durable disease remission; most will progress despite surgery, radiation therapy, and/or chemotherapy, with the most common site for metastatic disease being the lung [50].

Summary

The wide array of clinical manifestations of neuroendocrine tumors forms the basis for textbooks of internal medicine and cannot be covered in detail in this chapter. However this summary has provided a simple overview that emphasizes the importance of careful clinical history, sophisticated biochemical investigations, and thoughtful consideration of the potential for germline predisposition syndromes that are common in these disorders that are discussed as individual pathological entities on the following chapters of this book.

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Functional and Radiological Imaging of Neuroendocrine Neoplasms

3

Clarisse Dromain, John O. Prior, and Niklaus Schaefer

Introduction

Imaging plays a major role in the work-up of the primary tumor, its characterization and prognosis determination, the local and distant staging, the diagnosis of a cancer predisposition syndrome, as well as the evaluation of treatment and therapy response prediction, e.g., in nuclear medicine therapies. Imaging of neuroendocrine neoplasms (NENs) is extremely rich and varied. Conventional techniques of morphological imaging (ultrasound, CT, MRI) are complementary to other imaging techniques such as endoscopic explorations and functional imaging using radiopharmaceutical imaging techniques.

NENs have some common characteristics on morphological imaging. Most of primary and metastatic tumors are highly vascularized tumor requiring a specific acquisition at the arterial phase after contrast injection in addition of the standard venous phase. The volume of metastatic disease often important is contrasting with a small size of primary tumor. Moreover, the sites of metastatic disease depends from the primary tumor. Most of well-differentiated NENs are slow-growing tumors that can confound the accurate assessment of progression.

NENs have very distinct functional characteristics, which make this disease an ideal target for functional molecular imaging. In the early 1990s, first reports on radiolabeled forms of somatostatins have been published by Reubi et al. [1], and

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first series of indium-111-DTPA-D-Phe-1-octreotide scintigraphy scans (OctreoScan™) have been published by the group of Krenning and Kwekkeboom [2]. Despite the proven usefulness of OctreoScan™ imaging in NEN, the clinical workflow is relatively complicated with delayed imaging after 24/48 hours after bowel voiding and a relatively high radiation burden for the patient.

Modern PET/CT (positron emission tomography combined with inline computed tomography) scans as Ga-68 DOTATOC (DOTA(0)-Phe(1)-Tyr(3))-octreotide, Ga-68 DOTATATE (DOTA-(Tyr3)-octreotate), or Ga-68 DOTANOC (DOTA,1-Nal(3)-octreotide), targeting the somatostatin receptors (mostly SSTR2, less SSTR3 and SSTR5) [3], have mostly replaced scintigraphy due to higher imaging resolution, lower radiation burden, and a more patient convenient technical protocol [4].

Functional imaging in general plays a crucial role in the assessment of NEN in initial tumor distribution (staging), disease assessment after therapy (restaging), disease follow-up, and planning for SSTR2-based radiopeptide treatment which has shown to be beneficial in progression-free survival and quality of life in midgut G1/G2 neuroendocrine tumor (NET) [5, 6].

Other tracers, as 123I-metaiodobenzylguanidine (123I-MIBG) [7] scintigraphy or 18F-DOPA (dihydroxyphenylalanine) [8] and the SSTR2-antagonist (NODAGA-JR11) [9] PET/CT, were developed for specific indications or to gain in sensitivity and specificity. More aggressive, poorly differentiated forms of NEN (NEC) tend to have less SSTR2 receptor expression, and the tumor cell metabolism shifts toward anaerobic glycolysis. In these patients receptor-based imaging is less performant and should be complemented or replaced by metabolic 18F-FDG (18F-fluorodeoxyglucose) PET/CT [10] (Fig. 3.1). The continuum from

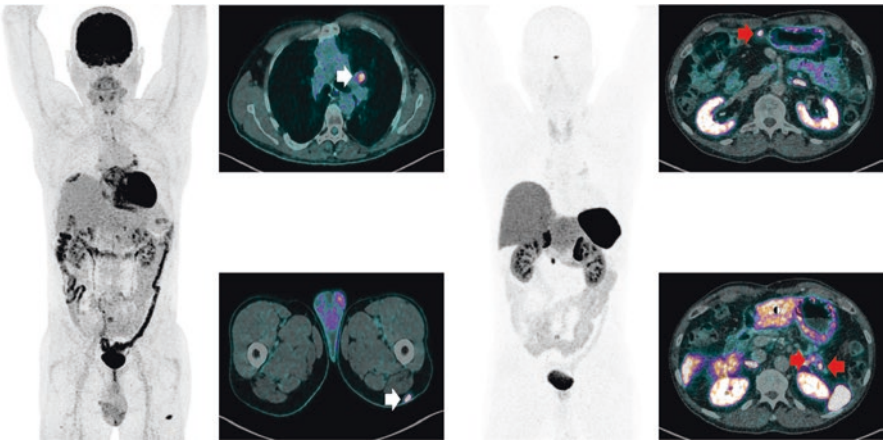

Fig. 3.1 Aggressive, poorly differentiated forms of NEN (NEC) tend to have less SSTR2 receptor expression, and the tumor cell metabolism shifts toward anaerobic glycolysis. In these patients receptor-based imaging is less performant and should be complemented or replaced by metabolic 18F-FDG (18F-fluorodeoxyglucose) PET/CT

well-differentiated NETs to the more aggressive NEC makes functional imaging sometimes challenging. In the following chapter, we describe the indications and limitations for each subtype of NEN.

Lung Neuroendocrine Neoplasms

Bronchial NEN

General Considerations

Neuroendocrine neoplasms of the lung (bronchial NEN) are heterogeneous diseases, which vary from well-differentiated, low-grade carcinoids to poorly differentiated, high-grade large or small cell neuroenodcrine carcinomas with poor prognosis. All subtypes of lung NEN need imaging to delineate localized versus metastatic disease at staging and restaging and in case of suspected recurrence. Contrast-enhanced CT is the cornerstone in all of these indications. Functional SSTR2 imaging versus metabolic FDG PET/CT is chosen according to the disease aggressiveness (NET versus NEC). In general, well-differentiated NENs are depicted with SSTR2 receptor imaging, whereas aggressive subtypes as small or large cell neuroendocrine carcinomas are visualized best with metabolic FDG imaging.

Radiological Imaging

The CT with injection of iodinated-based contrast agent is the imaging of reference for bronchial NEN. Typical and atypical bronchial NETs (carcinoids) have similar imaging features, which mainly depend on the tumor location. Radiologic features include hilar or perihilar masses, endobronchial nodules, findings related to bronchial obstruction, and peripheral nodule [11].

Hilar or perihilar mass is usually a unifocal well-defined round lesion ranging from 2 to 5 cm that narrows or obstructs adjacent airways. When obstruction is complete, CT images demonstrate a peripheral atelectasis and postobstructive pneumonia. Eccentric calcifications are also a common finding. As most of NEN, bronchial NETs are highly vascularized with strong and homogeneous enhancement that can mimic a pulmonary varix or a pulmonary artery aneurysm. However, not all bronchial NETs enhance.

Another presentation is an endobronchial nodule extending into the adjacent parenchyma (Fig. 3.2). Such tumor can display a dominant extraluminal component with a very small endoluminal component so-called the iceberg lesion.

Finally, bronchial NETs may have a peripheral distribution presenting as a solitary pulmonary nodule with lobulated contour [12] (Fig. 3.3). The mean size of peripheral tumors has been reported to be 14 mm (range, 9–28 mm).

CT imaging may also suggest the diagnosis of diffuse idiopathic primary neuroendocrine cell hyperplasia (DIPNECH) that is considered as a preinvasive lesion for lung carcinoid tumors and is found in 5.4% of patients with resected lung carcinoid tumors [13]. Because DIPNECH is characterized in pathology by cell proliferation into the bronchial wall, CT features are those of airway-related diseases

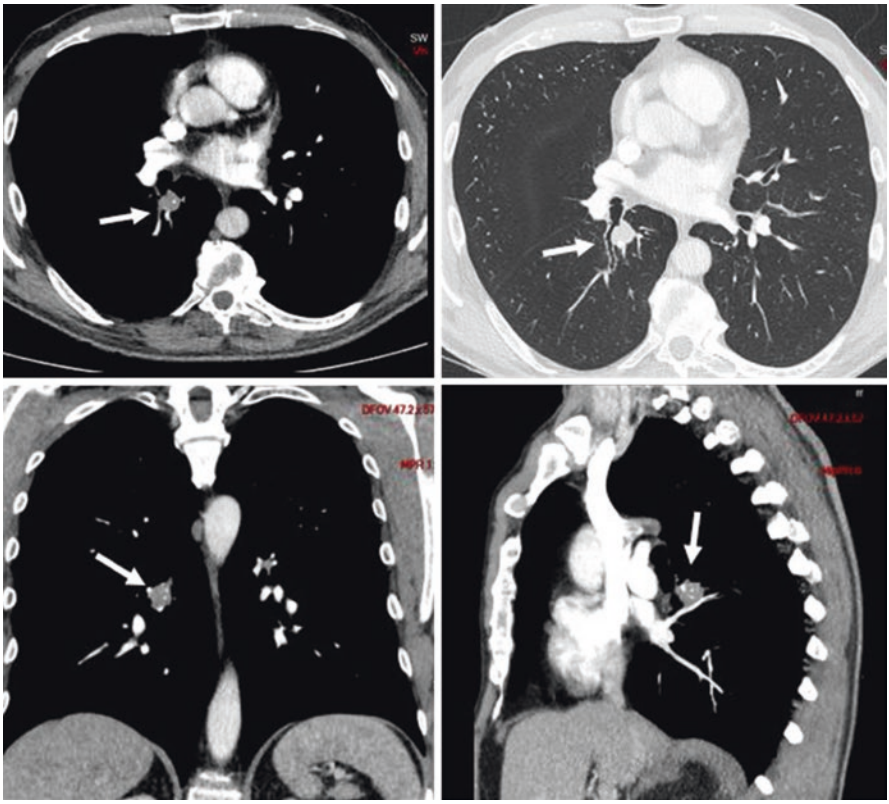

Fig. 3.2 Endobronchial NET corresponding to a typical carcinoid tumor. Axial CT images with coronal and sagittal reconstruction show a well-delineated lesion with calcification located into the airway with extraluminal component

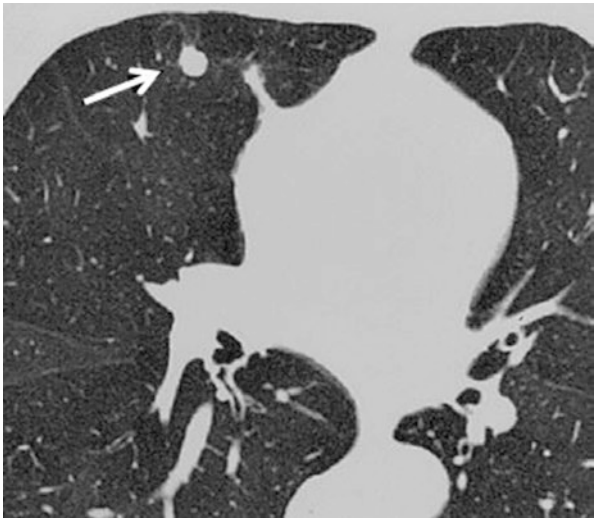

Fig. 3.3 Solitary lung nodule corresponding to a typical lung carcinoid (NET)

including bronchial wall thickening, mild bronchiectasis, mucoid impactions, and mosaic perfusion [14].

Both typical and atypical bronchial carcinoids (NETs) may be associated with hilar and/or mediastinal lymph nodes metastases, which are more frequently associated with atypical bronchial carcinoid.

Nuclear Medicine Imaging

Already in the mid-1990s, scintigraphy with indium-111 octreotide was reported to be useful in lung carcinoids [15]. It was described as especially useful to identify sites of ectopic ACTH production in patients with Cushing's syndrome [16]. Today, scintigraphy is mainly replaced by DOTATATE/DOTATOC/DOTANOC (SSTR) PET/CT imaging due to higher imaging resolution, faster and more patient convenient imaging protocols, and lower radiation burden for the patients. FDG PET/CT plays a role in more aggressive subtypes [17], and already atypical carcinoids have a significantly higher uptake of FDG than typical carcinoids [18]. Further studies revealed FDG as independent prognostic marker for progression-free survival after resection. In this relatively large study of 65 patients, no patient with low uptake of FDG relapsed, and patients with higher uptake had a significantly worse relapse-free survival [19]. In patients with small and large cell neuroendocrine carcinoma, FDG PET/CT is recommended to exclude distant metastasis to choose between localized radio-chemotherapy and palliative chemotherapy alone [20].

Digestive NEN

General Considerations

Digestive NEN comprises a large variety of different neoplasms from different sites and can arise from esophageal, gastric, duodenal, small bowel, colon, or rectum origin. The role of imaging in these subtypes varies, since the prognosis, treatment, and metastatic spread are different. In very early gastroduodenal or colorectal NEN subtypes, cross-sectional imaging might play less of a role. In small bowel NEN, imaging already in early tumors is mandatory due to early local metastasis and associated imaging findings due to local serotonin secretion (e.g., local mesenteric fibrosis).

Radiological Imaging

Primary NEN of the small bowel is usually small and difficult to diagnose. CT enteroclysis is the imaging of reference for small bowel primary tumor detection. Indeed, since most of these tumors are small (<2 cm), conventional CT is most often negative for the primary tumor. By combining small bowel distension and intraluminal low contrast, CT enteroclysis maximizes contrast between the lumen and small bowel wall facilitating the assessment of the small bowel wall. The sensitivity of CT enteroclysis has been shown to be 100% and a specificity of 96% in identifying small bowel NENs [21].

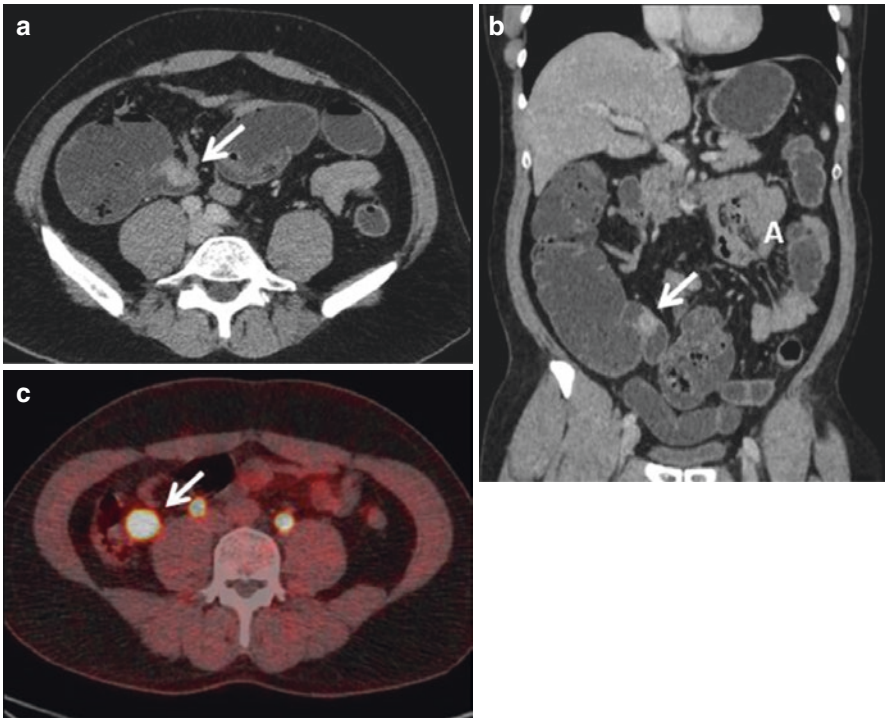

Fig. 3.4 Digestive NET: Axial CT image (**a**) with coronal reconstruction shows a 12 mm polypoid lesion located in the terminal ileum demonstrating a significant enhancement after injection. The corresponding F-DOPA PET/CT image (**c**) shows a marked tracer uptake into the lesion

Imaging feature of small bowel NENs in CT enteroclysis is a focal nodular mass located in the small bowel wall (Fig. 3.4) or an intraluminal polypoid mass showing marked enhancement after injection [21]. The median size of lesion is 14 mm ranging from 5 to 30 mm. Most of lesions are located in the terminal part of the ileum. Digestive NENs are multifocal in 30% but often underestimated during the initial work-up.

Other very common associated imaging features are a *mesenteric fibrotic mass*, small bowel ischemia, and enlargement of mesenteric lymph nodes. CT detection of a mesenteric mass might be the first clue suggesting the need to search for a primary tumor within the small bowel. Mesenteric fibrotic mass appears on CT images as a 3 cm (median size) round or oval well-defined soft-tissue mass, with radiating linear strands with stellate or spoke-wheel configuration, and thickened adjacent bowel loops (Fig. 3.5) [22]. Small, stippled, or coarse calcifications are present in 70% of cases.

The *linear radiating strands* observed in the mesentery on CT scans are due largely to the fibrotic process. Moreover, it has been found that the degree of histologic fibrosis tended to be directly related to the degree of radiating strands detected

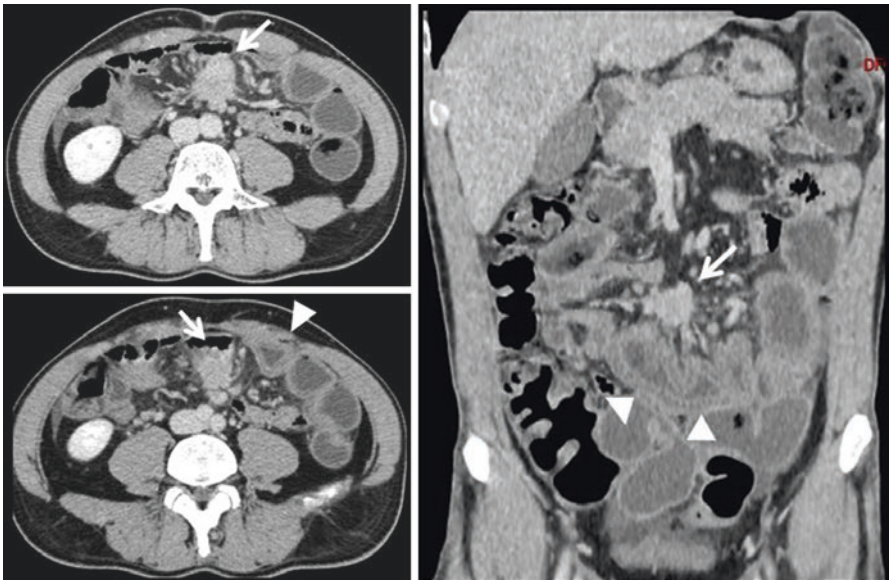

Fig. 3.5 Mesenteric fibrotic mass from a digestive NET. Axial CT images with coronal reconstruction show a well-defined soft-tissue mesenteric mass with homogeneous enhancement (arrows). The association with radiating linear strands and thickened adjacent bowel loops with target appearance (arrowheads) is highly suggestive of mesenteric ischemia due to development of elastic mesenteric vascular sclerosis induced by tumor-produced hormones in particular the release of serotonin by the tumor

by CT scans [22]. This profound desmoplastic response is caused by hormonally active substances, especially serotonin, secreted by NETs. The same hormones also provoked desmoplastic effects within the bowel wall.

Small bowel wall thickening observed on CT images is the result of fibrosis within the bowel wall and edema due to mesenteric ischemia. Mesenteric ischemia is a result of obstruction of the mesenteric artery due to infiltrated paraaortic lymph nodes or more often the development of elastic mesenteric vascular sclerosis induced by tumor-produced hormones in particular the release of vasoconstrictive substances [23]. These elastic mesenteric vascular changes are only noted in cases in which there is spread of tumor in the mesentery, suggesting that this vascular lesion may be caused by the local effect of substances secreted by the tumor. Imaging features of small bowel ischemia include bowel wall thickening, dilatation of bowel lumen, and with sometimes a halo target appearance representing hyperemia and hyperperfusion associated with surrounding mural edema.

Capsule endoscopy provides a more complete evaluation of the small bowel with an absence of irradiation and a minimal patient discomfort. However, lesion location and measurement is difficult. Other disadvantages are the absence of extraluminal abnormalities evaluation as well as distant metastases, the time of the examination procedure, and its contraindication in patients with an obstruction or stricture.

Nuclear Medicine Imaging

The correct indication for functional imaging techniques needs to be adapted to the primary site, the grading, and the size of the primary tumor. Small gastric NETs (gNETs) are divided into subtypes with different prognosis and risk of progression, and the data for functional imaging in gNET is relatively sparse. 68-Ga DOTATOC may be further helpful in localizing occult gastrinomas in the setting of type 2 gastric NET [24]. Aggressive type 3 tumors need FDG PET/CT for staging and restaging under therapy or surgical procedures as gastrectomy [25, 26]. Small bowel NETs tend to metastasize very early in local lymph nodes and later liver metastasis. Since most of the well-differentiated (G1/G2) NETs of the small bowel express SSTR2, DOTATATE/DOTATOC PET/CT is recommended in all patients [27]. Different prospective studies have shown the superiority of DOTATATE/DOTATOC PET/CT imaging to contrast-enhanced computed tomography [28], and it has to be considered as standard of care. SSTR receptor-based imaging therefore is standard and mandatory in all patients with NEN of the small bowel. 18F-DOPA plays a minor role in functional, well-differentiated NET and is superior to octreotide scintigraphy in small studies [29]; however SSTR2-based PET/CT remains standard of care in small bowel NET. In colon and rectal NET, functional imaging plays a minor role in tumors larger than 2 cm or higher grade according to the above referenced ENETS guideline.

Pancreatic NEN

General Considerations

pNEN can occur as sporadic tumors or in the context of genetically caused disease as, e.g., multiple endocrine neoplasia type 1 (MEN-1). pNEN can further be divided into functionally active tumors (e.g., insulinoma, VIPoma, glucagonoma, or gastrinoma) with specific hormonal hypersecretion syndromes or nonfunctioning pancreatic tumors which cause morbidity by invading local tissue and metastases. Endoscopic ultrasound in combination with fine-needle biopsy is mandatory in most patients to stage and diagnose local disease. Contrast-enhanced MRI, CT, and specific functional imaging methods in pNEN subtypes are optimally suited to stage localized disease and systemic metastases.

Radiological Imaging

Endoscopic ultrasonography (EUS) is particularly suited to detect small-size (2–5 mm) pancreatic lesions such as gastrinomas and insulinomas with reported detection rates from 79% to 94% [30]. Due to the proximity of the endoscope, the sensitivity is, however, higher in the head than in the tail. The addition of contrast agent injection has been shown to increase potential detection of small pancreatic tumor by their ability to detect hypervascular enhancement [31]. EUS is also used to survey patients at increased risk of developing pNETs in particular in multiple endocrine neoplasia type 1. A prospective multicentric study in 90 patients with MEN-1 comparing EUS and pancreatic EUS has shown that 48 (53.3%) patients

had at least 1 tumor ≥10 mm. EUS detected 86 tumors ≥10 mm vs. 67 tumors for MRI. EUS failed to identify 15.7% of patients with pancreatic tumors ≥10 mm, vs. 19.3% of patients for MRI. The authors concluded that EUS and MRI are complementary and should be performed at initial evaluation in multiple endocrine neoplasia type 1 patients [32].

CT is the first-line imaging modality in the evaluation of patients with suspected pNENs allowing the investigation of the pancreas as well as the assessment of the disease extension. CT imaging should include four-phasic imaging including unenhanced phase, arterial/pancreatic phase, venous phase, and delayed phase. The late arterial (30 sec) or pancreatic phase (40 sec) is mandatory allowing an increased detection of small functioning pNET in particular insulinoma [33]. Moreover, it also increases the detection of hepatic metastases [34, 35]. The delayed phase is complementary of the arterial/pancreatic and the venous phase allowing the detection of delayed enhancement of some fibrous tumors [36].

MR imaging protocol should include a T1W and T2W sequence with fat suppression, dynamic 3D sequence before and after Gd-chelate contrast agent injection with multiarterial, venous, and delayed (>5 min) acquisition and DWI sequence. Fat suppression on T1W and T2W images is useful to emphasize the signal intensity differences between the pancreatic tumor and the normal pancreatic tissue. Similarly to CT, T1W delayed (>5 min) images are required to improve both characterization and detection [36]. Diffusion-weighted images increase the sensitivity for the detection of the primary pancreatic tumor as well as associated liver metastases [37].

Imaging features are depending on either the tumor is functioning or nonfunctioning. Functioning pNETs are most often manifested by endocrine symptoms with an established or highly suspected clinical and biological diagnosis. The challenge of imaging is to localize the tumor that is often of small size. Insulinomas are the most frequent functioning pNEN. Most insulinomas are under 2 cm in size, solitary and indolent. They are located all over the pancreatic gland. On CT images typical insulinomas are well defined and hypervascular and show intense enhancement during arterial/pancreatic phase (Fig. 3.6). The enhancement is usually uniform. Sometimes, a rim enhancement is depicted highly suggestive of the diagnosis [38]. Gastrinomas are also small pancreatic tumor (1–3 cm) arising in 80% within the "gastrinoma triangle" defined as the confluence of the cystic and common bile duct superiorly, the second and third portions of the duodenum inferiorly, and the neck and body of the pancreas medially. Gastrinomas are the pNET more often associated with a MEN-1. In these cases, they are often multicentric and are associated with a major morbidity and mortality. After contrast injection, gastrinomas have more often a delayed enhancement persistent on delayed phase due to presence of fibrosis. Other presentations of functioning pNET include purely cystic tumors in 10% that is a common pattern of pNET associated with MEN-1, complex solid and cystic pattern, and calcified tumors in less than 5% [39].

On MRI most of functioning pNETs show low signal intensity on T1W, high signal intensity on T2W images, and intense and early enhancement on dynamic T1W sequence after injection (Fig. 3.6). Hypervascular tumors (typically, insulinoma) are often better depicted on the T2W with fat suppression, whereas

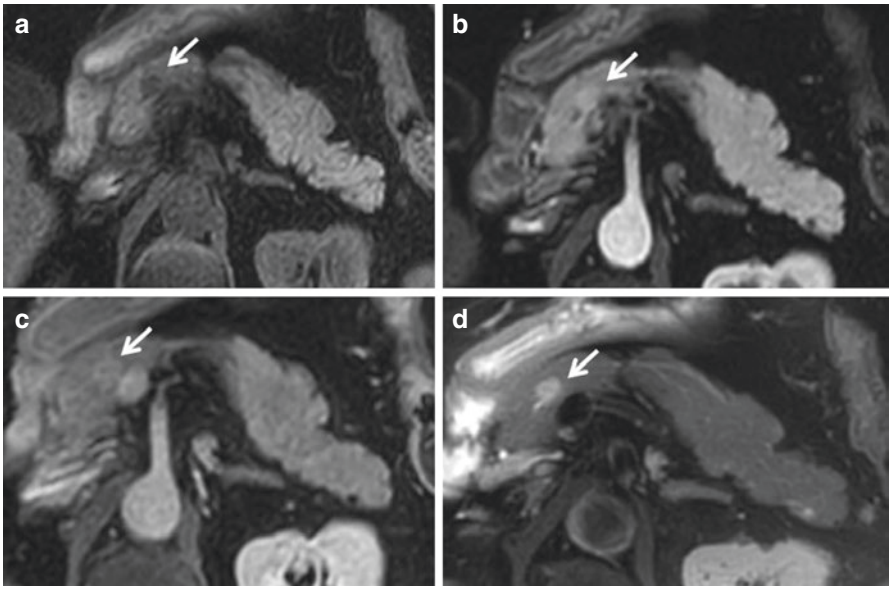

Fig. 3.6 MR images of an insulinoma (well-differentiated grade 1). T1-weighted MR images before (**a**) and after injection of gadolinium-based contrast agent at the arterial phase (**b**) and the portal venous phase (**c**) show an 8 mm nodule hypointense on T1, with significant enhancement on the arterial phase and no washout on the portal phase. On T2-weighted image (**d**), the tumor is depicted as a small well-demarcated hyperintense nodule

hypovascular tumors are better depicted on the T1W sequence in the arterial phase. This is probably explained by the high enhancement of the pancreas in the arterial phase which concealed hypervascular tumors, whereas non-hypervascular tumors were surrounded by the enhanced normal pancreatic parenchyma [40]. Diffusion-weighted imaging is helpful to depict small pNET due to its greater image contrast. ADC values have been shown to be lower than adjacent pancreatic parenchyma in all cases of solid nodules [40]. However, higher ADC values can be obtained in case of cystic pattern [41].

Differential diagnoses of hypervascularized pNET are pancreatic metastases (coming most often from a renal cell carcinoma (RCC)) and intrapancreatic accessory spleen. In addition, a splenic artery loop should not be misinterpreted as small pNET. With multiplicity, the relative percentage of washout of the tumor on CT could be helpful for differentiating pancreatic metastases from RCCs from hypervascular pNETs [42]. However, multiple hypervascular pNET is frequent in case of MEN-1.

Nonfunctioning pNETs are often detected incidentally or announced by nonspecific symptoms. The challenge of imaging is not to detect the pancreatic tumor that is more often large but to differentiate this tumor from ductal adenocarcinoma or other types of pancreatic neoplasm and to determine the extent and the potential of resectability. On CT and MR images, nonfunctioning pNET appears as a large

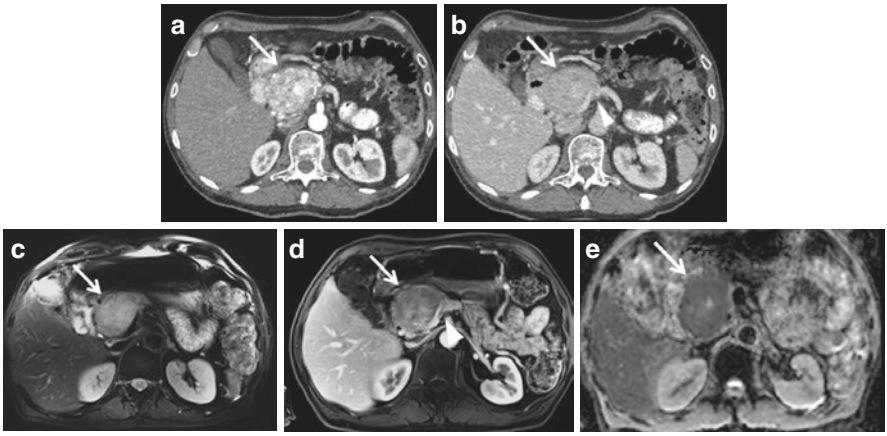

Fig. 3.7 Pancreatic NET grade 2. CT images acquired at the arterial (**a**) and portal phase (**b**) show a large mass with heterogeneous enhancement at the arterial and venous phase. On MR images the lesion is hyperintense on T2-weighted images (**c**) and hyper during the arterial phase of the dynamic study. MR images also depicted a tumoral vein thrombosis in the superior mesenteric veins but the absence of vascular encasement. No dilatation of the upstream pancreatic and common bile duct is shown

pancreatic mass with heterogeneous enhancement due to necrotic and hemorrhagic changes. On MR images, in contrast to pancreatic adenocarcinoma, most of pNETs are hyperintense on T2W images and hyper- or isointense during the arterial/pancreatic phase of the dynamic study [43] (Fig. 3.7). Moreover pNETs tend to have a higher rate of tumoral vein thrombosis (splenic, portal, and superior mesenteric veins) and a lower rate of vascular encasement than pancreatic adenocarcinoma [40, 43]. Dilatation of the upstream pancreatic and common bile duct is rarer than in pancreatic adenocarcinoma present in 33% of cases for the main pancreatic duct and less than 9% for the common bile duct [44].

Nuclear Medicine Imaging

Most pNETs express the SSTR subtype 2 (>80%), with the exception of insulin-producing tumors (insulinoma) (<50%) [28, 45]. Thus, Ga-68 DOTA-SSTR PET/CT has become the mainstay of pancreatic molecular imaging of pNEN with high sensitivity and specificity (>90%) [46] (Fig. 3.8).

A recent meta-analysis in 383 metastatic patients [47] has shown that for unknown primary tumor, SSTR PET/CT was able to find the primary tumor in 56% of the patients with the most common primary site being the bowel and the pancreas, with a change of management in 20% (95%CI 10–33%) of the patients. A word of caution should be given to finding the primary tumor in case of patient in whom clinical or biochemical findings suggest a NET suspicion with only about 10%, respectively, 1.5% of cases being a NEN, while this number amounts to 32% for a suspicious lesion on conventional imaging [48].

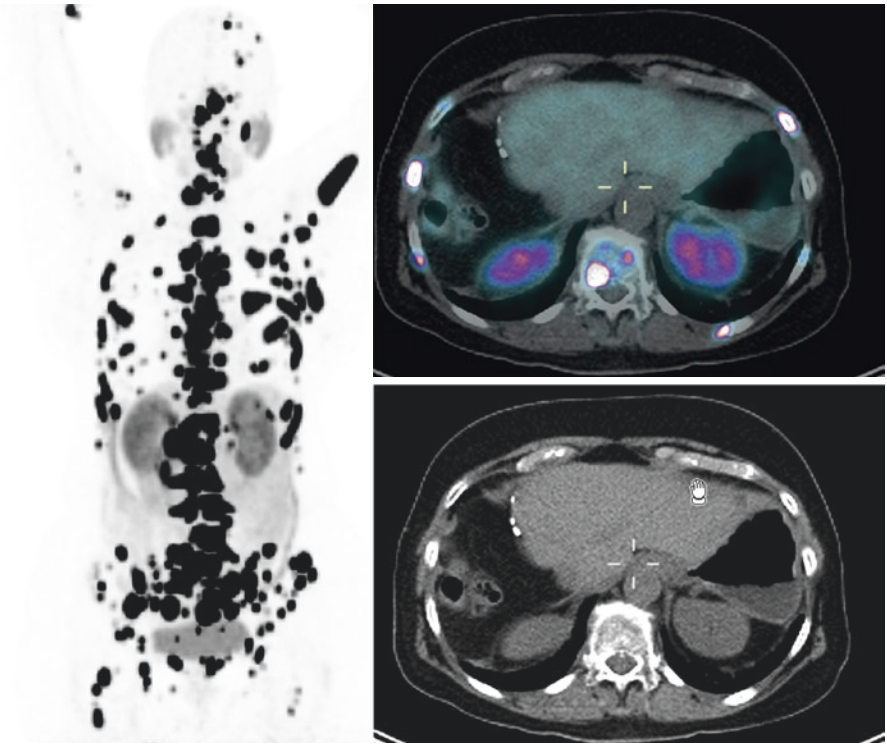

Fig. 3.8 Most pNETs express the SSTR subtype 2 (>80%), with the exception of insulin-producing tumors (insulinoma) (<50%). Thus, Ga-68-DOTA-SSTR PET/CT has become the mainstay of pancreatic molecular imaging of pNEN with high sensitivity and specificity (>90%)

Therapy monitoring with PERCIST for Ga-68 PET for pNET has not been evaluated systematically. Decreased SSTR PET/CT after the first PRRT can predict time to progression and improved clinical symptoms in one study [49], although ΔSUV tumor/spleen ratio was superior to ΔSUVmax. Interestingly, a novel structured framework for SSTR PET/CT has been proposed to standardize reporting called the SSTR-RADS [50, 51]. In its latest form SSTR-RADS Version 1.0, the level of SSTR expression on PET/CT on the baseline scan is graded on a 3-point scale using internal organs as reference. The SSTR-RADS system can be used for baseline SSTR PET to help guiding work-up and therapy with Lu-177- or Y-90-based PRRT for the referring physician (Table 3.1).

The NETPET grade is based on a dual-tracer SSTR/FDG PET/CT approach evaluated on a 5-point scale (Table 3.1) [52]. In this setting, Kaplan-Meier curves for the least aggressive P1 clearly differed from the P2–P4 groups and the most aggressive P5 tumors. When both NETPET and SSTR-RADS standardized frameworks are used in structured reporting, this may change the reader confidence in interpreting PET/CT, especially if they are already familiar with the PSMA-RADS or NI-RADS frameworks [50].

Table 3.1 Standardized frameworks for neuroendocrine neoplasias (NENs)

System (Reference)	Grade	SSTR PET/CT lesion intensity[a]	Intensity comparison	FDG PET/CT lesion intensity[a]
NETPET [52]	P0	–		–
	P1	+		–
	P2	+	>	+
	P3	+	=	+
	P4	+	<	+
	P5	–		+
		Significance	PET/CT imaging correlates	
STTR-RADS, Version 1.0 [51]	1	Benign	Benign or biopsy-confirmed	
	2	Likely benign	Atypical for metastases	
	3	Undetermined lesion	Requires further work-up	
	4[b]	NEN highly likely	High intensity, typical site, but lack CT anomaly	
	5[b]	NEN certainly present	High intensity, typical site, with CT anomaly	

[a]Threshold for positivity: SUVmax SSTR ≥15 g/mL, SUVmax FDG ≥7 g/mL [39]
[b]PRRT can be considered in these patients [51]

Novel whole-body parametric imaging PET/CT technology allows to compute Ga-68-SSTR PET-measured net influx rate according to Patlak ($K_{i\ Patlak}$), which shows better image contrast and potentially better measures tumor uptake for therapy monitoring [53, 54], although one study found that SUV_{max} correlated very well with absolute K_i values and might be sufficient to reflect the SSTR expression in NEN [55].

Normally, glucose metabolic rate is low in well-differentiated NEN, and elevated 18F-FDG avidity identifies patients with poorer progression-free survival and higher Ki-67 index [45], even in patients with less than G3 tumors. In an international survey [28, 45], about 10% (72 centers) described performing routinely a FDG PET/CT at the time of diagnosis (Table 3.2). Due to tumor heterogeneity, positive FDG PET/CT can already be observed in NEN with Ki-67 of <2% and can help guiding biopsies [56]. Patient with documented NET can develop a second malignancy and suspicion can be raised on a CT/MR follow-up imaging. In this case, a FDG PET/CT can be recommended and compared to DOTA-peptide PET/CT for identification and eventually biopsy planning [45, 57]. Furthermore, patient's survival and response to Lu-177-PRRT has been correlated to FDG PET/CT uptake, even for G1 and G2 pNET [58].

NENs take up and decarboxylate amine precursors such as F-18-dihydroxyphenylalanine (FDOPA) or C-11-5-hydroxytryptophan (5-HTP) with a slight advantage of the latter for pNEN (Fig. 3.9), although it is available only to centers with an onsite cyclotron [45]. Based on retrospective data, FDOPA/5-HTP PET/CT seems to be less sensitive than DOTA-peptide PET/CT, although these tracers can be of use in specific cases of tumors with low-SSTR expression. Some authors recommend using carbidopa (an inhibitor of peripheral amino acid

Table 3.2 Radiopharmaceuticals used for pancreatic neuroendocrine neoplasias (pNENs)

Radiopharmaceutical	Principle	Sensitivity, advantage	Remark
Ga-68-DOTA-conjugated peptides (DOTATOC, DOTATATE, DOTANOC, DOTANOCATE, etc.)	Binding to somatostatin receptors	82–100% Very sensitive for well-differentiated disease Superior to FDG in G1 and G2 tumors	False-positive lesions in case of the uncinate process, inflammatory disease, or accessory spleen
F-18-Fluorodeoxyglucose (FDG)	Glucose analog entering the cell and trapped inside, after phosphorylation	92% for Ki > 15% Detects patients with aggressive disease and poor outcome	Useful in lesions with Ki > 10–15% or when patient develops a second malignancy
F-18-Dihydroxyphenylalanine (FDOPA)	Active uptake by NEN and decarboxylation of amine precursor	80% Detects small tumors and recurrence, especially in SSTR-negative, serotonin-secreting NEN	F-18-FDOPA less sensitive than 5-HTP in pNEN (in contrary to gastrointestinal NEN)
C-11-5-Hydroxy-L--tryptophan (5-HTP)	Active uptake by NEN and decarboxylation of amine precursor	96% Detection of small tumors and recurrence 5-HTP more sensitive than FDOPA in pNEN	Available only in centers with onsite cyclotron
Ga-68-glucagon-like-peptide-1 (GLP-1)	Binding to glucagon-like-peptide-1 receptor	100% Localization of benign insulinoma	Available only in few centers worldwide

decarboxylase) for FDOPA PET of PNEN to damper FDOPA physiologic uptake by the mature exocrine pancreas and performing 5-min postinjection early images [59].

Upcoming innovative SSTR antagonists (Ga-68-NODAGA-JR11, also known as Ga-68-OPS202) might further improve imaging pNEN, as they are much more frequent on the cell surface and display PET uptake severalfold higher than conventional SSTR agonists radiopharmaceuticals [56]. Ga-68-glucagon-like-peptide-1 (GLP-1) represents a promising tool for detecting benign insulinomas, and Ga-68-exendin-4 PET/CT is currently being used in clinical trials [56]. Evidences are raising that SSTR PET/MR could be a promising method to detect pNEN with equal or better sensitivity and specificity than SSTR PET/CT or MR alone [56, 60–62], while its cost-effectiveness will need to be evaluated.

Liquid biopsy technology (called NETest) seems promising to accurately diagnose pNEN using multianalyte signature with significantly increased level as compared to normal controls [63]. Furthermore, NETest values were significantly higher for progressive vs. stable disease.

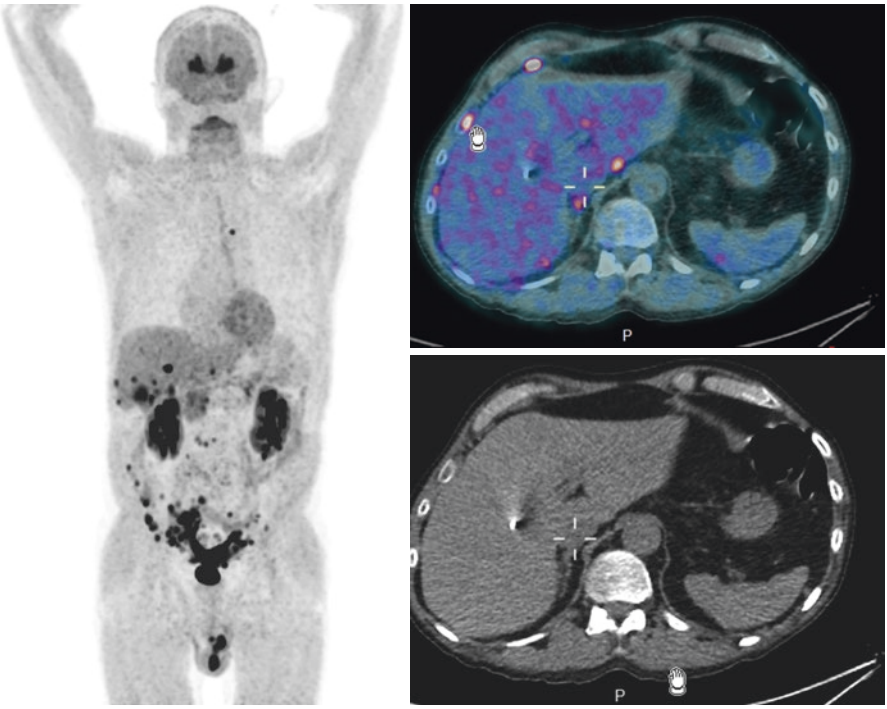

Fig. 3.9 NENs take up and decarboxylate amine precursors such as F-18- dihydroxyphenylalanine (FDOPA) or C-11-5-hydroxytryptophan (5-HTP) with a slight advantage of the latter for pNEN, although it is available only to centers with an onsite cyclotron

Finally, all molecular imaging PET/CT studies should be interpreted and reviewed in conjunction with conventional imaging (CT/MR) and discussed in a multidisciplinary tumor board.

Colorectal NEN

General Considerations

Colorectal NENs are described as a uniform entity in many guidelines, e.g., the ENETS guidelines [64]. NENs of the colon tend to be more aggressive, whereas rectal NENs tend to be of lower grade and form usually small tumors. Due to the specific anatomic circumstances of the rectum versus the colon, relevant questions for imaging are slightly different (e.g., infiltration of adjacent structures in the pelvis). In both subtypes, endoscopy associated with endoscopic ultrasound is mandatory for assessing local infiltration. In colon NEN, cross-sectional imaging is indicated for larger tumors (>1 cm); in rectal NEN, MRI can assess tumor infiltration in larger primaries (>1 cm). Functional imaging in colorectal NEN is reserved for larger tumors (>2 cm) and more aggressive histology.

Radiological Imaging

The majority of lesions in the rectum is diagnosed endoscopically. Imaging of reference for preoperative staging of rectal NEN is the endorectal ultrasonography (EUS) that can accurately assess the tumor size and the depth invasion and look for the presence of lymph node metastases, which are the most important parameters to determine the adequate treatment modality.

On EUS, colorectal NEN appears as well-demarcated homogeneous iso- or hypoechoic lesion most often localized in the submucosa. EUS is particularly adequate for evaluating the depth of invasion of small lesion.

MRI is recommended for patients with tumors >10 mm (>T1) in size and/or node-positive tumors, in tumor not completely removed at endoscopy, or if metastatic disease is suspected [65]. Rectal NEN is seen on MRI as a single submucosal mass with a homogeneous and marked contrast enhancement after contrast injection.

Nuclear Medicine Imaging

Colorectal NETs are usually SSTR positive and staging, restaging, and follow-up using functional imaging is useful in larger tumors (>2 cm). To the knowledge of the authors, no direct comparison study between CT/MRI and functional imaging has been performed. SSTR2 imaging can be of further use to separate adenocarcinoma versus neuroendocrine origin [66]. In more aggressive subtypes, especially in colon NET/NEC, FDG PET/CT is the imaging of choice in the case of suspected distant metastases.

Appendix NEN

General Considerations

The correct staging of appendix NEN is sometimes challenging due to local infiltration and micrometastases in local lymph nodes and the possible consecutive indication for right hemicolectomy. However, in the absence of large randomized studies, only considerations and not scientific valid recommendations can be given.

Radiological and Nuclear Medicine Imaging

In a recent multicenter analysis of more than 400 patients, size, local invasion, and grade were predictive for the development of later lymph node metastases in patients follow-up [67]. Cross-sectional imaging therefore plays a role in a subset of patients with larger tumors (>15.5 mm), lympho-vascular invasion, and grade higher (>G1). However, it is unclear if right hemicolectomy in this setting leads to an overall survival benefit. In absence of prospective controlled data, no definitive recommendation can be given, but guidelines recommend contrast-enhanced CT in tumors >1 cm and SSTR2-based PET/CT in tumors larger than 2 cm and higher grades [68].

Imaging of Rare Neuroendocrine Neoplasms

General Considerations

Rare neuroendocrine neoplasms sometimes have specific biological features and warrant particular imaging techniques. Central and pituitary NENs, either of primary or metastatic nature, need contrast-enhanced MRI and bone CT to depict local extent and bone involvement. In the very rare case of distant metastasis of a primary central NEN, lung CT, SSTR imaging, or FDG PET/CT according to the tumor aggressiveness might play a role in selected cases. Medullary thyroid cancer arises from C-cells either as sporadic form or in the context of MEN-2. At staging, all patients need neck ultrasound of the central, lateral, and posterior neck compartment to identify suspicious lymph nodes prior to surgery [69]. MRI and CT of the mediastinum and the chest is recommended in all patients to complement staging prior to surgery [69]. In cases with high calcitonin levels (>400 ng/l), contrast-enhanced liver MRI is recommended to exclude liver metastases [69]. The role of FDG PET/CT in MTC is not yet defined, but might play a role in patients with aggressive disease, e.g., in the case of rapidly rising tumor markers and/or suspected distant metastases [69]. 18F-DOPA PET/CT is a valid option for staging and restaging in patients with suspected recurrence [70]. In patients with paraganglioma (PGL) or pheochromocytoma (PCC), metastatic disease needs to be excluded by imaging prior to surgery or other local interventions. For staging, patients need contrast-enhanced CT and MRI in specific cases (e.g., in head and neck sympathetic PGL). 123I-MIBG is a recommended staging method in these patients to exclude distant metastases [71]; however, more recently 18F-DOPA [71] and Ga-68 DOTATATE/DOTATOC [72] PET/CT have replaced scintigraphy/SPECT due to higher imaging resolution and lower radiation burden for patients as well as radiology technical personal. Due to the higher efficacy of PRRT versus I-131 MIBG, SSTR imaging will further gain of importance [73]. The role of FDG PET/CT is reserved for more agressive disease, and it might play a role in patients with SDHB germline mutation suggesting metabolic reprogramming [74].

Characterization and Pre-therapeutic Staging

General Considerations

Besides the diagnosis of the primary tumor, imaging has a major role in the staging, the diagnosis of a predisposition syndrome, the work-up of multiple tumors, the prognosis characterization, the monitoring, and the prediction of response therapeutic. TNM staging and the extension of distant metastases especially in the liver constitute the second most important prognostic factor. Moreover, liver involvement at diagnosis in contrast to either metastatic disease at other sites has been shown to be correlated to the prognosis [75, 76] and is a parameter of importance for the treatment management.

Other sites of NET metastases are the abdominal and mediastinum lymph nodes, peritoneum, bone, more rarely lungs, and even more rarely spleen, brain, thyroid, pituitary, breast, heart, meninges, and orbit. The frequency of metastatic sites depends on the primary tumor, the stage of the disease, and the differentiation of the primary tumor [77].

Radiological Imaging

CT scan complements nuclear medicine imaging for NEN staging. MRI has several advantages over CT for evaluating NET liver metastases, in addition to its high sensitivity: (1) it is a non-radiant technique that can be repeated over time without any risk of cumulative irradiation; (2) high MRI contrast between metastases and normal liver enables precise measurement of liver metastases on unenhanced sequences, independently of metastasis enhancement; (3) MRI is the imaging technique with the best interobserver agreement and is more sensitive than ultrasonography (US), CT scan, or SRS for liver metastases detection [78]; (4) adding DW to standard liver MRI yielded additional findings for 45% of the patients with 1.78 times more new lesions, mainly infracentimetric; it induced a management change for 18% of the patients [37]. Its sensitivity is similar to that of intraoperative US assessment. However, about half of the liver metastases are not detected by any pre- and intraoperative imaging technique [79].

Peritoneal carcinomatosis is less often mainly depicted in ileal primary NEN. It is best explored by abdominopelvic CT. Bone metastases are more frequent in lung NETs but may also be present in other primary tumors in advanced disease associated with huge hepatic involvement >25%. Spine MRI or whole-body MRI is here indicated.

Few studies have addressed the value of imaging for assessing *tumor aggressiveness and predictors of the biological tumor behavior* in order to tailor the most appropriate treatment. Some parameters of importance, correlated with the patient prognosis in NENs, are particularly important to mention in imaging reports:

- The size of the primary tumor: it has been reported to be correlated with aggressiveness in nonfunctioning pNEN [43]. In Manfredi et al. study, parameters associated with higher risk of malignant behavior nonfunctioning pNET were size >30 mm, irregular margin, absence of cleavage plane with the main pancreatic duct, and vascular encasement [80]. Other imaging predictors of malignancy in pNET are vascular encasement, ill-defined margins, main pancreatic duct and common bile duct dilatation, complex cystic morphology, and presence of calcification [39, 43].
- Vascularization of the tumor. In NET mean vascular density has been reported to be higher in well-differentiated neuroendocrine tumor, small lesion <2 cm, tumor with Ki-67 <2%, and non-metastatic tumor and in patients without disease progression [81]. Similar findings have been reported on CT and MR images. Well-circumscribed hypervascular mass with homogeneous enhancement is more

common in grade 1 tumor (Fig. 3.5). At the opposite ill-defined hypovascular tumor on arterial and portal phase with heterogeneous enhancement is more common in grade 2 or NEC [80, 82]. In agreement DCE-CT parameters were significantly correlated with prognostic histological characteristics of pancreatic NET [65]. Indeed, significant correlations existed between high blood flow and differentiation, proliferation index, or microvascular density and between longer mean transit time and lymph node or liver metastases. A link between blood flow and OS was also suggested but remains to be confirmed [83].

- ADC values have been recently identified as a biomarker of tumor aggressiveness correlated with the histological grade on pNET. In a recent paper, low ADC was a strong predictor of high tumor grade. A cutoff of 1.19 10^3 mm^2/s was associated with a sensitivity of 100% and a specificity of 92% [84] (Fig. 3.7).
- Tumor burden: the percentage of liver involvement and the number of metastatic sites are also prognostic parameters [75].
- The spontaneous tumor progression slope. In the study of Durante et al., an independent statistical correlation was found between tumor slope before treatment and survival of metastatic GEP tumors [76]. This parameter was found to better reflect tumor aggressiveness than disease-free interval or proliferative index. Most authors consider a low slope if RECIST sum <20% over 1 year. Another way to assess the spontaneous tumor growth is to measure the TGR (tumor growth rate) defined as an estimate of the increase/decrease of the tumor volume over time. TGR is expressed as the percentage change in tumor volume over 1 month. In post hoc analyses, tumor measurements from CLARINET were reevaluated to explore the clinical utility of TGR [85]. A pretreatment TGR >4%/month was associated with a 4.1-fold greater risk of progression than TGR ≤4%/month in the overall population (HR 4.1 [95% CI 2.5, 6.5]; $p < 0.001$, $n = 187$). This study also suggests a higher prognostic role of TGR than the histologic grade. The TGR at 3 months from the start of treatment was also identified as an early biomarker able to predict PFS at 1 year in grade 1 and 2 GEP-NEN [86].

Nuclear Medicine and Theranostic Imaging

Nuclear medicine encompasses different therapeutic strategies for patients with neuroendocrine tumors. Recently, peptide-related radiotherapy (PRRT) using lutetium-177 DOTATATE was tested in patients with small bowel NET versus high-dose long-acting octreotide [5, 6]. This multicentric controlled phase III trial showed an important prolongation of progression-free survival in patients treated with PRRT [5]. The same study also showed minimal side effects and significantly better quality of life in the PRRT patients group [6]. Apart from this landmark trial, many phase I and II as well as observational series showed activity in many other SSTR2 disease types as pNET [87], lung carcinoid [88], or paraganglioma [73] using lutetium-177 DOTATATE. Ongoing studies test other radiopharmaceuticals as lutetium-177 DOTATOC in pNET against standard treatment. As nuclear medicine comprises both diagnostic and therapeutic radiopharmaceuticals, the same peptide can be used

either for PET/SPECT imaging using short-lived isotopes as gallium-68 or for therapy using long-acting electron-emitting isotopes as lutetium-177. The combination of diagnostics and therapy using the same peptide is called theranostics. Using the information of theranostics, therapy outcome and side effects can be foreseen, and therefore patients can be chosen accordingly. Retrospective, large case series show that uptake in OctreoScan imaging according to simple grading systems (less than liver, less than spleen, and more than spleen uptake; Krenning score) is predictive for further patients outcome [89]. On the other hand, 18F-FDG PET/CT as metabolic marker can predict resistance against PRRT due to more malignant, metabolically active tumor metastases [90]. SRS imaging is therefore mandatory before PRRT, and theranostic imaging gains more and more attention in modern oncology outside neuroendocrine neoplasms [91].

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Pituitary Neuroendocrine Neoplasms

4

Sylvia L. Asa and Ozgur Mete

Historical Background

The history of pituitary tumors dates back to biblical times; the documentation of gigantism with visual disturbances is exemplified by the story of Goliath who was a giant compared to David, but could not see him well and was susceptible to apoplexy when hit in the head with a stone. Pituitary tumors were likely implicated in other historical events, but the full extent of this small organ on the history of mankind remains uncertain.

Indeed, the ancient Greeks and early scientists thought of this important endocrine gland as only a source of phlegm, giving rise to its name that derives from the word meaning "slime." It was only in the late eighteenth century that the endocrine nature of this structure became apparent. In 1886, Pierre Marie described the features of acromegaly [1], and 1 year later, Minkowski associated this disorder with a pituitary tumor [2].

Progress in the field was expedited by the work of Harvey Cushing who recognized the importance of pituitary tumors composed of basophilic cells of the pituitary in the genesis of the syndrome that bears his name [3].

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The 1950s saw the recognition of a syndrome of galactorrhea and amenorrhea by Forbes [4]. The identification of pituitary and hypothalamic hormones followed rapidly. The association of pituitary hyperfunction with other glandular disorders in McCune-Albright syndrome as well as the description of a multiple endocrine neoplasia syndrome with pituitary involvement gave rise to concepts of genetic predisposition to pituitary neoplasia. By the 1970s, with the identification of prolactin as a discrete hormone, the many functions of all the pituitary hormones and much of their regulation by the brain were elucidated [5], and the 1980s saw further identification of additional hypothalamic peptides that regulate their secretion [6–8].

The classification of pituitary tumors was one of the most sophisticated among human tumors in the 1980s when Kovacs and Horvath initiated the concept of routine immunohistochemistry and electron microscopy to determine structure-function correlations [9]. This small gland that is composed of at least six cell types that make six main hormones and multiple additional bioactive peptides was the focus of intense study that proved the importance of fidelity to normal structure and the correlates of promiscuous hormone expression. With the advent of molecular tools, the additional information obtained from lineage tracing provided the ability to use transcription factors in the classification of pituitary tumors as proposed in 1998 [10, 11].

The last two decades have seen additional progress in the identification of mutations and germline predisposition accounting for the development of some pituitary neuroendocrine tumors [12–14] that will be discussed in the appropriate sections of this textbook. But perhaps one of the most significant recent events was the concept that these are not simply benign neoplasms referred to as pituitary adenomas, as they were previously characterized; instead, these are epithelial neuroendocrine neoplasms (neuroendocrine tumors) analogous to those at other body sites [15]. The proposal for the new terminology "pituitary neuroendocrine tumor" or "PitNET" anteceded but was reinforced by a WHO/IARC proposal for a common framework for neuroendocrine tumors at all body sites [16]. Thus we have reached the stage where we clearly recognize that these lesions have significant impact on quality of life and longevity, and while they rarely have the potential to metastasize to distant sites, they nevertheless have critical impact that should be respected as potentially lethal neoplasms.

Epidemiology

The epidemiology of pituitary tumors has been the subject of many studies. In older literature, they were thought to be rare; data obtained prior to 1969 provided an annual incidence rate of 1.85 per 100,000 population [17]. Several surgical series reported that they represented from 10% to 25% of intracranial tumors [18], but

these numbers were criticized as reflecting referral bias to pituitary neurosurgeons who published their data. A few autopsy studies with careful histologic assessment identified "incidental" pituitary tumors in as many as 22.5–27% of people [19–21], and radiologic evaluation of asymptomatic patients using high-resolution computed tomography (CT) or magnetic resonance imaging (MRI) identified that approximately 20% of "normal" patients harbor an incidental pituitary lesion measuring 3 mm or more in diameter [22]. A meta-analysis of published data up to 2003 suggested that a pituitary tumor can be found in about 20% of the general population [23]. It was recognized that the majority of these tumors are asymptomatic, likely represented hormonally inactive lesions, with a smaller proportion of prolactin-producing pitNETs (also known as prolactinomas) that may cause clinical symptomatology [24, 25]. These studies found no gender predilection; some studies showed increasing incidence with age.

Increased awareness and improved diagnostic techniques have resulted in a higher prevalence in more recent epidemiological studies that have identified clinically diagnosed pituitary tumors in 78 to 116 cases per 100,000 people [26–29]; one very small Swedish study found only 3.9 tumors per 100,000 [30]. In data from 2011 to 2015 obtained from the Central Brain Tumor Registry of the United States (CBTRUS), pituitary tumors represent 17.5% of reported tumors, with an incidence of 4.12/100,000 population; again this figure is likely far lower than the actual incidence since these tumors are not usually reported to such cancer registries and these numbers almost certainly do not include prolactinomas and other pituitary tumors that are not operated [31].

Indeed the most common pituitary neuroendocrine tumor is the prolactinoma [23, 26–30], a lesion that is usually treated medically [9, 32–34]; the actual incidence of this type of PitNET is unknown. In contrast, among surgically resected PitNETs, more than a third are unassociated with clinical evidence of hormone excess and present instead with symptoms of an intracranial mass [35, 36]. The majority of these "clinically nonfunctioning" tumors are of gonadotroph lineage, accounting for 42.5% of PitNETs in a large surgical series [36]. Tumors of PIT1 lineage comprise about 30% of surgically treated PitNETs; more than half are associated with growth hormone excess. Tpit lineage tumors represent approximately 15% of PitNETs [9, 26, 27, 32, 36, 37].

PitNETs are more common in women who also usually present at a younger age; female predominance is most striking in tumors that secrete prolactin or ACTH. In contrast, men present at older ages and more often have clinically nonfunctioning tumors [9, 36]. PitNETs are rare in children [38] and less that 10% are diagnosed before the age of 20 years [39, 40] and they tend to be associated with hormone excess [36, 41].

The incidence of multiple PitNETs is increasing over time [16, 42–49], likely because of the increased sophistication of morphologic classification using immunohistochemistry to localize hormones and transcription factors.

Tumor Classification and Morphology

There are many types of tumors that occur in the sella turcica. The most common are PitNETs, but other lesions in the differential diagnosis include craniopharyngiomas and the rare malignant pituitary blastomas that arise from the pituitary anlage and resembling fetal pituitary gland of 10–12 weeks of gestation; tumors of the brain including meningiomas, gliomas, and hypothalamic neurons (which can occasionally cause hormone-excess syndrome mimicking adenohypophysial tumors); paragangliomas (non-epithelial neuroendocrine neoplasms) and Schwannomas; tumors of bone and soft tissue; lymphoid neoplasms; germ cell tumors; and many tumor-like lesions including inflammatory processes, cysts, and hormonally active hyperplasias [13]. This chapter will discuss only PitNETs.

Tumors of adenohypophysial cells are usually classified clinically based on their hormonal activity. They are also classified based on their size and invasiveness; radiologists use one of multiple classifications initially proposed by Hardy [50] but subsequently revised by Knosp and others [51, 52] that distinguish microtumors (≤1 cm) from macrotumors (>1 cm) and characterize the degree of invasion upward into the suprasellar space and hypothalamus, laterally into the cavernous sinuses, and downward into the sphenoid sinuses.

The morphology of PitNETs is exceptionally complex. The nontumorous adenohypophysis is composed of at least six distinct hormone-secreting cell types, and each of these cell types can give rise to at least one but often multiple tumor types [13] as shown in Table 4.1. The classification of these tumors is of prognostic and predictive value [53]. Previous classifications based on cytoplasmic acidophilia, basophilia, or chromophobia and other histochemical stains were replaced by immunohistochemical localization of hormones, a classification that was refined by electron microscopy [9]. The addition of immunostains for the transcription factors that define adenohypophysial cell lineage (Fig. 4.1) has allowed the development of a sophisticated approach to the recognition of plurihormonal PitNETs that may be well differentiated or poorly differentiated (i.e., lacking terminal differentiation toward a distinct adenohypophysial cell phenotype) and to distinguish hormonally active and hormonally silent tumors of various cell types that have distinct pathogenetic mechanisms which determine optimal therapeutic approaches [10, 12, 13].

Adenohypophysial tumors may secrete hormones in excess or may be clinically hormonally inactive. When hormonally active, they may be monohormonal or plurihormonal. These tumors also grow within the confined space of the cranium, and as they enlarge, they cause mass effects, usually resulting in headache, visual disturbances, and hypopituitarism. Involvement of cranial nerves other than the optic chiasm is unusual and, when present, should point to a tumor of another type.

Tumors that secrete *adrenocorticotropic hormone (ACTH)* in excess cause Cushing disease. The manifestations of this disorder result from excess

Table 4.1 Classification of adenohypophysial cells and pituitary neuroendocrine tumors

Cell type	Transcription factor(s)	Hormone(s)	Keratin (CAM 5.2 or CK18)	Tumor variant	Hormone-excess syndrome[a]
Corticotroph	TPIT (TBX19), NeuroD1/ β2	ACTH and other POMC derivatives	Strong	Densely granulated	Florid Cushing, often microtumor
			Variable	Sparsely granulated	Subtle Cushing, often macrotumor
			Intense ring-like perinuclear	Crooke cell	Variable, Cushing but may be unusual
Somatotroph	PIT1	GH, α-subunit	Perinuclear	Densely granulated	Florid acromegaly
	PIT1	GH	Fibrous bodies (>70%)	Sparsely granulated	Subtle acromegaly
Mammosomatotroph	PIT1, ERα	GH (often predominant), PRL, α-subunit	Perinuclear	Mammosomatotroph	Acromegaly and hyperprolactinemia[b]
	PIT1, ERα, GATA2/3	GH (often predominant), PRL, α-subunit, βTSH	Perinuclear	Well-differentiated Pit1-lineage plurihormonal tumor	Acromegaly, hyperprolactinemia[b], and hyperthyroidism
Lactotroph	PIT1, ERα	PRL	Weak or negative	Sparsely granulated	Hyperprolactinemia[b]
	PIT1, ERα	PRL	Weak or negative	Densely granulated	Hyperprolactinemia[b]
Acidophil stem cell[c]	PIT1, ERα	PRL (predominant), GH (focal/ variable)	Scattered fibrous bodies		Hyperprolactinemia[b] and subclinical acromegaly
Poorly differentiated PIT1[c]	PIT1, ERα, GATA2/3	GH, PRL, α-subunit, βTSH	Focal/ variable		Acromegaly, hyperprolactinemia[b], and hyperthyroidism
Thyrotroph	PIT1, GATA2/3	α-Subunit, βTSH	Weak or negative		Hyperthyroidism
Gonadotroph	SF1, ERα, GATA2/3	α-Subunit, βFSH, βLH	Variable		Hypogonadism
Null cell	None	None	Variable		None

[a]Any tumor type can be clinically nonfunctioning

[b]Hyperprolactinemia that is moderate can occur with any sellar mass that has suprasellar extension, interrupting hypothalamic tonic dopaminergic inhibition; however this rarely exceeds 150 ng/ml; lactotroph tumors usually show a characteristic correlation between tumor size and PRL levels, whereas other tumors that secrete PRL do not

[c]Uncertainty if normal counterpart exists

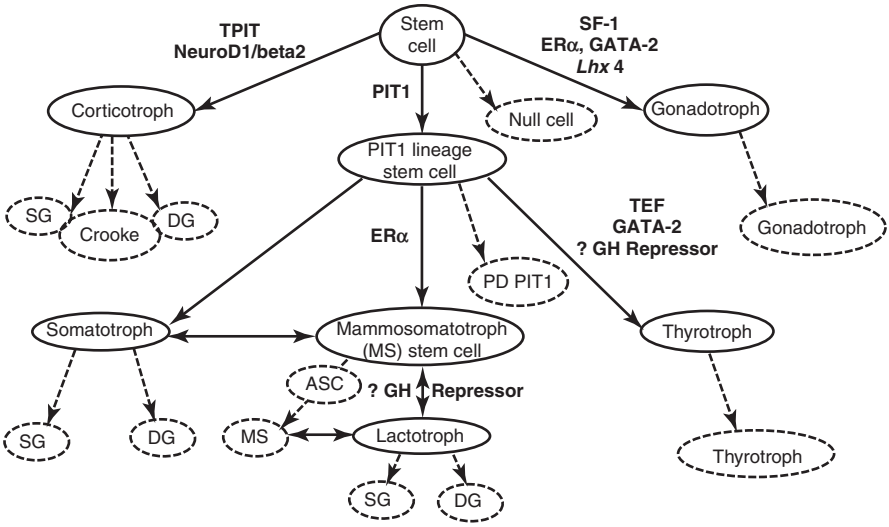

Fig. 4.1 Cytodifferentiation pathways in adenohypophysis and related pituitary neuroendocrine tumors. (Modified from Mete et al. [36])

glucocorticoid secretion from the adrenal cortex: patients are obese with moon facies, buffalo hump, striae, and, in females, hirsutism. Long-term complications include osteoarthritis and immunosuppression. The disease also is implicated in emotional disturbances. There are three variants of corticotroph tumors. Densely granulated tumors are composed of cells that resemble normal "basophilic" corticotrophs that are typically diffusely positive for PAS and ACTH (Fig. 4.2); these are usually small tumors associated with florid Cushing disease and high levels of ACTH. Sparsely granulated tumors are composed of cells that lack the usual large number of secretory granules (focal to weak diffuse staining with PAS and ACTH) but otherwise resemble corticotrophs (Fig. 4.3); these tumors cause less florid Cushing disease, explaining the delayed diagnosis until the tumor is larger at diagnosis. Most functional corticotroph tumors show loss of p27 expression (Fig. 4.2); retained p27 expression is more frequent in nonfunctional corticotroph tumors (Fig. 4.3) [36].Crooke cell tumors are composed of unusual cells that show accumulation of keratin filaments that almost completely fill the cytoplasm (Fig. 4.4); this is a response to elevated glucocorticoid levels and is seen in the nontumorous adenohypophysial corticotrophs of patients with pituitary Cushing disease or any other cause of Cushing syndrome. When the change occurs in corticotroph tumor cells, the clinical presentation can vary, often either cyclical or atypical Cushing presentations or clinically silent tumors that can be very large and highly invasive.

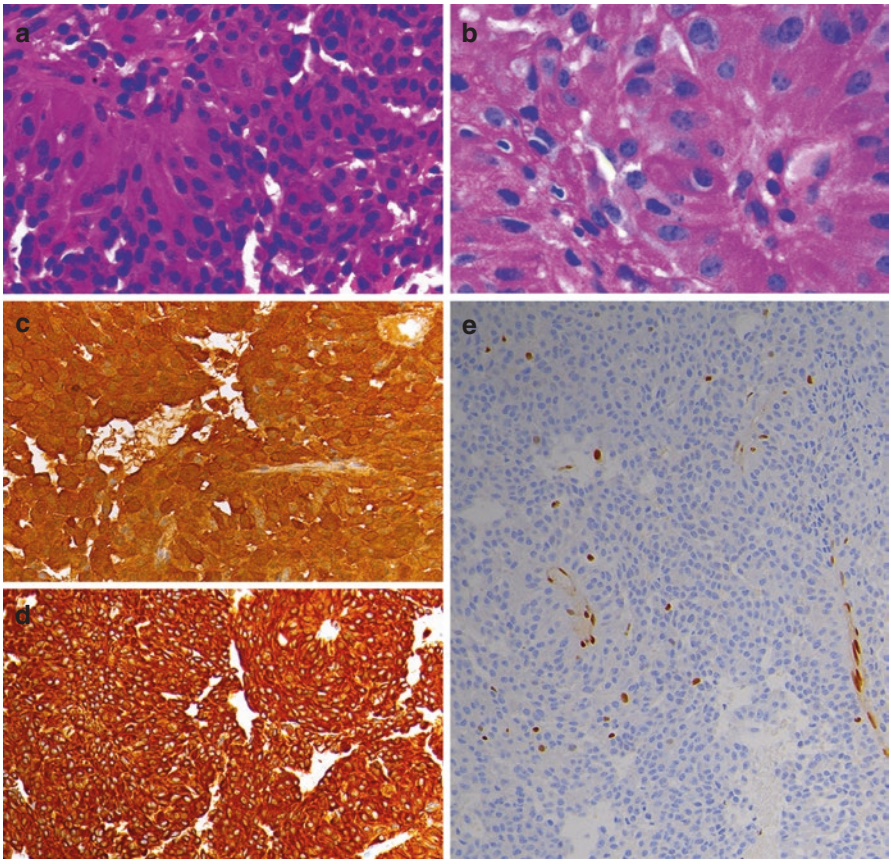

Fig. 4.2 Densely granulated corticotroph tumor. These tumors are distinguished by their distinctive basophilic appearance on hematoxylin and eosin-stained sections (**a**). PAS (**b**) and ACTH (**c**) highlight the numerous secretory granules. These tumors are diffusely positive for CAM5.2 (**d**). Functional corticotroph tumors often show loss of p27 expression (**e**)

Tumors that secrete *growth hormone (GH)* in excess give rise to acromegaly in adults and/or gigantism in children who have onset of the disease before epiphysial fusion. GH excess causes not only the gradual and insidious but often severe disfigurement caused by prolonged soft tissue and bone overgrowth including frontal bossing, prognathism, and acral hypertrophy but also complications that include osteoarthritis, carpal tunnel syndrome, and dental problems, as well as metabolic abnormalities due to persistent excess of insulin-like growth factor-1 (IGF-1) that include diabetes mellitus, cardiac complications, and an increased incidence of cancer. Like other pituitary hormone-induced disorders, there is an emotional component to this disease as well. There are several different types of pituitary

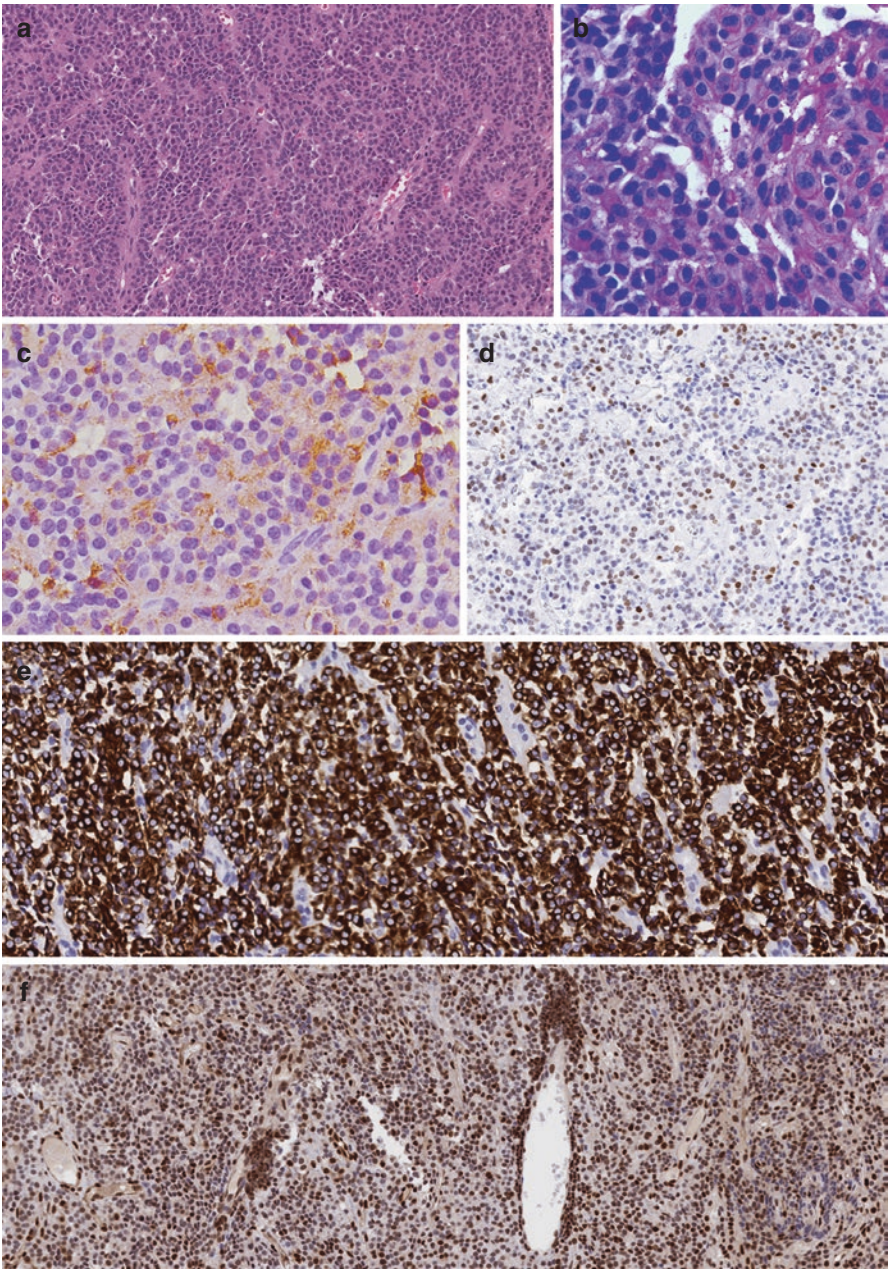

Fig. 4.3 Sparsely granulated corticotroph tumor. Unlike their densely granulated counterparts, these tumors are less basophilic on hematoxylin and eosin-stained sections (**a**). Staining for PAS (**b**) and ACTH (**c**) is variable. Regardless of the cytoplasmic granulation pattern, all corticotroph tumors are positive for TPIT (**d**). Diffuse CAM5.2 reactivity is frequently encountered in sparsely granulated corticotroph tumors (**e**). This composite photomicrograph illustrates a nonfunctional sparsely granulated corticotroph tumor (also known as silent corticotroph tumor, type 2). This tumor shows retained p27 expression (**f**)

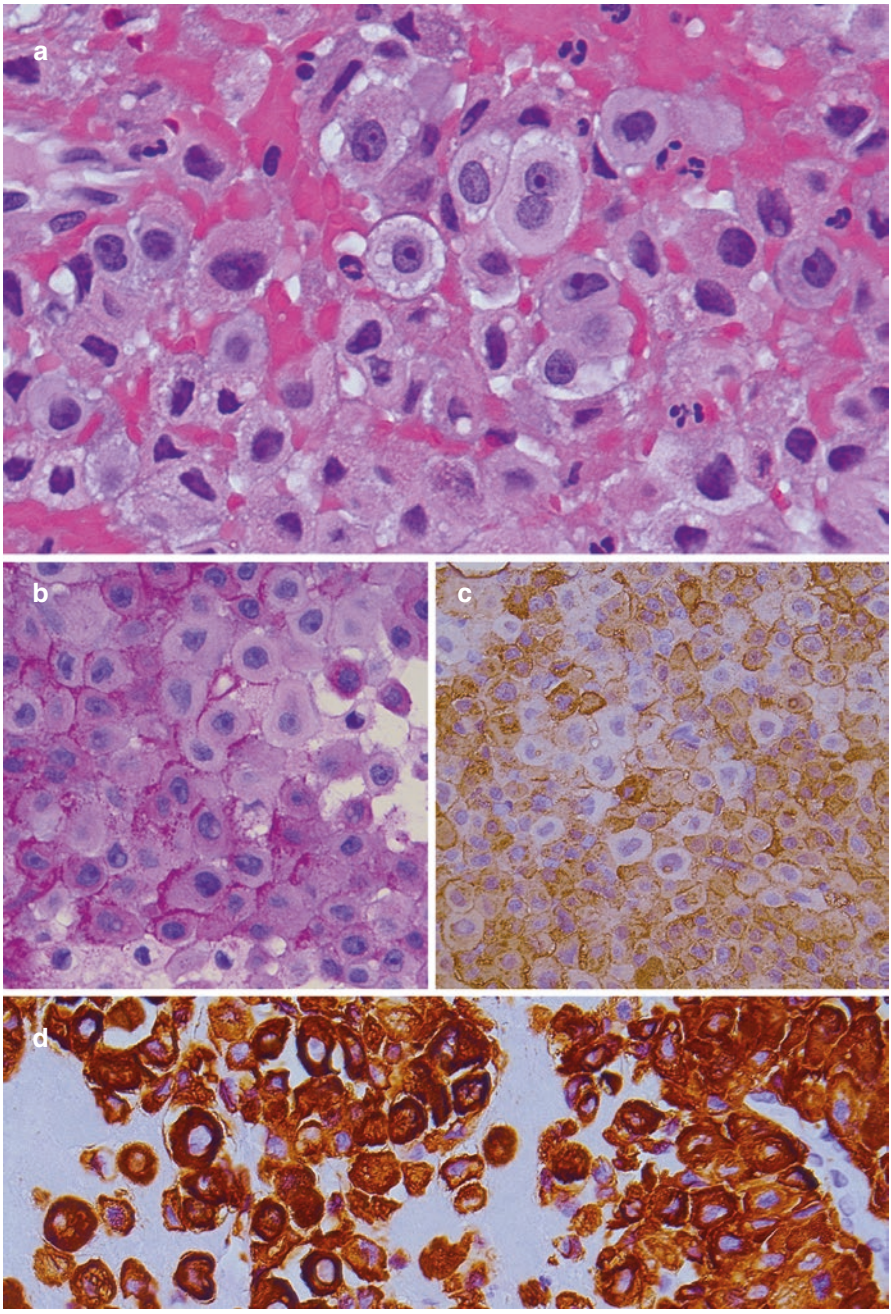

Fig. 4.4 Crooke cell tumor. Corticotroph tumors showing Crooke's hyaline change are aggressive tumors that are distinguished by their characteristic cytomorphologic (**a**) and staining patterns on PAS (**b**), ACTH (**c**), and CAM5.2 (**d**). The basis of this distinct pattern is related to relocation of PAS- (**b**) and ACTH- (**c**) positive secretory granules to the cell periphery and juxtanuclear region. CAM5.2 shows a ring-like staining pattern (**d**)

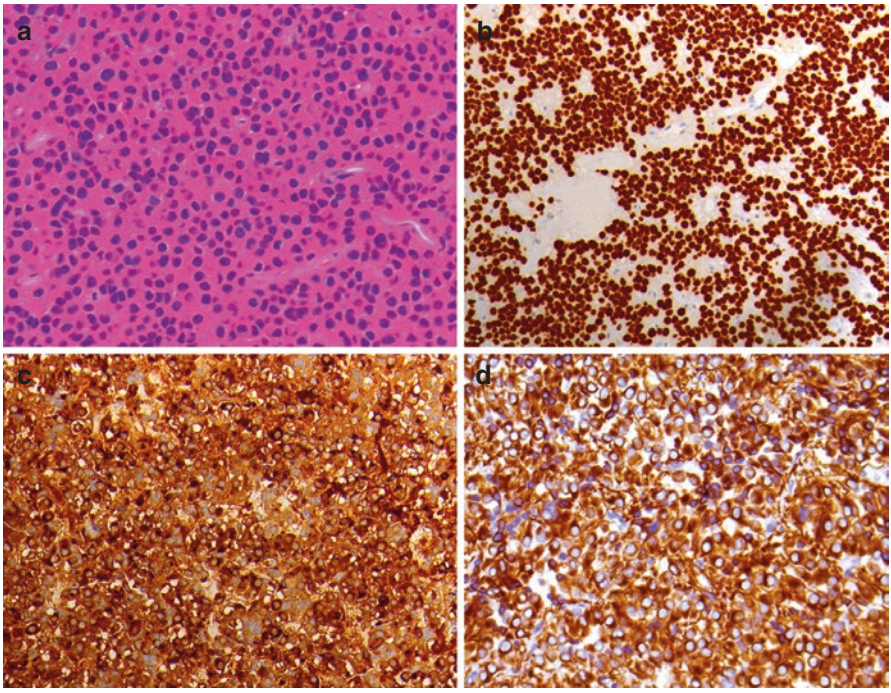

Fig. 4.5 Densely granulated somatotroph tumor. This tumor type consists of acidophilic cells with bright cytoplasmic eosinophilia (**a**). Diffuse staining for PIT1 (**b**), GH (**c**), and alpha-subunit (not illustrated herein) and perinuclear CAM5.2 staining pattern (**d**) are characteristics of this tumor

neuroendocrine tumors that can cause GH excess. The commonest are the densely granulated tumors that are composed of cells that resemble normal somatotrophs (Fig. 4.5); they are diagnosed in older patients and are associated with florid acromegaly and very high GH and IGF-1 levels. Slightly less common are sparsely granulated somatotroph tumors that are composed of atypical somatotrophs that have an abundant accumulation (>70% of the tumor) of keratin filaments in juxtanuclear aggregates known as "fibrous bodies" (Fig. 4.6); these tumors are diagnosed in younger patients and often present as larger tumors than their densely granulated counterparts but with less elevation of GH and IGF-1. Rare examples of densely granulated somatotroph tumors with variable fibrous bodies accounting for less than 70% of the tumor cells have been recognized. These tumors are also known as intermediate granulated somatotroph tumors (Fig. 4.7) and are often classified as densely granulated somatotroph tumors as they are biologically closer to their densely granulated counterparts [54]. Rare cases of acromegaly are caused

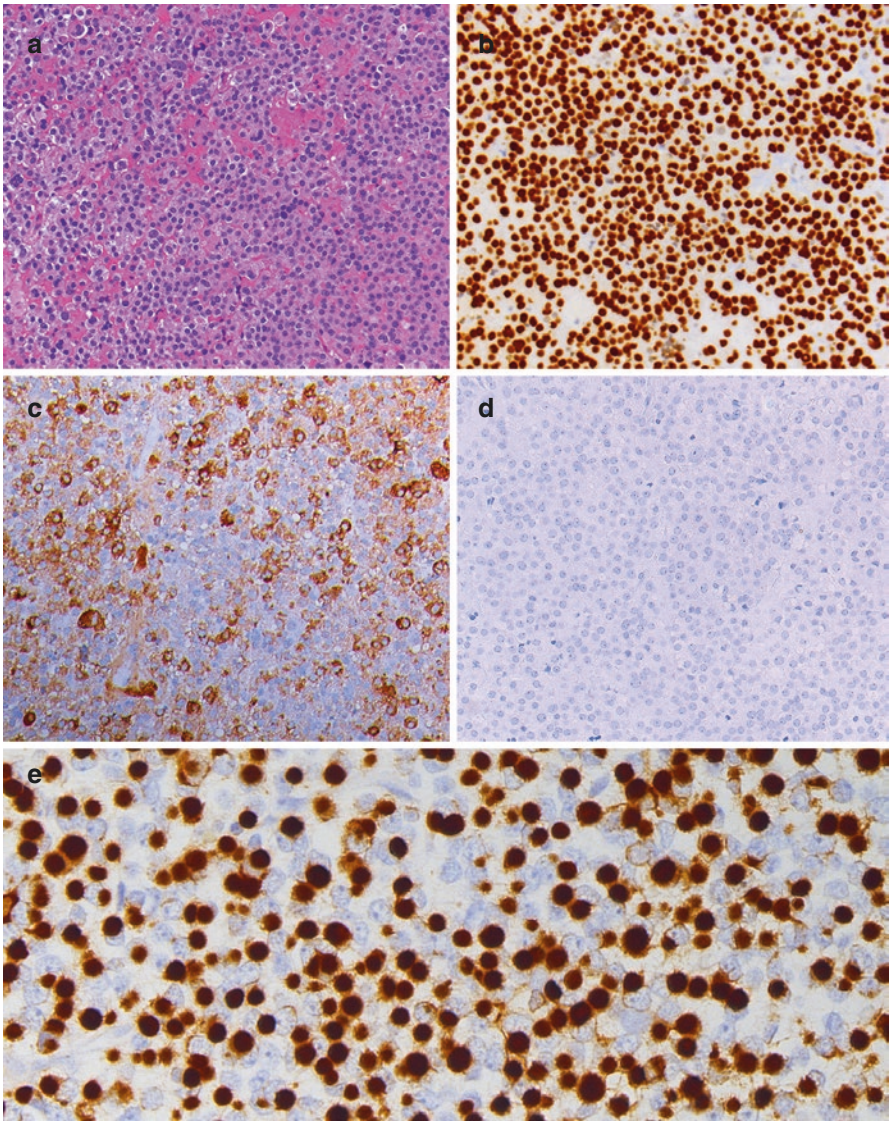

Fig. 4.6 Sparsely granulated somatotroph tumor. Unlike their densely granulated counterparts, these tumors contain sparse granulation that results in a less acidophilic, often chromophobic appearance (**a**). Diffuse PIT1 expression (**b**), variable GH expression (**c**), and absence of alpha-subunit expression (**d**), as well as abundant fibrous bodies on CAM5.2 (**e**), are characteristics of this tumor type

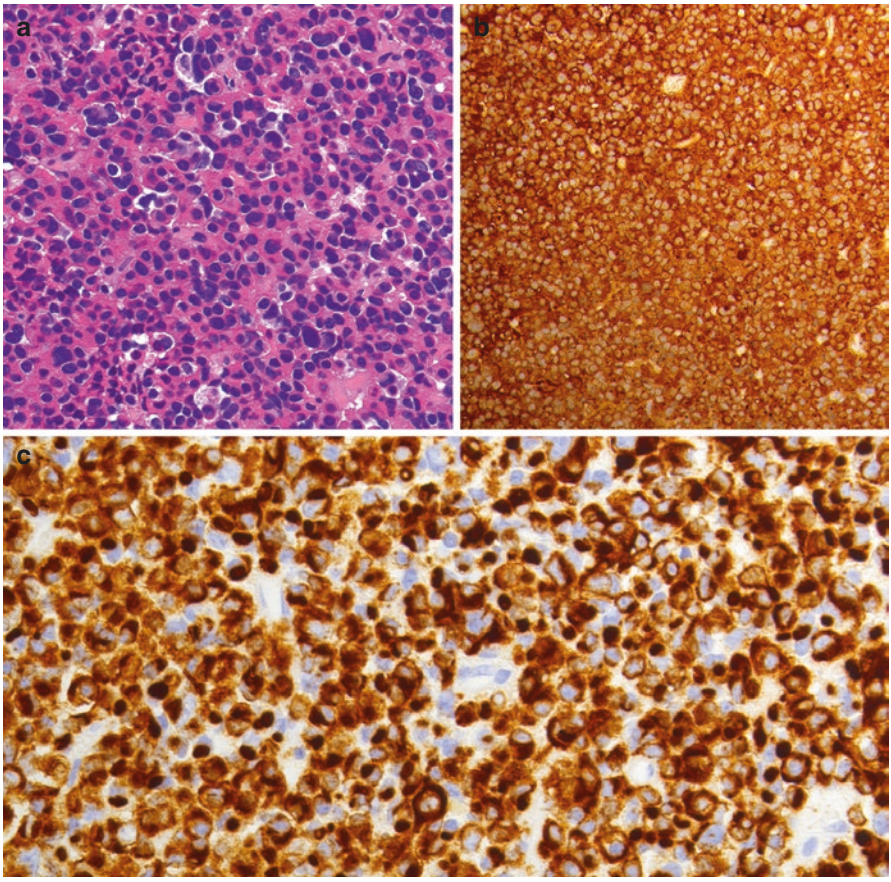

Fig. 4.7 Intermediate granulated somatotroph tumor. This tumor is considered to be a morphologic variant of densely granulated somatotroph tumors. Intermediate granulated somatotroph tumors display variable cytoplasmic acidophilic appearance (**a**) and are often diffusely positive for GH (**b**) and alpha-subunit (not illustrated here). CAM5.2 shows perinuclear keratin as well as scattered fibrous bodies that are present in less than 70% of tumor cells (**c**)

by mammosomatotroph tumors that make both GH and PRL (Fig. 4.8) or even less often well-differentiated plurihormonal PIT1-lineage tumors that resemble mammosomatotrophs but also make TSH.

Tumors that secrete *prolactin (PRL)* in excess resulting in hyperprolactinemia cause gonadal insufficiency resulting in infertility, menstrual irregularities in women, loss of libido, reduction in bone and muscle mass, and emotional sequelae. In severe cases, galactorrhea may occur. The most common pituitary neuroendocrine tumor is a prolactinoma that is usually highly responsive to dopamine antagonism, resulting in correction of hormone levels and tumor shrinkage; these tumors are therefore treated medically and do not come to surgery. Those that are resistant and excessively large or arise in patients who cannot tolerate the medication come

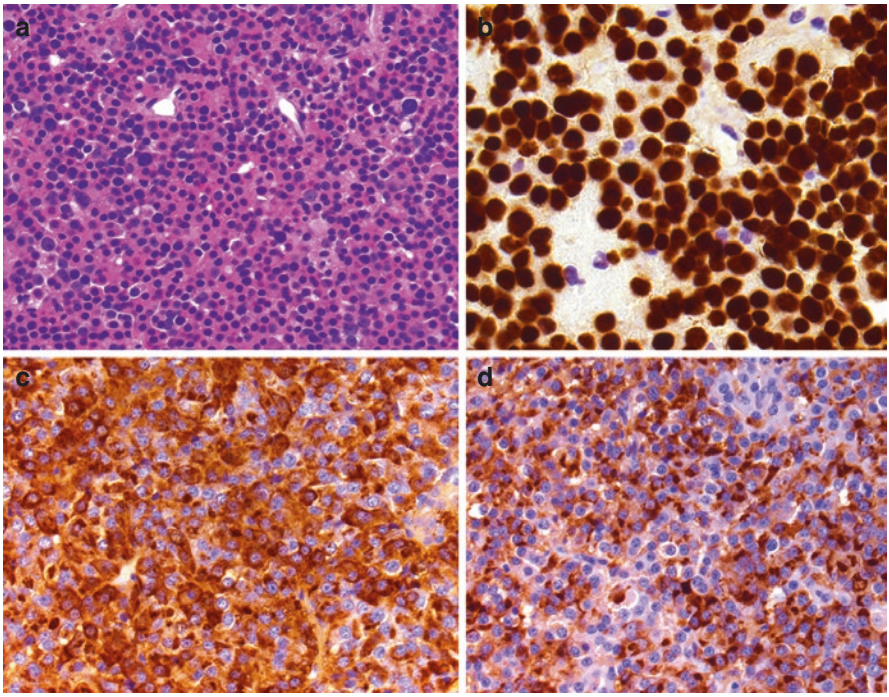

Fig. 4.8 Mammosomatotroph tumor. These tumors are variably acidophilic on hematoxylin-eosin-stained sections (**a**). Diffuse PIT1 (**b**) and GH (**c**) expression along with variable PRL (**d**) expression are characteristic features of these neoplasms

to surgery, and at resection, they are usually sparsely granulated lactotroph tumors that are composed of cells that resemble normal active lactotrophs that have a characteristic juxtanuclear staining pattern of hormone in the Golgi apparatus (Fig. 4.9). Very rarely, a lactotroph tumor may be densely granulated. Many other tumors can also cause hyperprolactinemia; it must be noted that any sellar mass that has suprasellar extension can interrupt hypothalamic tonic dopaminergic inhibition, resulting in elevated PRL levels; however this rarely exceeds 150 ng/ml. Lactotroph tumors usually have a tight correlation between tumor size and PRL level. However other tumors can also synthesize and secrete PRL that is not proportional to tumor size; these may be associated with acromegaly (mammosomatotroph or plurihormonal PIT1-lineage tumors) or even mixed tumors that are composed of two discrete populations of densely or sparsely granulated lactotrophs and somatotrophs (Fig. 4.10). An unusual tumor is the acidophil stem cell tumor that is composed of oncocytic cells that resemble lactotrophs but may also synthesize GH (Fig. 4.11); these tumors do not show the appropriate size and PRL level correlation and are usually resistant to dopaminergic inhibition.

Tumors that secrete *thyrotropin (thyroid-stimulating hormone, TSH)* in excess cause hyperthyroidism. These rare thyrotroph tumors tend to be large and aggressive

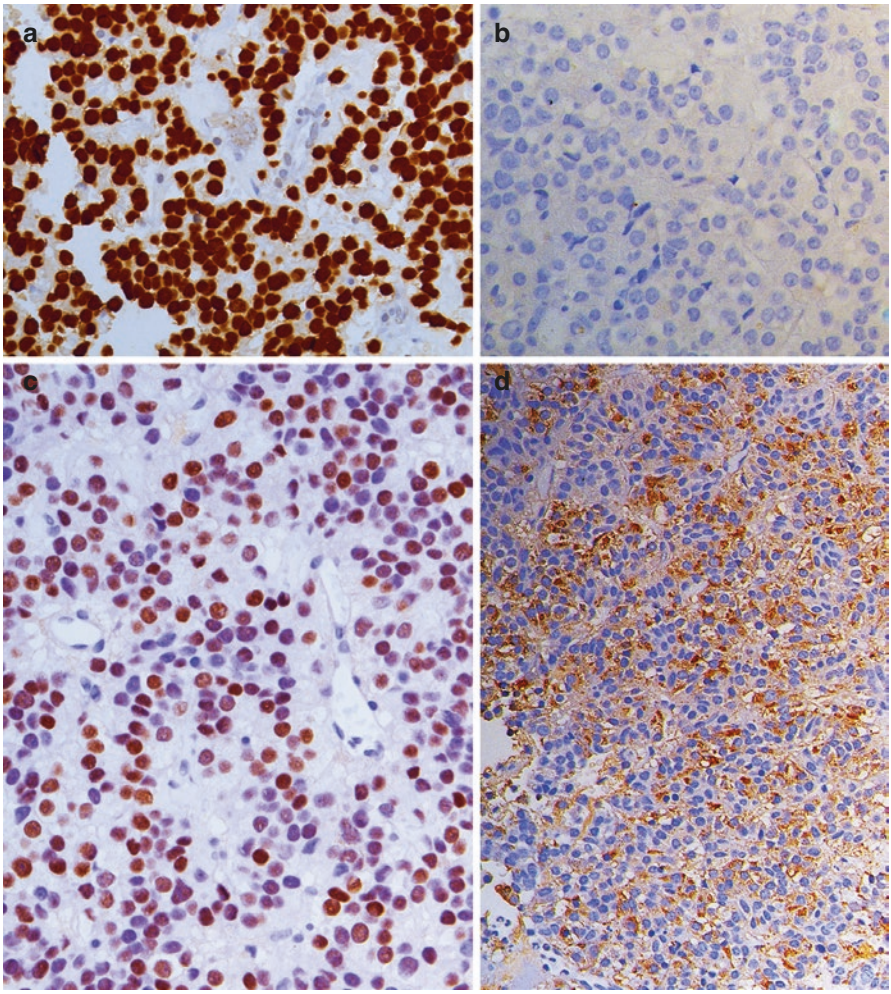

Fig. 4.9 Sparsely granulated lactotroph tumor. This is the common histologic subtype among pituitary neuroendocrine tumors causing PRL excess. Positivity for PIT1 (**a**) and absence of alpha-subunit expression (**b**) are features of lactotroph cells. Most lactotroph tumors are also positive for ER-alpha (**c**). A paranuclear PRL staining pattern distinguishes sparsely granulated tumors (**d**)

with intense fibrosis that makes resection difficult. They are composed of polygonal and spindle-shaped cells that resemble normal thyrotrophs (Fig. 4.12). A primitive form of tumor that resembles thyrotrophs but is often plurihormonal is the poorly differentiated PIT1-lineage tumor that may cause hyperthyroidism but may also cause acromegaly or hyperprolactinemia or be clinically silent (Fig. 4.13). These tumors are characteristically more aggressive and infiltrative, resulting in an inability to secure complete resection at surgery.

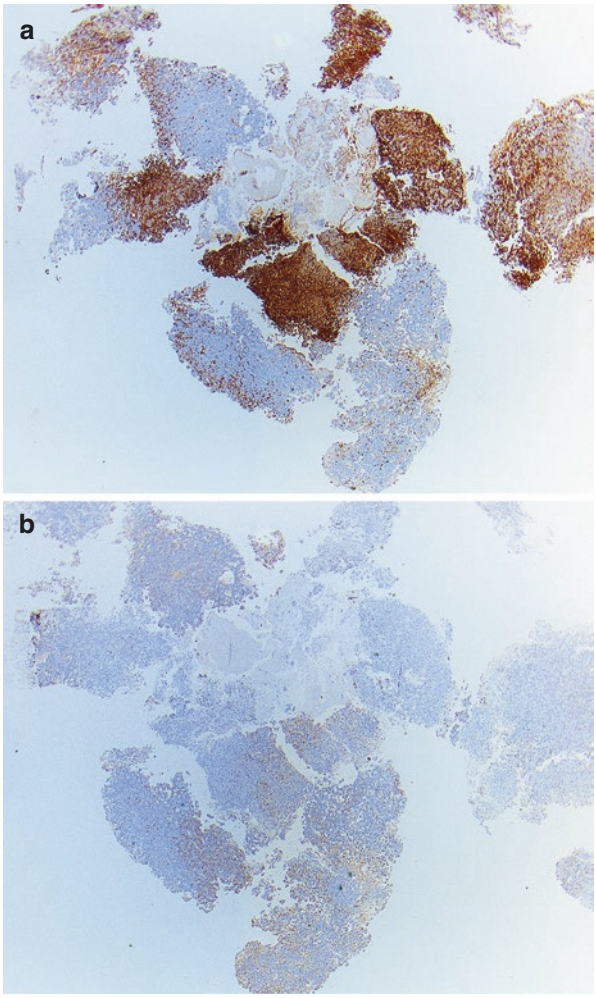

Fig. 4.10 Mixed densely granulated somatotroph and sparsely granulated lactotroph tumor. This composite photomicrograph illustrates the distribution of GH-expressing densely granulated somatotroph tumor (**a**) and PRL-expressing sparsely granulated lactotroph tumor (**b**) components

Tumors that secrete *gonadotropins (follicle-stimulating hormone and/or luteinizing hormone (FSH, LH)* in excess are rarely clinically functioning, instead resulting in paradoxical hypogonadism. These gonadotroph tumors are the most frequent tumors in surgical series, being less common than prolactinomas, but because they do not respond to medical therapy, they require surgery. Morphologically they resemble normal gonadotrophs (Fig. 4.14). The routine use of pituitary transcription factors underscored the presence of gonadotropin-immunonegative gonadotroph tumors [36]. In our experience, this subgroup constitutes around 40% of all gonadotroph tumors [36].

Rare *unusual plurihormonal tumors* occur; they are usually mixed or "composite" tumors that have individual components as described above [49], but true plurihormonal tumors that cross lineage boundaries do occur [55].

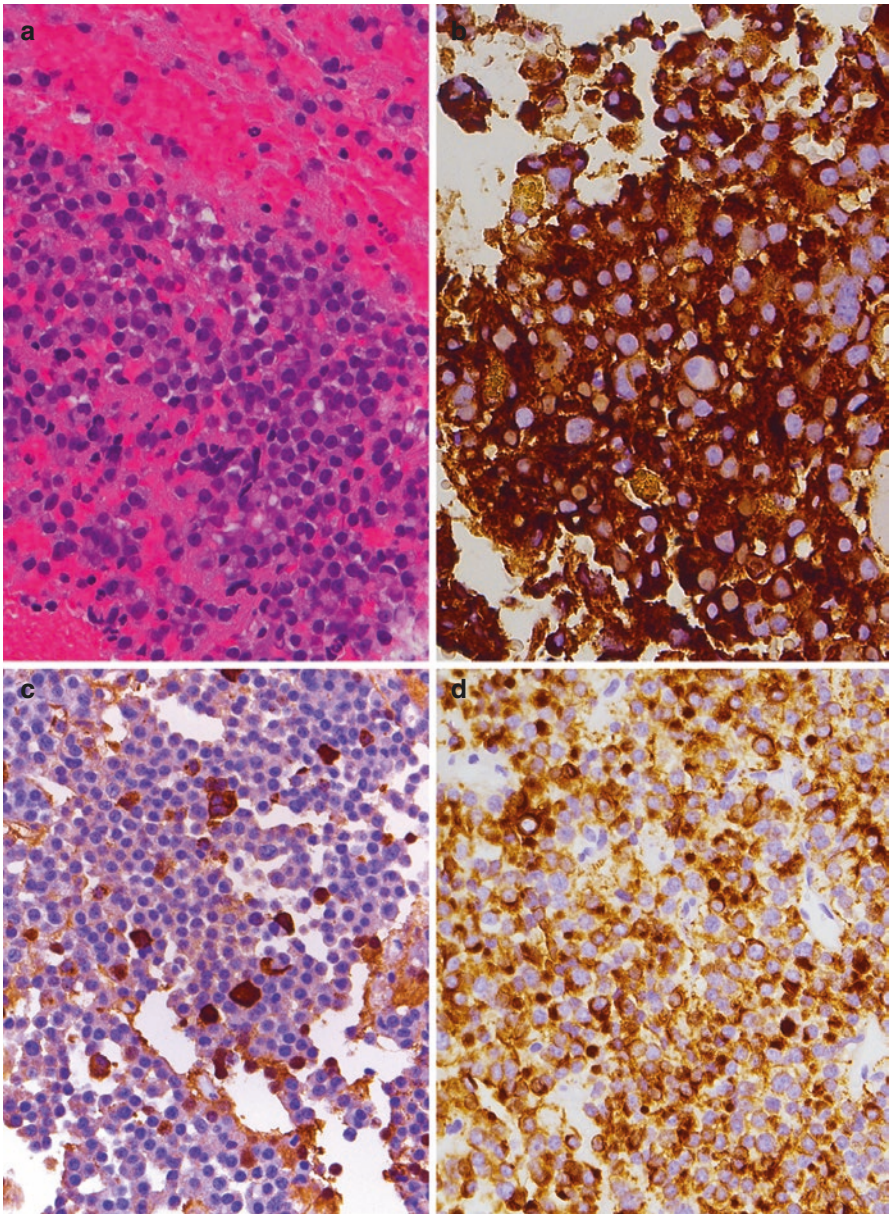

Fig. 4.11 Acidophil stem cell tumor. This is a rare PIT1-lineage family tumor with variable oncocytic change and intracytoplasmic vacuoles (**a**). Most cases show a predominant PRL reactivity, which can sometimes be diffuse as seen in densely granulated lactotroph tumors (**b**). Variable GH expression (**c**) and scattered fibrous bodies on CAM5.2 (**d**) are common in these neoplasms

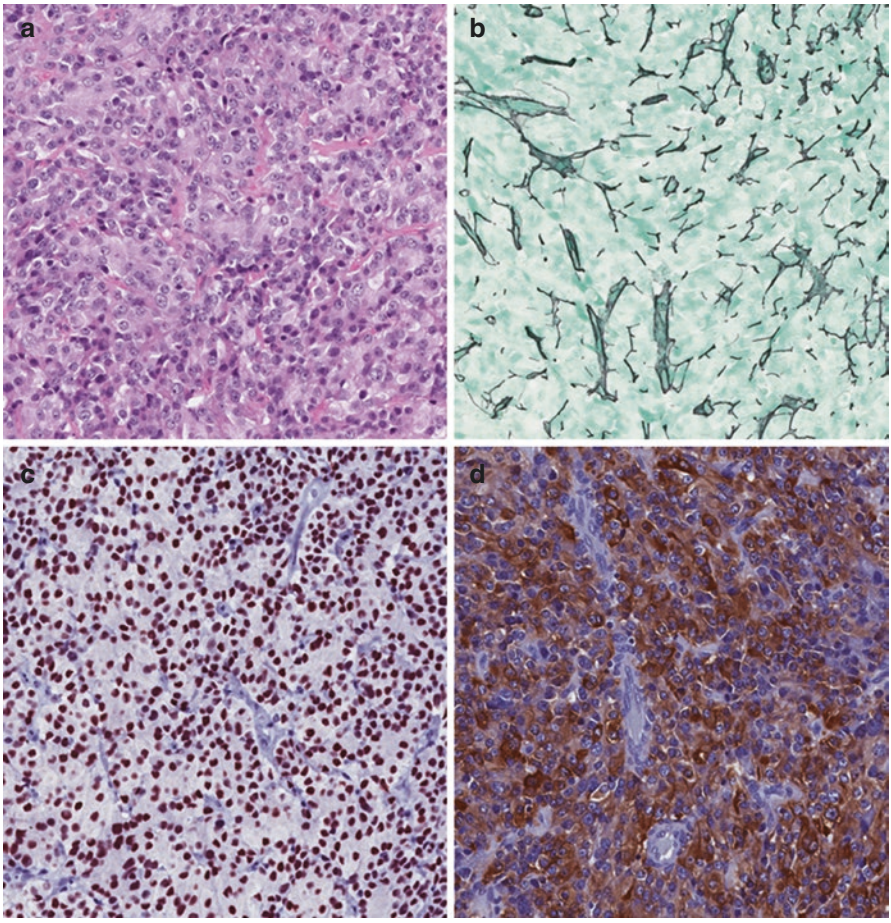

Fig. 4.12 Thyrotroph tumor. These tumors are composed of polygonal cells that often have abundant cytoplasm (**a**). It is important to perform a reticulin stain to exclude the possibility of thyrotroph hyperplasia; the complete breakdown of reticulin is the hallmark of neoplasia (**b**). These tumors have strong nuclear reactivity for PIT1 (**c**) and GATA3 (not shown), and staining for TSH shows diffuse cytoplasmic positivity (**d**)

Tumors that are clinically unassociated with hormone excess can be of any of the above morphologic types as well as in the setting of null cell tumors. By far the most common are gonadotroph tumors; they tend to be indolent, and even if not resectable, they regrow slowly and, because they are soft, rarely cause dramatic local effects. In contrast, other clinically silent tumors, in particular silent corticotroph tumors (Fig. 4.6), silent somatotroph tumors, and silent plurihormonal tumors, are considered to be more aggressive; they are often more invasive and tend to be hard, causing significant hypopituitarism and local mass effects, and they are usually diagnosed when they are large and not surgically resectable.

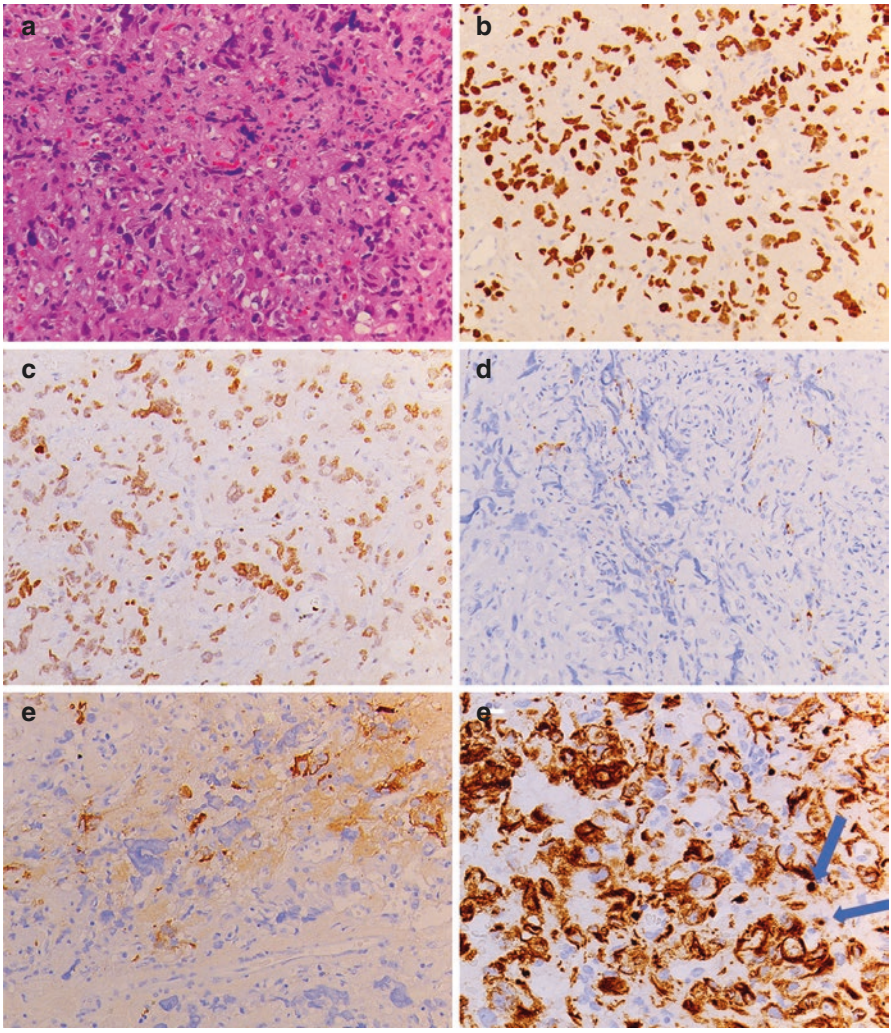

Fig. 4.13 Poorly differentiated PIT1-lineage pituitary neuroendocrine tumor (formerly known as silent subtype 3 pituitary adenoma). These tumors often show mild to severe atypia (**a**). Diffuse PIT1 expression (**b**) along with focal/variable staining for one or more than one PIT1 lineage hormone is a characteristic finding. These tumors can also express GATA3 (**b**). Some cases can be hormone negative. In this composite photomicrograph of a single tumor, there is scattered/focal staining for PRL (**c**) and beta-TSH (**d**). Scattered fibrous bodies can also be a feature of this tumor (**e**; arrows indicate fibrous bodies)

Pituitary carcinoma is defined as a PitNET with cerebrospinal or distant metastasis [13] (Fig. 4.15). This is a very rare occurrence and therefore these tumors are exceptional.

Pituitary blastoma is a malignant triphasic pituitary neoplasm consisting of cells resembling rosette- or gland-making Rathke's epithelium admixed with small folliculostellate cells and large secretory adenohypophysial cells. Most of the affected patients manifested with Cushing syndrome [56, 57] (Fig. 4.16).

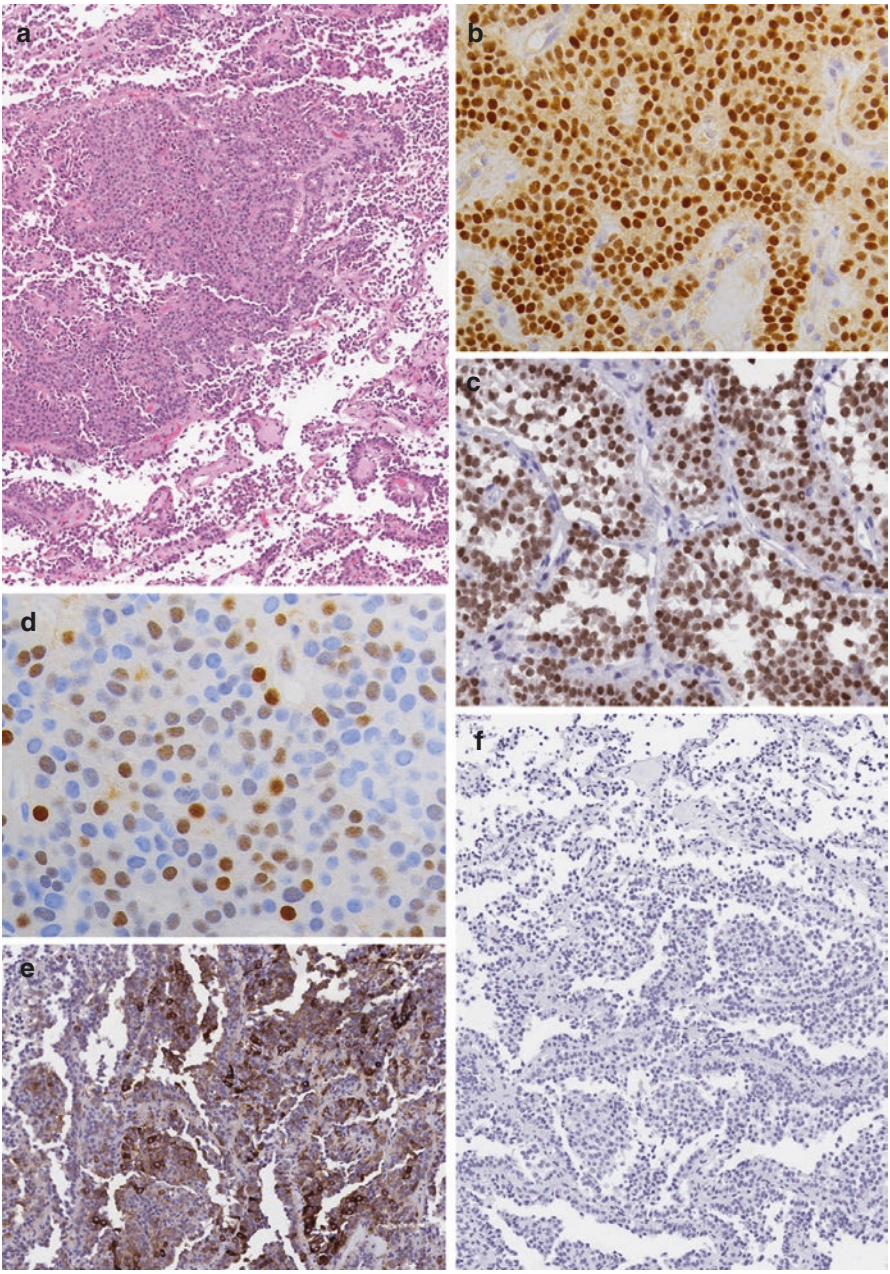

Fig. 4.14 Gonadotroph tumor. Pituitary neuroendocrine tumors of gonadotroph cell lineage have a characteristic histologic pattern with perivascular rosette formation (**a**); they are distinguished by positivity for SF1 (**b**), GATA3 (**c**), ER-alpha (**d**), and gonadotropins (**e**; here beta-FSH is illustrated). SF1 stands out as the best performing biomarker in the distinction of gonadotroph cell lineage, as gonadotropins can sometimes be negative in these tumors. Since GATA3 and ER-alpha expression can also be seen in PIT1-lineage pituitary neuroendocrine tumors, the use of multiple biomarkers should be considered. CAM5.2 negativity is not an uncommon finding in gonadotroph tumors (**f**)

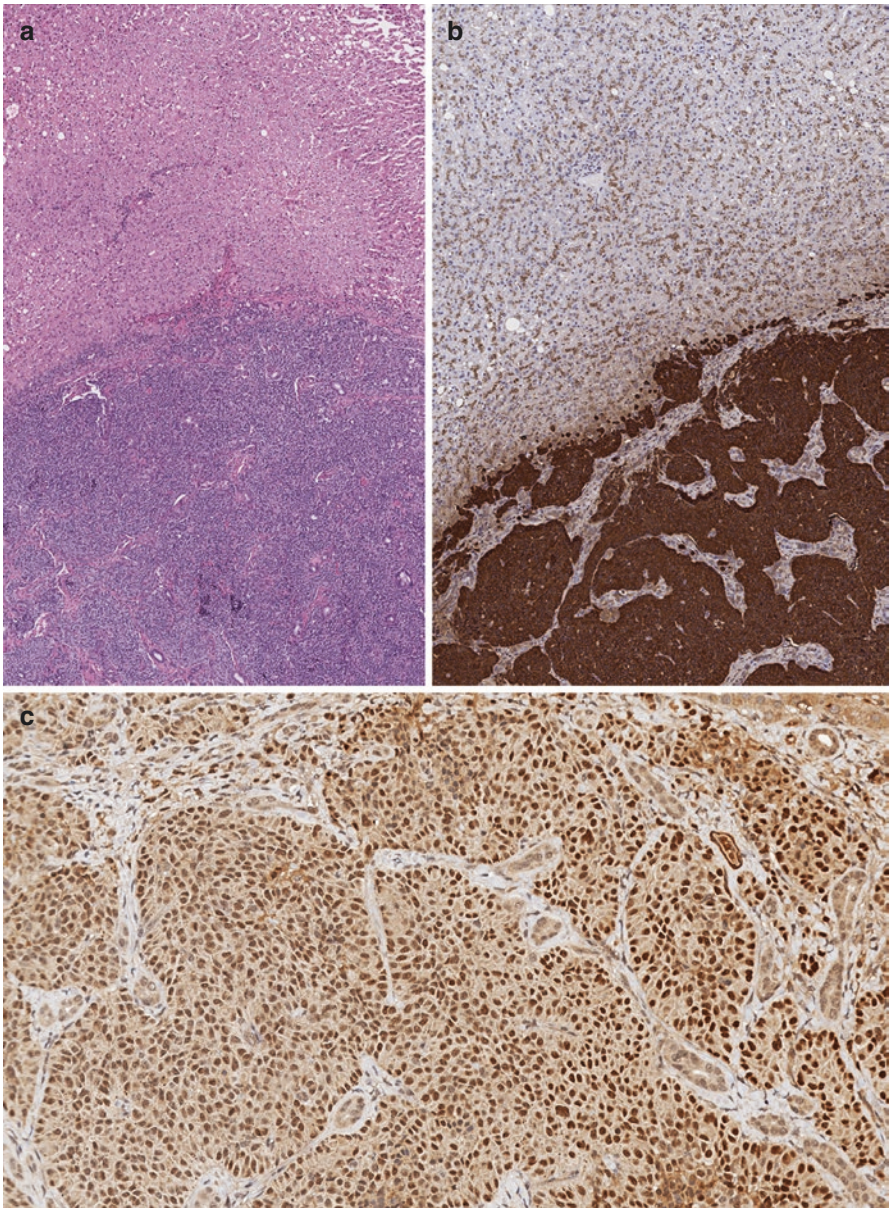

Fig. 4.15 Pituitary carcinoma. This composite photomicrograph illustrates metastatic corticotroph carcinoma in the liver (**a**). Positivity for ACTH (**b**) alone cannot be used to confirm corticotroph origin as several other neuroendocrine neoplasms can express ACTH. Positivity for TPIT confirms pituitary origin (**c**)

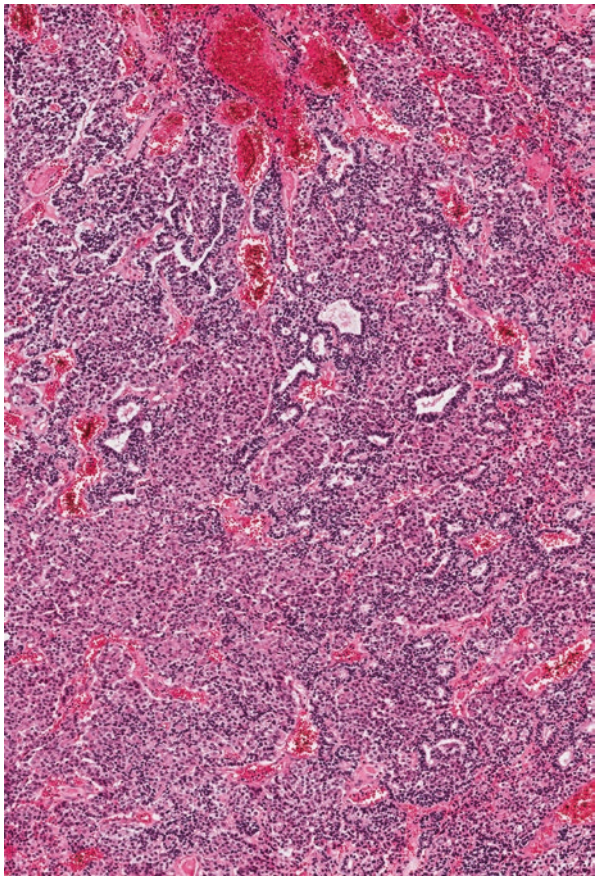

Fig. 4.16 Pituitary blastoma. This entity is a malignant pituitary tumor that stands out as one of the hallmarks of DICER syndrome. The tumor consists of cells resembling rosette- or gland-making Rathke's epithelium admixed with small folliculostellate cells and large secretory adenohypophysial cells

Molecular Pathogenesis

The pathogenesis of PitNETs is as complex as the classification. There are several genetic alterations that have been identified as causative of some tumors. However, the majority of PitNETs have no detectable genetic mutation and it appears that epigenetic changes are implicated in many cases.

The earliest genetic information comes from family studies that identified PitNETs as a component of the syndrome of multiple endocrine neoplasia type 1 (MEN1); when the *MEN1* gene was cloned, it was proven to show loss of heterozygosity (LOH) with loss of the normal allele in patients with one mutant allele [58, 59]; however it became rapidly evident that sporadic tumors do not show mutation and/or LOH [59, 60]. PitNETs are also a component of the MEN4 syndrome

associated with mutations in *CDKN1B* [61] and Carney complex due to mutations in *PRKR1A* [62], but these genes are not typically mutated in sporadic tumors. A familial syndrome of isolated pituitary tumors has been attributed to mutations in the *AIP* gene [63]; somatotropinomas are prevalent in this disorder but other PitNET types also occur. Again, mutations are not found in sporadic tumors; however there has been evidence of increased frequency of epigenetic silencing of *AIP* in sparsely granulated somatotroph tumors [64]. Other more rare familial predisposition syndromes include *succinate dehydrogenase (SDH) complex* mutations [65] and Xq26 microduplications and *GPR101* mutation that give rise to X-linked acrogigantism (X-LAG) with early childhood onset of somatotroph or mammosomatotroph hyperplasia and neoplasia [66, 67]. Isolated case reports of extra-colonic manifestation of Lynch syndrome [68], VHL disease [69], and germline *MAX* mutations [70] have expanded the possibility of pituitary neuroendocrine tumors in these circumstances.

Despite the frequent occurrence of PitNETs in these familial syndromes, the vast majority of tumors are sporadic. Causative mutations have been identified in a few specific types.

The first genetic alteration described in PitNETs was the family of G-protein oncogenes (called *gsps*) that were identified in a subset of somatotroph tumors as well as in other endocrine hormone-secreting tumors [71, 72]. These mutations of *GNAS,* which more frequently involve the maternal allele consistent with monoallelic imprinting of this gene [73]**,** result in constitutive activation of Gsα that raises intracellular cAMP levels, stimulating hormone secretion and cell proliferation. In the pituitary, *GNAS* mutations are characteristic of densely granulated somatotroph tumors [74, 75], and the high cAMP levels account for both the co-expression of α-subunit and the clinical responsiveness of these tumors to somatostatin analogue therapy [76–79]. Germline mosaic *GNAS* mutation is also the cause of the McCune-Albright syndrome [80] which is associated with somatotroph hyperplasia or tumor [81].

A subset of corticotroph tumors harbors mutations in the deubiquitinase *USP8* that are thought to impair the proteasomal degradation of EGF-R [82–84]. It appears that this mutation is characteristic of small, densely granulated corticotroph tumors and may predict responsiveness to pasireotide [84]. A recent study also showed another deubiquitinase *USP48* mutation in *USP8* wild-type corticotroph tumors which tend to manifest in female patients with smaller tumors [85].

A number of endocrine-specific genetic alterations have been described in occasional tumors of the various types; these alterations result in altered hormone regulation that can affect cellular activity and potentially result in cell proliferation [12, 13].

It is becoming clear that the vast majority of PitNETs have no detectable genetic coding alterations. Instead, it appears that these tumors are characterized by epigenetic alterations [86] that result in dysregulated expression of cell cycle proteins pRB1, p21, and p27 [86, 87]. The implicated factors include the DNA methyltransferase (DNMT) enzyme family [86] that regulate DNA methylation; chromatin remodeling by *Ikaros*, a factor that regulates multiple promoters through deacetylation, HDAC, non-HDAC, and methylation-dependent mechanisms; and the High

Mobility Group (HMG) proteins containing AT-hook domains (HMGA) that are also involved in DNA binding and chromatin remodeling [88–92]. MicroRNAs (miRs) may also play a role in this epigenetic landscape [86, 93]. The causes of such epigenetic changes are largely unknown, but environmental factors such as stress and pollution have been implicated.

The role of mutations in progression of pituitary carcinoma may be more convincing. *RAS* mutations are largely restricted to the rare pituitary carcinomas [94, 95]; in one study the mutation was identified in the metastatic deposits of but not the primary in three cases [94]. It appears that the disease progresses by accumulation of additional genetic alterations [96, 97]. The retinoblastoma gene (*RB1*) has also been implicated [98], as may be *TP53* [99–101], *ATRX*, and *PTEN* [102]. Pituitary carcinoma has been reported in a patient with *SDHB* mutation [103] and a patient with Lynch syndrome [104]. *DICER1* mutations constitute the hallmark of pituitary blastomas [57].

Prognosis

The prognosis of PitNETs is dependent on multiple factors. These tumors can be small, hormonally inactive, slow-growing lesions that may be detected only incidentally. They can be small and slow-growing but hormonally active and cause major sequelae; it has been suggested that untreated Cushing disease causes death in 4 years on average. Some are readily treated with medical therapy; most patients with prolactinoma respond to dopamine agonists with normalization of hormone levels and tumor shrinkage that may be permanent. However, the mainstay of therapy for the other types of PitNETs is surgical resection. When a pituitary tumor is diagnosed early and in the hands of an experienced surgeon, cure can be achieved by total resection. In contrast, many PitNETs are diagnosed when they are no longer amenable to surgical resection because of the degree of parasellar invasion into areas that cannot be removed. In some patients, usually those with clinically nonfunctioning tumors, they are readily treated by surgery for decompression, and with slow regrowth, they may or may not require a second or third operation to manage the mass effects. These patients can receive adequate hormone replacement and the role of external radiation is limited. For those with more aggressive tumors, such as true null cell tumors [105] or the poorly differentiated PIT1-lineage tumors [106], there may be a role for more aggressive surveillance and potentially for radiotherapy. The definition of an "aggressive" tumor remains contentious with some authors proposing the importance of morphology [107] and others emphasizing proliferation [108].

Patients with hormone-secreting tumors that cannot be resected are likely to require medical therapy to normalize hormone levels. Guidelines have been developed for patients with acromegaly [109, 110] with more recent recognition of the importance of tumor subtyping in guiding the management of this disorder [79]. This focused approach has not been taken for Cushing disease but guidelines are available for management of the syndrome in general [111].

The management of aggressive pituitary tumors and pituitary carcinoma may require the use of oncologic agents such as temozolomide [112, 113], concurrent temozolomide and capecitabine [114, 115], bevacizumab [116], and ipilimumab and nivolumab [115].

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Hypothalamic Neuroendocrine Neoplasms

5

Sylvia L. Asa and Ozgur Mete

Historical Background

If the pituitary functions like the "conductor of the endocrine orchestra," by analogy one could consider the hypothalamus to function as the composer of the music. The hypothalamus is now recognized to be the location where signals from the internal milieu and external stimuli are integrated to regulate endocrine homeostasis.

Descartes was the first to recognize that the brain controls the body (1649) [1], and although he initially placed the role of control center in the pineal, the functions that he identified as integrating physiology with external sensation are actually now known to be mediated by the hypothalamus and pituitary. Further evidence of this role emerged in the eighteenth century in the work of Morgagni (1733), Soemmering (1792), Meckel (1802), and Zander (1890) who identified the importance of the brain in the regulation of adrenal structure and function [2]. The hypothalamic regulation of water resorption was shown in the elegant 1849 publication by Claude Bernard who described "le piqûre diabetique" [3]. Hypothalamic regulation of the pituitary was clarified by Harris in 1948 [4], isolation of several important hypothalamic hormones led to a Nobel Prize for Guillemin and Schally in 1977 [5], and the

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early 1980s saw the isolation and characterization of several of the more elusive hormones [6–8].

The hypothalamic nuclei that are responsible for endocrine functions are organized in a specific fashion with centers responsible for circadian rhythm, temperature control, appetite, and emotional responses to stress that then impact pituitary function through the hormonal activity of hypothalamic neurons with axons that extend downward to form the posterior pituitary gland. Their products may be secreted into the portal vasculature so that they regulate adenohypophysial function or they may reach the systemic circulation to regulate endocrine targets elsewhere in the body. While some of these neurons act in true neuronal fashion through synapses to other neurons, those that secrete hormones into the bloodstream represent a hybrid model of neuroendocrinology that is one with true neurons having impact on cells that they do not directly touch, but rather signal through endocrine mechanisms.

The hypothalamic nuclear structure is complex [9, 10] and only the elements involved in endocrine regulation will be discussed here. The pulsatile secretion of growth hormone (GH) is regulated by neurons in the arcuate nucleus, previously called the infundibular nucleus, that synthesize and secrete growth hormone-releasing hormone (GHRH) countered by neurons in the supraoptic and paraventricular nuclei that produce somatostatin [6–8]. Prolactin (PRL) secretion is regulated by tonic inhibition by dopamine produced in the arcuate nucleus. Thyroid function is regulated by pituitary thyrotropin (thyroid-stimulating hormone, TSH) that is regulated by thyrotropin-releasing hormone (TRH) produced in the paraventricular nuclei as well as other smaller nuclei of the anterior hypothalamus [5]. Gonadal function is controlled by pituitary gonadotropins that are regulated by gonadotropin-releasing hormone (GnRH), also produced in the paraventricular nuclei as well as other smaller nuclei of the anterior hypothalamus [5]. The hypothalamus senses ambient glucocorticoid levels and stress and balances them through regulation of pituitary adrenocorticotropic hormone (ACTH) by corticotropin-releasing hormone (CRH) [6, 11]. Appetite regulation is mediated by the medial basal hypothalamus through production of glucagon and other peptides [12, 13]. A number of other complex mechanisms regulate appetite in association with diurnal rhythm and depression through secretion of galanin, gastrin, gastrin-releasing peptide (GRP), ghrelin, neuropeptide Y (NPY), serotonin, and vasoactive intestinal polypeptide (VIP) that are all produced in the basal hypothalamus. Appetite regulation is also a function of the ventromedial nucleus. Temperature regulation is involved in control of appetite, and the posterior hypothalamus is the site of temperature regulation [9, 10].

Two products of the paraventricular and supraoptic nuclei are secreted as hormones directly into the systemic circulation. Vasopressin, also known as antidiuretic hormone (ADH), is secreted into the bloodstream to act on the kidney which maintains normal serum osmolarity by regulating absorption of water. Oxytocin acts on the uterus to stimulate contractions during childbirth and on the breast to induce milk ejection during lactation; it is also called “the love hormone” because of its

roles in many physiological and emotional responses such as sexual arousal, maternal bonding, trust, and even social interactions [14].

The neuroendocrine tumors that arise from neurons in and around the hypothalamus give rise to clinical manifestations of mass effect as well as hormonal activity. Only those causing functioning as neuroendocrine tumors will be discussed in this chapter. Other tumors that arise in the hypothalamus and posterior pituitary, including gliomas, pituicytomas, ependymomas, and stromal tumors [15, 16], are beyond the scope of this review.

Epidemiology

Hypothalamic neuroendocrine neoplasms are exceptionally rare. They usually present as sellar masses and are considered to be variants of pituitary neuroendocrine tumors by some, but their unique characteristics as a hybrid type of tumor, composed of neurons that secrete hormones into the bloodstream, make them unique.

The extreme rarity of these lesions makes it impossible to determine if there are age- or gender-specific qualities. They occur at all ages and in both sexes. One early study identified a female predominance and an average age of diagnosis of about 40 years [17].

Tumor Classification and Morphology

There are two types of hypothalamic neuroendocrine neoplasms that likely reflect the two types of hypothalamic neurons – ganglion cells that are traditionally classified as magnocellular neurons and neurocytomas that may represent small neurons.

Hypothalamic NENs may secrete hormones in excess or may be clinically hormonally inactive. When hormonally active, they may be associated with vasopressin excess causing the syndrome of inappropriate diuresis (SIAD), or they may secrete hormones that impact on adenohypophysial cells resulting in acromegaly, Cushing disease, or hyperprolactinemia.

These mass effects of these tumors include headache, visual disturbances, hypopituitarism, nausea, vomiting, and hydrocephalus [17]. Disturbances of temperature regulation, appetite, diurnal rhythm, blood pressure, and breathing are exceptional and more common in other hypothalamic infiltrative diseases such as craniopharyngioma, but some have been manifest in patients with significant disease [18]. The psychological and emotional changes seen in patients with pituitary tumors, including anger, confusion, and depression, have not been specifically examined or reported in association with these rare tumors.

Gangliocytoma is composed of well-differentiated mature magnocellular neurons [17, 19]. Rarely, they may have an associated neoplastic glial component that

results in classification as "ganglioglioma" [20]. These tumors may arise within the hypothalamus, but many have been reported in a peri-hypothalamic location, sometimes attached to the hypothalamus by a thin stalk. The majority have been reported to have an intrasellar component and have therefore been called sellar gangliocytomas. While that terminology reflects their physical location, the tumor cell differentiation is that of hypothalamic neurons [17, 18, 21].

Gangliocytomas are composed of large mature ganglion cells that vary in size and shape; usually scattered binucleated or multinucleated cells are identified (Fig. 5.1) but mitoses are rare or absent. The stroma is composed of neuropil with variable collagen and glial elements as well as abundant vasculature. Some tumors have focal calcification but necrosis is highly unusual. Immunohistochemistry confirms neuronal differentiation with nuclear NeuN and cytoplasmic synaptophysin, microtubule-associated protein 2 (MAP2), and neurofilaments [22, 23] as well as chromogranin positivity that identifies secretory granules both in the cytoplasm of the tumor cell body and in axonal bulbs. Glial elements, if present, are identified with immunostains for glial fibrillary acidic protein (GFAP) and S100; the latter may stain neural elements with both nuclear and cytoplasmic reactivity. The Ki-67 labeling index is usually very low.

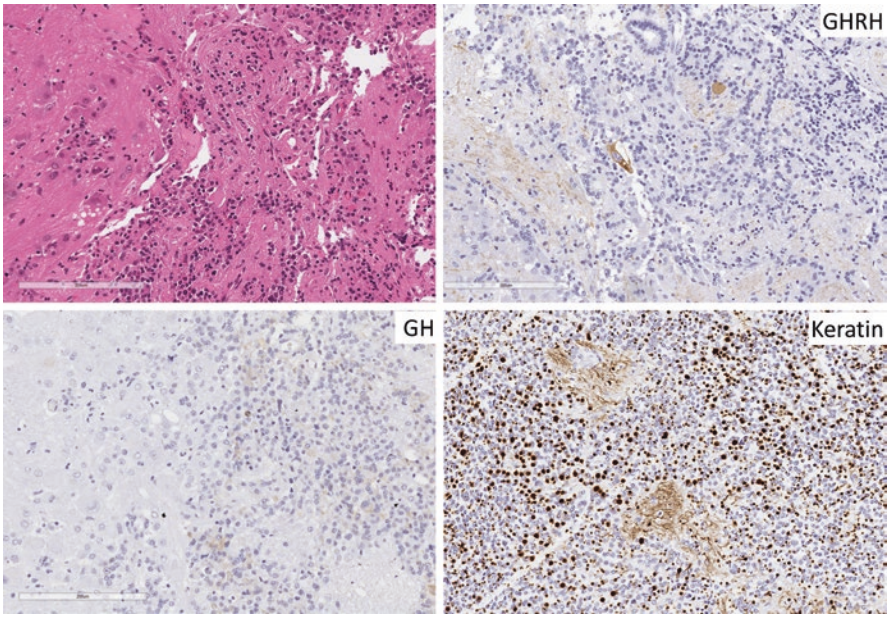

Fig. 5.1 A hypothalamic gangliocytoma associated with a pituitary sparsely granulated somatotroph tumor was the cause of acromegaly. In this image stained with H&E, on the left there are large neurons within neuropil and on the right are round adenohypophysial cells; trapped neurons are also seen scattered among the adenohypophysial cells. The neurons express GHRH, whereas the somatotroph tumor has weak cytoplasmic positivity for GH and the characteristic juxtanuclear globular staining for keratins in fibrous bodies

Electron microscopy confirms that the tumor cells are large neurons within neuropil and the stroma may also contain glia [18, 24–27].

The endocrine function of these tumors is related to their production of hormones that can be identified by immunohistochemistry. The most common clinical syndrome is acromegaly that is attributed to GHRH production [18, 24, 28]. Occasional gangliocytomas have been identified as the cause of Cushing disease, and these have been shown to secrete CRH [27] or, in a recent report, vasopressin that is known to stimulate pituitary corticotrophs [23]. Expression of GnRH by gangliocytomas is a recognized cause of precocious puberty when the lesion occurs in childhood [29–32]. Gangliocytomas have been reported to express other hypothalamic hormones including glucagon, somatostatin, gastrin, galanin, oxytocin, and serotonin [25, 30, 33–38], and a few have been reported to express adenohypophysial hormones such as prolactin [33, 39] and pro-opiomelanocortin derivatives. Hyperprolactinemia has been identified as the result of hypothalamic gangliocytomas producing endorphins and/or enkephalins, VIP, or even GHRH that stimulate prolactin secretion [33, 40, 41]; however, mild hyperprolactinemia is usually due to interruption of the tonic dopaminergic suppression of dopamine when these tumors impact the pituitary stalk. Other endocrine manifestations due to mass effect include hypopituitarism and diabetes insipidus.

An unusual feature of these tumors is their frequent association with adenohypophysial pathology [17, 19]. The adenohypophysis may be nontumorous and hyperplastic [23, 27], but the more frequent association has been with a pituitary neuroendocrine tumor. The commonest scenario is with acromegaly due to a mixed tumor that is a hypothalamic gangliocytoma producing GHRH and a sparsely granulated somatotroph tumor. In such cases, there is evidence of an intimate association between the neoplastic neurons and adenohypophysial cells that led to the theory of hypothalamic hyperstimulation as a cause of adenohypophysial cell transformation [18, 24–26, 28]. However another theory is that these mixed tumors may indicate divergent differentiation of a common precursor [28, 42, 43], a proposal supported by the identification of PIT1 nuclear reactivity in ganglion cells of a GHRH-producing hypothalamic tumor associated with a somatotroph tumor [22]. Other explanations for these mixed tumors implicate a common causative mechanism [28], such as a common exogenous transforming event or an endogenous cause, such as multiple endocrine neoplasia type 1 [44].

Neurocytoma is composed of small hypothalamic neurons; these tumors resemble neurocytomas at other sites in the central nervous system (CNS) that have been classified as "central" when they originate within the lateral ventricles and as "extraventricular" variant when they occur within the tissue of the CNS.

Hypothalamic neurocytomas are composed of solid nests, sheets, and occasional rosettes of small- to medium-sized cells that resemble pituitary neuroendocrine tumors except for their fibrillary neuropil (Fig. 5.2). The tumor cell cytoplasm is pale acidophilic or chromophobic; the nuclei are round to oval with granular chromatin and multiple nucleoli. The stroma usually contains scattered hyaline globules that are composed of dilated axonal terminals; in the normal posterior

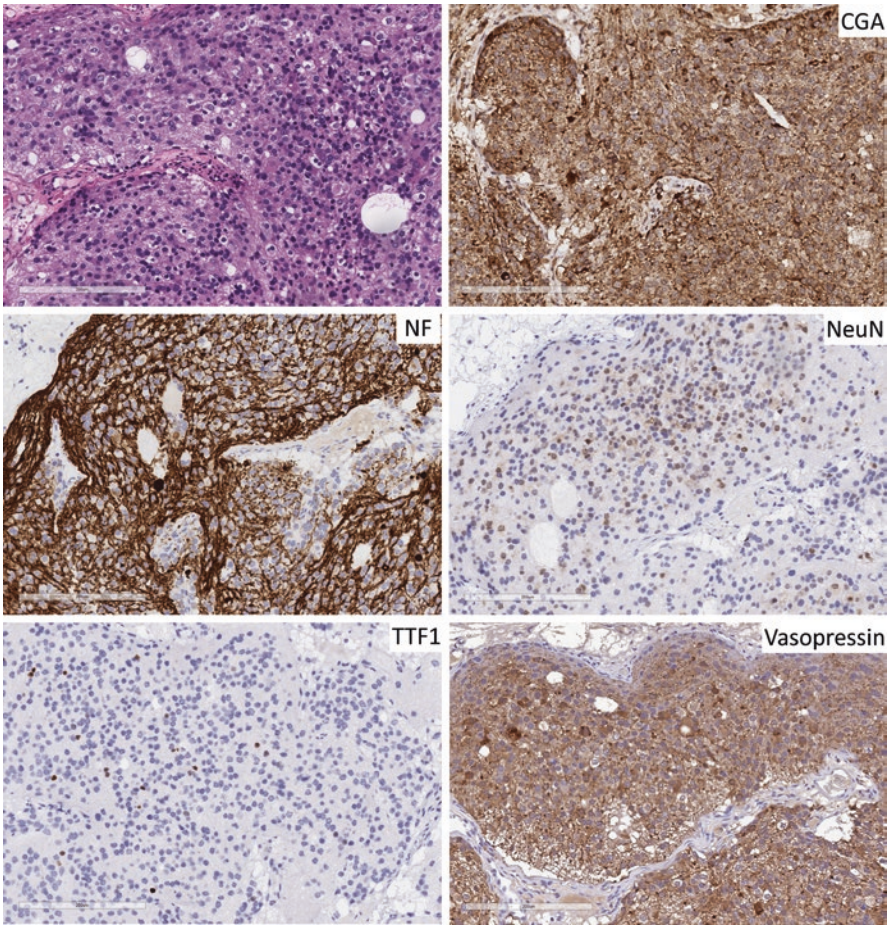

Fig. 5.2 A hypothalamic neurocytoma is composed of small round cells that resemble adenohypophysial cells, but the tumor has distinct neuropil at the periphery of the cell nests. The tumor cells are strongly positive for chromogranin A (CGA) and neurofilament; the nuclei show positivity for NeuN. These features confirm neuronal differentiation. Scattered positivity for TTF1 and diffuse cytoplasmic reactivity for vasopressin are features of hypothalamic neurons

pituitary, these are known as "Herring bodies." There may be stromal fibrosis and/or focal calcification. Mitoses are usually rare. Immunohistochemistry confirms neuronal differentiation with cytoplasmic positivity for CD56, synaptophysin, chromogranin A and neurofilaments, and variable nuclear NeuN [45, 46]; these tumors may also express S100 protein, calretinin, and CD99. The hypothalamic nature of these tumor cells is confirmed by at least focal nuclear TTF1, a biomarker of the medial basal hypothalamus, and cytoplasmic reactivity for hypothalamic hormones. These tumors are usually of low proliferative grade with Ki67 labeling indices below 3%.

In sites outside the hypothalamus, neurocytomas that exhibit vascular proliferation, necrosis, increased mitosis (three or more mitoses per ten high-power fields), or a Ki-67 labeling index >3% have been classified as "atypical neurocytomas." These tumors are associated with a worse prognosis [20, 47, 48]. While not reported as such, the same is likely to be true of neurocytomas in this location.

By electron microscopy, neurocytomas are composed of polygonal tumor cells with numerous elongated neuritic cell processes. The cytoplasm contains microtubules. The tumor cells have variable numbers of dense core secretory granules [49, 50].

The commonest endocrine manifestation of these tumors is SIAD due to production of vasopressin [23, 49, 50]. These patients have signs and symptoms of water overload including nausea and vomiting, tremors, muscle cramps, and even seizures. Gigantism due to a neurocytoma producing GHRH was reported in a single case [51]. Other symptoms due to mass effect include headache and visual field disturbances.

The differential diagnosis of sellar neurocytoma includes pituitary neuroendocrine tumors (see Chapter 4), paraganglioma (see Chapter 12), and olfactory neuroblastoma (also known as esthesioneuroblastoma; see Chapter 6). Indeed, it is likely that previous reports of sellar olfactory neuroblastoma causing SIAD or Cushing disease [52–58] were actually hypothalamic neurocytomas. While these tumors should be readily distinguished from pituitary neuroendocrine tumors that express keratins, pituitary transcription factors, and hormones and from paragangliomas that express GATA3 and tyrosine hydroxylase and are negative for keratins, the ability to distinguish them from olfactory neuroblastoma requires the identification of TTF1 and hypothalamic hormones.

Mixed neurocytoma and ganglioglioma has been reported but not in the region of the hypothalamus and sella turcica [59–62]. These tumors are most common in the fourth ventricle [61, 62] and are often cystic lesions [61]. A mixed tumor in the frontal lobe of a child was classified as a heterotopia [63].

Molecular Pathogenesis

The pathogenesis of hypothalamic NETs is unknown. Gangliocytomas have been associated with multiple endocrine neoplasia [44] but this is exceptional. The pathogenesis of hypothalamic neurocytomas is also unknown. Extraventricular neurocytomas do not have the genetic alterations found in other brain tumors including co-deletion of 1p/19q or mutations of isocitrate dehydrogenase enzyme isoform 1 (*IDH1*), *IDH2, alpha-internexin*, or *Tp53* [48]. Array-based comparative genomic hybridization analysis of two tumors identified different profiles of gain and loss of multiple chromosomal loci [48]. A single case has been reported to harbor polysomy of the epidermal growth factor receptor (*EGFR*) gene [48]. Only a single case has had methylation of the promoter of O6-methylguanine-DNA methyltransferase (*MGMT*) [64].

Prognosis

The prognosis of hypothalamic neuroendocrine tumor is highly variable and dependent on the tumor size and location that determine the ability to achieve complete surgical resection [17]. Patients with progressive severe disease have died of their tumors [18], while others with small tumors have been apparently cured [23]. The management of residual disease may involve medical therapy; some patients with acromegaly due to hypothalamic gangliocytomas have been treated with long-acting somatostatin analogues with some success. Hypothalamic neurocytomas have been shown to express somatostatin receptors [65], indicating the potential role for both medical therapy with long-acting somatostatin analogues and also for the use of radiolabeled somatostatin agents, known as peptide receptor radiotherapy (PRRT) [66]. Reports of external beam radiotherapy have shown variable results.

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Neuroendocrine Neoplasms of the Upper Aerodigestive Tract, Ear, and Salivary Glands

6

Silvia Uccella and Stefano La Rosa

Background

Neuroendocrine neoplasms (NENs) of the head and neck (H&N) region include a heterogeneous group of neoplastic proliferations arising in the nasal cavity, paranasal sinuses, nasopharynx, larynx, salivary glands, middle ear, and skin. In addition to epithelial neoplasms, H&N paraganglioma and olfactory neuroblastoma can be included in this group. Since paraganglioma and Merkel cell carcinoma are treated in Chaps. 12 and 15, respectively, they will not be discussed in this chapter.

The morphological and clinical features of H&N NENs depend on several different factors, including their degree of differentiation, site of origin, and molecular background. Indeed, H&N NENs encompass a wide spectrum of neoplasms, ranging from indolent tumors to highly aggressive neuroendocrine carcinomas.

The terminology used over the last years to define epithelial NENs of the cervicocephalic region has been a matter of debate [1]. In the WHO classification published in 2005, they were generally subdivided into typical carcinoid, atypical carcinoid, and neuroendocrine carcinoma (small and large cell subtype) following the criteria used to classify NENs of the lung [2]. In the last WHO classification, published in 2017, a change in the nomenclature has been proposed, and all H&N NENs have been defined as *neuroendocrine carcinoma* and graded into well-differentiated (replacing typical carcinoid), moderately differentiated (replacing atypical carcinoid), and poorly differentiated (including small cell and large cell types) forms [3]. However, this terminology has been questioned in the light of clinical, molecular,

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and morphological data supporting the fact that low-grade and high-grade NENs do not represent a continuous spectrum of diseases. Indeed, a growing burden of evidences has been accumulating in favor of the concept that low-grade NENs, which have been defined neuroendocrine tumors (NET, G1, G2, and G3), are pathogenetically and biologically separated from neuroendocrine carcinomas (NECs), which are high grade by definition, despite the shared neuroendocrine phenotype. In fact, as NENs are virtually ubiquitous and any site of the body may be involved, a variety of different terminologies have been employed to designate them in the various locations over time. This has created some confusion among both pathologists and clinicians and has led to the need of a uniform classification. To meet this issue, the WHO has recently published a classification framework for all NENs, derived from a consensus conference held in November 2017 in Lyon [4]. This common classification underlines the distinction between NETs (designated in the H&N district as carcinoids or well-differentiated and moderately differentiated neuroendocrine carcinomas) and NECs. In the present chapter, this novel classification approach will be used (Table 6.1). In addition to pure neuroendocrine neoplasms, rare cases composed of neuroendocrine and nonneuroendocrine components have been described in the H&N region, but they are not currently classified as separate entities. The term mixed neuroendocrine-nonneuroendocrine neoplasms (MiNENs) has been recently proposed to designate such rare proliferations [5].

Olfactory neuroblastoma (ONB) is a rare non-epithelial nasal neoplasm showing neuroendocrine differentiation. The terminology and the diagnostic criteria of ONB have not changed over the years, so the most problematic issue in daily histopathologic practice is not represented by the terminology to use, but, rather, by the differential diagnosis with other neoplasms showing a neuroendocrine phenotype and, in

Table 6.1 Proposed classification for H&N epithelial NENs, according to the common classification framework proposed by WHO and IARC [4]

Diagnostic features	Entity
WD neuroendocrine morphology Necrosis absent <2 mitoses × 2 mm^2	Typical carcinoid (NET G1)
WD neuroendocrine morphology Necrosis present 2–10 mitoses × 2 mm^2	Atypical carcinoid (NET G2)
WD neuroendocrine morphology Necrosis present >10 mitoses × 2 mm2	Highly proliferative atypical carcinoid (NET G3)
PD neuroendocrine morphology >10 mitoses × 2 mm^2	Small cell NEC
	Large cell NEC
Neuroendocrine morphology + epithelial nonneuroendocrine morphology	MiNENs*

Legend: *WD* well differentiated, *NET* neuroendocrine tumor, *PD* poorly differentiated, *NEC* neuroendocrine carcinoma; MiNEN, mixed neuroendocrine-nonneuroendocrine neoplasm; *both neuroendocrine and nonneuroendocrine differentiation must be morphologically evident and demonstrated with immunohistochemical stains. The sole presence of positive immunostains in morphologically nonneuroendocrine cells is not sufficient to qualify for MiNEN

particular, with epithelial NENs. ONBs need to be distinguished from nasal NENs because of the different therapeutic approach and prognosis, especially between high-grade (grade IV) ONBs and NECs.

Neoplasms of the Nasal Cavity and Paranasal Sinus

Neuroendocrine Neoplasms of the Nasal Cavity

NENs of the nasal cavity are rare, representing about 3% of sinonasal tumors [3]. They include NETs (carcinoids) and NECs of small and large cell subtype. In addition, cases of mixed neoplasms composed of a neuroendocrine and nonneuroendocrine component (MiNENs) have been described [5], and they need to be taken into account, although they have not been included in the WHO classification [3].

Neuroendocrine Tumors (Carcinoids)

NETs of the nasal cavity and paranasal sinuses are the rarest NENs in this site, accounting for 4.1% of nasal NENs in a recently published series [6]. Patients' age ranges from 13 to 83 years without gender predilection. Common symptoms are nasal obstruction and epistaxis.

Tumors are characterized by a proliferation of uniform cells growing forming small nests, trabeculae, or pseudoglandular structures. Tumor cells have a moderately abundant granular and eosinophilic cytoplasm, uniform round nuclei with clumped or finely granular ("salt and pepper") chromatin, and small nucleoli (Fig. 6.1a, b). Lympho-vascular invasion and necrosis have been reported, but they are infrequent findings. Proliferation rate is generally low, with a mitotic index of <3 mitoses/2 mm^2 [6]. Ki67 labeling index is generally lower than 20% [7] (Fig. 6.1c). Tumor cells are positive for cytokeratins (CKs) and general neuroendocrine markers (synaptophysin and chromogranin) (Fig. 6.1d). TTF1 has been reported to be negative and no other site-specific transcription factor has been reported to be significantly expressed. Among the possible differential diagnoses, the most important includes pituitary neuroendocrine tumors of the nasal cavity, nasopharynx, sphenoid, and ethmoid sinuses that may be ectopic, arising in the sphenoid sinus, or may be invasive from the sella. Immunohistochemistry, including pituitary hormones (ACTH, prolactin, TSH, FSH, LH, and GH) and transcription factors (PIT1, SF1, and TPIT), is mandatory for the diagnosis.

Due to their rarity, definitive data regarding the outcome and the best therapeutic approach for such neoplasms are lacking. However, although they generally present at advanced stages, the survival is better than that of NECs with a 2-year overall and disease-specific survival rates of 50% [6].

Neuroendocrine Carcinoma

NEC is an aggressive poorly differentiated carcinoma associated with dismal prognosis. It is the second most frequent NEN of the nasal cavity, after ONB, representing 22% of all nasal NENs. NECs are more frequent in males with an average

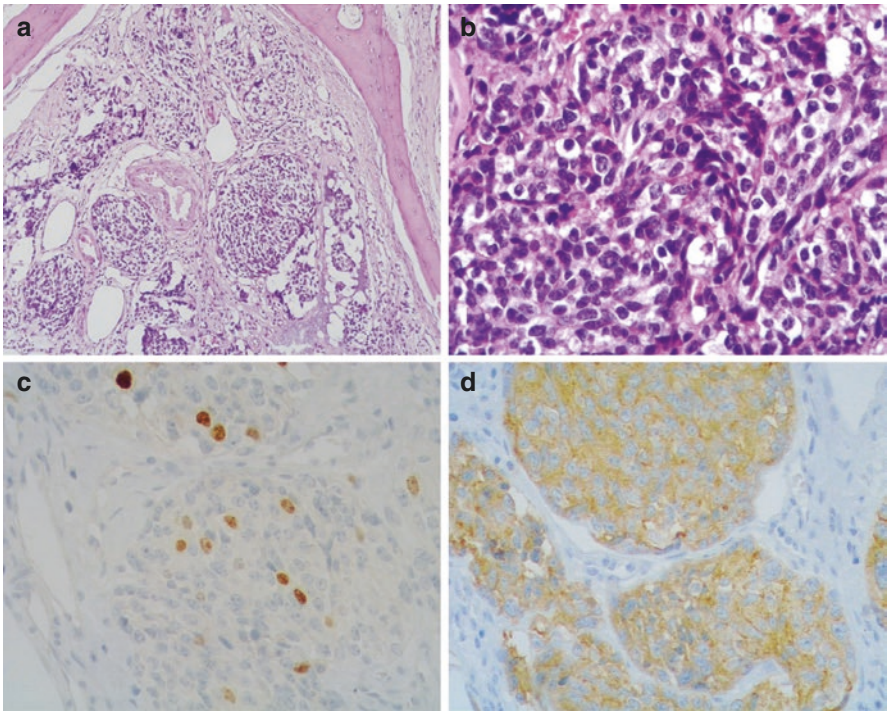

Fig. 6.1 NET G2 (**a**typical carcinoid) of the ethmoidal sinus. Tumor cells grow in solid nests infiltrating bony trabeculae (**a**. H&E ×40) and present moderately abundant eosinophilic cytoplasms, slight nuclear atypia, small nucleoli, and low mitotic index (**b**. H&E ×400). Ki67 proliferation index is around 3% (**c**. immunoperoxidase ×400) and synaptophysin is diffusely and intensely expressed (**d**. immunoperoxidase ×200)

age of 58.7 years at diagnosis [6]. An association with HPV infection and previous radiation exposure has been suggested, while smoking does not seem to play a major etiopathogenetic role [8–10]. Patients generally complain of nonspecific symptoms including nasal obstruction, epistaxis, and sinusitis. However, since most cases of NECs present at advanced stages, symptoms related to local dissemination or distant metastases may be present. Rare cases associated with paraneoplastic syndromes, such as syndrome of inappropriate secretion of ADH (SIADH) and Cushing syndrome, have been described.

Macroscopically, NECs are locally infiltrating large masses, with areas of necrosis and hemorrhage, frequently located in the superior portion of the nasal cavity. Histologically, they are characterized by a diffuse proliferation of cells, arranged in large nests or in sheets showing prominent necrosis. Similarly to their pulmonary counterpart, they are divided into small and large cell subtypes, a distinction based on the morphological features of the neoplastic cells. Small cell carcinomas are composed of small- to medium-sized (up to twice the size of a small lymphocyte), round to oval cells with scant cytoplasm, indistinct cell borders, and hyperchromatic

nuclei with inconspicuous nucleoli (Fig. 6.2a, b). Large cell subtypes are composed of cells with large vesicular nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm (Fig. 6.3a, b). Brisk mitotic activity (mean mitotic count, 16 mitoses x $2mm^2$) and vascular and/or perineural infiltration are frequently observed. Ki67 labeling index is >20%, with higher figures in small cell (Fig. 6.2c) than in large cell

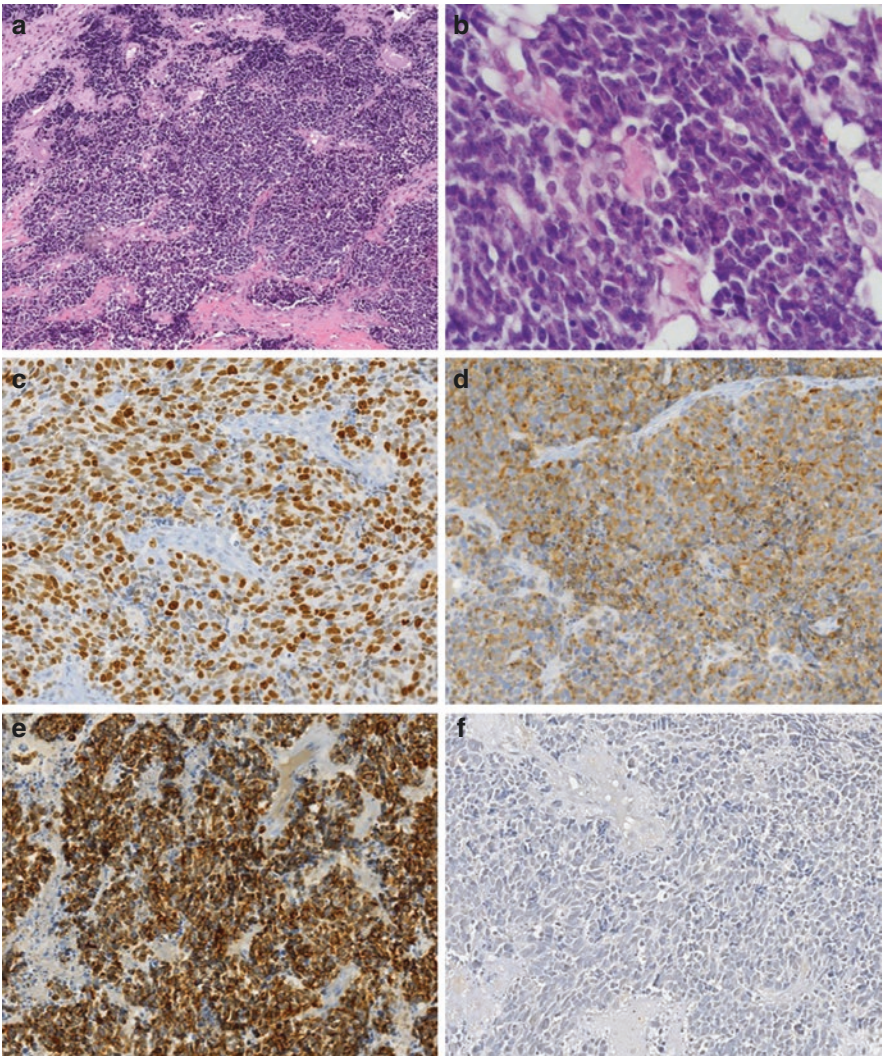

Fig. 6.2 Small cell NEC of the nasal cavity. Diffuse and irregularly trabecular proliferation (**a**. H&E ×40) of highly atypical cells with hyperchromatic nuclei, inconspicuous nucleoli, and scant cytoplasms showing nuclear molding and numerous apoptotic bodies (**b**. H&E ×400). Ki67 proliferation index is around 90% (**c**. immunoperoxidase ×200), synaptophysin (**d**. immunoperoxidase ×200) and cytokeratin 8/18 (**e**. immunoperoxidase ×200) are diffusely expressed, whereas S100 is negative (**f**. immunoperoxidase ×400)

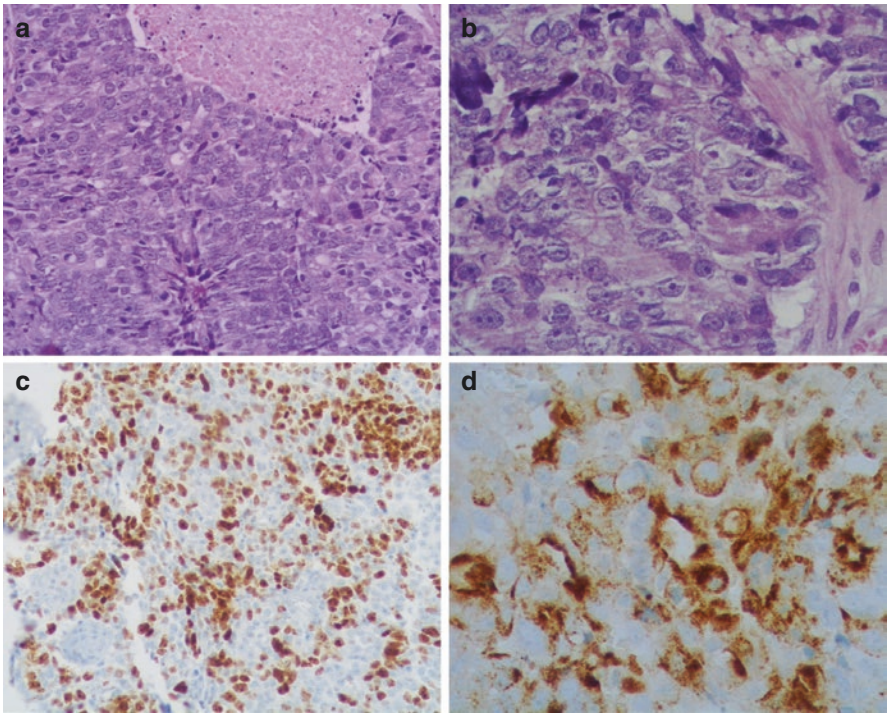

Fig. 6.3 Large cell NEC of the sphenoidal sinus. Neoplastic cells grow in large irregular nodules frequently showing large areas of necrosis (**a**. H&E ×200). Nuclei are large and vesicular and contain evident central nucleoli. Cytoplasms are eosinophilic and moderately abundant (**b**. H&E ×400). Ki67 index is high (**c**. immunoperoxidase ×200), and chromogranin A is intensely expressed in the majority of cells (**d**. immunoperoxidase ×400)

subtype (Fig. 6.3c). The diagnosis needs to be confirmed by immunohistochemical analyses using antibodies directed against general neuroendocrine markers (synaptophysin and chromogranin) (Fig. 6.2d and Fig. 6.3d) and CKs (i.e., CK AE1/AE3 and CK8/18). It is worth noting that NECs can be negative for CK AE1/AE3 leading to a misdiagnosis of ONB. However, CK8/18 was found to be positive in these cases, so CK8/18 immunohistochemistry is strongly recommended in the pathology work-up of morphologically suspected sinonasal NECs [6] (Fig. 6.2e). The possible differential diagnoses, besides ONB, include peripheral neuroectodermal tumors, Ewing sarcoma, desmoplastic small round cell tumors, lymphoid neoplasms, NUT midline carcinoma, adenoid cystic carcinoma, basaloid carcinoma, and other poorly differentiated carcinomas of the sinonasal tract.

NECs are associated with poor prognosis, with 1-year and 5-year DFS rates of 57.4% and 27.8%, respectively [6]. Although several prognostic factors have been

investigated (i.e., sex, age, stage histological subtype, positive surgical margins, and Ki67 index), the only independent prognostic marker seems to be the response to induction chemotherapy [6]. Consequently, the correct pre-operatory diagnosis represents one of the most important factors influencing the therapeutic approach and prognosis. Noteworthy, the cutoff of 55% for Ki67 labeling index, which is an important prognostic marker in digestive NECs [11], does not have a role in the prognostic stratification of patients with nasal NEC [6].

Mixed Neuroendocrine-Nonneuroendocrine Neoplasm (MiNEN)

Nasal MiNENs are extremely rare, with less than 20 cases published in the English literature. In a recent series including sinonasal NENs and ONB, they accounted for 5.1% of cases [6]. They are more frequent in males and diagnosed at a mean age of 58.8 years. Patients generally present unspecific symptoms including nasal stuffiness, epistaxis, rhinorrhea, and headache without any relationship with professional exposure to carcinogens.

Histologically, the nonneuroendocrine component can be represented by squamous cell carcinoma, adenocarcinoma (Fig. 6.4), adenosquamous carcinoma, or inverted papilloma. The neuroendocrine component often consists of NEC, generally showing bone infiltration and angioinvasion. At least one case in which the neuroendocrine component was represented by a NET (atypical carcinoid) has been described [5]. The immunophenotype of the nonneuroendocrine component depends on the tumor type: adenocarcinoma components are immunoreactive for carcinoembryonic antigen (CEA) and may be variably positive for CK7, CK8/18, CK20, and CDX2, according to the intestinal or non-intestinal differentiation, while the squamous cell component expresses CK5 and p63. The neuroendocrine component

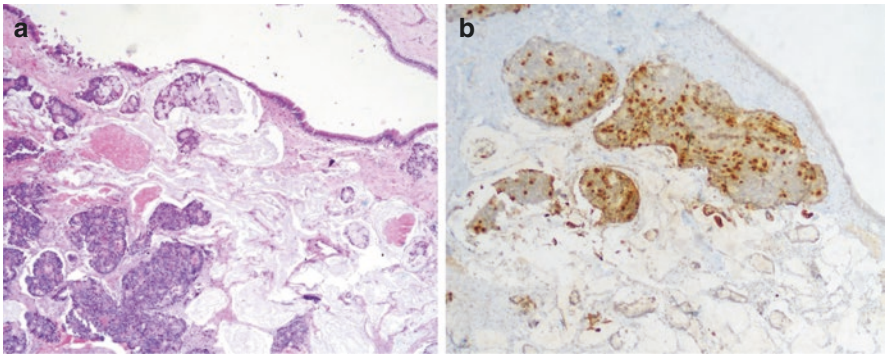

Fig. 6.4 MiNEN of the nasal cavity composed of a NEC (**a**, bottom left) and a mucinous adenocarcinoma (**a**, up right. H&E ×40). Chromogranin A is intensely positive in the neuroendocrine neoplastic cells (**b**. immunoperoxidase ×100), whereas the adenocarcinoma component is negative (**b**)

is positive for general neuroendocrine markers (synaptophysin, chromogranin A). The molecular profile of nasal and paranasal MiNENs has not been extensively analyzed. In a case of mixed intestinal-type adenocarcinoma/NEC, concurrent copy number changes in both components at the *TP53*, *MLH3*, and *KLK3* regions have been found. It has been suggested that MiNENs derive from the proliferation of a single precursor cell with divergent differentiation and that the molecular and morphological progression implies a pathway going from a nonneuroendocrine toward neuroendocrine cell pathway and not vice versa [12].

Nasal and paranasal MiNENs are generally locally advanced (T4a or T4b) and aggressive cancers with poor survival despite the employment of multimodal therapies, including surgery, radiotherapy, and platinum-based chemotherapy. Three-year OS and DFS is 40% and 26%, respectively, with no patient alive after 5 years of follow-up [6].

Olfactory Neuroblastoma

ONB is a rare malignant neoplasm arising in the upper portion of the nasal cavity. It accounts for about 2–3% of nasal neoplasms, with an estimated annual incidence of 0.4 cases per million population [3, 13]. There is not gender predilection, and a bimodal age distribution has been noted, with peaks in the second and sixth decades of life, although ONBs can be observed in almost all ages, ranging from 2 to 90 years [14]. There are not well-identified etiological agents, and, to date, there are not data suggesting an association with wood dust or other occupational exposure.

Patients frequently present epistaxis and symptoms of unilateral obstruction, which generally precede the diagnosis by 6–12 months. Other less frequent symptoms are mainly related to the extent of the disease and include anosmia, headache, proptosis, visual field defects, and epiphora [15].

ONBs are typically located in the upper portion of the nasal cavity, although rare cases in other nasal regions including lower nasal cavity and maxillary sinus have been described. Moreover, cases of intracranial and intrasellar ONBs without an apparent intranasal component have also been reported [16, 17], but these are more likely to be hypothalamic neurocytomas [18]. The classic radiological picture of the true ONB is a “dumbbell-shaped” mass extending across the cribriform plate [14].

Macroscopically, tumors present as a mucosa-covered highly vascularized polypoid soft red-gray mass with variable size, ranging from 1 cm to large masses occupying the nasal cavity with possible extension to the paranasal sinuses and in more advanced cases to the orbit and/or nasal fossa.

Histologically ONBs are submucosal proliferations growing in lobules with a more or less well-represented neurofibrillary matrix, separated by a richly vascularized fibrous stroma. Vessels frequently show a peculiar plexiform or glomeruloid appearance. There is a great case-to-case variability in morphological features. In

low-grade cases, tumor cells are generally uniform in size with scant cytoplasm and small round nuclei showing coarsely to finely dispersed chromatin with the typical "salt-and-pepper" appearance. Nucleoli are generally absent or inconspicuous. Necrosis is absent and mitotic activity is usually very low. Tumor cells show tangles of neuronal cell processes which appear as a neurofibrillary matrix, around which pseudorosettes of the Homer-Wright type can be observed. Much more rare are true rosettes forming gland-like structures (the so-called Flexner-Wintersteiner-type rosettes). High-grade ONBs are composed of cells with nuclear pleomorphism and prominent nucleoli, high mitotic activity, and necrosis. The combination of architectural structure, nuclear pleomorphism, presence of neurofibrillary matrix, mitotic activity, and necrosis is currently used to grade ONBs according to the four-tiered Hyams' grading system (Table 6.1, Fig. 6.5). Grade 1 ONBs represent the most differentiated, while grade 4 are the least differentiated. The Hyams' grading system has been demonstrated to have a good correlation with prognosis [6, 19].

Immunohistochemistry is mandatory in the work-up of ONB to confirm the diagnosis. Tumor cells typically show a diffuse and intense positivity for synaptophysin and chromogranin A, whereas they are typically negative for cytokeratins, although some cases may show focal immunostaining. S100 immunostaining is typically

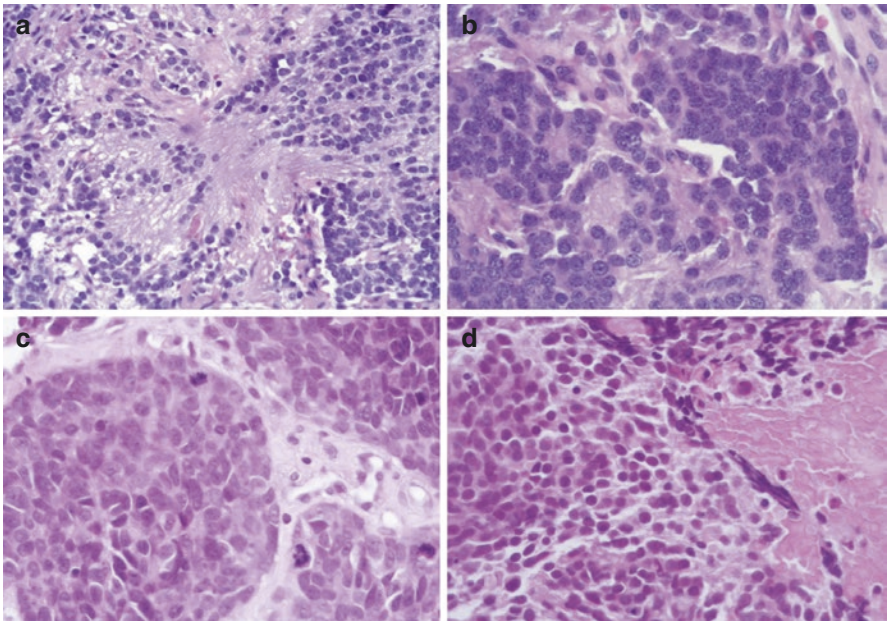

Fig. 6.5 Hyams' grading system of olfactory neuroblastoma. Grade I (**a**), grade II (**b**), grade III (**c**), and grade IV (**d**). H&E × 200 and 400. See text and Table 6.2 for comments

Table 6.2 Hyams' grading system

	Hyams' grade			
Microscopic features	I	II	III	IV
Pleomorphism	–	+	++	+++
Lobular architecture	+	+	+/–	+/–
Neurofibrillary matrix	+++	+	+/–	–
Rosettes	+	+	–	–
Homer-Wright	–	–	+/–	+/–
Flexner-Wintersteiner				
Mitoses	–	–	+	+++
Necrosis	–	–	+	+++

limited to sustentacular cells, which are located at the periphery of tumor lobules (Fig. 6.6). Somatostatin receptor 2A ($SSTR_2$) is positive in the majority of cases, and their assessment can represent the rationale for the employment of somatostatin analogues in diagnosis and therapy [20, 21]. CD99, desmin, HMB45, and hematolymphoid markers are negative and can help in the differential diagnosis with tumor mimickers, including lymphoma, rhabdomyosarcoma, Ewing sarcoma, and melanoma.

Molecular alterations of ONB are not well clarified, yet. Somatic *TP53* mutations have not been documented, although p53 immunohistochemical expression has been demonstrated in some cases [22, 23]. It has been suggested that p53 alterations probably occur at late stage of tumor growth and progression [23]. ONBs seem to show high levels of chromosomal instability which seems to be paradoxically associated with a relatively indolent behavior [24]. Deletions of *dystrophin* have recently been identified, but the pathogenetic role of this alteration still remains unclear [25]. Using a multi-omic approach, two molecular classes of ONBs with different clinicopathologic features have recently been identified: "basal" and "neural" ONBs. The basal subtype, which derives from basal cells, shows IDH2 R172 mutant genotype and harbors a CpG island methylator phenotype, reminiscent of IDH2 mutant gliomas. The neural subtype, which derives from immature olfactory neuron progenitors, shows genome-wide reprogramming with loss of DNA methylation at the enhancers of axonal guidance genes [26]. Basal cell subtype shows more aggressive morphological features than the neural subtype including higher proliferation rates (both mitotic and Ki67 indexes), necrosis, intratumor CD8+ lymphocytes, and reduction of S100 sustentacular cells. This reflects the worse outcome of basal subtype compared to the neural one [26].

One-year DFS is 94% and it decreases to 78.8% and 62.5% after 5 and 10 years, respectively. Several prognostic markers have been investigated, but only Hyams' high grade and Ki67 > 20% seem to be independent predictors of poor survival [6].

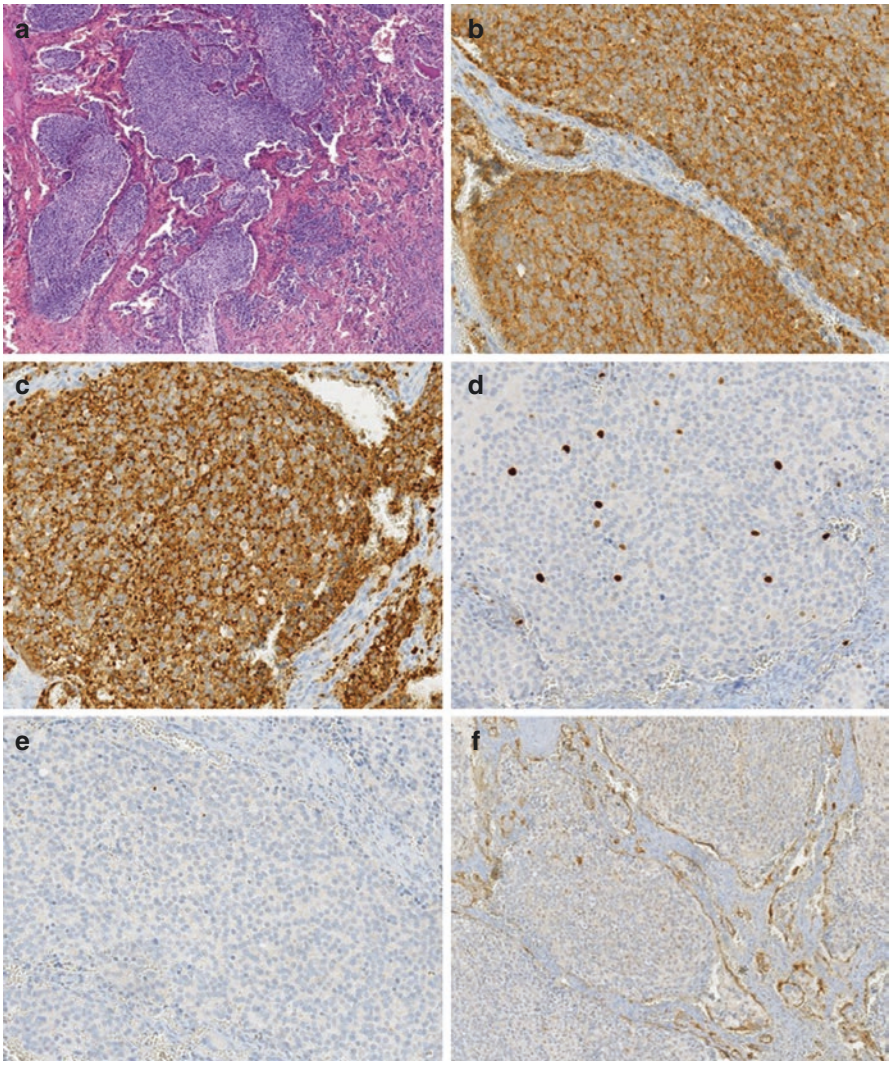

Fig. 6.6 Morphological and immunophenotypical aspects of olfactory neuroblastoma, grade II. Irregularly organoid proliferation of tumor cells in a fibrotic and highly vascularized stroma (**a**. H&E ×40). Immunostainings for synaptophysin (**b**. immunoperoxidase ×200) and chromogranin A (**c**. immunoperoxidase ×200) are intensely and diffusely positive. Ki67 proliferation index is very low (**d**. immunoperoxidase ×200). Pan-cytokeratin is negative (**e**. immunoperoxidase ×200) and S100 is expressed in sustentacular cells (**f**. immunoperoxidase ×200)

Neuroendocrine Neoplasms of the Larynx

NENs are the second most frequently diagnosed neoplasm of the larynx, after squamous cell carcinoma and its variants. NET G2 (atypical carcinoid) is the most frequent laryngeal NEN, followed by NEC, paraganglioma, and NET G1 (typical carcinoid) [27], although the heterogeneous nomenclature and classification criteria used along the years prevent the exact quantification of each subtype. Laryngeal NENs are more frequently diagnosed in elderly males (in their sixth and seventh decades), whereas paraganglioma generally occurs in females [27]. Among possible risk factors, only smoking seems to be relevant, while alcohol intake or exposure to environmental carcinogenic substances do not seem to play a pathogenetic role [28]. However, due to the rarity of these neoplasms, genetic and molecular studies are lacking, and no conclusive data support the existence of a specific pathogenetic pathway.

Clinical symptoms include hoarseness, dysphonia, sore throat or throat irritation, hemoptysis, and neck mass. Paraneoplastic syndromes are rare but can cause severe symptoms [28].

As already mentioned in paragraph 6.1 (Background), the terminology of laryngeal NENs has been very heterogeneous since they have been firstly described [29]. In the last WHO classification, published in 2017, the terms typical and atypical carcinoid have been replaced by the terms well-differentiated and moderately differentiated neuroendocrine carcinoma, respectively, while neuroendocrine carcinoma is defined as poorly differentiated neuroendocrine carcinoma [3]. We disagree with this terminology, and, as discussed in paragraph 6.1, the classification scheme proposed by the WHO after the consensus conference held in November 2017 in Lyon will be used in the present chapter (Table 6.1) [4].

Neuroendocrine Tumors (NETs, Carcinoids)

NET G1 (Typical Carcinoid)

This is a very uncommon neoplasm accounting for about 5% of laryngeal NENs [3]. Clinical symptoms include hoarseness and dysphonia, but some patients are asymptomatic, and laryngeal lesion is found incidentally during laryngoscopy or intubation for unrelated procedures [28].

Macroscopically, NETs present as polypoid, nodular, pedunculated, exophytic, fungating masses ranging in diameter from 0.3 to 4 cm, typically arising in the supraglottic region [3]. Histologically, they are composed of nests or chords of uniform polygonal cells with centrally placed round or oval nuclei, finely dispersed chromatin, small nucleolus, and granular eosinophilic cytoplasm. This organoid growth is accompanied by fibrovascular or hyalinized stroma. Mitotic figures are fewer than 2 per 2 mm^2 and necrosis and cellular anaplasia are absent. Oncocytic and mucinous

changes may be observed, as well as the focal presence of "Zell-ballen," rosettes, and foci of squamous differentiation. Ki67 index is generally <20%, and this feature may be of help in the preoperative evaluation of small biopsies, as it allows the differential diagnosis with NEC, particularly when morphology is not fully evaluable due to crush artifacts. Tumor cells are positive for general neuroendocrine markers and may express neuropeptide markers, such as serotonin, bombesin, calcitonin, and somatostatin. Low-molecular-weight cytokeratins (CK7–8–18-19-20), epithelial membrane antigen (EMA), and carcinoembryonic antigen (CEA) are consistently expressed [30].

The prognosis is difficult to evaluate, due to the rarity of this entity, but a 5-year survival of approximately 80% after conservative surgical resection has been reported [31]. Nevertheless, the clinical course may not be indolent, as cases with distant metastasis have been described, frequently involving the liver [32]. Lymph node metastases are infrequent and elective routine neck dissection is not indicated. Irradiation and chemotherapy have been proved to be ineffective.

NET G2 (Atypical Carcinoid)

This is the most frequent type of laryngeal NEN. It occurs more frequently in men than in women (male-female ratio of 2.4:1), with the highest incidence in the sixth and seventh decades.

Macroscopically, they are similar to NET G1 (typical carcinoid), presenting as submucosal nodules or polyps of the supraglottic region, sometimes with ulcerated surface. Histologically, they are composed of nests or chords of polygonal cells with oval or round nuclei often containing visible nucleoli. Chromatin may be more clumped than in NET G1 cells. Mitotic index ranges from 2 to 10 per 2 mm^2, Ki67 index is generally >3%, and punctate necrosis and lymphatic vessels invasion are often present (Fig. 6.7). Tumor cells are positive for synaptophysin and chromogranin A. It is worth noting that some laryngeal NETs can be positive for calcitonin and CEA, creating some difficulties in the differential diagnosis with medullary carcinoma of the thyroid. TTF1 immunohistochemistry can be useful since this marker is constantly expressed in medullary carcinoma, while it is frequently absent in laryngeal NETs. Careful clinical evaluation, with accurate imaging study, is advisable in such cases.

About 20% of patients present with advanced disease, and about 60% of cases recur after first-line therapy. The gold-standard treatment is surgical excision, in the form of partial or total laryngectomy depending on the site, size, and extent of the tumor. Elective neck dissection followed by adjuvant chemo- and/or radiotherapy is indicated in patients with locoregional lymph node metastases. Chemoradiotherapy alone does not appear to be effective. According to a meta-analysis of 436 reported cases published in 2015, 30% of patients have distant metastasis at presentation, and 5-year DFS and OS are 52.8% and 46%, respectively [1, 33]. Long-term follow up is indicated, as late recurrences have been reported [33].

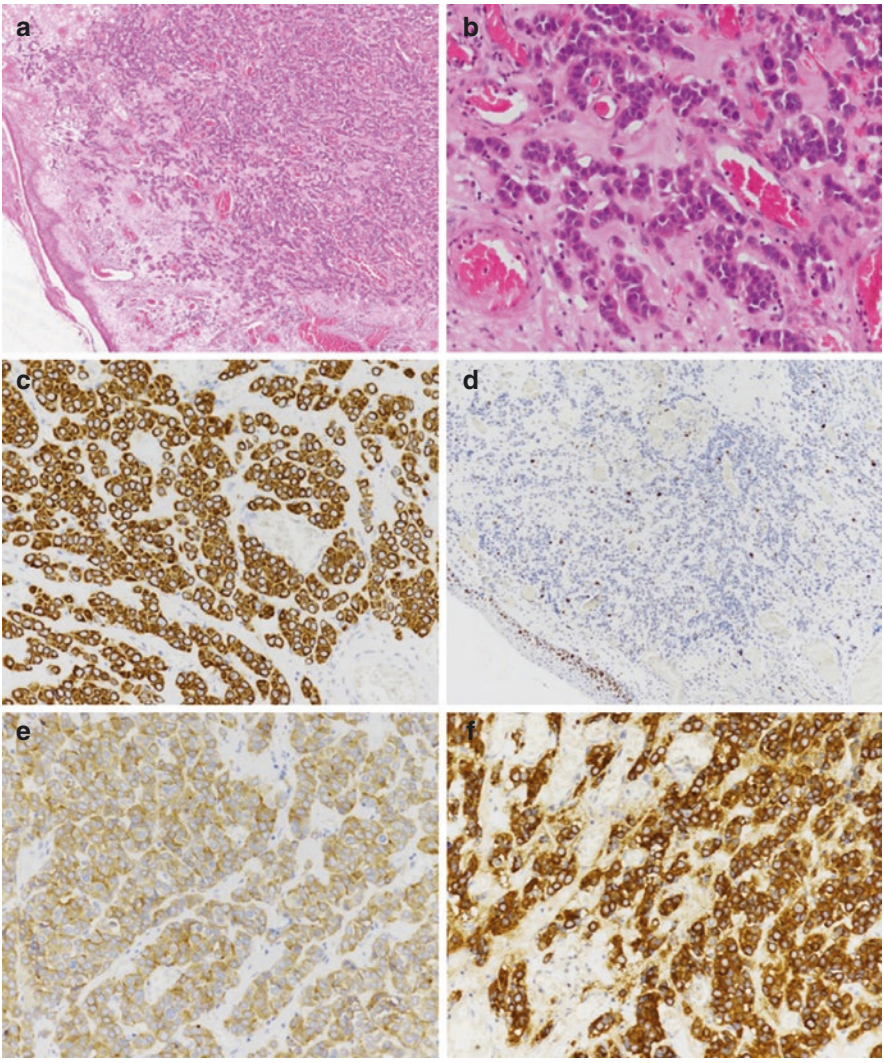

Fig. 6.7 Atypical carcinoid of the larynx. Tumor cells proliferate in chords and nests under the epithelial layer (**a**. EE ×40). Nuclei show bland atypia, cytoplasms are eosinophilic, and mitotic index is low (**b**. H&E ×200). Cytokeratin 7 is strongly expressed in all cells (**c**. immunoperoxidase ×200), Ki67 proliferation index is around 5% (**d**. immunoperoxidase ×100), and general neuroendocrine markers chromogranin A (**e**. immunoperoxidase ×200) and synaptophysin (**f**. immunoperoxidase ×200) are diffusely positive

Neuroendocrine Carcinoma

NEC is the second most common NEN of the larynx after NET G2. It frequently affects elderly men, with a mean age at presentation of 60 years. Most of the patients have a history of cigarette smoking.

These neoplasms are macroscopically undistinguishable from squamous cell carcinomas and present as fleshy ulcerated masses that can reach a large size (up to 5 cm). Histologically, small cell and large cell variants are recognized, although they are not associated with different prognoses [33]. Small cell NECs are composed of sheets or, occasionally, interconnecting ribbons of small- to intermediate-sized cells with high nuclear/cytoplasmic ratio; hyperchromatic oval, round, or spindle-shaped nuclei with delicate chromatin; small inconspicuous nucleoli; and a minimal rim of cytoplasm. Mitotic figures and apoptotic body are numerous. "Geographic chart" necrosis is the rule and vascular and/or perineural invasion are commonly seen. Rosette formation may be observed. The differential diagnosis of small cell NEC includes basaloid squamous cell carcinoma and the solid variant of adenoid cystic carcinoma. Large cell NEC of the larynx is a newly recognized entity with peculiar morphologic and clinical features [34, 35]. The histopathological criteria for recognizing large cell NEC in the larynx are the same used in the lung: tumor cells with neuroendocrine morphology and immunophenotype, showing moderate to abundant cytoplasm, vesicular nuclei with prominent nucleoli, mitotic activity >10 per 2 mm^2, and zonal to extensive necrosis [36]. Besides morphology, the most important parameter that distinguishes large cell NEC from NET G2 (atypical carcinoid) is the mitotic index. Until now, there is no definite evidence that Ki67-related proliferative index may be used in the distinction between these two neoplasms [37]. Immunohistochemistry is mandatory for the diagnosis, and it has to demonstrate the epithelial nature of the lesion (CKs) and its neuroendocrine phenotype (general neuroendocrine markers). Noteworthy, the immunostaining for p16 has been reported to be positive in a large number of laryngeal NECs, as well as in other H&N NECs, although HPV DNA is consistently absent in these neoplasms. The pathology should be aware of this feature to avoid a misdiagnosis with poorly differentiated squamous cell carcinomas [38]. Staining for p63 may be of use in this differential diagnosis, as only rare cases of laryngeal NEC show immunostaining for this marker, which is consistently expressed in squamous cell carcinoma [38].

Laryngeal NEC is an aggressive disease, with high rates of metastasis and a 5-year survival rate of 5–20% [3]. Multimodal treatment with combined surgery, chemotherapy, and radiation therapy is the most effective therapeutic approach, assuring a median survival of 55 months, which is significantly longer than that reached by any other approach.

Mixed Neuroendocrine-Nonneuroendocrine Neoplasm (MiNEN)

Laryngeal MiNENs are extremely rare, with only 19 cases reported in the English literature [5]. They typically affect males in their fifth to sixth decades of life, although the age range is wide and cases arising in female patients have been reported [5]. Symptoms can be unspecific and frequently include worsening hoarseness.

Macroscopically, MiNENs are not different from other high-grade laryngeal malignancies, with large exophytic or ulcerated masses, presenting areas of necrosis and hemorrhage. Histologically, most cases are composed of squamous cell carcinoma and small cell NEC (Fig. 6.8). A single case in which the neuroendocrine

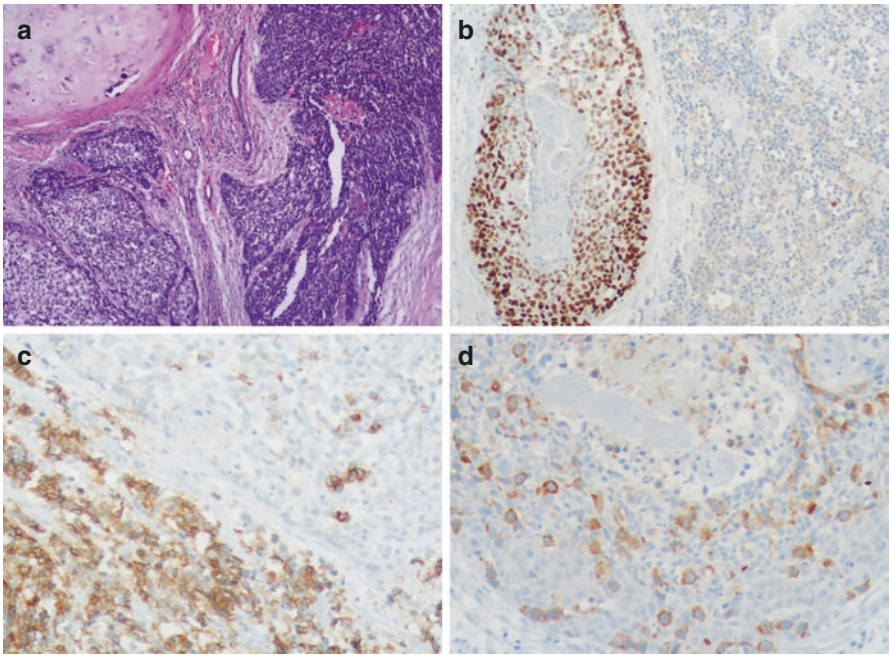

Fig. 6.8 MiNEN of the larynx composed of a poorly differentiated squamous cell carcinoma (**a**, bottom left) and a small cell NEC (**a**, right. H&E ×100). The squamous cell component is p63-immunoreactive (**b**. immunoperoxidase ×200), whereas the neuroendocrine cells express synaptophysin (**c**. immunoperoxidase ×400) and chromogranin A (**d**. immunoperoxidase ×400)

component was represented by an atypical carcinoid has been reported [39]. The identification of the NEC component is crucial for predicting patient's outcome and management. When the squamous cell carcinoma component is poorly differentiated, the identification of the NEC component can be challenging and needs immunohistochemical analysis. In this setting, an appropriate panel includes general neuroendocrine markers, p63, p40, and CKs (Fig. 6.8) [1].

Neuroendocrine Tumor of the Middle Ear

Literature published in the last years has indicated that the so-called middle ear adenoma and middle ear carcinoid [40, 41] represent the same entity [1]. This tumor is composed of both glandular (exocrine) and solid (neuroendocrine) components, making the neuroendocrine tumor of the middle ear a mixed neoplasm, for which the term MiNENs may be more appropriate [1].

This is a very rare neoplasm accounting for <2% of ear tumors. It shows equal sex distribution and occurs more frequently in the third to fifth decades of life (range 20–80 years). The most common symptom is unilateral conductive hearing loss. Pain, discharge from the external auditory canal, and facial nerve paralysis are rare

but, when present, demonstrate locoregional extension and are associated with aggressive behavior. No etiologic factors have been identified to date.

The tumor appears as a gray-white to red-brown firm mass, can arise anywhere in the middle ear cavity, and occasionally extends into the mastoid, Eustachian tube, or external auditory canal [3].

Histologically, the tumor is unencapsulated showing a commingling of glandular/tubular and solid/trabecular structures (Fig. 6.9). Rarely, a predominant papillary architectural pattern can be observed. Tumor cells are cuboidal with eosinophilic cytoplasm and round to oval nuclei with "salt-and-pepper" aspect, sometimes containing eccentrically located small nucleoli. Mitoses are absent or rare. Tumor cells are positive for CKs and express neuroendocrine markers including synaptophysin and chromogranin A [42]. Interestingly, middle ear neuroendocrine tumor can show an immunophenotype similar to that of hindgut-derived well-differentiated neuroendocrine tumors including the expression of pancreatic polypeptide-related peptides, glucagon-related peptides, serotonin, CAR5, and prostatic acid phosphatase, but the reason of this is not clear [43].

Data on patients' prognosis are limited by the rarity of such neoplasms. Recurrence has been reported for cases with incomplete local surgical excision and a metastatic potential may exist.

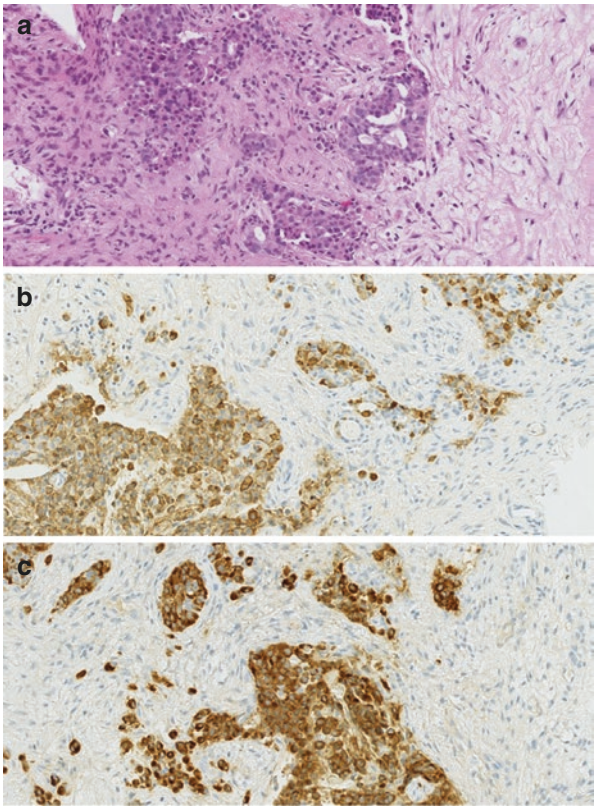

Fig. 6.9 NET of the middle ear. Pseudoglandular, trabecular, and solid structures of neuroendocrine neoplastic cells (**a**), showing diffuse positivity for synaptophysin (**b**) and chromogranin A (**c**). H&E and immunoperoxidase ×200

Neuroendocrine Neoplasms of the Salivary Glands

Most of salivary gland NENs are NECs of the small cell and large cell types with only a few reported NETs [1]. In the 2017 WHO classification of salivary gland neoplasms, NETs are not included as specific entity [3].

Neuroendocrine Tumors (NETs, Carcinoids)

NETs of the salivary glands are exceedingly rare, with only a few cases reported to date, including both typical and atypical carcinoids [44–47]. They have been observed in both males and females, although, due to their rarity, epidemiological data are lacking.

Macroscopically, they can be well- or poorly circumscribed masses ranging from 1 to 5.5 cm in size [46]. Histologically, they show an organoid growth, with cords, nests, or pseudoglands, composed of uniform neoplastic cells with moderately abundant eosinophilic cytoplasm, round nuclei with "salt-and-pepper" chromatin, and small nucleoli. In typical carcinoids, mitotic figures, pleomorphism, and necrosis are characteristically absent, whereas atypical carcinoids may show a low number of mitoses, punctate necrosis, and slight to moderate pleomorphism. Strong and diffuse immunoreactivity for neuroendocrine markers and pan-CKs is a consistent feature [1]. The differential diagnosis of salivary glands carcinoids includes metastatic carcinoids from other sites, which need to be excluded on a clinical basis, and large cell NECs, in which proliferative index and cytologic atypia are greater. In addition, metastatic melanoma can be ruled out using appropriate immunostainings, as well as other primary tumors of the salivary glands, first of all adenoid cystic carcinoma.

Due to the small number of the published cases, no definitive prognostic information is available, but reported follow-up data suggest that salivary gland NETs are less aggressive than NEC of this site [43–47].

Neuroendocrine Carcinoma

NEC is the most frequent NEN of the salivary gland; affects males more frequently than females, at a median age of 64 years; and occurs almost exclusively in the parotid gland [3]. Patients generally present with a painless mass in the parotid region, but in some cases they show facial nerve paralysis. In more than 50% of cases, locoregional lymph node metastases are present.

Macroscopically, NECs are poorly circumscribed nodules of 2–5 cm in the greatest dimension with infiltrating borders and a variegated cut surface showing areas of necrosis and hemorrhage. Histologically, NECs are separated into small cell and large cell subtypes. *Small cell NEC* is very similar to small cell carcinoma of the lung and is composed of sheets, ribbons, or nests of round, oval to spindle cells measuring as large as or up to twice the size of a small lymphocyte diameter, with scant cytoplasm. High mitotic index, zonal necrosis, and invasion of vascular and

perineural spaces are common features. Tumor cells are immunoreactive for at least one general neuroendocrine marker and CKs, which usually shows a dot-like paranuclear pattern [48]. The expression of CK20 is observed in more than 70% of cases and has led to the concept that these CK20-immunoreactive neoplasms can be related to Merkel cell carcinoma. For this reason, two types of salivary gland small cell NECs are recognized: the Merkel cell type, which is CK20-positive, and the pulmonary type, which is CK20-negative [49]. Merkel cell subtype seems to behave less aggressively than the pulmonary type, suggesting that CK20 immunostaining may represent a useful prognostic marker. There is an obvious overlap between the so-called primary Merkel cell carcinoma of the salivary glands and the Merkel cell type of small cell NEC of these sites. Small cell NECs may also express CK7, epithelial membrane antigen (EMA), and neurofilaments. Vimentin may be positive, whereas immunoreactions for S100 and HMB45 are always negative. The differential diagnosis of small cell NECs includes a number of epithelial and non-epithelial malignancies, both primary and metastatic. Immunohistochemistry is a useful tool, in addition to morphology, to distinguish these neoplasms from other blue cell tumors, such as non-Hodgkin lymphomas, basaloid carcinomas, and the solid variant of adenoid cystic adenocarcinoma. In contrast, the distinction from metastatic small cell neuroendocrine carcinomas of other sites, in particular from the skin or from the lung, may be challenging. In this context immunohistochemistry is not sufficient to identify the primary site of the NEN, as CK20 positivity does not discriminate between cutaneous and salivary gland NECs, and TTF1 may be expressed both in pulmonary and in parotid small cell carcinoma. In addition, Merkel cell carcinoma polyomavirus may be present both in cutaneous and in salivary gland NECs [1]. For these reasons, accurate imaging and clinical analyses are mandatory.

Large cell NECs are characterized by an organoid growth of polygonal or, rarely, fusiform cells with a well-defined cell border, abundant eosinophilic cytoplasm, and vesicular nuclei with prominent nucleoli. Brisk mitotic activity is often seen, as well as perineural and/or vascular invasion and necrosis. Giant tumor cells with bizarre or anaplastic nuclei and poorly formed ducts may be also found. Neuroendocrine differentiation is confirmed by the immunoreactivity for general neuroendocrine markers. Pan-cytokeratin is expressed, but CK20 immunoreactivity is consistently absent, whereas immunostainings for Bcl-2, p53, epidermal growth factor (EGF), cyclin D1, EMA, and carcinoembryonic antigen (CEA) may be positive [1]. The differential diagnosis of LCNEC of the salivary glands includes poorly differentiated squamous cell carcinoma and adenocarcinoma, high-grade lymphomas, and melanoma. Also in this case, metastatic localization of LCNECs of other sites must be considered and can be ruled out only with a thorough clinical examination.

NECs of the salivary gland are aggressive neoplasms, with a high rate of local recurrence and distant metastases. Hematogenous spread is more frequent than locoregional lymph node involvement. The 5-year survival rate ranges from 36% to 50%, without significant differences in survival between small and large cell subtypes [1].

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Thyroid Neuroendocrine Neoplasms

7

Sylvia L. Asa and Ozgur Mete

Historical Background

The history of the neuroendocrine component of the thyroid dates back to 1894 when Karl Hürthle identified clear cells within the basement membrane of follicles in the thyroid [1]; unfortunately this has been long forgotten, and today many pathologists mistakenly call oncocytes "Hürthle cells." The cells that Hürthle identified became known as parafollicular or clear cells (C cells) and were largely discounted for more than half a century. Indeed, in the 1953 AFIP Fascicle on Tumors of the Thyroid Gland, there is no mention of these cells or their tumors [2]. However, in 1961 there was a report of an unusual tumor with distinctive morphology [3], and 8 years later Hazard coined the term "medullary" for these solid tumors [4].

The hormone produced by C cells, calcitonin, was purified in 1962 by Copp and Cheney at the University of British Columbia [5]; they thought it was of parathyroid origin and named it for its role in maintaining calcium levels. In 1964 it became clear that calcitonin was secreted by the thyroid and by the parafollicular C cells of Hürthle. In 1966 William proposed that medullary thyroid carcinoma was derived from these calcitonin-producing C cells [6].

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Thyroid C cells are prototypic neuroendocrine cells and were thought to be derived from the neural crest [7] but subsequently were shown to be epithelial neuroendocrine cells and, like others in the respiratory and gastroenteropancreatic tract, derive from the endoderm [8, 9]. They produce calcitonin as their main hormone product but also produce calcitonin gene-related peptide (CGRP), somatostatin, gastrin-releasing peptide (GRP), serotonin, and thyrotropin-releasing hormone as well as being a rich source of carcinoembryonic antigen (CEA).

The association of thyroid C cell pathology with pheochromocytoma in a familial disorder was recognized by Williams in 1965 [10]. This description was classified as multiple endocrine neoplasia (MEN) type 2 syndrome. The characterization of this syndrome underwent multiple changes with division into types 2A and 2B or types 2 and 3, but with the recognition that this disease is due to mutations in a single gene, *RET*, that encodes a tyrosine kinase involved in the migration of neural and neuroendocrine cells [11], the classification has become more complex. Known as MEN2, there are several variants associated with mutations that alter conformation of the molecule in the extracellular and transmembrane domain, all classified as MEN2A, and a more aggressive variant associated with activation of the kinase known as MEN2B [12].

Traditionally diagnosticians have considered medullary thyroid carcinoma (MTC) to be the only NEN of the thyroid gland; however, there is a morphological spectrum of NENs that can be seen in this gland. Thyroid NENs include the following entities: (i) MTC that originates from parafollicular C cells, (ii) mixed neuroendocrine and non-neuroendocrine neoplasms (MiNENs) that often manifest as a composite MTC and papillary thyroid carcinoma, (iii) paraganglioma that originates from dispersed microscopic elements of the laryngeal paraganglia (see Chapter 12), (iv) NENs originating from intrathyroidal parathyroid gland (see Chapter 8), (v) NENs originating from intrathyroidal thymic remnants (i.e., intrathyroidal thymic NENs) (see Chapter 9), and (vi) metastatic neuroendocrine neoplasms. From a patient management perspective, it is important to be aware of these various differential diagnoses and be able to distinguish these neoplasms given their distinct clinicopathologic characteristics.

Epidemiology

Medullary thyroid carcinoma has traditionally been thought to represent about 5% of all thyroid carcinomas [13] and in some series up to 10%, but more recent data suggest that a more accurate number is 1–2% [12]. Despite this low incidence, it is responsible for more than 13% of thyroid cancer-related deaths [12, 14]. Familial syndromes are responsible for a significant proportion of these; in earlier studies, 30–40% were considered to be familial; however more recent studies show a lower incidence of 25–30% suggesting that screening and prophylactic thyroidectomy is causing this proportion to decrease.

Tumor Classification and Morphology

Medullary thyroid carcinoma has characteristic neuroendocrine histologic and cytologic features that should make it an obvious diagnosis in a gland that is otherwise not neuroendocrine. Despite its unique features, it is often misdiagnosed [15, 16], especially on cytology where only about half of cases of this entity are accurately identified [17, 18].

The morphology of medullary carcinoma includes a spectrum of architecture and cytology [15]. Most commonly, these tumors have a typical neuroendocrine pattern of solid nests in a vascular stroma; they are usually infiltrative but can sometimes be well delineated (Fig. 7.1) [19]. Rarely, tumors can have complete or partial encapsulation, but most tumors lack a true capsule as seen in a subset of thyroid follicular epithelial-derived neoplasms. Other tumors can display a nested "zellballen" pattern that can simulate paragangliomas, and such tumors are referred to as paraganglioma-like variants of this disease [20]. They frequently have palisading at the periphery of the solid nests, and occasionally central degeneration results in a pseudopapillary growth pattern that mimics papillary thyroid carcinoma [21, 22], and they can even be cystic [23, 24]. The tumor usually infiltrates around adjacent follicles, and these tumors can sometimes be mistaken for follicular carcinoma; true glandular variants also occur.

The tumor cells are usually round, polyhedral, or spindle-shaped but they may also be oncocytic or have clear cytoplasm [25, 26] (Fig. 7.2). Absence of distinct

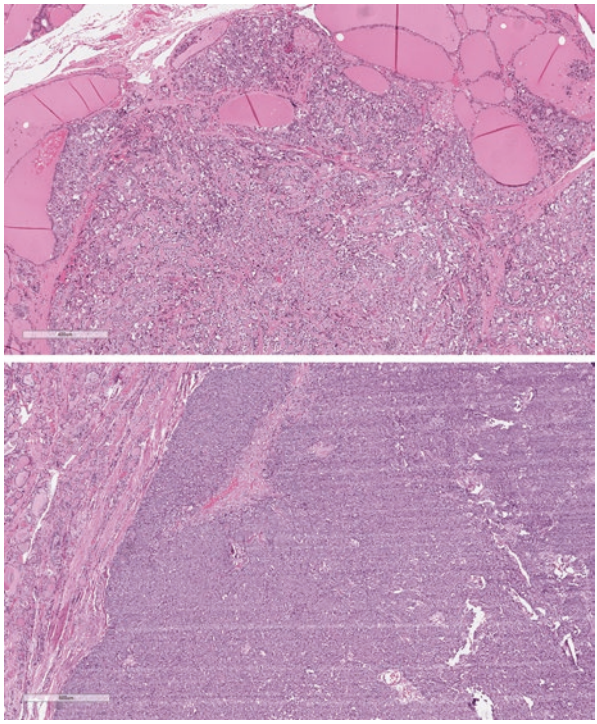

Fig. 7.1 Patterns of growth of medullary thyroid carcinoma. These tumors are usually infiltrative and grow around the follicles of the nontumorous thyroid (top), but occasionally they are well-delineated and expansile lesions that mimic thyroid follicular lesions (bottom)

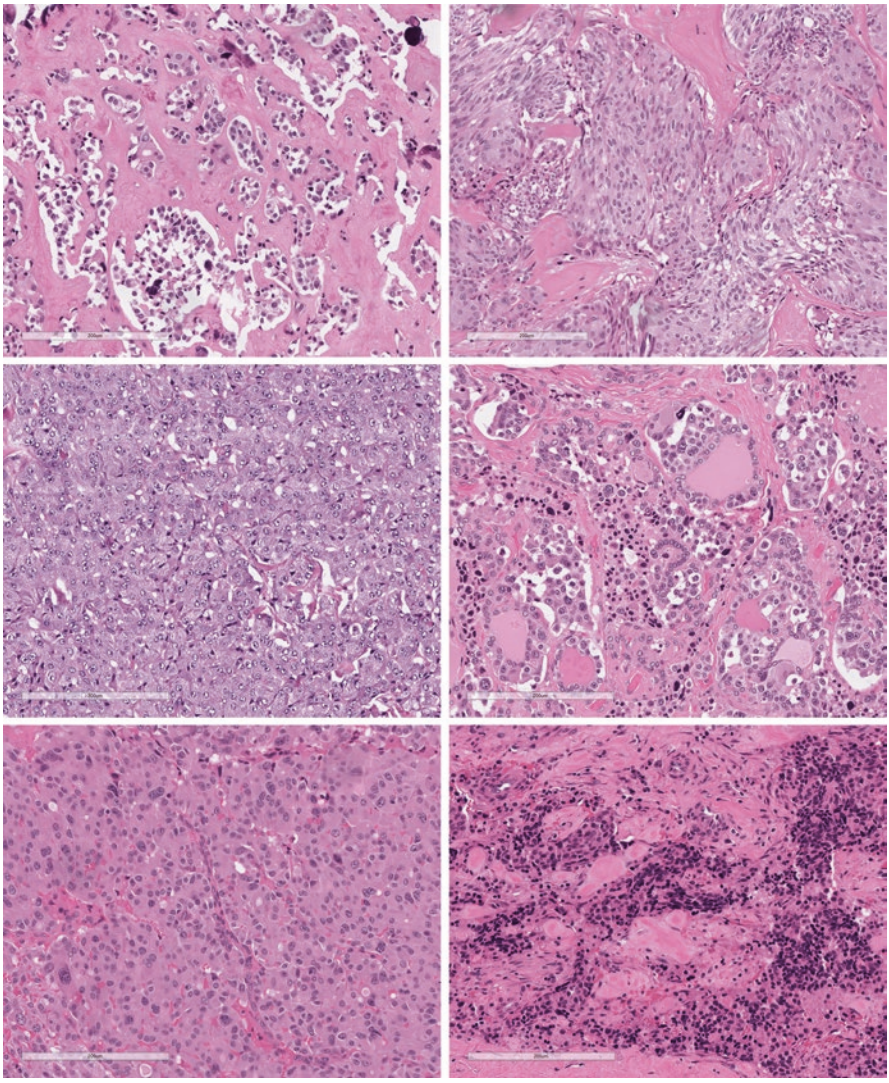

Fig. 7.2 Architecture and cytology of medullary thyroid carcinoma. The classical variant of this tumor is composed of small nests of discohesive cells in a stroma with amyloid and may have focal calcification (top left). Tumors with less amyloid usually have a more spindle cell morphology (top right). Some tumors are composed of epithelioid cells that appear to be more cohesive but lack the well-defined cell borders of follicular epithelial cells (middle left). When they trap nontumorous follicles, they can be mistaken for follicular cell-derived lesions, but the tumor cells have distinctive morphology including giant cell formation (middle right). Some medullary thyroid carcinomas are composed of oncocytic cells (bottom left) that should be characterized appropriately using immunohistochemistry so that they are not misdiagnosed as "Hürthle cell carcinoma." The small cell variant of medullary thyroid carcinoma (bottom right) is a more aggressive and less well-differentiated form of this disease

cell membranes and discohesive or loosely cohesive appearance with basophilic or amphophilic cytoplasmic granularity are distinctive features. The nuclei are usually bland with a "salt and pepper" appearance, but in some tumors, they develop grooves, resembling papillary thyroid carcinoma or hyalinizing trabecular tumor [27]. Medullary thyroid carcinoma is usually a relatively well-differentiated neuroendocrine tumor, but there is a small cell- or neuroblastoma-like variant that can be mistaken for small blue round cell tumors including but not limited to hematologic malignancy or neuroblastoma [28] and a giant cell variant as well [29]. Pigmented melanin-producing cases occur and rare tumors have an angiosarcoma-like morphology [15, 16, 30, 31].

A distinctive feature of this tumor type is the formation of amyloid, beta-pleated sheets of a preprocalcitonin molecule (Fig. 7.3). Amyloid can be identified by its characteristic apple-green birefringence with polarized light that is enhanced by Congo Red staining but can be seen on unstained sections and on tissue stained with H&E. Amyloid is present in just over half of medullary thyroid carcinomas, and it may be only very focal, limited to intracytoplasmic globules. Because of this, it is not a reliable marker of this tumor type. Moreover, amyloid may also be found in benign amyloid goiter and associated with other tumors [32–36].

Calcification is rare in medullary thyroid carcinomas, and even more rare is the identification of psammoma bodies that have been reported in this tumor type.

Immunohistochemistry is required to confirm the diagnosis (Fig. 7.4). These tumors, as members of the family of neuroendocrine tumors, express synaptophysin and chromogranins as well as the transcription factor regulating neuroendocrine differentiation insulinoma-associated protein 1 (INSM1). They are epithelial NENs and therefore express keratins as seen in other NETs of endodermal origin. Some express TTF1 as detected by the SPT24 antibody; however about one quarter of these neoplasms can be negative for TTF1. The diagnosis must entail identification of the biomarkers that are often considered specific to this entity: calcitonin, CGRP, and carcinoembryonic antigen (CEA) that should be stained using a monoclonal antibody. However, there are several pitfalls that diagnosticians should recognize when using these biomarkers.

Calcitonin and CGRP are considered by many to be the specific biomarker of MTC; however a small fraction of MTCs do not express calcitonin and/or CGRP, and more importantly, the expression of these hormones is not specific to this disease; several other neuroendocrine tumors, including parathyroid neoplasms, thymic neuroendocrine neoplasms, head and neck NENs, pancreatic NETs, and paragangliomas, can also express these hormones [20, 37–41]. In addition, tyrosine hydroxylase, which is often used to confirm the paraganglioma diagnosis in a cytokeratin- and transcription factor-negative NEN, can also be expressed in medullary thyroid carcinomas [20, 42]. However, GATA3, which is also expressed in paragangliomas and parathyroid and pituitary NETs, is typically negative in medullary thyroid carcinomas. Since some MTCs can display overlapping features with follicular epithelial neoplasms and these tumors can be positive for TTF1, it is critical to use appropriate tools to distinguish these entities. PAX8 expression in medullary

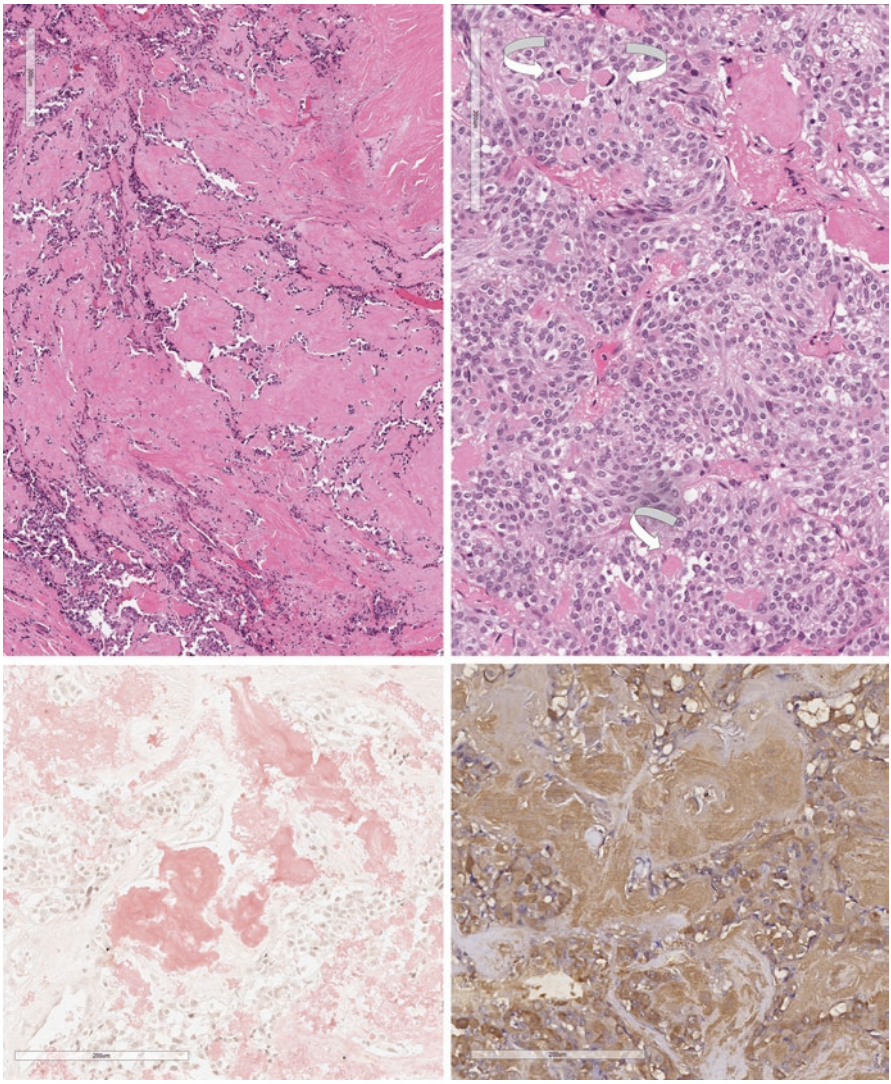

Fig. 7.3 Amyloid in medullary thyroid carcinoma. The presence of amyloid is identified in approximately half of these tumors. It is usually abundant (top left) but may be scattered and scant (top right); it may be found only within tumor cells that accumulate the material and rupture (top right, arrows). It stains with Congo Red (bottom left), but this stain is not required to elicit the apple-green birefringence with polarized light that is characteristic of amyloid. The amyloid material is composed of preprocalcitonin molecules and stain for calcitonin (bottom right)

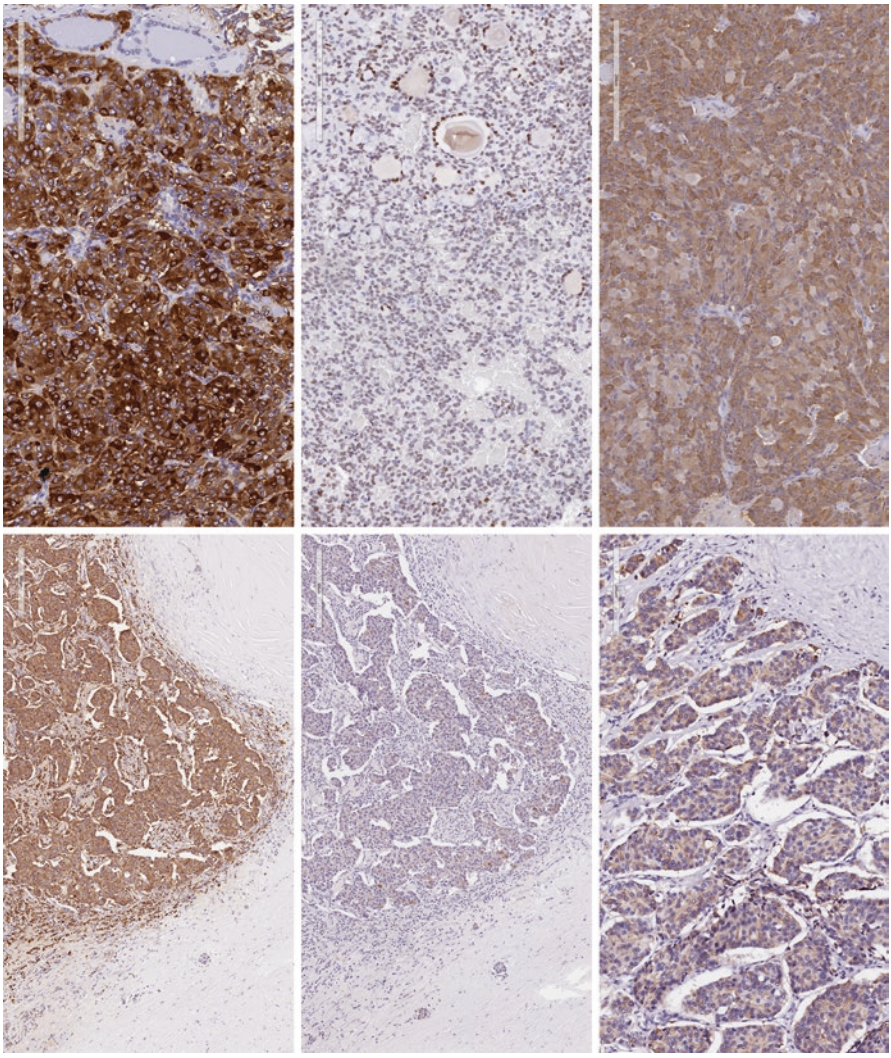

Fig. 7.4 Immunohistochemical profile of medullary thyroid carcinoma. These tumors stain strongly for chromogranin (top left) and may have variable nuclear reactivity for TTF1 (top middle). They usually have cytoplasmic positivity for calcitonin (top right), and they stain diffusely for carcinoembryonic antigen (CEA) (bottom left); the importance of CEA cannot be overemphasized, as sometimes aggressive tumors lose expression of calcitonin (bottom middle), while CEA is retained as a valuable tumor marker. Some tumors express hormones ectopically, most commonly ACTH (bottom right) as in this tumor that caused ectopic Cushing syndrome

thyroid carcinomas occurs in an antibody-dependent manner that is likely due to cross-reactivity; polyclonal PAX8 antisera and some N-terminus-specific PAX8 monoclonal antibodies can be positive in MTCs, whereas C-terminus-specific monoclonal PAX8 antibodies (clones BC12 and PAX8R1) and N-terminus-specific monoclonal PAX8 (clone MRQ50) are negative in these neoplasms [43].

The importance of monoclonal CEA immunohistochemistry cannot be overemphasized; as discussed earlier other NETs can express calcitonin and/or CGRP and be variably positive for CEA. However, diffuse strong reactivity using a monoclonal antibody to CEA is characteristic of medullary thyroid carcinoma, and while calcitonin can be reduced as tumors dedifferentiate, CEA is typically retained. For this reason, circulating CEA is of clinical value in surveillance, and a reduction in calcitonin levels with persistent or increasing CEA is a feature of tumor progression and dedifferentiation [44, 45]. Other peptides can also be expressed, including somatostatin and some that can give rise to clinical syndromes, for example, derivatives of proopiomelanocortin including ACTH that can cause ectopic Cushing syndrome and serotonin that can be a cause of carcinoid syndrome that can be mimicked by calcitonin. Other unusual hormonal products include glucagon, gastrin, cholecystokinin, vasoactive intestinal peptide (VIP), bombesin, and α-hCG [13, 46–48].

Like other neuroendocrine tumors, medullary thyroid carcinomas should have a formal Ki67 labeling index [49], but unlike other neuroendocrine tumors, there is no classification scheme for grading. Prognostic and predictive markers have been identified but are not in routine clinical use [50–52]. The poorly differentiated forms such as small cell types tend to be more aggressive [53], and significant tumor necrosis is a feature of more aggressive biology (Fig. 7.5). Angioinvasion, defined as the presence of tumor cells within vascular channels associated with thrombus (Fig. 7.5), is an important predictor of recurrence and distant metastasis [50]. Expression of somatostatin receptors (SSTRs) may be of value in determining therapy including administration of somatostatin analogues to inhibit hormone secretion and restrain growth and somatostatin-based peptide receptor radiotherapy (PRRT) in the treatment of unresectable disease [54–57]. Somatostatin-labeled imaging is also useful to identify metastatic deposits [58, 59].

The differential diagnosis includes intrathyroidal paraganglioma that can be identified by nuclear reactivity for GATA3, cytoplasmic staining for tyrosine hydroxylase, and lack of keratin and monoclonal CEA reactivity. Since tyrosine hydroxylase reactivity can sometimes be focal or absent depending on functional status of a paraganglioma, the use of a panel approach combining GATA3, TTF1, keratins, and monoclonal CEA should be used in the diagnostic workup.

The rare intrathyroidal thymic NEN can pose diagnostic challenges [41]. These tumors are often thought not to express diffuse monoclonal CEA, but can be positive for CGRP and calcitonin.

Medullary thyroid carcinomas with clear cell change and/or oncocytic change can simulate an intrathyroidal parathyroid neoplasm. Parathyroid tumors express GATA3, GCM2, keratins, and parathyroid hormone. Rarely, calcitonin and CGRP can be expressed in parathyroid neoplasms [40] (references); however, the

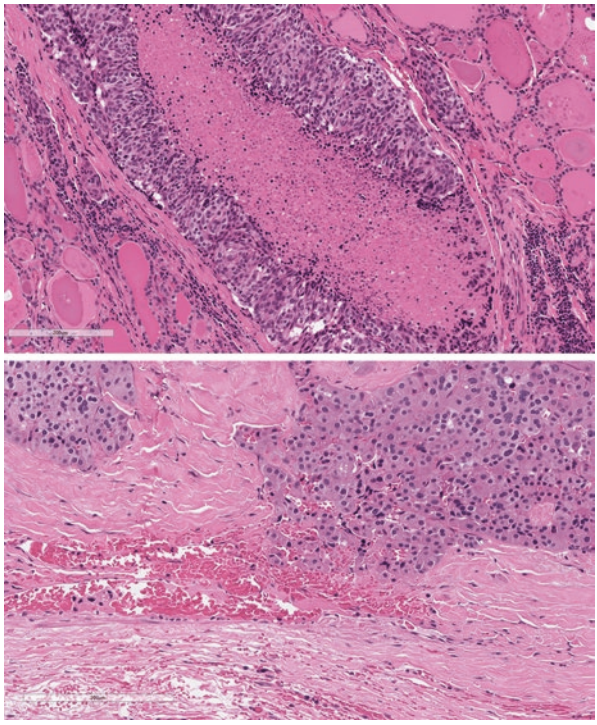

Fig. 7.5 Prognostic features in medullary thyroid carcinoma. The presence of extensive tumor necrosis (top) and angioinvasion, defined by tumor cells within vascular channels associated with thrombus (bottom), are adverse features seen within the primary tumor

parathyroid-specific transcription factors and lack of monoclonal CEA expression distinguish parathyroid origin.

Mixed follicular-C cell tumors constitute the only well-recognized mixed neuroendocrine and non-neuroendocrine tumor (MiNEN) of the thyroid gland. These unusual neoplasms may be composite or collision tumors [60–64] or the exceptional monomorphous proliferations with dual differentiation [65–67]. It is important to recognize that most medullary thyroid carcinomas have trapped nontumorous thyroid follicles (Fig. 7.6) and the follicular epithelium may show reactive atypia, but this does not qualify as a composite tumor. There must be clear evidence of two malignant components, and when in doubt, rely only on the presence of metastasis of both components to a regional node (Fig. 7.6) [63, 64, 68]. The application of biomarkers of malignancy of follicular epithelial neoplasms (e.g., HBME-1, galectin-3 *NRASQ61R*- or *BRAFV600E*-mutation-specific antibodies) [69] can assist in proving malignancy of the follicular component, but this must be coupled with the clear knowledge that the medullary thyroid carcinoma may express some of these markers.

The familial nature of medullary thyroid carcinoma can be detected by careful pathologic examination to identify *C cell hyperplasia to neoplasia* that may be associated with progression to *multifocal primary microtumors (medullary microcarcinomas)* (Fig. 7.7) [70, 71]. Normal C cells are present as scattered single cells at the junction of the upper third and lower two thirds of the lateral thyroid lobes. C cell

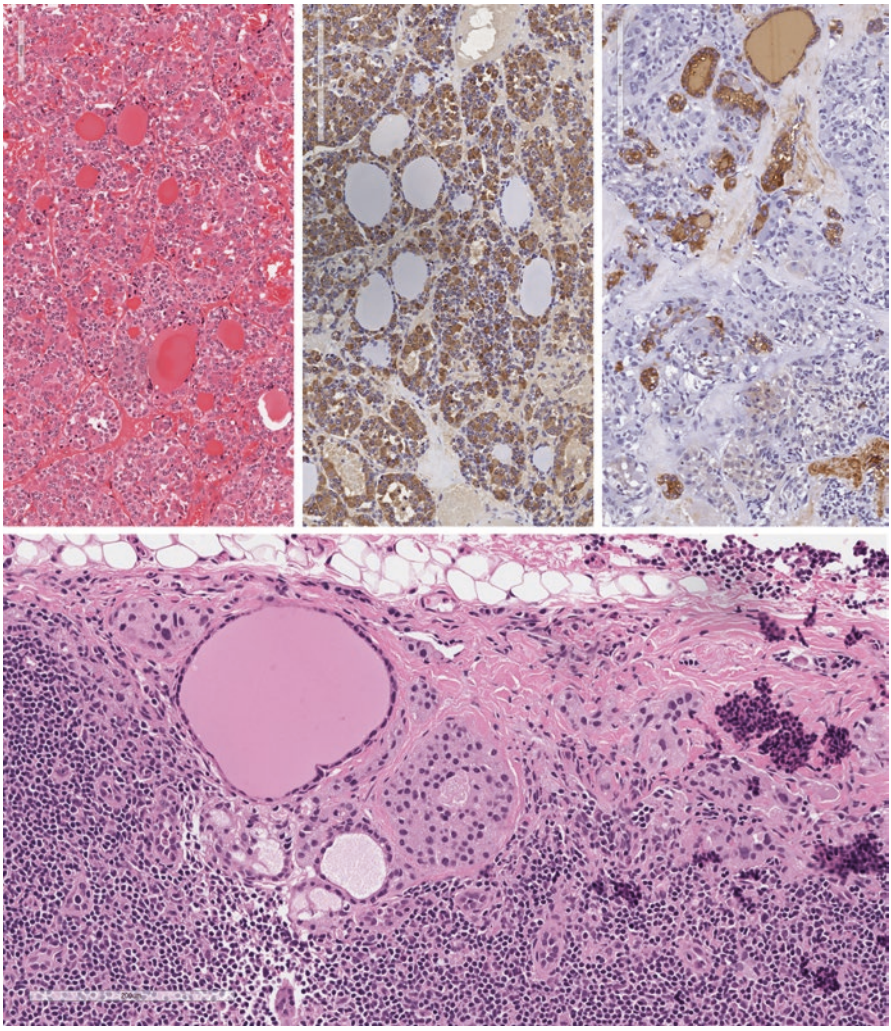

Fig. 7.6 Trapped thyroid or composite tumor? The presence of thyroid follicles within a medullary thyroid carcinoma (top left) does not indicate the presence of a composite tumor, since these lesions grow by surrounding the adjacent nontumorous gland. The follicular cells may even exhibit nuclear atypia that resembles papillary thyroid carcinoma but this is usually reactive. Careful examination will confirm the presence of cells that are negative for calcitonin (top middle) and monoclonal CEA and positive for thyroglobulin (top right) in such cases, confirming that these are two separate populations of cells. However, when a lesion metastasizes to a lymph node with both C cell and follicular cell components (bottom), that confirms that the tumor was indeed a composite lesion

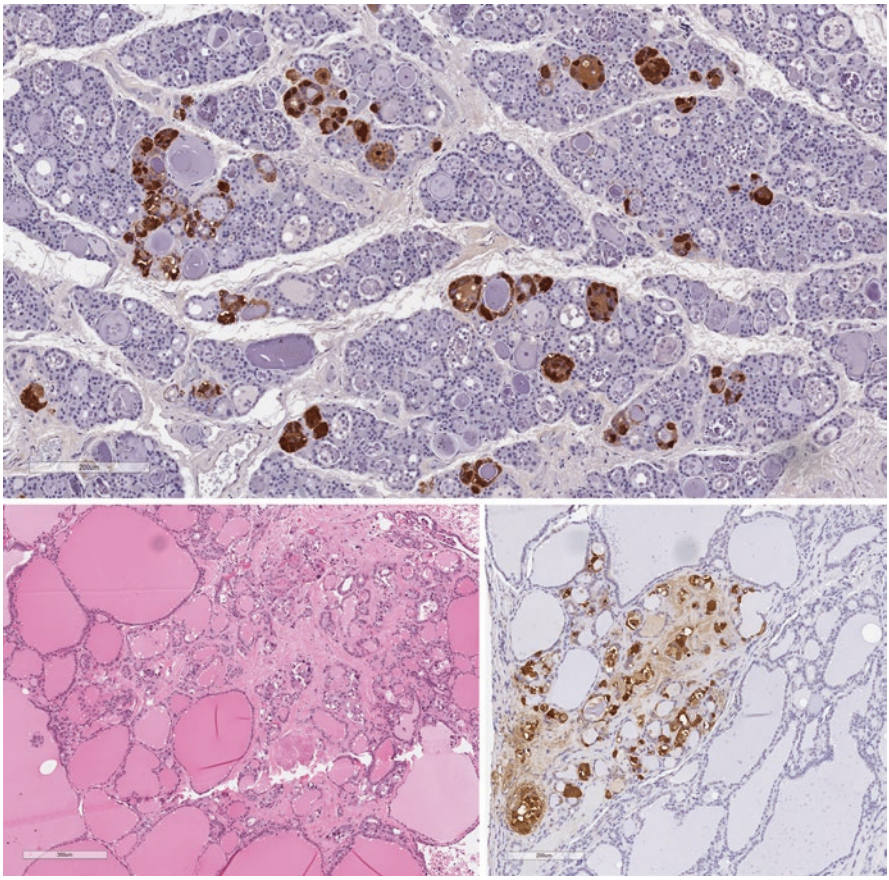

Fig. 7.7 C cell hyperplasia and micromedullary thyroid carcinoma. In patients with germline *RET* mutations, these precursor lesions develop multifocally throughout the thyroid. C cell hyperplasia is difficult to see on routine H&E staining, but immunohistochemistry for calcitonin (shown, top) or monoclonal CEA will identify the increased number of C cells forming clusters and completely surrounding follicles. With progression, they form small tumors that are visible on H&E (bottom left) and stain for calcitonin (bottom right) and monoclonal CEA (not shown)

hyperplasia, an increase in the population of C cells, has two clinicopathologic variants: (i) reactive or secondary and (ii) precursor or primary forms. The criteria used to define C cell hyperplasia are variable and include an increased number of C cells with (i) more than 7 cells per cluster leading to 50 C cells per low-power field, (ii) complete follicles surrounded by C cells, or (iii) C cells outside the normal location, including in the lower pole of the thyroid lobes and isthmus [72]. The two cytomorphologic variants are linear and nodular hyperplasia; the former is usually

associated with reactive (secondary) to other lesions, whereas nodular C cell hyperplasia is a feature of germline *RET* mutation and can progress to microtumors (medullary microcarcinomas) and clinical medullary thyroid carcinoma. The potential for metastasis is thought to be achieved once C cells invade the basement membrane of a follicle. The distinction of medullary microcarcinoma from nodular C cell hyperplasia can be challenging. The identification of stromal desmoplasia and single cell infiltration can help in this distinction. Collagen type IV immunohistochemistry can also facilitate the assessment of basement membrane breakdown in microinvasive tumors [69, 72].

It is important to note that C cell hyperplasia cannot be assessed in the nontumorous tissue surrounding a medullary thyroid carcinoma, since this may represent invasive tumor; therefore, this analysis should be carried out on the lobe opposite a tumor. Other causes of C cell hyperplasia including chronic hypercalcemia, thyroiditis, and reaction to nodular follicular lesions [73–76] as well as PTEN hamartoma tumor syndrome (PHTS) usually are characterized by linear C cell hyperplasia [77] that does not appear to progress to malignancy. Interestingly, in animals, antidiabetic incretins (glucagon-like peptide-1 analogues such as exenatide, liraglutide, and taspoglutide) have been implicated as causing C cell hyperplasia and MTC [78], but the data in humans have not supported this finding.

Since RET and RAS mutations are mutually exclusive in MTC, immunolocalization of *NRASQ61R* using the mutation-specific SP174 antibody [79] can assist in screening for sporadic MTCs (see pathogenesis below).

Molecular Pathogenesis

A significant proportion of MTCs are hereditary [12] as integral components of MEN2 syndrome. In MEN2A, they are associated with pheochromocytomas and parathyroid proliferations. In MEN2B, the thyroid, adrenal, and parathyroid proliferations are also associated with mucosal ganglioneuromas and a Marfanoid habitus. Some patients with MEN2A also have cutaneous lichen amyloidosis (CLA) and/or Hirschsprung's disease [12]. The syndrome formerly known as "familial medullary thyroid carcinoma" (FMTC) is now classified as a variant of MEN2A syndrome that rarely is associated with parathyroid disease or pheochromocytoma, but screening for these other entities is still warranted as it may occur [12]. These syndromes are all caused by germline mutations in the *RET* proto-oncogene. Familial transmission of MEN2A is associated with activating mutations in the ligand-binding regions of the extracellular domain or in the transmembrane or cytoplasmic domains. The most common mutations are in exon 10, codons 609, 611, 618, and 620; exon 11, codons 630 and 634; and exons 8, 13, 14, 15, and 16. In contrast, MEN2B is not usually familial, but rather is due to sporadic (de novo) germline mutation, most frequently in codon 918 of exon 16 and occasionally in codon 883 in exon 15 [12].

The identification of germline *MET* mutations in two siblings with wild-type *RET* harboring inherited medullary thyroid carcinomas has expanded germline

correlates of this disease and can open potential use of MET-inhibitor therapies in affected patients [80].

The management of patients with this disorder includes assessment of relatives, and members of known kindreds should undergo genetic screening early in life. As this represents a unique situation of inheritance of an activated oncogene (unlike most familial cancer syndromes that involve a mutant tumor suppressor requiring a second hit), affected individuals have an almost 100% chance of developing medullary thyroid carcinoma. For this reason, screening is critically important and affected individuals should undergo prophylactic thyroidectomy. The age at which this procedure is undertaken should be determined by the specific mutation and family history; however there is also occasional "genetic anticipation" which can cause earlier onset of tumors in following generations [12, 81, 82].

Sporadic medullary carcinomas may harbor mutations of *RET,* usually in codon 918 encoding the cytoplasmic tyrosine kinase domain, providing a target for therapy. The majority of sporadic tumors that lack *RET* mutations harbor *RAS* mutations, many of which can be identified using an immunohistochemical assay for mutant *NRASQ61R* [79]. Rare tumors have been reported with a *RET* fusion [83–85], *ALK* fusion [86], sequence variants of *NTRK1* [87], *BRAF* mutations or fusions [88, 89], telomerase activation [90], and microRNA abnormalities [91].

Prognosis

The prognosis of patients with medullary thyroid carcinoma varies with a number of parameters including age at diagnosis and tumor stage including extrathyroidal extension, lymph node status, and distant metastases [92].

Surgical resection is the only hope for cure of this disease, and patients with the diagnosis of MTC should undergo total thyroidectomy with central-compartment lymph node dissection if there is no evidence of disseminated disease biochemically and on imaging. There is a significant role for lateral neck dissection if there is any evidence of involved cervical lymph nodes or if the patient's calcitonin level is >200 ng/L [12]. Those with evidence of local residual disease after surgery or high-risk findings on pathology are thought to benefit from postoperative external beam radiation therapy (EBRT) to the neck.

Distant metastasis requires a tailored approach to therapy. Surgical resection has been used for solitary metastasis to the lung, brain, or liver. Radiofrequency ablation is recommended for hepatic metastases. Metastatic disease to brain or vertebral lesions that result in spinal cord compression may require glucocorticoid therapy in addition to surgical decompression and/or EBRT. Systemic therapy with somatostatin analogues is used to restrain tumor growth and alleviate symptoms of hormone excess. The tyrosine kinase inhibitors vandetanib or cabozantinib can provide a significant increase in progression-free survival as shown in prospective randomized double-blind clinical trials [12] but may have adverse effects. The use of a specific RET inhibitor LOXO-292 is currently under investigation (libretto trial, NCT03157128 at https://ClinicalTrials.gov/) and BLU-667 (arrow trial, NCT03037385). The role of

somatostatin-based peptide receptor radiotherapy (PRRT) offers promise for the treatment of unresectable disease [54–57].

Symptomatic relief of diarrhea induced by calcitonin can be obtained with antimotility agents such as loperamide or codeine, and the pain from bone metastases can be treated with denosumab or bisphosphonates. Patients with ectopic hormone excess such as Cushing syndrome due to tumor production of ACTH and/or CRH should be treated with medical therapies to inhibit glucocorticoids (e.g., ketoconazole, mifepristone, aminoglutethimide, metyrapone, or mitotane) or may be helped by bilateral adrenalectomy.

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Parathyroid Neuroendocrine Neoplasms

8

Sylvia L. Asa and Ozgur Mete

Historical Background

The history of the parathyroid glands is interesting because these tiny glands were not recognized until 1850 when Owen, working on the Indian rhinoceros, identified "a small, compact yellow glandular body attached to the thyroid at the point where the veins emerge"; he published this 12 years later [1]. In the interim, Remak and Virchow described similar structures in the cat [2] and human [3]. The terminology "glandulae parathyroidae" was provided by Sandström in 1880 [4]. Kohn proposed the term "Epithelkörperchen" as he recognized that these glands had a distinct embryology from that of the thyroid [5, 6].

Their functional relevance and tumor biology awaited the accurate biochemistry that is required to understand them. Early studies by Gley and Erdheim associated tetany with lack of parathyroids [7–9]. The work of Hanson [10] and Collip [11] proved their importance in calcium regulation; parathyroid hormone was not discovered until 1959 [12].

The parathyroid glands are the site of the most benign NETs. Metastatic disease is very rare, and local growth does not have significant sequelae, so unlike the pituitary NETs, large non-metastatic tumors are easily resected.

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There has been significant progress in understanding the genetics of these tumors and their association with familial endocrinopathies. The latter is further discussed in the chapter on inherited neuroendocrine neoplasia syndromes.

Epidemiology

The diagnosis of parathyroid tumors is increasing over time. This is attributable to the increasing recognition of "asymptomatic" primary hyperparathyroidism based on biochemical testing; it is now rare to see symptomatic disease with nephrolithiasis and peptic ulcers or the more severe osteitis fibrosis cystica and psychiatric/cognitive symptoms that characterized this disease prior to routine serum calcium testing.

Primary hyperparathyroidism now is thought to have an incidence of approximately 1:1000 population. It increases with age, reaching a peak in the seventh decade [13–16]; it is five times more common in older women than in older men, but this gender difference is not evident in patients under the age of 45 years.

By far the vast majority of cases (approximately 80–85%) is due to an adenoma involving a single parathyroid gland. Hyperplasia and carcinoma account for the remainder with carcinoma representing <1% of cases. Although rare, the incidence of parathyroid carcinoma has been increasing in the last few decades [17]. Secondary and tertiary hyperparathyroidism are being diagnosed more frequently as patients with renal failure live longer on dialysis.

Tumor Classification and Morphology

The majority of parathyroid NENs consists of benign parathyroid adenomas that are readily diagnosed clinically and morphologically and can be resected surgically, resulting in cure of primary hyperparathyroidism. These usually involve only a single gland of the four or more glands that are normally present; up to 13% of people have a supernumerary gland [16, 18, 19] which, if not identified, may be a reason for failed surgery [16]. The two superior glands give rise to tumors that are usually located posterior to or within the thyroid at the junction of the upper and middle third of the lateral lobes, near the cricothyroid junction, superior to the inferior thyroid artery and usually deep to the recurrent laryngeal nerve [20]; occasionally they may be retropharyngeal or retroesophageal. The inferior glands give rise to tumors that are more anterior and below the recurrent laryngeal nerve [20]; they may be in the inferior thyroid pole, intrathymic, mediastinal, or higher in the neck near the hyoid bone or carotid bulb.

The diagnosis of a *parathyroid adenoma* involves the identification of an enlarged (>6–8 mm) and cellular gland with increased glandular weight (>40–60 mg) that has uniform architecture and a normal rim of uninvolved parathyroid tissue [21, 22]

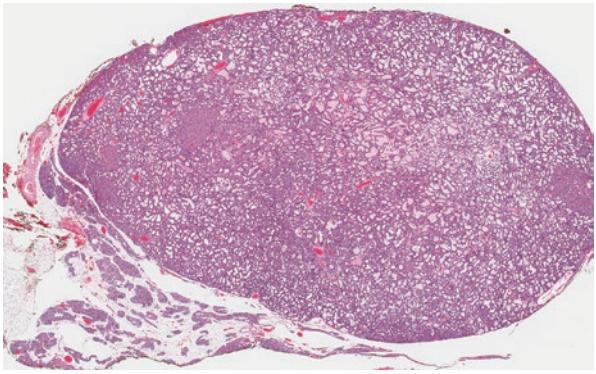


Fig. 8.1 Parathyroid adenoma. A rim of atrophic parenchyma surrounding a nodular chief cell proliferation is the hallmark of parathyroid adenoma in the appropriate clinical and biochemical setting

(Fig. 8.1). These tumors may be composed of solid nests, sheets, glands, or trabeculae of cells; the cells may be uniform chief cells, clear cell or oxyphils, or even the rare water clear cells (Fig. 8.2). There may be mixtures of the various cell types. The tumors are usually well delineated and may be encapsulated; they rarely have any evidence of invasive growth. There may be cystic structures, hemorrhage, and peliosis. Mitoses are usually inconspicuous. Occasional tumors have lipomatous stroma and are known as "lipoadenoma" [23] (Fig. 8.3).

Occasionally a single enlarged gland has worrisome morphologic features; there may be fibrosis with the formation of fibrous bands or a pseudocapsule with tumor cells trapped in the fibrosis; there may be tumor necrosis, and mitotic activity may be brisk. In this setting, the possibility of carcinoma must be excluded. *Parathyroid carcinoma* is rare but can be diagnosed with the identification of metastasis or invasive growth [24] including unequivocal angioinvasion, defined as the presence of tumor cells within a vascular lumen associated with thrombus [22] (Fig. 8.4). Other components of invasive growth include lymphatic invasion, perineural invasion, as well as locally gross malignant invasion into the surrounding/adjacent tissues. If any of these is identified, the diagnosis is clear. However, if other worrisome features (e.g., increased mitotic activity, necrosis) are seen without invasive growth (e.g., angioinvasion), the term "atypical parathyroid adenoma" has been suggested. This diagnostic category has now been referred to as diagnostic category of uncertain malignant potential in the 2017 WHO classification of parathyroid neoplasms [24]. In the experience of the authors, atypical changes are most often seen in patients who have undergone prior manipulation of the parathyroid lesion, usually fine needle aspiration, but occasionally more aggressive ethanol injection, and these features are reactive [25, 26] (Fig. 8.5). Fibrous bands can also be encountered in the setting of tertiary hyperparathyroidism as well as in some MEN1 syndrome-related parathyroids [22]. The use of immunohistochemistry for biomarkers of malignancy can be used to clearly distinguish parathyroid carcinoma from adenoma with degeneration and reactive atypia (Figs. 8.6, 8.7, and 8.8); these biomarkers include loss of expression of parafibromin encoded by the *CDC73/HRPT2* gene (this may be total

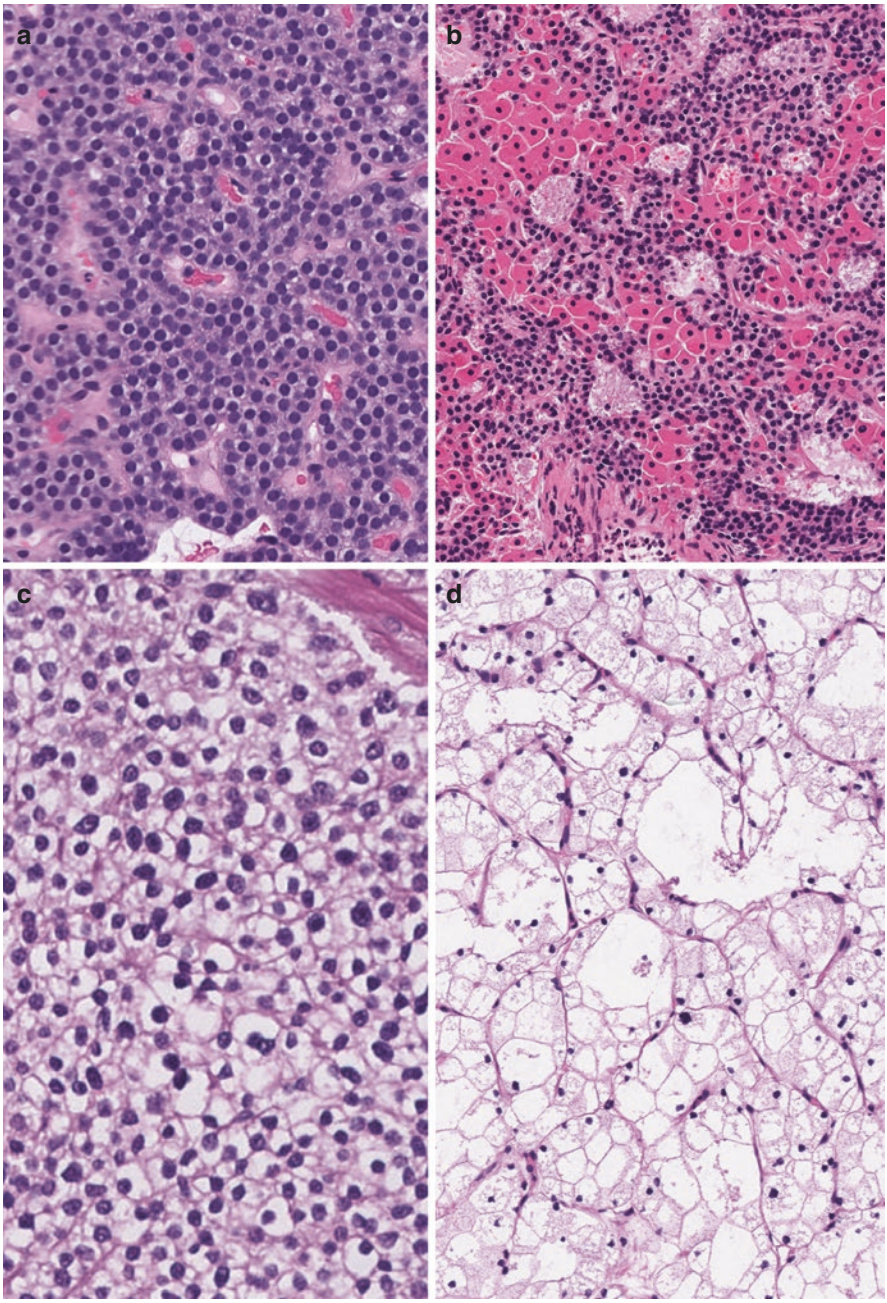


Fig. 8.2 Cell types in parathyroid adenomas. Various cell types can be seen in parathyroid proliferations. Chief cell proliferations are most commonly encountered (**a**). In some cases, mixed chief and oncocytic cells can be encountered (**b**). Rare examples of pure oncocytic proliferations (not shown), clear cell (**c**), and water clear cell (**d**) parathyroid proliferations comprise the cytomorphological spectrum of parathyroid proliferations

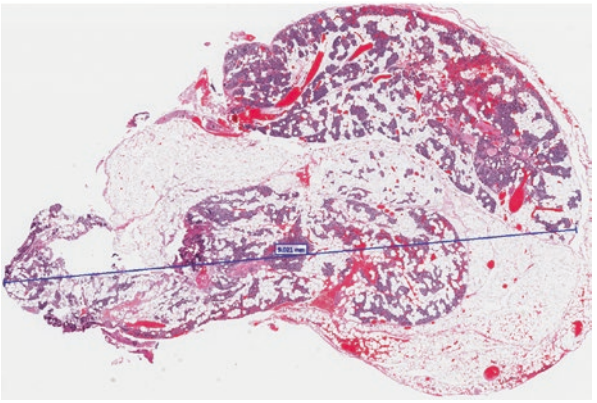


Fig. 8.3 Parathyroid lipoadenoma. This photomicrograph illustrates an enlarged gland displaying nests of chief cells admixed with adipocytes forming a lipomatous nodule in a patient with primary hyperparathyroidism. Removal of this gland resulted in biochemical cure

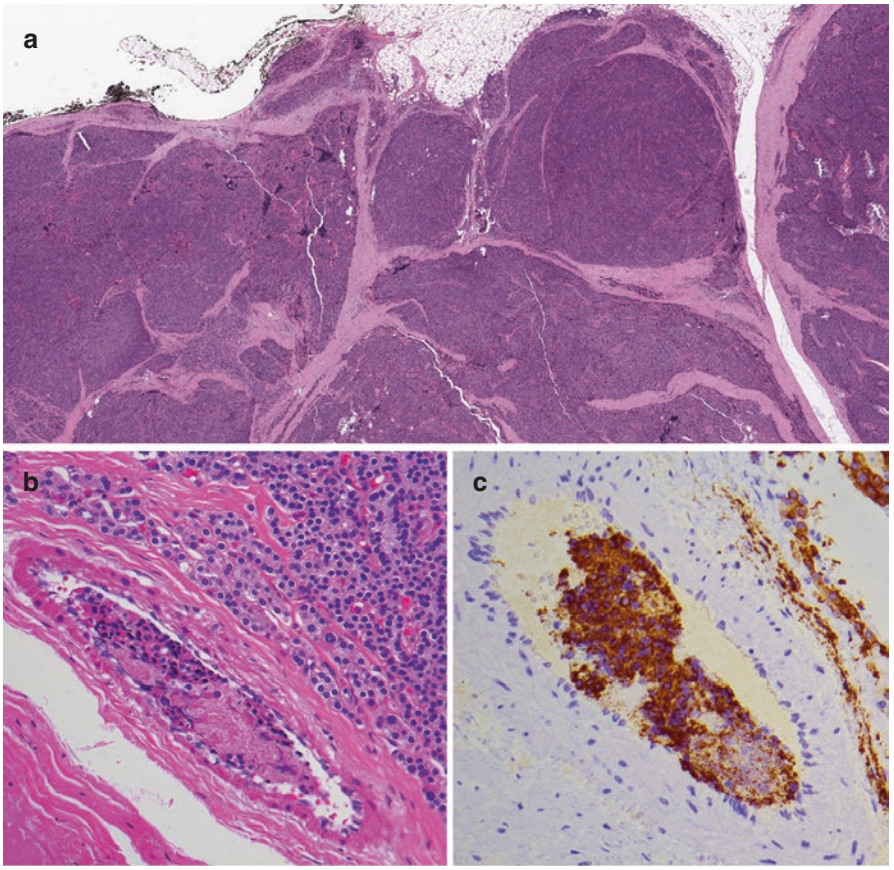
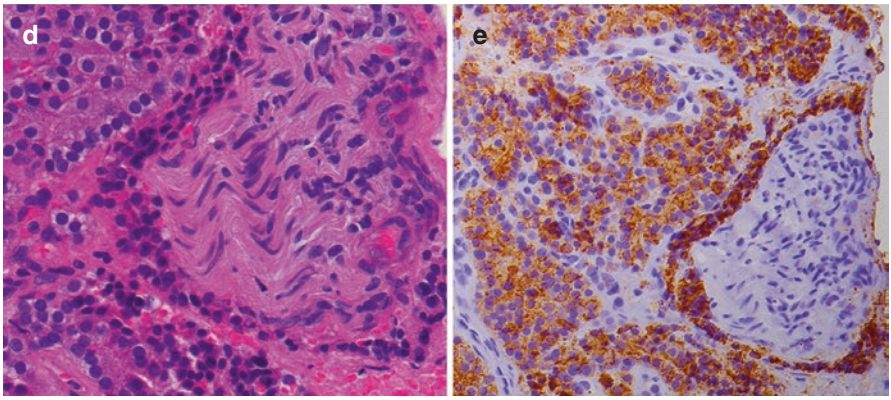


Fig. 8.4 Parathyroid carcinoma. The diagnosis of parathyroid carcinoma requires demonstration of metastatic spread or invasive growth. This composite photomicrograph illustrates some morphological features of parathyroid carcinoma: locally invasive growth with formation of fibrous bands (**a**), angioinvasion with intravascular tumor cells (**b**, **c**) and perineural invasion with infiltrating tumor cells in the epineurium (**d**, **e**) highlighted using PTH immunohistochemistry (**c**, **e**)



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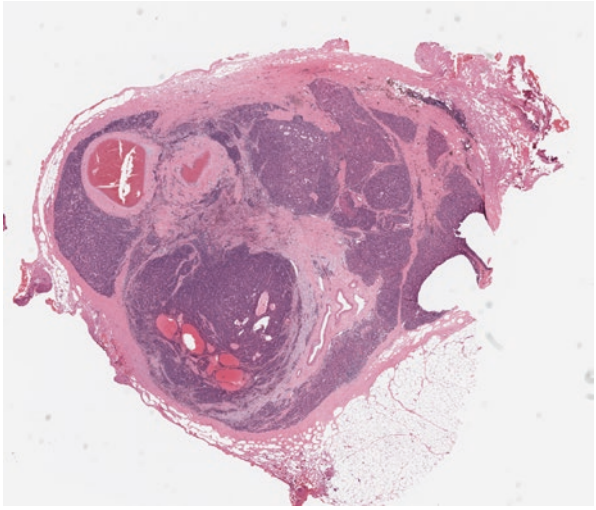

Fig. 8.5 Biopsy-induced atypia in a parathyroid adenoma. This photomicrograph illustrates a parathyroid neoplasm with post-biopsy worrisome histological changes

loss of nuclear staining or sometimes only more subtle nucleolar loss) (Fig. 8.6), loss of nuclear p27 [27] or retinoblastoma protein [27, 28], and loss of cytoplasmic BCL2 [27], abnormal expression of galectin-3 [27, 29] or PGP 9.5 [30, 31], and/or an abnormal pattern of p53 protein expression suggesting mutation (i.e., total negativity or diffuse strong positivity) [32] (Fig. 8.7). Other biomarkers of malignancy include loss of expression of MDM2 [33] and APC [34, 35]. Most parathyroid carcinomas tend to have a Ki67 labeling index exceeding 5% (Fig. 8.8). A high Ki67 labeling index also predicts aggressive disease [36].

Most patients with parathyroid carcinoma are diagnosed initially with primary disease, and then they go on to develop metastases. It is exceptional to have a patient present with metastases. Metastatic parathyroid carcinoma resembles other

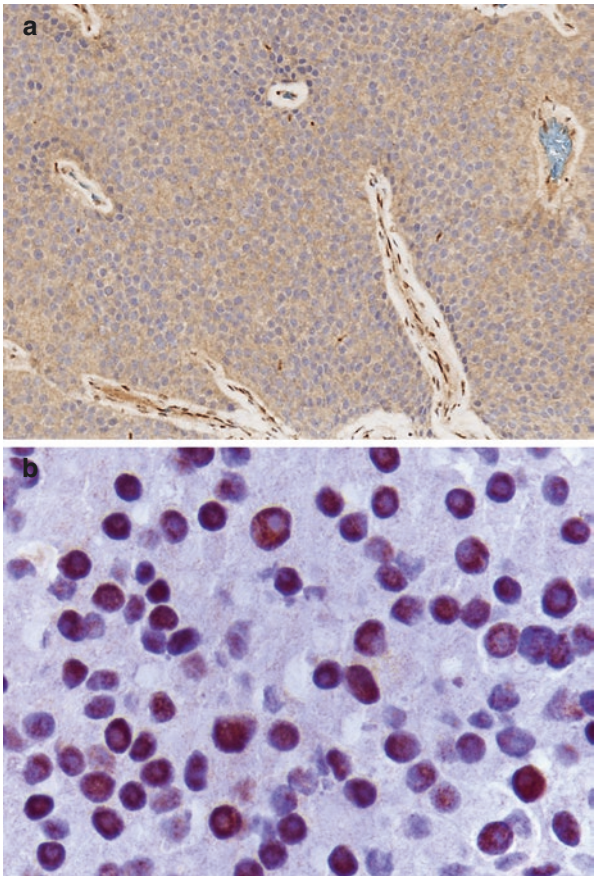

Fig. 8.6 Parafibromin immunohistochemistry. Loss of nuclear parafibromin expression supports the diagnosis of parathyroid carcinoma (**a**). Loss of nucleolar parafibromin expression (**b**) is also regarded as an abnormal staining pattern indicating parafibromin deficiency

epithelial NETs, but the diagnosis can be made by identifying expression of biomarkers of parathyroid cells: GATA3, GCM-2, and parathyroid hormone (Fig. 8.9). As in other NETs, with progression there may be dedifferentiation resulting in loss of parathyroid hormone expression [36], but strong nuclear GATA3 and keratin positivity support this diagnosis. The differential diagnosis of a NET with GATA3 expression includes paraganglioma (which should be negative for keratins) and the even more rare pituitary NENs of gonadotroph or thyrotroph differentiation [37]. However, focal GATA3 staining may be seen in any highly proliferative lesion; while these malignancies do show increased proliferation and there may be high and variable Ki67 labeling, it rarely reaches the levels associated with nonspecific GATA3 staining. One should also be aware of the fact that calcitonin (Fig. 8.10) and CGRP can be expressed in parathyroid neoplasms [38–40]. This should be taken into account when the differential diagnosis is medullary thyroid carcinoma. For this reason, the adoption of panel approach including but not limited to TTF-1 and monoclonal CEA should be considered when unusual morphological manifestations are encountered.

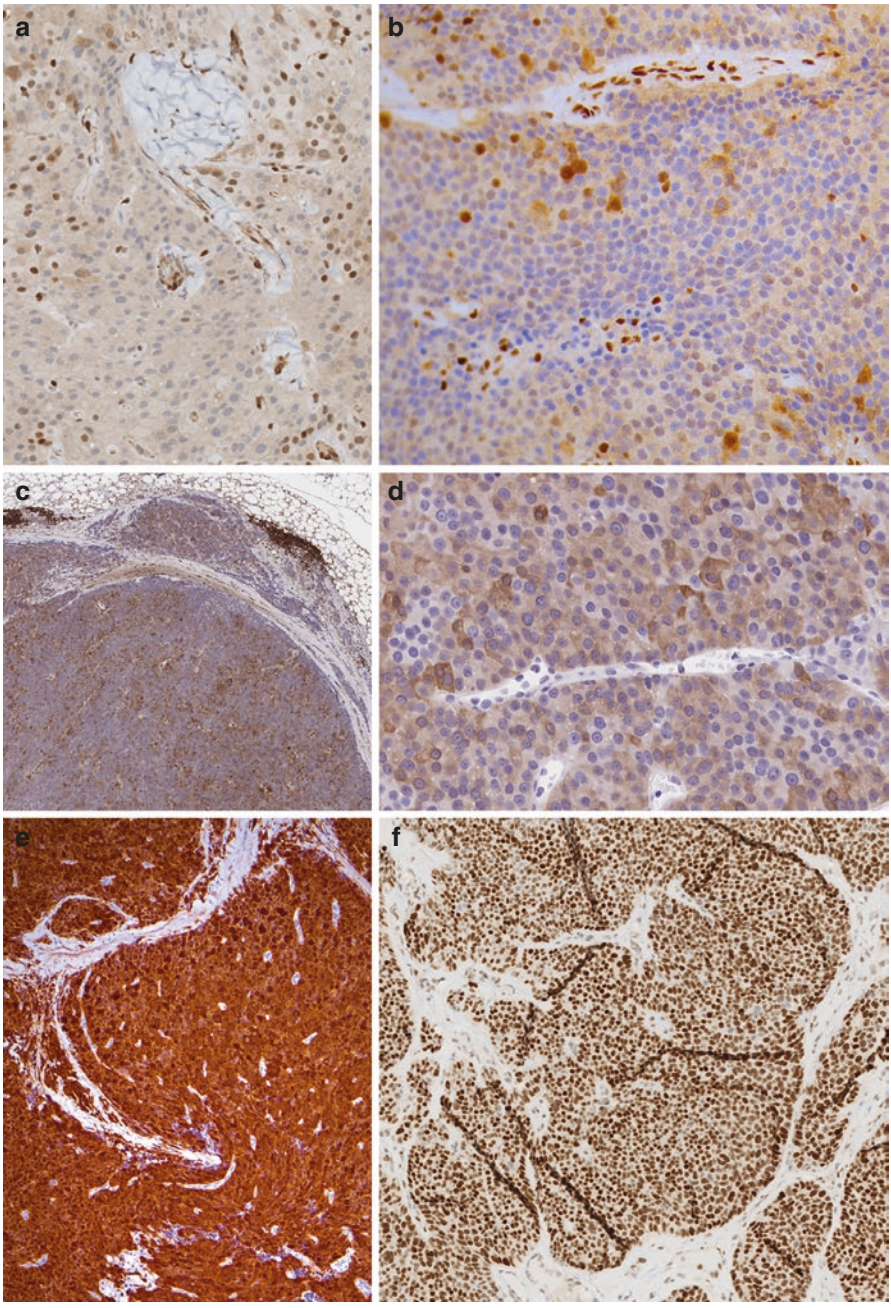

Fig. 8.7 Other biomarkers of parathyroid carcinoma. Several immunohistochemical biomarkers can assist in the diagnosis of parathyroid carcinoma. Loss/reduced expression of p27 (**a**), Rb (**b**), and BCL2 (**c**) and positivity for galectin-3 (**d**) and PGP9.5 (**e**) are features of parathyroid carcinoma. Although rare, p53 overexpression can also be identified in some cases (**f**)

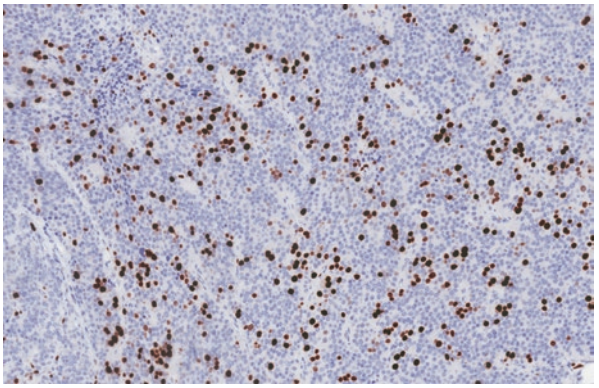

Fig. 8.8 Ki67 immunohistochemistry in parathyroid pathology. Most parathyroid carcinomas show a Ki67 proliferation index exceeding 5% as seen in this photomicrograph

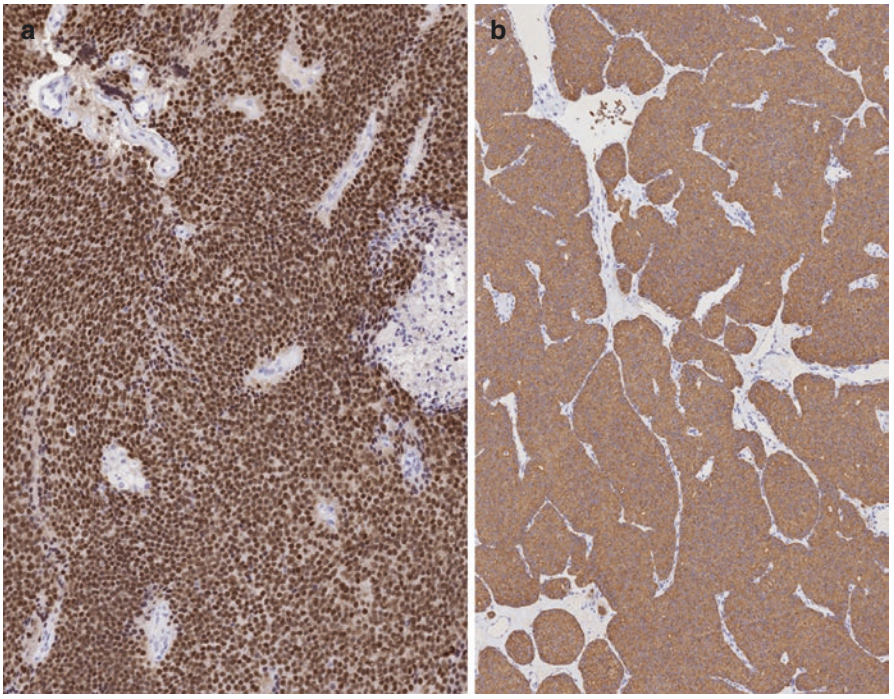

Fig. 8.9 Confirmation of parathyroid differentiation. GCM-2, GATA3, and PTH are biomarkers that can be used to confirm the parathyroid differentiation. Since GATA3 is not specific to parathyroid and can be expressed in other neuroendocrine neoplasms and PTH can rarely be seen in other neuroendocrine neoplasms, combined use of GATA3 and PTH is strongly recommended. The composite photomicrograph illustrates a parathyroid carcinoma (**a**, **b**) and water clear cell parathyroid adenoma (**c**, **d**) using GATA3 (**a**, **c**) and PTH (**b**, **d**) immunohistochemistry

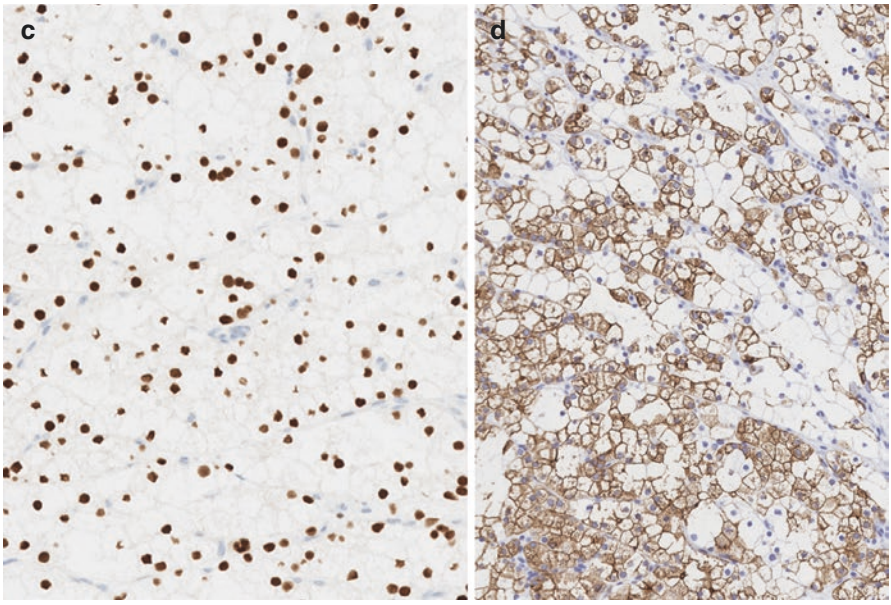

Fig. 8.9 (continued)

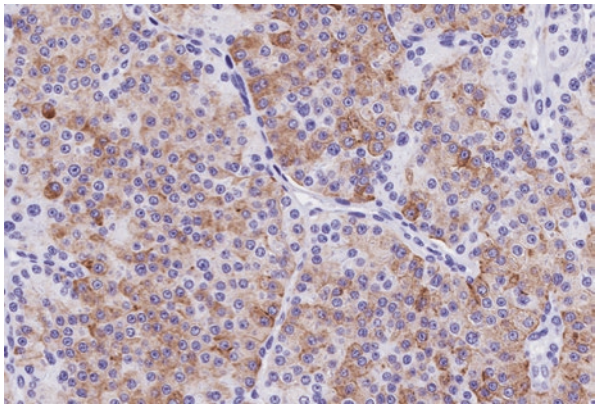

Fig. 8.10 Calcitonin expression in parathyroid neoplasms. The photomicrograph illustrates a parathyroid carcinoma that also expressed calcitonin (illustrated herein) along with diffuse GATA3 and PTH (not shown)

The involvement of more than one gland usually indicates a diagnosis of hyperplasia; however, multiple adenomas occur. True hyperplasia is usually a secondary phenomenon in patients with secondary hyperparathyroidism (that will not be discussed in this book on tumors), but patients with multiple endocrine neoplasia type 1 and type 4, who are described as having "hyperplasia," actually have multifocal

neoplasia involving multiple glands with multiple tumors in each gland. Other germline predisposition syndromes include MEN2 that results in a more conventional pattern of parathyroid hyperplasia with large cellular glands that do not have evidence of neoplasia despite the fact that this disorder is attributed to an activated oncogene.

Molecular Pathogenesis

Familial syndromes associated with parathyroid NETs have shed light on the pathogenesis of these tumors in some sporadic cases as well [41]. Patients with multiple endocrine neoplasia (MEN) type 1 and type 4 can be confirmed by documentation of loss of expression of the gene product of the *MEN1* gene, menin [42, 43], or global loss of the cyclin-dependent kinase inhibitor $p27^{kip1}$ [42, 44, 45]. Some sporadic parathyroid tumors also have somatic mutations and loss of heterozygosity in these genes [46].

Another familial syndrome associated with parathyroid neoplasia is the hyperparathyroidism-jaw tumor syndrome due to mutations in a tumor suppressor gene initially called *HRPT2*, now known as *CDC73,* which encodes the protein parafibromin [47]. Mutations in this gene are common in sporadic parathyroid carcinomas and can be identified by loss of parafibromin in tumor cell nuclei and even only in nucleoli [34, 42, 48]. Parafibromin loss may indicate germline predisposition, especially in a young patient. Parafibromin-deficient parathyroid neoplasms are more common in younger patients and tend to be large tumors with thick capsules, sheets of eosinophilic cells with coarse chromatin and perinuclear clearing, microcystic change, and arborizing vessels [31].

Alterations in other tumor suppressor genes, including *p27, Rb,* and *Tp53*, have been reported in parathyroid carcinomas [28, 32, 49–52]; novel mutations have been identified in genes that mediate chromosome organization, DNA repair, and cell cycle, and occasional mutations were found in in genes that regulate MAPK signaling and immune response [53]; some, such as *PTEN*, *NF1*, *KDR*, *PIK3CA*, and *TSC2*, may be targets for therapy of metastatic disease [54]. A recent series also underscored the occurrence of PI3K (*PIK3CA, TSC1, ATM*) and TP53 pathway-related mutations in advanced parathyroid carcinomas [55]. The same study also identified for the first time the occurrence of *SDHA, DICER1,* and *TERT* promoter mutations in parathyroid carcinomas [55]. As in other NETs, epigenetic modifications appear to also be implicated, and these tumors have changes in DNA methylation, histone modifications, microRNA dysregulation [56], and unusual circular RNAs [57].

In sporadic adenomas, there have been reports of *GNAS* mutation [58] and of overexpression of *cyclin D1 due to* the *CCND1/PRAD1* rearrangement that places cyclin D1 under the control of the PTH gene promoter, resulting in significant upregulation [50, 59].

Prognosis

The mainstay of treatment for all parathyroid NETs is surgery; complete resection offers cure for patients with localized carcinomas. In the case of familial disease that is multicentric, patients may require multiple surgical procedures if not anticipated at initial presentation.

Nevertheless, only a minority of patients with unequivocal malignancy achieve a durable remission from surgery [36]. Lung is the most common site of distant metastasis [36], but other sites include the bone and liver. In cases of carcinoma with more extensive disease, a number of therapeutic options are available. These include postoperative radiotherapy [36, 60] and chemotherapy with either cytotoxic agents such as dacarbazine and/or 5-fluorouracil (5-FU) and cyclophosphamide [36, 61] or the use of more targeted biological approaches such as sorafenib [36, 62]. Since parathyroid neoplasms are members of the larger family of neuroendocrine neoplasms and, like other NETs, they express somatostatin receptors [63], there is likely to be a role for peptide receptor radiotherapy (PRRT) in their management [36].

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Thoracic (Lung/Thymus) Neuroendocrine Neoplasms

9

Marco Volante and Giuseppe Pelosi

Foreword

Thoracic neuroendocrine neoplasms derive, at least in the majority of cases, from neuroendocrine cells, which are normally present as either single cells scattered in the ciliated epithelium of the airways or clusters (the so-called neuroepithelial bodies) in the lung, but that have not been recognized in the normal thymus, so far. Pulmonary neuroendocrine cells are identified as early as week 7 of gestation in large bronchi and derive from intrapulmonary stem cells through the activation of a specific transcriptional programming, being the human achaete-scute homologue 1 (hASH1) transcriptor factor, encoded by *ASCL1* gene in chromosome 12q, the most studied [1]. Lung neuroendocrine cells share the general morphological, ultrastructural, and immunophenotypic features described in the diffuse neuroendocrine system and possess specific physiological functions acting as sensory chemoreceptors involved in oxygen sensing. Lung neuroendocrine cell functions are mediated by an extraordinary variety of hormonal and receptor interactions. In fact, starting from the gestational phase, lung neuroendocrine cells produce several hormones including serotonin, gastrin-releasing, and bombesin-like peptides, ghrelin, obestatin, calcitonin, calcitonin gene-related peptide, and somatostatin. The role of neuroendocrine cells and neuroepithelial bodies is different in the course of development compared to adult life. In fetal and newborn lung, neuroendocrine cells participate to regulatory mechanisms of air tree branching and cell differentiation and maturation.

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Similar functions are also in place in adult lung during repair processes following injury [2]. In the young and adult population, the oxygen-sensing properties of neuroendocrine cells are tightly interacting with double sensory innervation [3]. A specific oxygen receptor was identified in the cell membrane of neuroendocrine cells of neuroepithelial bodies, belonging to the category of cytochrome b and of NAPDH oxidase [4]. The effects of oxygen sensing cells are automatically evident through the regulation and adaptation of bronchial/bronchiole wall tone, as well as breathing and blood flow control. Such effects are mediated by different neurotransmitters, including serotonin, acetylcholine, and ATP [5]. The complexity of these physiological functions, developmental processes, and hormone productions mirrors the heterogeneous conditions leading to neuroendocrine cell nonneoplastic and neoplastic proliferations and their wide variety of biological and clinical properties.

Classification of Thoracic NENs

The definition of thoracic neuroendocrine neoplasms, according to the most recent World Health Organization (WHO) classification of tumors [6], follows a scheme which encompasses four major categories. The classification scheme should be applied to surgical samples and relies mainly in the combination of morphological parameters which include the evaluation of mitotic index, of the presence of necrosis and of the cell size. Additional cytological and architectural features, such as the pattern of nuclear chromatin, the presence of nucleoli, or the pattern of growth, are descriptive of a given lesion but not definitional per se (Table 9.1). However, in some instances a clear-cut separation between entities encoded by the classification

Table 9.1 Pathological characteristics of thoracic neuroendocrine neoplasms

Parameter	Typical carcinoid	Atypical carcinoid	Large cell neuroendocrine carcinoma	Small cell carcinoma
Mitotic index[a]	<2 × 2 mm^2	2–10 × 2 mm^2	>10 × 2 mm^2	>10 × 2 mm^2
Necrosis[a]	Absent	Absent or present (punctate)	Usually present (extensive)	Usually present (extensive)
Cell size[a]	Variable (variants)	Variable (variants)	Large	Small (<3 small lymphocytes)
Nuclear features	Finely granular chromatin	Finely granular chromatin	Usually vesicular chromatin	Finely granular chromatin
Nucleoli	Occasional, small	Common, small	Present, large	Inconspicuous
Cytoplasm	Variable (variants)	Variable (variants)	Abundant	Scant
Pattern of growth	Organoid/ trabecular	Organoid/ trabecular	Organoid/trabecular/ cribriform	Sheetlike, diffuse
Ki-67[b]	Low (<5%)	Intermediate (<20%)	High (40–80%)	Very high (50–100%)

[a]Definitional parameters for classification
[b]Data on lung NENs

is worrisome since some cases may show borderline pathological features; moreover, the morphological criteria adopted in the classification are somehow subjective or potentially biased by not uniform sampling procedures, and therefore the reproducibility of the classification is not perfect among pathologists [7]. Finally, despite an overlapping classification scheme, neuroendocrine neoplasms in the lung or thymus possess different clinical, biological, immunophenotypic, and molecular characteristics that will be described in detail in this chapter.

Lung Neuroendocrine Neoplasms

Nonneoplastic Conditions and Preinvasive Lesions

The pathological features of neuroendocrine cell alterations in nonneoplastic and preinvasive conditions can be recapitulated by a spectrum of morphological changes ranging from linear hyperplasia to tumorlets whose clinical context of onset and morphological characteristics lack definitive criteria and in several instances coexist in the same tissue sample. The role of these lesions as precursors of lung neuroendocrine neoplasms is postulated for carcinoids [8], mainly those in peripheral location that may be associated with neuroendocrine cell hyperplasia in up to 75% of cases. By contrast, these lesions are probably not associated with the development of high-grade small and large cell carcinomas whose origin seems to be more complex and possibly linked also to other cell types (including type II alveolar cells) [9].

Neuroendocrine Cell Hyperplasia

Clinical Features

Increased number of neuroendocrine cells in the lung is associated with many causative factors.

In the pediatric age, neuroendocrine cell alterations are described in bronchopulmonary dysplasia and dysmaturity, in respiratory distress syndrome, in cystic fibrosis and cystic malformation, in pulmonary hypertension, and in sudden infant death. Bronchopulmonary dysplasia and cystic malformation are complex entities in which developmental errors bring to a disordered growth of different cell types, including epithelial and mesenchymal elements, together with neuroendocrine cells. A form of idiopathic neuroendocrine cell hyperplasia has been also recently described consisting of an obstructive airway disease of unknown etiology and pathogenesis, characterized by tachypnea, crackles, and hypoxia in infants aged less than 2 years [10]. The lung is hyper-expanded with ground-glass opacities [11] and contains hyperplastic bombesin-positive neuroendocrine cells in the alveolar and distal bronchiolar walls in the absence of developmental or inflammatory changes.

In the adult population, some overlapping with pediatric lesions exists, but alterations of the neuroendocrine cell compartment are generally associated with chronic obstructive diseases, smoking-related bronchiolar disease and pneumonia, or more generally to any condition leading to pulmonary injury and repair, as well as in interstitial inflammation and fibrosis [12, 13].

The mechanisms leading to neuroendocrine cell increase are only partly understood. On the one side, experimental mouse models showed that according to the type of induced injury cell regeneration is operated by different cell types, including progenitor cells and neuroendocrine cells [14] as a consequence of the need of expanding the regenerating cell pool in the setting of various basal pulmonary cell plasticities. In some other conditions, neuroendocrine cell hyperplasia is supposed to develop as a consequence of a hypoxic status, a hypothesis supported by experimental evidence in animal models and by the common occurrence of increased neuroendocrine cell number in normal individuals living at high altitudes, as well as patients suffering from hypoventilation syndromes [1].

Pathology

From a pathology viewpoint, neuroendocrine cell hyperplasia is not recognizable at gross examination. Histopathological patterns are recapitulated into two major types:

Linear hyperplasia is defined as an irregular overgrowth of typical triangular- or flask-shaped neuroendocrine cells, located in close contact with the basal membrane of small or large airways, intercalated with mucin and ciliated cells (Fig. 9.1a). Although without numerical cutoffs, in normal conditions neuroendocrine cells do not exceed 0.4% of all bronchial epithelial cells.

Nodular hyperplasia is defined by formation of small clusters of >10–20 neuroendocrine cells in contact with the basal membrane (larger than normal neuroepithelial bodies) (Fig. 9.1b) that may be associated or not with linear hyperplasia.

Tumorlets

Clinical Features

Tumorlets are proliferations of neuroendocrine cells in the bronchial or bronchiolar walls with submucosal extension, with a size of less than 5 mm. Any neuroendocrine cell proliferation of 5 mm or more is by definition a neuroendocrine tumor

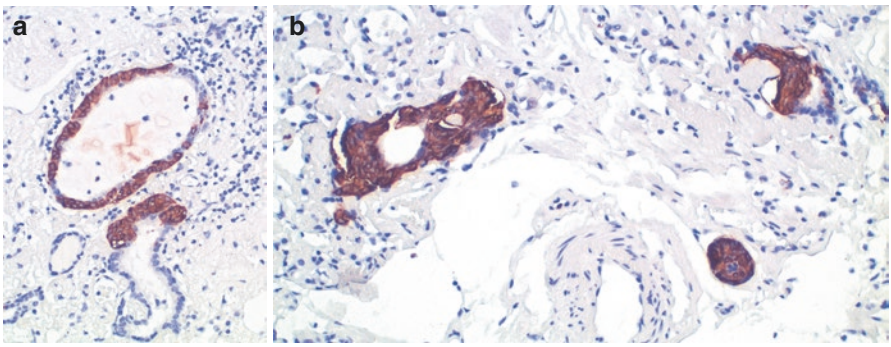

Fig. 9.1 Pulmonary neuroendocrine cell linear (**a**) and nodular (**b**) hyperplasia in a patient with multiple bronchiectasis and chronic inflammation (chromogranin A staining, immunoperoxidase; original magnification 10×)

(carcinoid) [6]. Tumorlets are rare, but their small dimensions probably result in underestimation of their real incidence, if appropriate serial sections are not performed during examination of the surgical specimen. Tumorlets are usually incidental findings at light microscopy, when a variety of pulmonary conditions are examined, including bronchiectasis, chronic inflammation and fibrosis, or tuberculosis, conditions where tumorlets were originally considered as reactive (rather than neoplastic) secondary lesions. However, they may also be occasionally encountered in the lung parenchyma surrounding carcinoid tumors (up to 8% in some series) [15], they can be exceptionally associated with Cushing's syndrome [16], and finally they can even more rarely be responsible for lymph node or distant metastases, thus sharing several features of classical carcinoid tumors of the lung [17]. No clinical relevance has been associated with tumorlets (except for the rare possibility of airway narrowing and/or obliteration), unless they are identified in the context of the rare DIPNECH (see below).

Pathology

Tumorlets can be recognized incidentally at macroscopy as single or multiple nodules. Histologically they are made of oval-, round-, or spindle-shaped cells with minimal atypia and scant, weakly eosinophilic cytoplasm, growing in a more or less dense fibrous stroma in the bronchial or bronchiolar walls, with submucosal extension (Fig. 9.2). Mitotic figures are exceptional and necrosis is invariably absent. Neuroendocrine cell clusters in tumorlets can spread into the surrounding parenchyma although true "spread through air spaces (STAS)" in these lesions is called into question [18]. Neuroendocrine cells in tumorlets do not differ morphologically nor immunohistochemically (production of GRP, serotonin, and calcitonin, as well as of "ectopic" ACTH) from those of lung carcinoids.

Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH)

Clinical Features

The term diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) may be used to describe a clinical-pathological syndrome, as well as an incidental finding on histological examination. According to the WHO classification [6], the definition of DIPNECH is purely histological. However, DIPNECH encompasses symptomatic patients with airway disease, as well as asymptomatic patients with neuroendocrine cell hyperplasia associated with multiple tumorlets/carcinoid tumors. Due to the lack of uniform classification criteria, its exact incidence and prevalence have not been established. Moreover, DIPNECH is recognized by the WHO classification of lung tumors [6] as a preneoplastic lesion although there are insufficient molecular data to depict pathways of progression from neuroendocrine cell hyperplasia to carcinoids, and this pathological condition represents, if real, a precursor of a small subset of carcinoids.

The term DIPNECH was first introduced in the 2004 WHO classification of lung tumors [19] in light of the observation by Aguayo and coworkers reporting on six

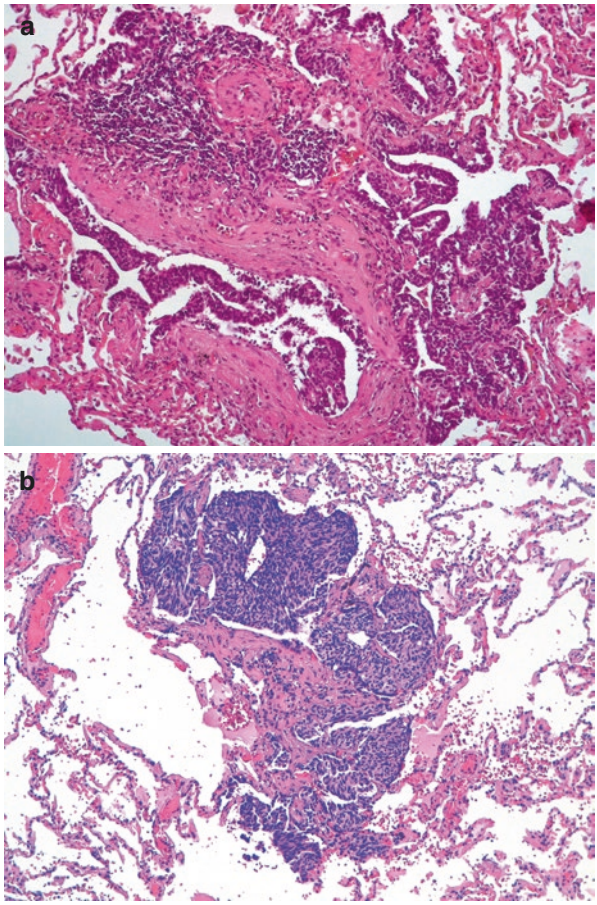

Fig. 9.2 **a**, **b** Examples of lung tumorlets, revealing in both instances (**a**, **b**) an invasive growth that is common to lung tumorlets in contrast to NE cell hyperplasia that is confined to the thickness of the bronchial epithelium (hematoxylin and eosin staining; original magnification 10×)

nonsmoker patients with diffuse hyperplasia of pulmonary neuroendocrine cells, multiple tumorlets and/or carcinoids, and peribronchiolar fibrosis obliterating the small airways [20].

When strict criteria of DIPNECH are applied, patients' characteristics are different from those of reactive neuroendocrine cell hyperplasia and of tumorlets/carcinoid tumors. DIPNECH occurs ten times more frequently in females than males, with a mean age of 58 years, it is not associated with smoking, and it is always symptomatic [21, 22]. DIPNECH has been also diagnosed in the setting of type 1 multiple neuroendocrine neoplasia [23].

DIPNECH presents with chronic symptoms including cough, dyspnea, and wheezing and is often misdiagnosed as asthma, gastroesophageal reflux disease, or chronic obstructive pulmonary disease [24]. Rare DIPNECH cases have been associated with ectopic secretion of adrenocorticotropic and growth hormone [25]. More than half of patients have an obstructive or mixed pulmonary function testing. At imaging, lung nodules are identified at CT scan in about 60% of cases.

DIPNECH-associated specific features are mosaic attenuation with air trapping, which is due to constrictive bronchiolitis, bronchial wall thickening, bronchiectasis, and mucoid impactions [26]. Patients with DIPNECH may remain stable for several years or rapidly deteriorate in few years.

Pathology

DIPNECH is defined by the WHO as "generalized proliferation of scattered single cells, small nodules (neuroendocrine bodies) or linear proliferation of pulmonary neuroendocrine cells" [6]. Although usually confined to the bronchial and bronchiolar epithelium, these proliferations can extend beyond the basement membrane to form tumorlets or carcinoid tumors (when 5 mm or more in diameter). Obviously from this definition, immunohistochemical detection of neuroendocrine cells using pan-neuroendocrine markers, such as chromogranin A and/or synaptophysin, is mandatory for DIPNECH diagnosis. As compared to carcinoids, DIPNECH expresses at a higher extent thyroid transcription factor-1, CD10, and gastrin-releasing peptide/bombesin-like peptide [22].

By some authors, the presence of at least five neuroendocrine cells, isolated or in clusters, located within the basement membrane of the bronchiolar epithelium of at least three bronchioles in combination with at least three carcinoid tumorlets (and in the absence of conditions that could result in secondary neuroendocrine cell hyperplasia), can be used to diagnose DIPNECH in surgical lung biopsy specimens [27]. In addition, in symptomatic cases bronchioles may show a fibrogenic constrictive process leading to constrictive bronchiolitis with mural scarring, luminal narrowing, and/or complete obliteration (Fig. 9.3). Bronchiectasis with mucostasis, emphysematous changes, and mild inflammation may be also present with a peculiar patchy involvement that suggests a thorough examination of the surgical biopsy for a correct pathological diagnosis. DIPNECH may be misdiagnosed with minute meningothelial-like nodules (either isolated or multiple in the setting of so-called meningotheliomatosis) [28], which are tiny aggregates of spindle-shaped cells characterized by nuclear grooving and occasional pseudoinclusions that express EMA, progesterone receptors, and CD56, thus for this latter marker potentially mimicking neuroendocrine cell proliferations.

Neuroendorine Tumors (Carcinoids)

Lung carcinoids are malignant epithelial neoplasms with well-differentiated neuroendocrine morphology and differentiation. They are subdivided into typical and atypical based on mitotic index and presence of necrosis, and histological typing represents the most important prognostic factor.

Epidemiology

Pulmonary carcinoid tumors comprise approximately 27% of all neuroendocrine tumors and account for 1–2% of all lung malignancies with an estimated age-adjusted incidence from 0.1 to 1.5 per 100.000, with a significant increase from 1973 to 2003

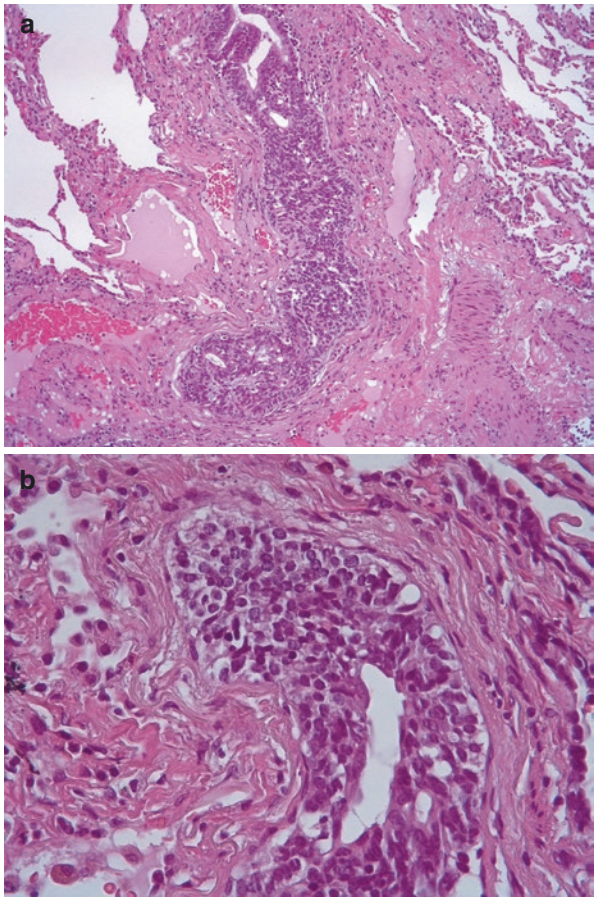

Fig. 9.3 Neuroendocrine cell hyperplasia in a patient with DIPNECH, female, aged 51 years old, with a long clinical history of asthma (hematoxylin and eosin staining; original magnifications: **a** 10×, **b** 20×). Multiple tumorlets and typical carcinoids, from 5.5 to 17 mm in size, were also present

[29, 30]. Typical carcinoids are 70–90% of lung carcinoids. Lung carcinoids more frequently develop in females patients, aged <60 years, and white. Smoking history is usually negative in typical carcinoids, but atypical carcinoids are associated with tobacco smoking in about half of patients. Lung carcinoids may develop in about 5% of patients with MEN1 syndrome [31] (see also section "Inheritance").

Gross, Clinical Presentation and Imaging

Both typical and atypical carcinoids may be central or peripheral and show peculiar pathological and immunohistochemical features depending on their location. In fact, peripheral lesions are associated with presence of spindle cell component, sustentacular cells, a female predominance, and strong association with neuroendocrine hyperplasia, whereas centrally located tumors have more polygonal cell morphology, acinar growth pattern, and only rare association with neuroendocrine hyperplasia [32]. At macroscopy lung carcinoids are relatively well-demarcated nodules, varying in color from yellow-whitish to tan-yellow or brown and ranging from 5 to 95 mm, with a mean size larger in atypical as compared to typical histotype.

About half of patients with carcinoid are asymptomatic, but even when symptoms occur, the tumor may require years before a definitive diagnosis is achieved. Symptoms are site-dependent with peripheral carcinoids usually being asymptomatic and incidentally discovered at imaging studies. Centrally located carcinoids are often symptomatic as a result of partial or complete bronchial obstruction or secondary to its high vascular supply. Cough, hemoptysis, and recurrent pulmonary infections in the same pulmonary segment or lobe are the most frequently reported symptoms. Unilateral wheezing, bronchial asthma refractory to medical therapy, chest pain, and pleural effusion have been occasionally reported [33]. A long-lasting bronchial obstruction can lead to focal bronchiectasis, resulting in partial or complete destruction of the distal lung tissue. Bronchial carcinoid can be associated with paraneoplastic syndromes due to the production and secretion into systemic circulation of several amino peptides and hormonal substances. Carcinoid syndrome occurs in about 8% of bronchial carcinoid, mainly in patients with bronchial carcinoid metastatic to the liver, and is caused by the systemic release of vasoactive substances, in particular serotonin [34]. In actively secreting carcinoids, bronchoscopic management or tumor manipulation during surgical procedures can precipitate the so-called carcinoid crisis: a life-threatening clinical situation characterized by a sudden systemic vasodilatation that leads to a severe cardiovascular collapse. Although bronchial carcinoids are the most frequent cause of ectopic ACTH secretion, Cushing's syndrome is found in 4% of ACTH-secreting carcinoids only. ACTH-functioning tumors are associated with younger age of onset and more advanced tumor stage, although they did not show an independent different survival [16]. GH secretion with acromegaly has been described rarely in pulmonary carcinoids [35].

At imaging, the chest radiograph is abnormal in most cases of bronchial carcinoid, but in approximately 10% it is negative. Centrally located tumors usually present with complete or partial atelectasis, and more rarely a hilar mass can be revealed at chest radiograph. These lesions appear at fibro-bronchoscopy as an esophytic, vascularized mass with smooth bloody surface. CT scan gives an excellent morphological characterization of peripheral and especially centrally located carcinoids that can be purely intraluminal (polypoid configuration), exclusively extraluminal, or more frequently a mixture of intraluminal and extraluminal components ("iceberg" lesion), although pathology still remains mandatory for their correct classification [36]. Bronchocele can be seen in small tumors involving the orifice of a bronchus, and calcifications are present in up to 30% of centrally located carcinoids. In light of the overexpression of somatostatin receptors in carcinoid tumors, somatostatin analog scintigraphy had a role in the past showing a high sensitivity for neuroendocrine cells (over 90% for both primary tumor and metastases), but low specificity because inflammatory conditions and other tumors can also be positive [37]. However, the development of functional imaging evaluation using nuclear medicine techniques during last the two decades provided novel tools for the detection and characterization of lung carcinoids. ^{68}Ga-DOTA-peptide has been shown to be superior to ^{18}F-FDG in terms of the detection rate of pulmonary carcinoids. Moreover, SUVmax ratio of ^{68}Ga-DOTA-peptide and ^{18}F-FDG was an accurate predictor of the carcinoid histotype compared with the SUVmax on ^{18}F-FDG-PET/CT alone [38].

Rate of lymph node metastasis is very different in typical versus atypical carcinoids. In a recent surgical series, positive nodal status was identified in 17.5 typical carcinoids and 45.9 atypical carcinoid cases [39]. Oncological surgical resections (lobectomy or limited sublobar resections in peripheral lesions) associated with regional lymph node sampling are the mainstay of therapy for localized or locally advanced lung carcinoids, either typical or atypical. Typical carcinoids have an excellent prognosis, but in the small proportion of cases with disease progression, both tumor recurrences and metastasis may occur even after 10 or more years from the diagnosis, due to the indolent biologic course of the disease. In atypical carcinoids, the local recurrence rate is also low in the case of limited resection, but the overall prognosis is affected by the high rate of lymph node involvement at diagnosis and by the extent of surgery [40]. Adjuvant treatment, chemotherapy or radiotherapy, has been considered in completely resected atypical carcinoid with mediastinal lymph node involvement, but their real efficacy has been recently called into question [41].

Treatment of metastatic disease is more problematic and no standard strategies have been developed. Various treatment options including somatostatin analogs, peptide receptor radioligand therapy, and biologic systemic therapy, specifically with the mTOR (mechanistic target of rapamycin) inhibitor everolimus, are now available, but the most appropriate treatment algorithms are still not completely designed [42].

In typical carcinoids, the overall 5-year and 10-year survivals range from 90% to 100% and 80% to 90%, respectively, whereas for atypical carcinoids the 5-year and 10-year overall survivals range from 61% to 88% and 35% to 67%, respectively. TNM stage, which has been applied for non-small cell lung cancer, is the key prognostic parameter for both typical and atypical carcinoids [43]. Of note, typical carcinoid with regional lymph node metastasis still have an excellent outcome, especially if with a diameter less than 2 cm [39].

Histopathology

Typical Carcinoid Typical carcinoids have fewer than two mitoses per 2 square mm (usually per 10 high-power fields) and lack necrosis.

Central tumors usually appear as a highly vascularized proliferation of polygonal/round cells with abundant granular and eosinophilic cytoplasm and a central to eccentric round-shaped nucleus with finely granular chromatin with a single, small, inconspicuous nucleolus. These elements are arranged in a mixture of growth patterns, including nesting, solid sheets, trabeculae, ribboning, insular configurations, and rosettes structures (Fig. 9.4). The tumor cell nests are generally dissected by a delicate fibrovascular stroma with dense collagen-rich hyaline stroma that may also contain calcifications, amyloid deposits, and more rarely metaplastic bone and/or cartilage. Cellular pleomorphism may be seen in typical carcinoid, but this feature does not seem to have a prognostic value and does not modify the diagnosis [44]. At intraoperative frozen section examination, a diagnosis of carcinoid can be made in most cases when the tumor has the usual morphological features. However, when there is significant cytological atypia and/or prominent spindle-shaped cell

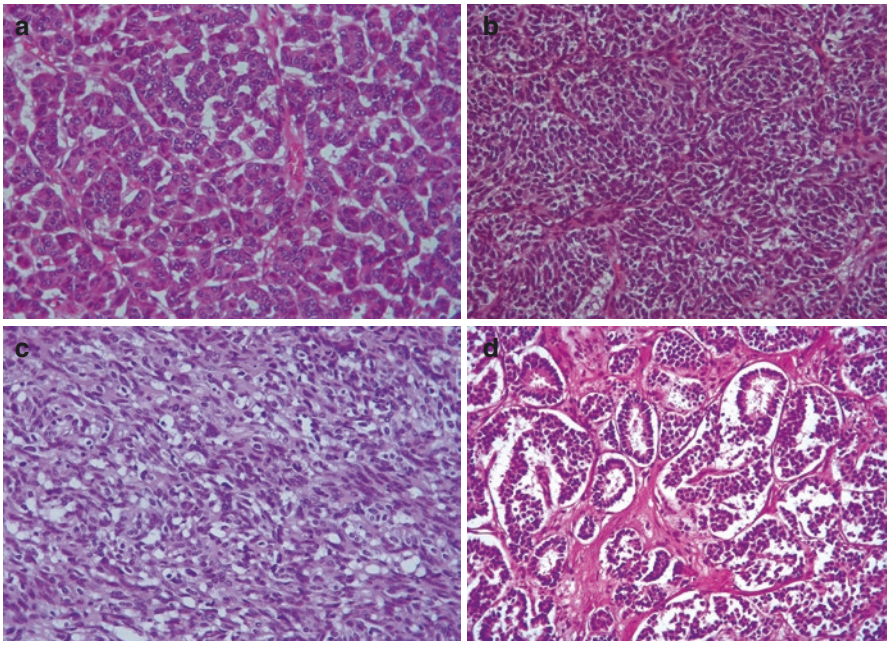

Fig. 9.4 Architectural patterns in typical carcinoid: trabecular (**a**), spindle (**b**), spindle with clear cells (**c**), and pseudo-glandular (**d**) (hematoxylin and eosin staining; all original magnifications 20×)

morphology, distinction from other tumors can be problematic, and the diagnosis may be deferred to prevent a misdiagnosis. The presence of an organoid pattern, stromal hyalinization, spindle-to-ovoid cell proliferation, and finely dispersed nuclear chromatin seems to support a diagnosis of carcinoid tumor [45]. By contrast, distinction between typical and atypical carcinoid may be very problematic at intraoperative consultation, and a diagnosis of pulmonary carcinoid tumor, not otherwise specified, would be preferable and sufficient for therapeutic purposes.

In cytology specimens, typical carcinoids are characterized by hemorrhagic smears containing uniform rounded-to-oval tumor cells, isolated or aggregated in cohesive sheets with the typical finely dispersed nuclear chromatin with inconspicuous nucleoli. The cellular background often shows fragments of delicately vascularized connective tissue with loosely attached tumor cells. However, some cytological features such as nuclear molding and crowding are not discernible features because they may be found on smears with increased cellularity; moreover crush artifact can occur in both carcinoids and high-grade neuroendocrine neoplasms and may cause a misinterpretation of small cell carcinoma. Other artifacts resulting from delayed fixation or poor processing and sampling error are potential causes of incorrect interpretations, leading to up to 49% of discordant diagnoses at definitive histology [46].

Atypical Carcinoid Atypical carcinoid has more than two and up to ten mitoses per 2 square mm (or per 10 high-power fields). Necrosis may be present with punctate foci, but never with large and/or geographic areas (Fig. 9.5).

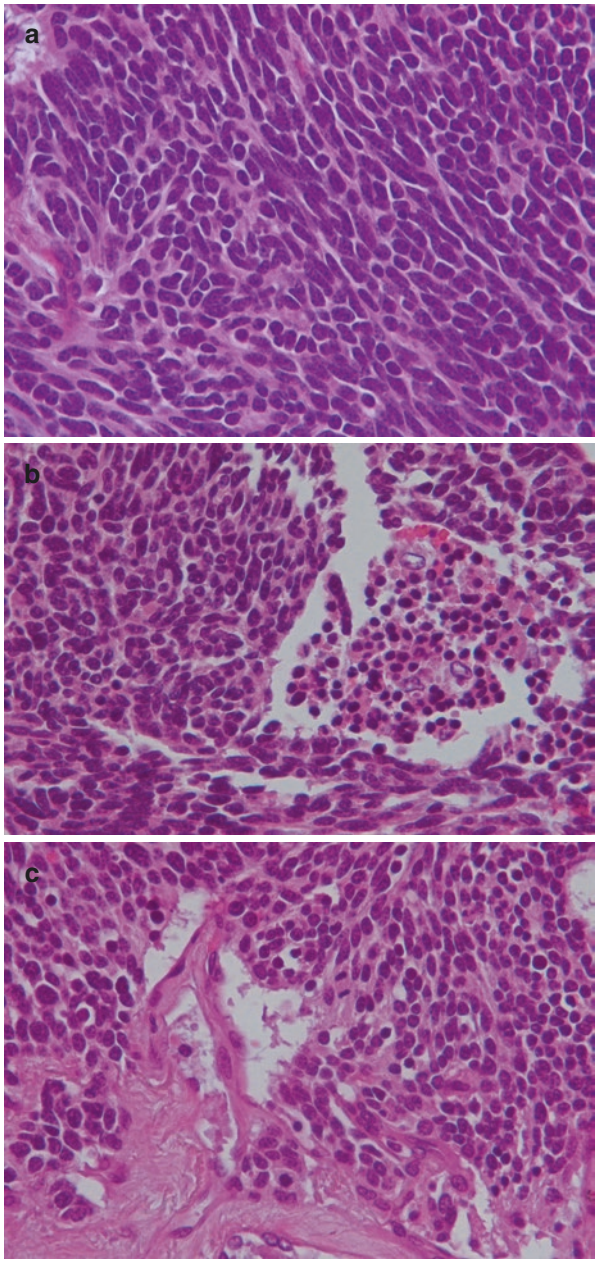

Fig. 9.5 Spindle cell morphology (**a**), punctate necrosis (**b**) and mitotic figure (**c**) in atypical lung carcinoid (hematoxylin and eosin staining; all original magnifications 20×)

Atypical carcinoid was first described as a carcinoid tumor with five to ten mitoses/10 HPF, necrosis, cellular pleomorphism, and increased cellularity [47]. With the recognition of large cell neuroendocrine carcinoma as a distinct high-grade neuroendocrine tumor entity (see section "Large Cell Neuroendocrine Carcinoma"), the criteria to define atypical carcinoid were modified, setting the mitotic index range as it is in the current WHO classification Scheme [6]. As in typical carcinoid, even atypical carcinoids may show several growth patterns including spindle cell, trabecular, palisading, solid/organoid, papillary, and follicular with rosette-like structures. Dense collagen, amyloid, bone, or melanin deposition may be seen. Although cellular pleomorphism, vascular or lymphatic invasion, and hypercellularity are not used in taking typical apart from atypical carcinoids, these features more frequently occur in atypical tumors. Recently, the spread through air spaces (STAS) pattern has been described in lung carcinoids with a higher frequency in the atypical histotype and was significantly correlated with unfavorable parameters, such as high tumor stage, positive nodal status, high Ki-67 index, presence of angioinvasion, and with adverse disease outcome, shorter overall survival, and time to progression [48, 49]. At cytology, atypical carcinoid cells have greater pleomorphism, more coarse chromatin, and more prominent nucleoli than those of typical ones, but these features are not consistent enough to clearly separate these two entities, and mitotic figures and a necrotic background are seen.

Carcinoid Variants

Several histologic variants of typical carcinoid have been described and depict the wide heterogeneity of cytological and architectural patterns in these lesions. Among the most common, the oncocytic variant is characterized by tumor cells with an ample amount of granular oncocytic cytoplasm (as a consequence of mitochondrial accumulation) that has a round-to-oval nucleus with coarse chromatin. Oncocytic areas may be pure or admixed with non-oncocytic ones (Fig. 9.6). Bone formation, the presence of giant cells, and tumor cells with a conspicuous nucleolus are more frequently observed than in conventional cases [50]. Other variants are on record and are mainly to be mentioned as potential pitfalls in diagnostic histopathology. Among those, mucin-producing, clear cell, large spindle, and melanocytic type have been described [51–54].

Immunohistochemical Profile

The use of immunohistochemistry in the diagnostic approach to lung carcinoids partly depends on the type of material available (Fig. 9.7). The definition of the presence of neuroendocrine differentiation in lung carcinoids is mandatory. It may be confirmed by means of several techniques, such as histochemistry (positive reaction with Grimelius or Fontana-Masson stains) and electron microscopy (presence of 30–300 nm electron-dense intracytoplasmic neurosecretory granules, with higher density in typical carcinoids), but immunohistochemistry (IHC) is nowadays the gold standard. Neuroendocrine markers such as chromogranin A (Fig. 9.8), synaptophysin, and CD56 are the most specific and sensitive neuroendocrine markers

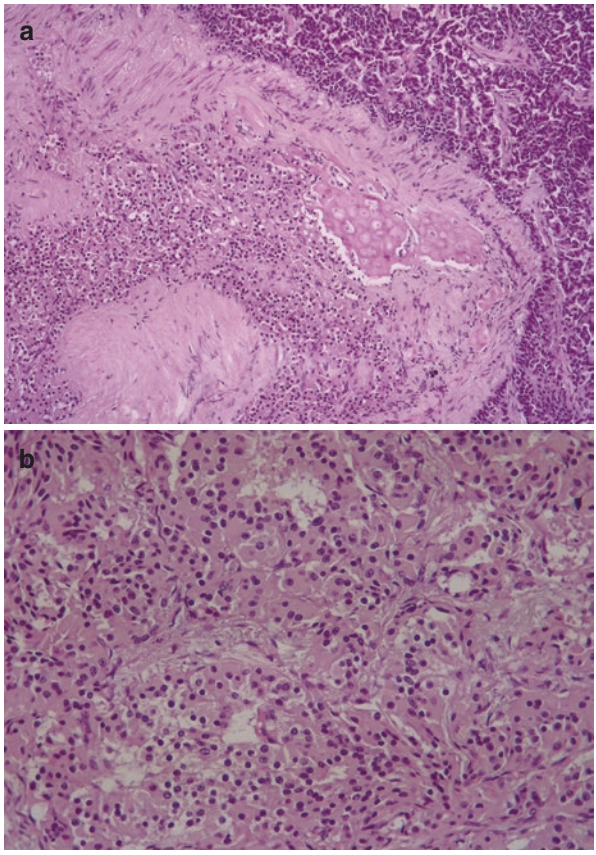

Fig. 9.6 Oncocytic atypical lung carcinoid, admixed with a non-oncocytic component and showing bone formation (**a**); oncocytic cells show the characteristic abundant granular eosinophilic cytoplasm (**b**) (hematoxylin and eosin staining; all original magnifications: a 10×, b 20×)

[55]. Lung carcinoids are also usually reactive for wide-spectrum cytokeratins and CK7, but not for high molecular weight cytokeratins (such as cytokeratin 34betaE12 and/or CK903) nor for napsin A, p40, or p63, features that are helpful to distinguish lung carcinoids from other non-neuroendocrine lung neoplasms. S100 protein may detect the presence of sustentacular cells, which are mainly observed in peripheral lesions. At variance with high-grade neuroendocrine carcinomas, lung carcinoids are almost always negative for PAX-5 [56]. As many other well-differentiated neuroendocrine neoplasms, lung carcinoids express at a high extent the different subtypes of somatostatin receptors, with loss of subtype 2A being associated with more aggressive disease outcome [57].

Different carcinoid histotypes in surgical samples are recognized by means of pure morphological parameters, only, and the use of immunohistochemistry once the neuroendocrine nature is proven is of scarce value. By contrast, in small biopsies or cytological samples, morphological parameters cannot be sufficient alone,

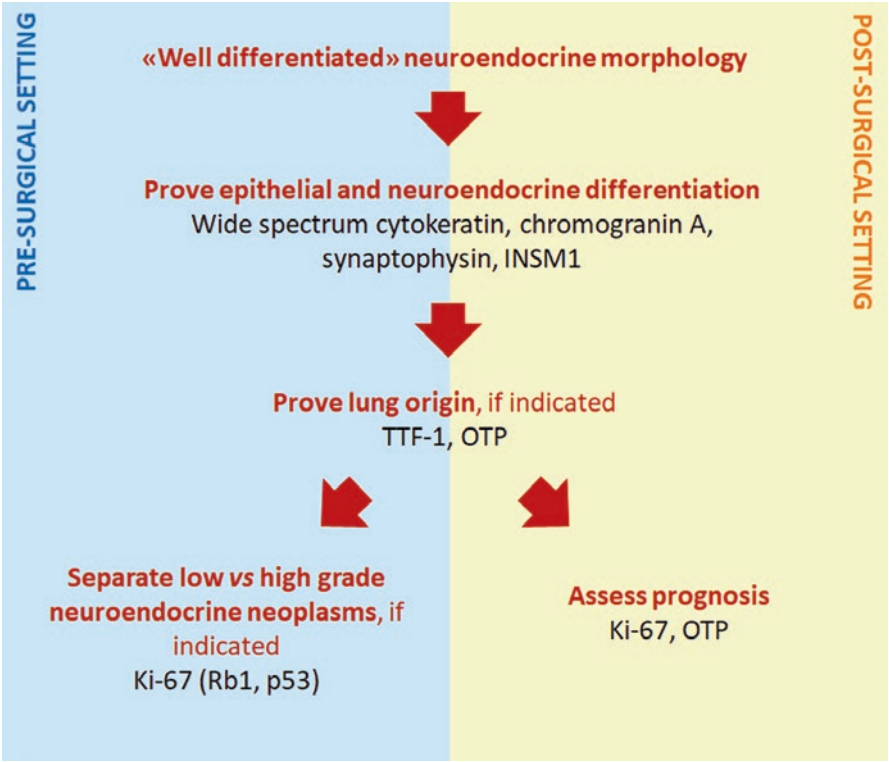

Fig. 9.7 Simplified algorithm of IHC use in lung carcinoids

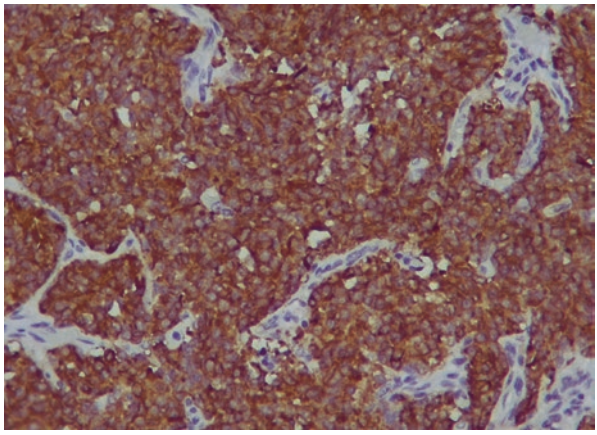

Fig. 9.8 Strong and diffuse chromogranin A staining in typical lung carcinoid (immunoperoxidase; original magnification 20×)

and the pattern of distribution and extent of neuroendocrine markers might be indicative although not supportive of a specific histotype. A diffuse and intense chromogranin A positivity favors a diagnosis of carcinoid, whereas a focal dot-like pattern is more indicative of a high-grade neuroendocrine carcinoma, mainly of the small cell type. The same holds true for hASH-1, a transcription factor whose prevalence of expression increases with the increase of aggressiveness being usually positive at a low prevalence in typical carcinoids and diffusely positive at the other side of the spectrum in small cell carcinoma [58, 59]. By contrast, synaptophysin is usually diffusely positive in both carcinoids and high-grade forms, as well as the novel neuroendocrine marker INSM1 [60]. An important clue in lung carcinoid diagnosis is the identification of the primary lung origin in advanced cases of well-differentiated neoplasms with multiple locations where the clinical definition of the primary site might be not straightforward, despite a strong impact on the management of the patient. Immunohistochemical panels should be specifically designed according to the clinical and radiological pictures and the morphological differentiation of the lesion. Lung carcinoids express TTF1 [61] although mostly in the peripheral location [62]. In this context, metastatic medullary carcinoma of the thyroid may represent a formidable diagnostic challenge, since this latter has morphological as well as immunophenotypic properties of a carcinoid tumor and calcitonin production has been rarely reported in lung carcinoids also [63]. In recent years, the novel marker orthopedia homeobox protein (OTP) has shown to be selectively expressed by lung carcinoids as compared to neuroendocrine tumors of other locations, with a sensitivity of 100% for the typical carcinoid histotype [64] that may be supportive of a lung origin also in cytological samples [65]. The positive expression of other location-specific markers, such as CDX-2, PAX-8, and PDX-1, is indeed supportive for extrapulmonary location and indicative of gastrointestinal or pancreatic origin, according to the phenotypical picture observed. Other differential diagnosis in lung carcinoids includes pulmonary paraganglioma [66] (which expresses neuroendocrine markers and S100 in sustentacular cells but is cytokeratin-negative), glomus tumor (which is positive for smooth muscle actin only), spindle cell neoplasms (especially mesenchymal tumors such as leiomyoma/leiomyosarcoma, schwannoma, and metastatic sarcoma or sarcomatoid spindle cell carcinoma), metastatic melanoma, primary or metastatic meningioma, and various metastatic tumors having a solid growth pattern. In all the above contexts, cytological and architectural features, as well as appropriate immunohistochemical panels, should be integrated to confirm or disprove the diagnosis of carcinoid tumor.

In terms of prediction of clinical behavior, several phenotypical markers have been proposed to be significantly associated with survival, but most of them are directly associated with carcinoid histotype and therefore although of biological interest are of not independent value and limited clinical value. Among the most recent are epithelial-to-mesenchymal transition markers [67], chemokine receptors [68], and IMP3 [69].

Data from gene expression profiling identified several markers potentially applicable in immunohistochemistry in lung carcinoids. However, as for those already mentioned above, most of them are differentially expressed in typical and atypical ones and lose their prognostic value when assessed in comparison to histotyping. Among those, the only biomarker strongly and independently associated to adverse outcome in lung carcinoids is OTP protein loss, either alone or associated with CD44 expression. Since the original publication [70], subsequently validated by the same authors in another independent series [7] and by other groups [71], OTP nuclear expression has been described as a strong independent prognostic factor for recurrence-free survival in carcinoids, including typical ones with locally advanced pathology stage. Among the few others, the lack of central cell cycle proteins KLF4 and p21 expression has been associated with an accumulation of aggressive features in typical carcinoids [72].

Evidence-Based Grading Proposals

Proliferation marker Ki-67, apart from histological type and TNM stage, is the most relevant prognostic indicator in lung carcinoids and has been widely studied and validated since several years [73], and although not coded in the WHO classification system as a prognostic determinant to be mandatory mentioned in the diagnostic report, its assessment is strongly recommended in the clinical practice [36]. Despite even recently called into question as an independent prognostic factor [74], a grading proposal was specifically designed in lung neuroendocrine neoplasms embedding Ki-67 with mitotic index and necrosis [75], and the reliability of this marker in the preoperative setting was recently proved by the high concordance – when carefully assessed – between corresponding presurgical and surgical lung samples [76]. However, no agreement has been reached at the current present on the definition of a grading system for carcinoids, with variable combinations of Ki-67 cutoff levels and morphological criteria [77].

Indeed, Ki-67 relative high expression (using a cutoff of 10% or 20%) further segregates a subgroup of lung carcinoid cases with distinct pathological features and significantly worse outcome independently from the typical or atypical histotype, which, at least in part, resemble the pancreatic “NET G3” group of neoplasms [78, 79] (Fig. 9.9). The presence of aggressive well-differentiated lung neuroendocrine neoplasms that do not have the morphological features of high-grade neuroendocrine carcinomas but exceed canonical proliferative and mitotic indexes of carcinoids has been also strongly suggested in a recent report on stage IV lung carcinoids. In the reported series, up to 27% of cases, mainly in metastatic sites, had mitoses and/or Ki-67 superior than the standard criteria for carcinoids; however, these cases retained well-differentiated morphology and conventional proliferation rates in other samples from same patient, lacked RB1/TP53 alterations (at variance with high-grade neuroendocrine carcinomas), and had a median overall survival of 2.7 years, as compared to <1-year survival of stage IV high-grade neuroendocrine carcinomas [80].

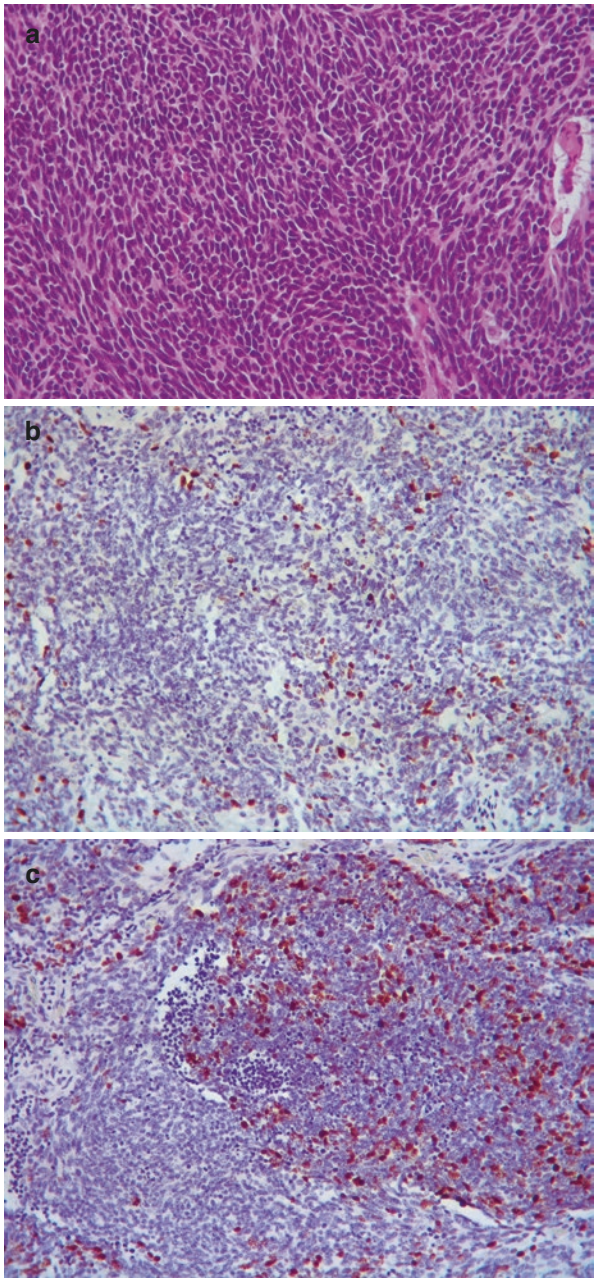

Fig. 9.9 Atypical lung carcinoid with spindle cell morphology (**a**) showing a heterogeneous pattern of staining for Ki-67, with intermediate (**b**) to high proliferation indexes (**c**, in close association with necrotic debris) (**a**, hematoxylin and eosin staining; **b** and **c**, immunoperoxidase; all original magnifications 20×)

Large Cell Neuroendocrine Carcinoma

Epidemiology

Large cell neuroendocrine carcinoma (LCNEC), in the past clustered together large cell carcinomas (LCC) as tumors presenting with neuroendocrine differentiation [81], accounts for 3% of less of all lung cancers, but its prevalence is destined to increase due to heightened diagnostic awareness and increased use of immunohistochemistry for refining poorly differentiated tumors. A recent study dealing with a large Surveillance, Epidemiology, and End Results dataset has reported on a 1–2% prevalence for LCNEC, with female gender, black race, surgery, radiation, and chemotherapy being protective factors for survival in these patients [82]. Early-stage LCNEC patients showed a higher risk of lung cancer-specific death and specific patterns of metastasis with a larger incidence of brain metastases than patients with early-stage non-small cell lung carcinomas (NSCLC) [83]. In particular, patients with isolated liver or brain metastasis or combined invasion patterns to other organs showed poorer survival rates, identifying LCNEC as an aggressive tumor subtype when investigated epidemiologically. Smoke and male gender are considered risk factors for the development of LCNEC, which usually affect elderly patients (with a median age of 65 years) [84]. However, fewer cases of LCNEC arising in non-smokers and/or younger people upon ALK [85] or ROS-1 [86] rearrangement or EGFR mutations [87] are increasingly on record especially in peripherally located lesions. These considerations witness the inherent biological heterogeneity of LCNEC, which may have important patho-biological and clinical implications [88]. According to a recently released common classification framework, LCNEC as defined by current criteria [6] are NENs belonging to the family of neuroendocrine carcinomas, typed as featuring large cells.

Gross, Clinical Presentation and Imaging

There are no specific macroscopic or clinical features of LCNEC compared to conventional NSCLC. At variance with small cell lung carcinoma (SCLC), paraneoplastic syndromes are uncommon, but single case reports of ectopic adrenocorticotropic hormone syndrome [89], Lambert-Eaton syndrome [90], or cancer-associated retinopathy [91] have been well documented. LCNEC present high rate of lymph node (60–80%) and distant metastasis (40%) at the time of diagnostic recognition, similarly to SCLC [84, 92] even if metastatic sites are less frequently reported than in the latter. These findings underline a potentially different natural history of LCNEC as compared to SCLC, as also documented by survival analysis [92, 93]. Tumors may feature central or, more frequently, peripheral location in the form of large, circumscribed, and abundantly necrotic masses infiltrating the pleura, the chest wall, or the adjacent structures (even with Pancoast tumors and Horner syndrome), while cavitation is uncommon. It has recently been observed

that peripheral LCNEC patients had better life expectation compared with central lesions and that the location inside the lung was an independent prognostic factors for overall survival [86]. Even this finding supports once again inherent differences in the origin cells and pathogenesis of LCNEC, also outside the lung, when they are considered as a unitary tumor category. CT scan evaluation usually shows a well-defined and lobulated tumor with no air bronchograms or calcification, where necrosis may cause an inhomogeneous enhancement of the contrast medium to appear especially when dealing with large-sized LCNEC, while this is less apparent in small-diameter (<33 mm) lesions even if they entail some amount of necrosis [94]. The maximum standardized uptake value (SUVmax) on positron emission tomography with 2-deoxy-2-[fluorine-18]fluoro-D-glucose (^{18}F-FDG PET) is commonly high, consistent with highly malignant tumors and correlated with shorter disease-free survival. LCNEC present also with somatostatin receptors, even if at lower levels in comparison with carcinoids [95], but scintigraphic imaging with OctreoScan (indium 111-tagged diethylenetriaminepentaacetic acid pentetreotide scintigraphy), ^{111}In-DOTA-TOC (111In-DOTA-Dphe1-Tyr3-octreotide), or ^{111}In-DOTA-LAN (111In-DOTA-lanreotide), albeit proposed in preoperative staging and in postoperative follow-up of LCNEC patients, did not enter the routine clinical practice.

Histopathology

The diagnosis of LCNEC is usually straightforward on resection specimens by applying the defining criteria settled in the 2015 WHO classification [6], but can be also supported on biopsy specimens by relying on immunohistochemistry findings [96]. Current guidelines stated that non-small cell carcinoma on biopsy samples with neuroendocrine morphology and neuroendocrine marker positivity supports a possible diagnosis of LCNEC; thus, such a diagnosis can be rendered on biopsy samples only if morphology actually suggests neuroendocrine differentiation. As a matter of fact, it has also been observed that neuroendocrine marker staining should not be performed and is not recommended its use for tumors with no obvious neuroendocrine morphological features [97], because some neuroendocrine markers can be even shared by tumors lacking overt neuroendocrine differentiation. Since neuroendocrine morphology may be yet frequently missed in biopsy and cytology samples, there is a potential for LCNEC diagnosis to be missed on small specimens. This is the reason why this tumor type is usually recognized on resection specimens only, even if this is the second most prevalent neuroendocrine tumor after SCLC. At variance with SCLC, there are no reliable criteria for this tumor to be diagnosed on cytological samples due to their large overlap with those of other neuroendocrine tumors or conventional NSCLC, although criteria such as tumor cell size, naked nuclei, thin nuclear membranes, nuclear streaking, neuroendocrine marker positivity, and a necrotic background have been proposed for LCNEC on cytological samples [98].

LCNEC as a tumor entity was proposed by Travis et al. in 1991 [99] by refining the previous Gould and Warren's definition of intermediate cell neuroendocrine carcinoma (intermediate in cell size between well-differentiated neuroendocrine carcinoma, i.e., atypical carcinoid, and SCLC) [100, 101]. LCNEC was described to exhibit neuroendocrine architecture (e.g., organoid and often palisading tumor

islands) and neuroendocrine marker expression, in pure or combined form with other NSCLC and with an intermediate prognosis between atypical carcinoid and SCLC but closer to the latter. These criteria have been largely maintained unchanged over the subsequent three WHO classifications until the last of 2015, with the only change regarding survival that now is considered to largely overlap with SCLC.

In its most classical description, LCNEC is a tumor showing neuroendocrine morphology featuring organoid aggregates or solid to trabecular pattern of growth (Fig. 9.10). Tumor cells are large as opposed to those of SCLC (typically more than three resting lymphocyte diameter), with abundant granular to variably clearer cytoplasm and well-defined cell borders realizing a prominent peripheral palisading or mosaic pattern. Nuclear molding is typically lacking likely due to the cytoplasm abundance that prevents tumor cells to closely juxtapose to each other causing nucleus shape deformation to arise. The chromatin pattern is typically coarse with abundance of heterochromatin and basophilic to amphophile prominent nucleoli (Fig. 9.11), and this is considered the single most important criterion to separate LCNEC from SCLC. Mitoses are plentiful (more than 10 per 2 mm^2, with no upper limits, but a median value of 70 mitotic figures) and may be atypical. The necrosis

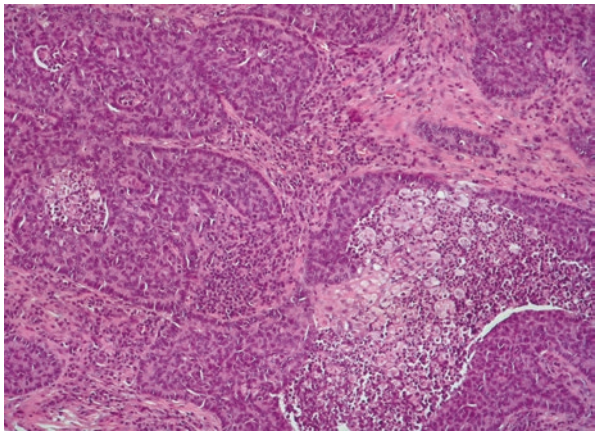

Fig. 9.10 Organoid growth pattern in large cell neuroendocrine carcinoma (hematoxylin and eosin staining; all original magnifications 10×)

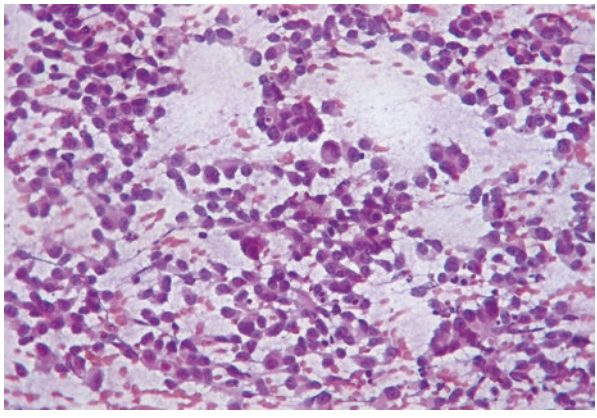

Fig. 9.11 Cytological features in a cytological smear of large cell neuroendocrine carcinoma, showing pleomorphic nuclei with vesicular chromatin and prominent nucleoli (hematoxylin and eosin staining; original magnification 40×)

is variably extensive, sometimes geographic, and peritheliomatous in appearance, with sheets of viable tumor cells being concentrically arranged to survive around vascular channels indicative of complex mechanisms of tumor necrosis [102]. A small subset of LCNEC features histological details that overlap atypical carcinoid, except for showing more mitoses exceeding the allowed number of 10 per 2 mm^2 and more necrosis, this indicating a wide spectrum of morphologic appearance in turn indicative of heterogeneity in cell composition and derivation. Combination of LCNEC with SCLC, for which a 10% percentage of either tumor type is required, is considered a combined variant of SCLC with LCNEC rather than a combined variant of LCNEC with SCLC likely because of the morphologic continuum existing in neuroendocrine carcinomas of small and large cells, which is in turn responsible for the disappointing diagnostic reproducibility between them [103].

The diagnosis of LCNEC is a stepwise process, in which at first neuroendocrine morphology must be recognized through identification of organoid nesting, trabeculae, rosettes, and peripheral palisading, and then LCNEC is identified according to mitotic count and necrosis extent to rule out atypical carcinoid and a combination of morphology and IHC to exclude NSCLC subtypes. Separation from SCLC may be challenging for either the continuous dimensional overlap of small and large cells around three resting lymphocyte/endothelial cell diameter in tumors sharing common neuroendocrine properties or the subjective application of defining criteria [104]. Although a constellation of features regarding cell size, chromatin patterning, and cytoplasmic amount has been advocated to distinguish LCNEC from SCLC, this separation continues to remain challenging and, to some extent, arguable on biologic bases. Difficulties in assessing cell size and cytological features including chromatin pattern may account for disappointingly low inter-observer reproducibility of LCNEC diagnosis even among experts that remain around 50% (just as little as a chance).

It may be useful to briefly comment here the possibility of facing with conventional NSCLC, where IHC and electron microscopy demonstrate neuroendocrine markers but neuroendocrine morphology is lacking by light microscopy and they feature conventional adenocarcinoma, squamous cell carcinoma, or large cell carcinoma realizing the so-called NSCLC with neuroendocrine differentiation. These tumors, which have not been included in the last 2015 WHO classification as independent tumor entities, should be rather classified as adenocarcinoma, squamous cell carcinoma, or large cell carcinoma but commenting on the presence of positive neuroendocrine markers [6]. As a matter of fact, the clinical implications on survival and chemotherapy response have been variably interpreted in the past [105–107], but more recent molecular data favor the biological relationship of NSCLC with neuroendocrine differentiation with the development of LCNEC upon evolution from these precursor lesions [108].

Immunohistochemical Profile

Although recent recommendations for diagnostic IHC on lung cancer have stated that LCNEC diagnosis should be made only when morphology and neuroendocrine markers can be simultaneously demonstrated in the same tumor [109], once obvious

squamous or adenocarcinoma has been reasonably ruled out, a positive decoration for two of three neuroendocrine IHC stains (chromogranin A, synaptophysin, CD56) is supportive on the diagnosis of LCNEC even in small samples. It could be also commented that the greater the expression of neuroendocrine markers, the greater the probability that also the neuroendocrine morphology is as patent as to allow the diagnosis of LCNEC to per rendered according to WHO criteria. For the ultimate diagnosis of LCNEC, the IHC confirmation of neuroendocrine differentiation is compulsory for distinguishing these tumors from mimickers such as conventional NSCLC, with a clear-cut identification of at least one out of two or the three classical and most used neuroendocrine markers (synaptophysin, chromogranin A, and CD56) [96]. There is no proposed clear cutoff value for the extent of tumor cells being positive for neuroendocrine markers to make a diagnosis of LCNEC, but any amount of positive staining of any of these markers should be considered meaningful, if neuroendocrine morphology is clearly patent. Dependency of LCNEC diagnosis on combined evaluation of morphology and IHC is also instrumental to reduce the inter-observer variability and increase the pathologists' diagnostic confidence [110]. Most recently, insulinoma-associated protein 1 (INSM1), an early inducer of NE/neuroectodermal differentiation during ontogenesis and in lung cancer, has been proposed as reliable and sensitive marker of neuroendocrine differentiation in thoracic neuroendocrine tumors, including LCNEC [111]. As the experience on such a marker is still limited, it should not be preferred yet to the other well-consolidated markers of neuroendocrine differentiation. Other IHC markers positive in LCNEC include TTF1 in about one half of instances, different cytokeratin pooling with either dot-like or diffuse cytoplasmic decoration, and rarely and focally p40 or napsin-A expression likely indicating an underlying inapparent keratinizing or glandular differentiation [112, 113].

Small Cell Carcinoma

Epidemiology

Small cell lung carcinoma (SCLC) accounts for about 15% of all lung carcinomas worldwide and for most neuroendocrine neoplasms arising in the lung. Its incidence rate has been decreasing for about the last two decades in both genders after peaking between the mid-1980s and the early 1990s in Western countries, reflecting major changes in smoking habit rather than substantial therapy or diagnosis improvements [110, 114]. Conversely, SCLC incidence is destined to further increase in countries where smoking habit is still largely prevalent in the population of both genders such as Eastern Europe [115]. Epidemiological evidence suggests that the proportion of elderly patients among all cases of SCLC has increased over the past 40 years, with a trend toward a shorter cancer-specific survival while increasing age in the subgroups from 70–74 to 85 or more years [116]. People younger than 40 years with SCLC are uncommon but show similar prognostic factors such as disease stage at clinical presentation, timely diagnosis, and performance status [117]. Female gender and hormone replacement therapy are protective factors for SCLC development

[118], with a prolonged overall and brain metastasis-free survival even in patients bearing limited disease [119]. As a matter of fact, SCLC has been staged for many years as either limited disease (primary tumor and regional lymph nodes within a tolerable radiation field) accounting for 25% of cases or extensive disease (anything beyond limited stage) accounting for 7% of instances. Currently, TNM classification (8th edition) is by far the most preferred and recommended tool for survival and clinical inferences in SCLC patients, because the M descriptors identifying stage IV-A, IV-B, and IV-C are of sure prognostic meaning in either presentation [120]. Most small cell carcinomas are associated with heavy smoking history, either current or former, with significant dose-response relationships for all quantitative smoking variables likely involving mechanistic pathways related to chronic obstructive pulmonary disease [121] and TP53 mutations [122]. Of note, reduction of the pretreatment FEV1/FVC ratio that in turn is diagnostic of obstructive pulmonary disease was independently associated with shorter overall and progression-free survival in limited disease patients, thus confirming once again such a close association with tobacco consumption. However, as many as 2–5% of SCLC patients are never smokers, who show a significantly longer progression-free and overall survival as compared with current or former smokers [123]. Since resected SCLC, whether elective or incidental, exhibit a more favorable clinical course than patients not undergoing surgery [124], it is tempting to speculate that even SCLC may encompass a case mix of diversely behaving tumors not predicted by morphology. According to a recently released common classification framework, SCLC as defined by current criteria are NENs belonging to the family of neuroendocrine carcinomas, typed as featuring small cells [125].

Gross, Clinical Presentation and Imaging

Most SCLC affect major bronchi presenting as hilar/para-hilar mass and huge involvement of regional lymph nodes and vascular channels, whereas 5% or less of them arise in the pulmonary parenchyma most often in the form of low-stage peripheral nodule. In major bronchi, rarely SCLC grow as an endoluminal polypoid tumor, but rather spread in a subepithelial and radial pattern causing diffuse increase of the bronchial wall thickness for concentric stenosis (an airway stenting may be also beneficial) and massive involvement of adjacent structures (nerves, vessels, lymph nodes, lung parenchyma). Clinical symptoms may be local, systemic, or related to paraneoplastic syndromes. Suffice it to say that SCLC make up the most frequent lung cancer histology associated with paraneoplastic syndromes [126], which can be caused by either ectopic hormone production (hyponatremia, Cushing's syndrome) or autoimmune-mediated destruction upon onconeural neoantigen expression by cancer cells (paraneoplastic encephalomyelitis, Lambert-Eaton myasthenic syndrome) [127], the former being associated with poorer outcome, the latter with more prolonged clinical prognosis [128]. Most SCLC are extended diseases at clinical presentation with widespread metastases (liver, bone, brain, adrenal grand, lymph nodes), along with pleural and pericardial effusions. Staging assessment is at the best performed by using TNM classification, as the prognosis of oligometastatic

patients (≤5 metastases in a single organ that tended to locally recur) was significantly superior to patients with polymetastases, thus paving the way to local and systemic combination therapies. No consensus exists on standard imaging modalities for pretreatment staging of SCLC, and there is only low-strength evidence suggesting that FDG-PET/CT is more sensitive than CT alone and bone scintigraphy for detecting osseous metastases [129]. Active magnetic resonance imaging surveillance of brain metastases in SCLC patients has recently been proposed in opposition to the simple prophylactic cranial irradiation to prevent declines in cognitive function [130]. CT scan of SCLC shows characteristically a large solid and lobulated mass in hilar/para-hilar region with bulky mediastinal lymph nodes and invasion of great vessels and mediastinal fat, whereas cavitation is rare. SCLC can also be variably found (6–13% but 34% of all interval cancers) [131] in screening programs with low-dose computed tomography, but prognosis of these patients remains disappointing with no survivors at 3 years after diagnosis. These findings support the widely held belief that low-dose computed tomography screening is ineffective in reducing SCLC-related mortality in an age- or smoking status-independent manner, whereas there was evidence of a differential benefit by female sex. Somatostatin receptor scintigraphy with 111In-pentetreotide (OctreoScan) scintigraphy showed optimal specificity but lower sensitivity for primary SCLC, mediastinal lymph nodes, and distant metastatic disease likely due to variable and inconsistent expression of somatostatin transmembrane receptors by poorly differentiated tumor cells [132].

Histopathology

SCLC diagnosis con be usually rendered on small samples (cytology and biopsy) and surgical resection specimens. As most SCLC are widespread metastatic at clinical presentation, cytology and biopsy samples are most often the only material investigated for clinical purposes of treatment. Small-sized cells, round to spindle shape, irregular nuclear outlines, naked or small clustered nuclei with evenly distributed fine chromatin, no prominent nucleoli, scant to stripped out cytoplasm, chromatin streaking, and apoptotic debris are the typical traits that can be observed in cytological preparations [133]. It has been observed that treatment facilities rather than patients' demography or clinic traits may affect the prevalence ratios of cytology as a confident diagnostic tool in SCLC patients [134], even if a judicious use of IHC improved the inter-observer agreement to good in most cases of small biopsy samples [110]. Cytology of SCLC was not specifically tested with IHC for inter-observer reproducibility, but it correlated well with histopathology, and it is well known the essential role played by IHC in the cytological subtyping of lung cancer [135]. Crush artifacts in both cytology and biopsy samples may hamper diagnostic recognition of SCLC, exposing to the risk of misdiagnosing carcinoid as SCLC (with major diagnostic pitfalls for the clinical handling of patients). Such a situation, however, can be easily overtaken by addressing IHC staining for Ki-67: carcinoids, either typical or atypical, present with a Ki-67 labeling index ranging up to 20–25%, while SCLC exceed to a large extent 50% easily arriving at 90–100%

[136]. Necrosis is variably seen in both cytological and biopsy samples, but mitotic figures are not easily recognizable as one would expect in such proliferating tumors, especially when crush artifacts concur.

Histopathology of SCLC is generally highlighted by small-sized cells not exceeding three resting lymphocytes or endothelial cells, with scant cytoplasm, finely granular to evenly dispersed nuclear chromatin, small or inconspicuous nucleoli, frequent and abundant necrosis up to featuring geographic distribution, and plentiful mitoses (more than ten mitotic figures per 2 mm^2, with a median value of 80). Round-, oval-, and/or spindle-shaped tumor cells are variably admixed with each other in a solid growth pattern with ill-defined borders and prominent nuclear molding sometimes resembling hematologic malignancies, undifferentiated NSCLC, or sarcoma. Giant tumor nuclei may also be seen. Peripherally located tumors show instead more developed neuroendocrine morphology featuring prominent trabecular sheets, organoid solid growth, rosette formation, and more abundant cytoplasm, even if these tumors do not differ in terms of nuclear features and mitotic count [137]. Azzopardi phenomenon [138], featuring basophilic DNA stratification around vascular channels or extracellular matrix collagen fibers, may be noted, albeit it is unspecific, concurrently with geographic necrosis or severe tissue crushing due to fragility of tumor cells. In general, these SCLC characters are sufficiently maintained over tissue samples to ensure inter-observer reproducibility of diagnosis, even in challenging settings such as frozen section examination during surgery. Rarely, in about 5% of instances, SCLC may be observed as asymptomatic peripheral tumor (solitary pulmonary nodule) on routine chest radiography, usually as low-stage tumor with no regional lymph node metastases upon surgery (Fig. 9.12). The survival of these stage I SCLC, after multi-organ scanning and lymph node sampling prior to thoracotomy, is similar to survival of surgically treated stage I NSCLC patients [139]. Interestingly, while these tumors fulfil diagnostic criteria for SCLC, instead they show organoid neuroendocrine patterns of growth with nesting, palisading, trabecular features and rosette formation at variance with centrally located and early aggressive SCLC, mostly presenting as extended disease, which show diffuse, solid, and/or sheetlike patterns simulating hematologic malignancies, thus suggesting a different underlying pathogenesis.

Immunohistochemical Profile

Even if IHC is not strictly required for the diagnosis of SCLC, it is warmly recommended due to the large number of histologic mimickers of this tumor (mainly poorly differentiated NSCLC of squamous lineage, NUT carcinoma, hematologic malignancies, melanoma, sarcomas) [110]. Of minor clinical relevance could seem distinguishing SCLC from LCNEC, because they share similar life expectation and many molecular alterations, but emerging data on the different susceptibilities of LCNEC to diverse chemotherapy regimens [9] and their widely recognized molecular heterogeneity [140, 141] strongly advice performing this separation. Thus, our discussion on IHC will imply two aspects, which are also strictly interconnected with the issue of differential diagnosis: diagnosis of SCLC from other tumor types and separation of SCLC from LCNEC.

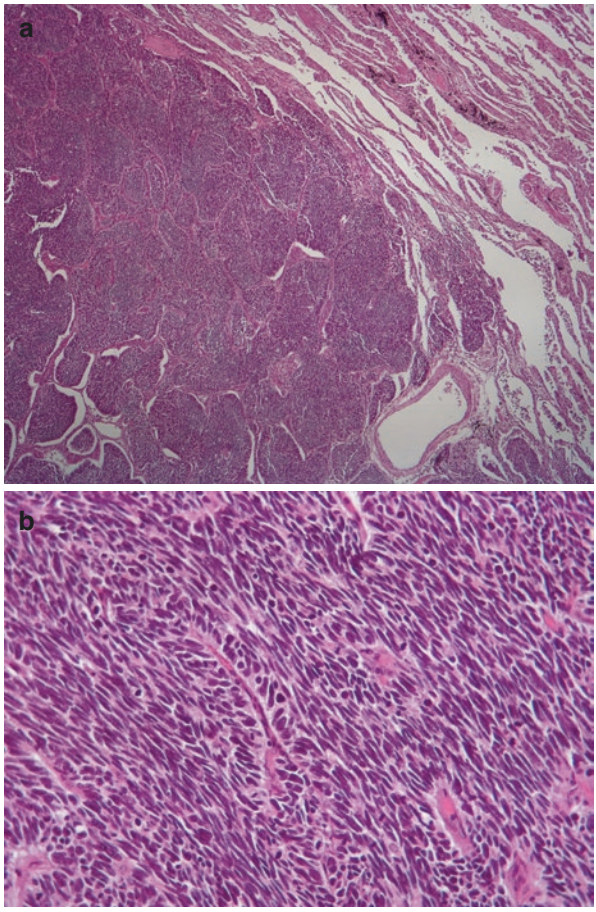

Fig. 9.12 A peripheral small cell lung cancer case, with well-defined borders (**a**) and small cell cytology with numerous mitotic figures (**b**) (hematoxylin and eosin staining; original magnification: **a** 4×, **b** 20×)

A reasonable antibody panel reacting to low and high molecular weight cytokeratins, TTF1, p40, chromogranin A, synaptophysin, retinoblastoma, CD56, NUT protein, and Ki-67 is useful to confirm SCLC diagnosis the morphological impression of facing with SCLC [110]. Low molecular weight cytokeratins highlight epithelial differentiation of tumor cells, with either paranuclear dot-like or cytoplasmic diffuse staining pattern, while high molecular weight cytokeratins or p40 but not p63 are always negative if not in the event of combined variant with squamous cell carcinoma [113, 142]. Pan-NE markers are consistently positive in 85–90% of SCLC, especially synaptophysin and CD56, whereas chromogranin A may be so faint and scattered to require close observation at high power magnification. CD56 is very sensitive in recognizing SCLC [143], but its lack of specificity toward unrelated neoplasms (e.g., small cell sarcomas, melanoma, or NUT carcinoma) obliges a cautious interpretation on the basis of the proper clinical and morphological context. About 10–15% of SCLC may lack overt NE differentiation likely due to different cell lineage derivations as assessed on the basis of differential gene expression:

yes-associated protein 1-SCLC (SCLC-Y) and POU class 2 homeobox 3-SCLC (SCLC-P), both lacking insulinoma-associated protein 1 (INSM1), an early embryonic inducer of NE differentiation, with SCLC-P recapitulating an expression profile closely resembling the rare pulmonary chemosensory tuft cells [144]. These SCLC missing NE differentiation have been called variant subtypes (not to confound with combined variant of SCLC), which are characterized by epithelial-to-mesenchymal transition leading to vimentin accumulation and lack of cytokeratin filaments [145]. At least the SCLC-Y phenotype was found to be associated with shorter patient survival and increased chemoresistance, while the clinical outcomes for SCLC-P patients have not been well defined [146]. INSM1 is accumulated in the nuclei of most SCLC apart from SCLC-P and SCLC-Y and seems the most specific marker, but its sensitivity is not superior to composite marker CD56 plus TTF1 and p16 [147]. It has been proposed, in the appropriate clinical and morphological context, a diagnostic algorithm comprising at first INSM1, then CD56, and lastly p16 and TTF1, in that order, if all previously applied markers were negative. In any case, the lack of NE markers or even cytokeratin filaments should not prevent performing diagnosis of SCLC, provided other alternatives have been reasonably ruled out according to the proper clinical and morphological context. TTF1 reactivity is found in about 90% of SCLC, but its expression is not related to the pulmonary lineage establishment, inasmuch as most extrapulmonary small cell carcinomas are also consistently positive for this marker [148]. TTF1 expression in SCLC is related to the activation of the achaete-scute family bHLH transcription factor 1 (hASH1, product of *ASCL1* gene)/TTF1/ nuclear factor IB (NFIB) axis that potentially contributes to the tumorigenesis and metastatic potential of most SCLC [149] (Fig. 9.13). TTF1 closely correlates with NE differentiation the inhibitory Notch ligand Delta-like protein 3 (DLL3) expression especially in the ASCL1-positive SCLC subset (SCLC-A), which account for at least 70–80% of all SCLC [144]. The truncated form p40 (DNp63) of *p63* gene is consistently negative in SCLC and in general neuroendocrine tumors as a whole, thus making this marker a useful tool in the differential diagnosis with basaloid and nonkeratinizing squamous cell

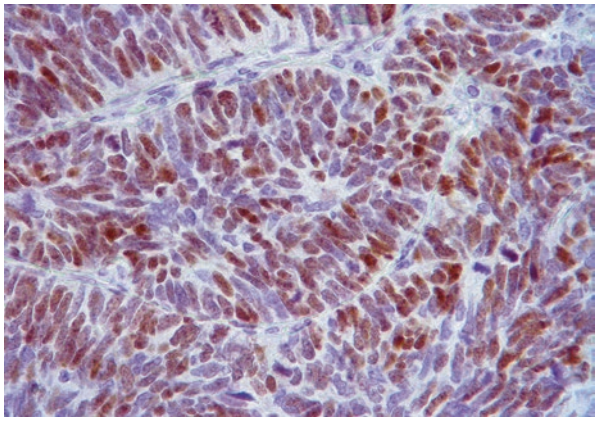

Fig. 9.13 Nuclear staining for hASH-1 in small cell lung carcinoma (immunoperoxidase; original magnification 40×)

carcinomas, which turn out strongly positive for p40 even in biopsy/cytology samples [150]. Retinoblastoma is frequently lost in classical SCLC while is strongly expressed in most NSCLC, but is retained in the variant subtype of SCLC with intermediate to large cell morphology (e.g., SCLC-P) where it is linked to decreased patient survival and increased chemo-refractory tumor response. The antigen Ki-67 is diffusely expressed in SCLC as one would expect from a highly proliferating tumor, with positivity rates approaching 100% [6], even though may sometimes present with some heterogeneity in intratumor distribution that is likely to play some role in histogenesis and pathogenesis of SCLC. Although Ki-67 is not per se diagnostic of SCLC outside its appropriate morphological context, the consistent huge positivity in either classical or variant subtype of SCLC makes Ki-67 a practical marker in the differential diagnosis from low- to intermediate-grade NE tumors (i.e., carcinoids) especially in the setting of limited and/or crushed diagnostic material as seen in biopsy or cytology samples to avoid major pitfalls in the management of patients [136]. In this type of material, beyond carcinoids, SCLC should be differentiated from reactive or neoplastic lymphocytic proliferations, Merkel carcinoma, Ewing sarcoma family tumors (ESFT), and even small cell melanoma. An integration of clinical data with an antibody panel approach including cytokeratins (including cytokeratin 20 for Merkel cell carcinoma), polyomavirus, neuroendocrine markers, CD99 (for ESFT), leukocyte common antigen (for lymphomas), S100 protein/HMB45 (for melanoma) and, if needed, fluorescence in situ hybridization for the relevant gene translocations are fruitful tools in this scenario. An IHC tool that never should miss in the antibody panel approach to SCLC is the nuclear-in-testis (NUT) protein, whose expression in the totality of tumor cells is diagnostic of NUT carcinoma, a rare but deadly form of lung cancer [151]. This tumor, which shows different histologic features and challenging expression profiles, including neuroendocrine differentiation and small blue round cell tumor appearance [152], should always be comprised among diagnostic options while examining small round cell tumors. Differentiating SCLC from LCNEC may be difficult and to some extent a subjective exercise, but is largely based on cytological criteria, such as larger nucleoli, smaller cell size, and lower nuclear-to-cytoplasmic ratio in LCNEC. A panel of three antibodies (BAI3, CDX-2, and VIL1) has been proposed as a useful adjunct to distinguish SCLC (more positive for BAI) from LCNEC (more positive for CDX-2 and VIL1) [153]. Retinoblastoma protein is preserved in about 50% of LCNEC along with cyclin D1 overexpression and p16 loss as opposed to SCLC displaying loss of retinoblastoma and cyclin D1 and hyperproduction of p16 [154] at least in its classical and more frequent form displaying neuroendocrine differentiation (SCLC-A and SCLC-N).

Combined Neuroendocrine-Non-neuroendocrine Carcinoma

Combined variants of LCNEC and SCLC refer to the presence of any other non-neuroendocrine tumor component, such as adenocarcinoma, squamous cell carcinoma, or giant/spindle cell carcinoma [6, 155], for which no cutoff is required for

the non-neuroendocrine components because they are easily recognizable as such, even if IHC characterization may help in diagnosis [156] (Fig. 9.14). However, for SCLC a 10% cutoff is required for LCNEC (see above) or large cell carcinoma to subclassify SCLC as combined variant according to combined (separate/juxtaposed) or composite (intermingled) manners due to the continuity in cell size and nuclear chromatin changes. Combined variant is rare in LCNEC but accounts up to one third of SCLC. In contrast, carcinoid tumors combined with non-small cell lung carcinomas are very rare and supposed to be collision tumors, rather than sharing a common clonal origin, although this remains to be proven by molecular studies since anecdotal cases have been reported sharing a common genetical profile [157]. The neuroendocrine and non-neuroendocrine cell population of combined carcinomas has the same immune-profile as their pure counterparts with regard to the expression of neuroendocrine and lineage-specific markers.

Combined variants of LCNEC and SCLC share the same epidemiology, clinical presentation, prognosis, and neuroendocrine properties as their pure counterparts

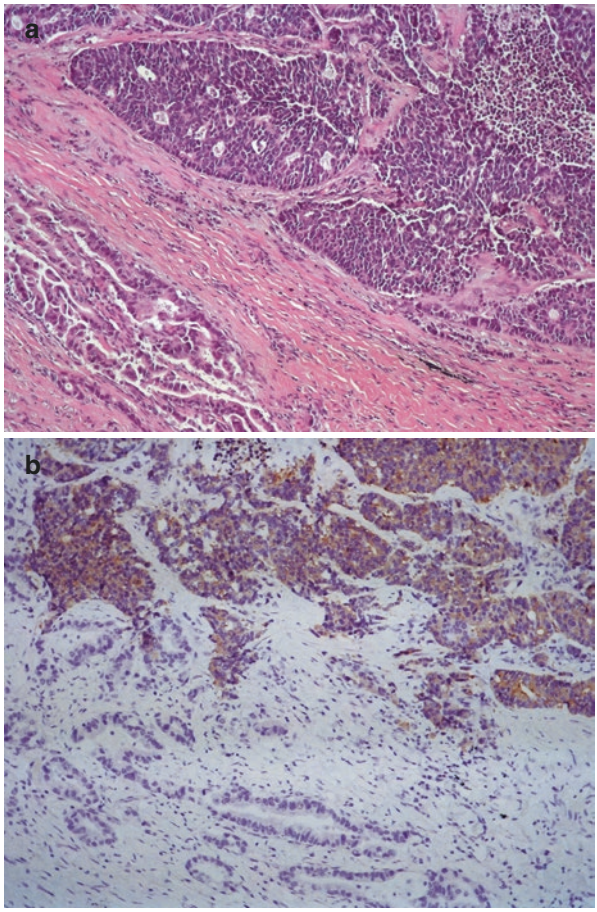

Fig. 9.14 A case of combined lung carcinoma, with acinar adenocarcinoma component and large cell neuroendocrine carcinoma component with necrosis (**a**) and synaptophysin staining (**b**). (**a**, hematoxylin and eosin staining; **b**, immunoperoxidase; all original magnifications 20×)

even if it has been suggested that combined SCLC could have a worse prognosis than pure SCLC, possibly because of a relative chemoresistance of non-SCLC components, which could emerge after therapy on recurrent or metastatic tumors.

Combined variants of LCNEC and SCLC may arise de novo or being the consequence – in the cases of an associated adenocarcinoma component – of histologic transformation as a mechanism of acquired resistance after epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) treatment [158], although data are still scarce and limited to very small series. In both these situations, the genomic alterations in neuroendocrine and non-neuroendocrine components of combined carcinomas are mostly homogeneous, with a high prevalence of *TP53* and *RB1* mutations in the non-neuroendocrine population [159]. Interestingly, mixed high-grade neuroendocrine carcinomas with non-neuroendocrine components (including those of the lung), when studied for regulators of DNA synthesis, repair, or recombination and chromosome disentanglement (such as ribonucleotide reductase, DNA excision repair protein ERCC-1, topoisomerase II-A, and thymidylate synthase), did not show differences for all genes but Topo-IIA between both components, with the thymidylate synthase content, predominant non-NE component, and chemotherapy acting as independent predictors for better prognosis [160].

Thymic Neuroendocrine Neoplasms (T-NENs)

Epidemiology

Neuroendocrine neoplasms of the thymus (T-NENs) make up a heterogeneous family of uncommon middle-aged mediastinal neoplasms accounting for 2–5% of all thymus tumors [161, 162]. T-NENs are classified according the same terminology as the homonymous neoplasms of the lung, i.e., typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma (SCC), and the same unbiased diagnostic criteria, i.e., number of mitoses per 2 mm^2, presence and extent of necrosis, and a constellation of morphologic and immunohistochemical features [163]. However, their biological behavior is quite different, at least for well-differentiated tumors, including TC and AC, which behave on average more aggressively than their pulmonary counterpart (Table 9.2). Pediatric

Table 9.2 Comparative features of carcinoids of lung and thymus

		Lung	Thymus
Age (mean)		40–60 years	45 years
M/F		1:2	3:1
Clinical syndrome	*Cushing*	2%	30–40%
	MEN1	10%	25%
Tumor size (mean)		3 cm	8–10 cm
Histotype	*Typical carcinoid*	90%	10%
	Atypical carcinoid	10%	90%
Lymph node metastases		5–10%	30–45%

and young people instances, mostly but not always belonging to the category of carcinoids, have also been recorded, but most of T-NENs affect adults or elderly patients. T-NENs are not strictly analogous tumors to the pulmonary counterpart, because they present a larger prevalence of AC and LCNEC over TC and SCC [164], a higher association rate with ectopic adrenocorticotropic hormone Cushing's syndrome [165], a more variable dependency on smoking also in female and MEN1 patients [166], and a lower MEN1 genotype-phenotype correlation suggesting the involvement of other genetic factors [167]. As a matter of fact, about one fourth of patients with T-NENs is MEN1-related as opposed to 1–8% of patients bearing such a syndrome who develop T-NENs during life [168]. Most MEN1-related T-NENs correspond histologically to carcinoids, but even poorly differentiated NE carcinomas or purported carcinoids with gross areas of necrosis have been recorded. LCNEC and SCC account for about 15–35% of all T-NENs, with a relative prevalence rate of LCNEC over SCC. Risk factors are largely unknown, inasmuch as high-grade NENs of the thymus are not associated with MEN1 syndrome. It has been estimated that LCNEC and SCC have an incidence of 1 case/20 million individuals and 1 case/50 million individuals, respectively, testifying their substantial rarity as compared with the corresponding neuroendocrine carcinomas of the lung.

Gross, Clinical Presentation and Imaging

Carcinoids usually present with space-occupying mass causing local symptoms (pain, cough, superior vena cava syndrome) to arise according to mediastinal tissue infiltration. Systemic symptoms due to paraneoplastic syndromes are most often due to ectopic hormone secretion, such as Cushing's syndrome (ectopic adrenocorticotropic hormone) [169], hypercalcemia/hypophosphatemia (parathyroid hormone-related protein) [170], acromegaly (antidiuretic hormone or atrial natriuretic peptide) [171], or, exceptionally, carcinoid syndrome (serotonin and other peptides) [161], but paraneoplastic limbic encephalitis [172] and late-onset myasthenia gravis [173] are also on record in thymus carcinoids. TC are unencapsulated and calcified lesions, either circumscribed or locally invasive, while AC are locally infiltrating and metastasizing tumors in most cases. When compared with their pulmonary counterpart, thymus carcinoids present with no significant differences between them in major risk factors, a male preponderance, difficult (delayed) preoperative diagnosis, a higher rate of lymph node and distant metastasis, a larger tumor size on average (delayed detection), low postoperative survival, and a lower rate of carcinoid syndrome as opposed to a higher rate of association of Cushing's syndrome. LCNEC and SCC are detected owing to local symptoms due to infiltration (lung, pericardium, major vessels) or occurrence of distant metastases (bone, liver, lung, brain, adrenal glands, lymph nodes) at the time of clinical presentation. Computed tomography, magnetic resonance, and ^{18}F-fluorodeoxyglucose positron emission tomography imaging play a major role in the identification, staging, preoperative biopsy planning, and follow-up monitoring of thymic epithelial neoplasms, including T-NENs: Furthermore, scintigraphy techniques based on the

bioavailability of somatostatin receptors have been developed in T-NENs by using (68)Ga-DOTA-TOC PET/CT, 111In-OctreoScan, or 99mTc-EDDA/HYNIC-octreotate. LCNEC and SCC have no particular gross presentation, which is the same as in other T-NENs in the form of variably sized (up to 10 cm or more) tumors, usually without the characteristic lobulated growth pattern of thymomas. Of note, cases associated with Cushing's syndrome tend to be smaller likely due to their earlier detection. Cytological criteria do not distinguish TC and AC, which in both instances present as round to oval cells, either single or in small clusters, with scanty cytoplasm, interspersed with some larger cells with moderate to abundant, granular cytoplasm [174]. On cytological grounds, it is not possible to separate TC from AC, while defining criteria for SCC are the same as the pulmonary counterpart with common crush artifacts, nuclear breakdown, and apoptotic bodies. There are no established cytological criteria for thymus LCNEC due to either their rarity or similarities of findings with other T-NENs or more common thymic epithelial cell tumors. From a clinical perspective, TC and AC are low- to intermediate-grade and well-differentiated tumors, while LCNEC and SCC high-grade tumors with similar dismal prognosis. The 10-year actuarial survival rates are 77.92% (median survival 126 months) for TC, 54.55% (median survival 52 months) for AC, and nihil for LCNEC or SCC [161]. As compared with thymic carcinomas, thymic carcinoids show no substantial prognostic differences [175], with younger patients, completeness of resection, adjuvant radiotherapy, no adjuvant chemotherapy, and TNM stage being independent predictors of better overall and/or disease-free survival.

Histopathology

Defining diagnostic criteria for T-NENs settled by 2015 WHO classification are the same as the pulmonary counterparts. The descriptive terms of well-differentiated neuroendocrine carcinoma to indicate carcinoids (Fig. 9.15), either TC or AC, and poorly differentiated neuroendocrine carcinoma to refer to LCNEC and SCC, as stated in the 2004 WHO classifications [19], have been abandoned in the new 4th edition of 2015, inasmuch as LCNEC and even SCC may be highly differentiated in terms of neuroendocrine features. In a perspective of clinical behavior in the decision-making process, TC are considered low-grade tumors, AC intermediate-grade tumors, and the group of LCNEC and SCC high-grade tumors or neuroendocrine carcinomas [163]. Histologically, TC are characterized by less than 2 mitoses per 2 mm^2 and no necrosis, with different growth patterns (trabecular, resetting, lobulated, solid, pseudoglandular, gyriform, festooned) and histologic variants (spindle cell, pigmented, oncocytic, amyloid stroma, angiomatoid), which do not impact on tumor behavior and can be disregarded in a clinical perspective provided that defining criteria are strictly respected but should be accounted for in the differential diagnosis. AC share the same architectural features as TC, with the differences consisting in higher mitotic count (2–10 mitoses per 2 mm^2) and occurrence of even small punctate foci of necrosis. Nuclear pleomorphism may be observed, along with calcifications, diffuse growth pattern, or extensive desmoplastic stroma

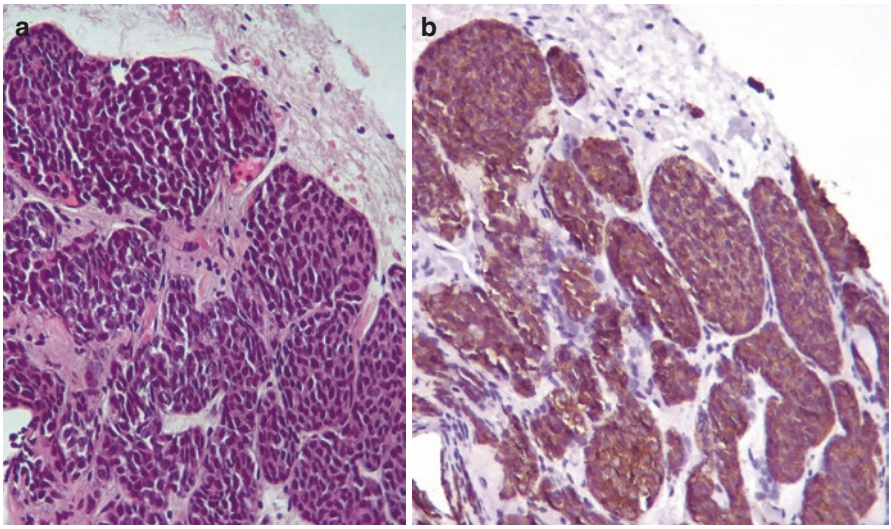

Fig. 9.15 Mediastinoscopic biopsy of a thymic carcinoid with insular arrangement (**a**) and diffuse chromogranin A immune-labeling (**b**). (**a**, hematoxylin and eosin staining; **b**, immunoperoxidase; all original magnifications 20×)

with Indian-file arrangement of tumor cells, which can be relevant to differential diagnosis [164]. The main differential diagnoses of carcinoids include spindle cell type A thymoma (missing diffuse neuroendocrine marker decoration), parasympathetic paraganglioma (missing cytokeratins) [176], extrathyroidal medullary carcinoma in amyloid-rich carcinoid (strong reactivity for calcitonin and carcinoembryonic antigen), metastatic mucinous carcinoma in mucinous carcinoid (missing neuroendocrine markers), and hemangioma in the angiomatoid variant of thymus carcinoid with pseudovascular spaces lined by tumor cells [177].

LCNEC exhibit non-small cell morphology with large tumor cell size, a mitotic rate by far exceeding 10 mitoses per 2 mm^2 (on average 45 mitoses) and extensive necrosis. Some tumors look like AC in terms of general architecture and cell morphology, but differ from them for having too many mitoses and more necrosis [178]. LCNEC co-express epithelial (cytokeratins, often with dot-like staining pattern) and neuroendocrine markers (usually in more than 50% tumor cells and with clear-cut decoration) alongside CD117, TTF1 and, rarely, CD5. The main differential diagnosis of LCNEC is toward thymic carcinomas, which can share reactivity for neuroendocrine markers, usually fainter and focal, more consistent CD5 and CD117 immunoreactivity and extensive positivity for p40, which is always missing in LCNEC. SCC appearance in the thymus is identical to that of the homologous tumors arising anywhere, especially in the pulmonary counterpart. In this regard, TTF1 is not helpful in the differential diagnosis, since it is frequently positive even in extrapulmonary neuroendocrine carcinomas [148]. Therefore, SCC remains basically a histological diagnosis, where expression of neuroendocrine markers is often detectable but not strictly required for the ultimate diagnosis to do. At variance, in

LCNEC the demonstration of neuroendocrine markers is tautologically required for diagnosis, once other histologic mimickers of small blue round cell tumors, either primary or secondary, have been convincingly ruled out. In SCC, mitoses exceed by far the number of 10 per 2 mm^2 (on average, there are 110 mitoses per 2 mm^2) along with small cell morphology (typically less than three times the size of a small resting lymphocyte) and extensive or geographic necrosis. Tumor cells are round to oval or spindle, with evenly distributed chromatin, inconspicuous nucleoli, nuclear molding, and plentiful apoptotic bodies. Most SCCs in the thymus stain for cytokeratins, but negative cases make its separation from other small blue round cell tumors particularly challenging. SCCs are consistently negative for p40, as usually happens for T-NENs. The main differential diagnosis is to distinguish thymus primaries from pulmonary small cell carcinoma, for which an accurate clinicpathologic and imaging correlation is required.

Immunohistochemical Profile

On immunohistochemistry grounds, carcinoids of the thymus exhibit reactivity for epithelial markers (cytokeratins), often with dot-like, paranuclear labeling pattern. Neuroendocrine markers are strongly expressed in TC, with more focal or dispersed distribution in AC [179]. Hormones, such as ACTH, human chorionic gonadotropin, or calcitonin) may be detected in carcinoids of the thymus, usually in a limited amount of tumor cells with no relationship with clinical symptoms of paraneoplastic syndromes. The differentiation of lung and thymus carcinoids proves to be particularly challenging in the setting of low- to intermediate-grade tumors displaying large unresectable or metastatic lesions at the time of diagnosis. TTF1 is a useful marker of pulmonary lineage only when positive in the group of well-differentiated NETs. In this regard, some T-NETs may be reactive for TTF1 even when using the most specific clone 8G7G3/1; thus, TTF1 may not be a reliable maker to exclude the thymic origin in thoracic well-differentiated NETs [180]. Reactivity for PAX-8 in thymus carcinoids helps to differ them from the pulmonary counterpart.

Origin of T-NENs and Combined Tumors

The origin of T-NET is unclear, but evolutionarily conserved neuroendocrine-committed thymus epithelial cells have been detected in the subcapsular region, cortex, and medulla of the thymus gland of reptiles, birds, mice, and humans [181]. Interestingly, subsets of thymus epithelial cells express a variety of neuroendocrine self-proteins belonging to neurohypophysis (oxytocin), tachykinin (neurokinin A), and insulin (IGF1, IGF2, insulin) family peptides, which are likely to be engaged in the self-recognition for immune-tolerance of T lymphocytes toward endocrine organs [182]. Furthermore, ACTH-immunoreactive thymus epithelial cells have been unveiled in the subcapsular region, cortex, and medulla of the human thymus gland [183]. Beyond T-NENs, neuroendocrine differentiation has also been

documented in tumors with no clear-cut neuroendocrine morphology, such as thymic squamous cell carcinoma [184] and, more rarely, thymoma [185], although this finding does not bear direct clinical implications on tumor behavior. These findings, however, account for the great plasticity of thymus epithelial cell ancestors of endoderm derivation, which are also likely to be involved in the development of combined tumors in keeping with similar phenomena occurring in lung NENs. The current 2015 WHO classification identified combined thymic carcinoma as any thymic carcinoma associated with any thymoma or carcinoid, thus excluding SCC and LCNEC. The most frequent combination is thymus squamous cell carcinoma and type B3 thymoma, but also papillary adenocarcinoma or sarcomatoid carcinoma in addition to type A thymoma has been recorded, while combination of different subtypes of thymic carcinomas with each other is quite rare. At variance with lung NENs, where carcinoids are exceptionally found along with non-small cell carcinomas, in the thymus it is possible to face with such a combination of carcinoids with thymoma, thymic carcinoma, or sarcoma-like elements of whatever size or percentage [186]. These combined thymic carcinomas should be listed in their components in 10% increments, starting from the predominant one. Moreover, in the setting of combined thymic carcinomas, associations of LCNEC or SCC with any other thymoma and/or thymic carcinoma are also on record, which yet are considered combined variants of either tumor type, featuring gradual transition or sharp separation from each other. These cases should be listed in their components, but their behavior is expected to be as aggressive as the homologous pulmonary tumors. T-NENs comprising transition forms between TC/AC and LCNEC/SCC within individual tumors have been documented in the past, but have remained an orphan category with only descriptive terminologies being reported on. Diversely graded T-NETs have been interpreted as high-grade NE carcinoma evolving from preexisting carcinoids rather than chance or collision tumors [187].

Molecular Pathology

Inheritance

Most NENs are sporadic in their distribution, in either the lung or the thymus, but about 10% of them are familial or inherited. The most common inherited genetic syndrome underlying NENs development is MEN1 [188], but familial carcinoid tumor syndromes due to rare germline mutation other than MEN1 have been reported in the lung [189]. Likewise, in T-NENs there is a lower MEN1 genotype-phenotype correlation suggesting the involvement of other genetic factors [190]. As a matter of fact, about one fourth of patients with T-NENs is MEN1-related [166] as opposed to 1–8% of patients bearing such a syndrome who develop T-NENs during life. Approximately 50% of patients from MEN1 families will develop the syndrome and the distribution between genders is equal, suggesting an autosomal dominant trait. MEN1 syndrome is due to inactivating mutations (over 1300 different mutations are known) of the tumor suppressor gene *MEN1* mapping to 11q13.1,

whose scaffold protein menin functions in chromatin remodeling through histone modification and epigenetic gene regulation via binding to and inhibition of JunD's (an AP-1 transcription factor) activation of transcription (https://www.ncbi.nlm.nih.gov/gene/4221). About 30–60% of patients bearing MEN1 germline mutations is destined to develop endocrine-neuroendocrine tumors (17% of whom before aging 21 years) [191], which affect the pancreas, parathyroid glands, hypophysis, lung, thymus, thyroid, adrenal glands, and ovaries, beyond meningioma, facial angiofibroma, collagenoma, and lipoma. Less common than the MEN1 syndrome is the von Hippel-Lindau disease (VHL), a dominantly inherited familial cancer syndrome whose germline mutations predispose to a variety of malignant and benign lesions, including hemangioblastomas of the central nervous system, renal clear cell carcinoma, pheochromocytoma, endolymphatic sac tumors, and pancreatic, renal, epididymal, and broad ligament cysts. The VHL gene product encodes protein VHL, which binds to elongin C, elongin B, cullin-2, and Rbx1 to form a complex catalyzing the polyubiquitinylation of specific proteins and targeting them for degradation by proteasomes (https://www.genecards.org/cgi-bin/carddisp.pl?gene=VHL). Neuroendocrine tumors usually affect the pancreas, while pulmonary carcinoids are quite uncommon in VHL [192] and thus far undescribed in the thymus. Neurofibromatosis type 1, inherited as autosomal dominant trait with biallelic inactivation of NF1 gene mapping to 17q11.2 that functions as negative regulator of the RAS signal transduction pathway (https://www.ncbi.nlm.nih.gov/gene/4763), is rarely associated with the development of carcinoids in the thymus while missing in the lung [193]. Tuberous sclerosis complex (TSC) is due to mutations in either the TSC1 or TSC2 gene, which map to 9q34.13 and 16p13.3, respectively, and regulate mammalian target of rapamycin complex 1 (mTORC1) signaling via stimulation of specific GTPases (https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=7248). Although TSC has not been linked to the development of hereditary carcinoids in either the lung or the thymus, somatic mutations of TSC1 or TSC2 genes with upregulation of p-mTOR and ribosomal p70S6-kinase (S6K) indicating PI3K/AKT/mTOR pathway activation have been yet observed in pulmonary carcinoids [194]. Interestingly, high-grade neuroendocrine carcinomas are not components of MEN1, VHL, or NF1 syndromes, but somatic MEN1 mutations have been identified in carcinoid-looking LCNEC and even SCC along with upregulation of eukaryotic initiation factor 4E-binding protein 1 (4EBP1), a downstream activator of mTOR pathway, indicating that this pathway can be engaged even in this subset of patients [195].

Molecular Classification

The current interpretation of lung NEN pathogenesis supports the view that there are major differences in gene alterations between TC/AC on the one hand and SCLC/LCNEC on the other hand, with minor or no differences inside each tumor group. In other words, there should be a close relationship between morphology and underlying molecular alterations making the spectrum of lung NENs a

clinical-pathological but not a pathogenetic one. As a matter of fact, when comparison is performed by means of morphology-based supervised analysis, a statistically significant separation is obtained among the diverse categories of lung NENs in terms of gene mutation and copy number variation (CNV) distribution [196].

In the lung, our current knowledge says that TC and AC show very low mutation rates and recurrent alterations in mechanisms of epigenetic regulation (chromatin remodeling, SWI/SNF complex-dependent DNA packaging, histone methylation and acetylation), with no relevant histology-dependent differences to support a causal relationship of at least some TC with the development of AC. Recurrently altered in carcinoids are chromatin remodeling genes, such as *MEN1*, *PSIP1,* and *ARID1A, with MEN1* mutations also bearing poor prognosis in the setting of AC [197]. Intra- or intertumor heterogeneity of carcinoids is a poorly explored issue due to their relative rarity and reduced metastatic potential at presentation, but incremental proliferation rates have been documented at metastatic sites in the lung with retention [80] of RB1 expression and carcinoid morphology. Conversely, SCLC exhibit high mutation rates and recurrent mutations/deletions in cell cycle regulators (especially *TP53* and *RB1*), chromatin remodeling (*CREBBP*, *EP300, MLL*), copy number variations (MYC family, *FHIT*, *SOX2*, *FGFR1*), somatic genomic rearrangement (*TP73*), and alterations in mechanisms of neuroendocrine differentiation (NOTCH family), with *KMT2D* gene (a histone modifier) mutations correlating with longer survival [196, 198]. In turn, LCNEC share with SCLC the highest mutation rates ever seen in pulmonary NENs, but make up the most heterogeneous tumors on molecular grounds, with some of them resembling carcinoids, some overlapping with SCLC, and some linking to NSCLC (especially adenocarcinoma but also squamous cell carcinoma) on the basis of their patterns of gene alterations. A recent study by George et al. on 75 cases of LCNEC found three main molecular subgroups: one resembling SCLC different from LCNEC type I and LCNEC type II groups. LCNEC type I presented with high neuroendocrine expression (ASCL1^{high}/DLL3^{high}/NOTCH^{low}) and *TP53* mutation similar to SCLC group but with additional *STK11/KEAP1* mutations and lack of *RB1* inactivation; and LCNEC type II, with low neuroendocrine expression (ASCL1^{low}/DLL3^{low}/NOTCH^{high}), combined *TP53* and *RB1* mutations and an upregulation of immune-related pathways [141]. Similarly, Simbolo et al. performed a comparative analysis of AC and LCNEC by means of next-generation sequencing alongside immunohistochemistry for menin and RB1 protein [199]. Transcriptomic and genomic investigation distinguished three separate clusters: (a) cluster 1 showed a large prevalence of LCNEC along with *TP53* and *RB1* gene inactivation while missing MEN1 mutations and Rb1 protein; (b) cluster 3 included especially AC with *RB1*, *MEN1,* and *TP53* mutations while missing menin and RB1; and (c) cluster 2 comprised slightly more AC than LCNEC with intermediate molecular findings. Expectedly, cluster 1 patients run a worse clinical course than the other two ones. These two studies not only support molecular classifications, which are quite independent of morphology but clinically relevant to targeted therapy, but also suggest models of malignancy progression from carcinoids to LCNEC, which are likely to depend on common risk factors.

About 10–15% of human SCLC, SCLC cell lines, and genetically engineered mouse models lack, or express at low levels, neuroendocrine markers: they have been called the "variant subtype of SCLC" with downregulation of neuroendocrine differentiation. A recent reappraisal of SCLC has identified four different subsets of patients according to their molecular profiles: SCLC-A (expressing achaete-scute homologue 1, ASCL1) and SCLC-N (expressing neurogenic differentiation factor 1, NeuroD1) are the neuroendocrine-differentiated forms of SCLC, while SCLC-Y (expressing yes-associated protein 1, YAP1) and SCLC-P (expressing POU class 2 homeobox 3, POU2F3) are the non-neuroendocrine-differentiated ones corresponding to the variant subtype [144]. The first two categories of SCLC make up about 80–85% of all SCLC, with SCLC-Y as the least frequent one with about a 2% prevalence, but virtually all SCLC would be composed of multiple subtypes revealing a still unexplored intratumor heterogeneity. Variant subtypes are characterized by intermediate cells, sometimes resembling NSCLC or LCNEC; downregulation of *TTF1 and DLL3;* upregulation of *REST*, NOTCH, and Hippo/TGFβ pathway; and MYC amplification, with vimentin-expressing epithelial-mesenchymal transition. These phenotype patients have a poorer response to chemoradiotherapy with shorter patient survival and increased chemoresistance (especially SCLC-Y) but vulnerability to Aurora kinase inhibitors as compared to the high-neuroendocrine classical SCLC counterparts [200]. Transformation of high-neuroendocrine classic subtype to low-neuroendocrine variants has been described upon MYC amplification leading to NOTCH pathway and REST activation in tumor cell subsets, which act as transcriptional repressors of neuroendocrine gene expression. These pathways provide a trophic/feeding microenvironment to classical SCLC cells and reveal a high plasticity of cancer stem cells, with a pro-tumorigenic role in the development of SCLC and, to some extent, a linking to NSCLC precursors [201]. Of note, while intra-/intertumor NSCLC genomic heterogeneity resulting from branching evolution is a well-known phenomenon responsible for acquired resistance to targeted treatments, SCLC usually maintain most of mutations in both primary and metastatic foci suggesting a different and linear model of evolution [202]. Many genetic alterations affecting lung NENs involve mechanisms of chromatin opening or gene transcription regulation, such as DNA methylation, histone deacetylation and deubiquitination, and miRNA up-/down-expression. These events include promoter hypermethylation of *RASSF1A* (paralleling tumor grade) [203] and *P15INK4b* [204]; histone modifications by downregulation of H4KM20 and H4KA16 [205]; upregulation of microRNA-129, microRNA-323-3p, microRNA-487b, microRNA-410, microRNA-369-3p, and microRNA-376a; and downregulation of miR-203, miR-224, miR-155, miR-302, miR-34b, miR-181b, miR-193a, miR-5p, and miR-34b [206].

The molecular landscape of T-NENs is largely unknown, but several chromosomal imbalances and aneuploidy status were found in 51–81% and 12% of instances, respectively [207]. Chromosomal losses and gains are differentially distributed among the diverse subtypes of T-NENs, with imbalances per tumor averaging 0.8 in TC (31% aberrant cases), 1.1 in AC (44% aberrant cases), and 4.7 in the LCNEC/SCLC group (75% aberrant cases) [208]. The most frequent overlapping

alteration across histologic variants maps to *MYC* locus-containing 8q24, a downstream target of ß-catenin involved in the development of some thymic and pulmonary neuroendocrine carcinomas. These findings support the current view that TC/AC are different and separate tumor entities with their own specific molecular drivers as opposed to LCNEC/SCC, when tumor separation of T-NENs is accomplished by using the 2015 WHO defining criteria. However, a recent low-coverage whole-genome sequencing study dealing with 63 T-NENs belonging to all different histologic subtypes has found that molecular classification by means of copy number instability (CNI) scores was prognostically effective to identify three tumor categories, somewhat independent of morphology [209]. Moreover, there was a subgroup of tumors fulfilling criteria for LCNEC, which featured carcinoid morphology, strong expression of neuroendocrine markers, Ki-67 averaged 29.5%, and negativity for p53 and enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), a promoter of cell cycle and apoptosis inhibition in SCLC models [210]. This subgroup was defined NET G3, in keeping with homologous lesions recently described in pulmonary and gastroenteropancreatic NENs, where progression of malignancy from low-grade to high-grade histology was noted between primary tumors and paired metastases. Accordingly, a morpho-molecular grading system was devised for better patient stratification and prognostication by identifying T-NET G1 to G3 based on an integrated evaluation of scored CNI and immunohistochemistry findings (Ki-67, chromogranin A, and EZH2 staining).

Novel Insights on the Molecular Pathways of Progression

Data are emerging that at least a certain number of NENs in both the lung and the thymus can take rise from progression of low-grade tumors to neuroendocrine carcinomas/high-grade tumors. This phenomenon holds particularly true in tumor patients undergoing surgical resection, probably because lesions amenable of surgery at presentation are inherently less aggressive. Accordingly, it is expected that resection specimens will capture in large majority TC and most of AC, presumably many LCNEC but only a minority of SCLC. The occurrence of common genetic traits shared by carcinoids and neuroendocrine carcinomas in the lung, thymus, and even gastroenteropancreatic tract supports secondary evolution of NETs to NECs in tumor resection specimens. On the basis of literature data reappraisal on NENs arising in the lung and the thymus, we have recently proposed an alternative and innovative interpretation by identifying a tripartite separation into early aggressive/primary high-grade neuroendocrine tumors (HGNETs), differentiating or secondary HGNETs, and indolent NETs [211].

- P-HGNETs (70–75% of lung NENs; 13% of lung tumors) are the most aggressive ones with widespread metastases at presentation, feature classical SCLC or variant subtype, are usually diagnosed on biopsies of male heavy smokers, present with minimal intertumor heterogeneity indicative of linear mechanisms of

evolution, and are characterized by biallelic inactivation of *TP53* and *RB1*. These tumors would originate through de novo or basal-like mechanisms of carcinogenesis with no intermediate/dysplastic lesions, deriving from cancer stem cells out of a neuroendocrine niche undergoing very early differentiation block. Ki-67 is uniformly high, even approaching 100%. These tumors exhibit high mutation burden.

- S-HGNETs (20–25% of lung NENs; 6% of lung tumors) are less aggressive tumors with longer survival; feature AC, LCNEC, or even SCLC; are usually diagnosed on resection specimens of male smokers; present with marked intertumor heterogeneity indicative of branching mechanisms of evolution; and comprise a variety of different molecular alterations even in common with conventional NSCLC (*TP53* and *RB1* mono-/biallelic inactivation, NOTCH inactivation, *KRAS/LKB1/MEN1* mutation, MYC family gene, *TERT*, *SDHA*, *RICTOR* amplification, and epithelial-mesenchymal transition). These tumors would originate from preexisting lesions (neuroendocrine cell hyperplasia/DIPNECH, neuroepithelial bodies, carcinoids, NSCLC) according to luminal-like mechanisms of sequential acquisition of gene alterations over time, with possibility of intermediate/dysplastic lesions. These tumors would originate from cancer stem cells within a neuroendocrine niche as carcinoids or their precursors or non-neuroendocrine cancer stem cells acquiring neuroendocrine differentiation as NSCLC. Ki-67 is typically heterogeneous within the tumor mass, ranging from 20–25% to 90% or more, with high staining areas intermingled with low staining areas. These tumors exhibit high mutation burden.
- Lastly, I-NETs (5% of lung NENs; 1% of lung tumors) are indolent behaving lesions with long-term survival, feature TC or low-mitotic count AC, are always diagnosed on resection specimens of female nonsmokers, occur in MEN1 or other inherited/familial syndromes, and comprise chromatin remodeling gene/epigenetic alteration mechanisms. These tumors are likely to derive from a neuroendocrine stem cell niche of preinvasive lesions (DIPNECH) through chromatin remodeling gene/epigenetic alteration mechanisms. Ki-67 is uniformly low, typically 10% or less, and these tumors exhibit low mutation burden.

The issue of malignancy progression or transition from low-grade to high-grade histology can be applied even to T-NENs, as previously suggested or more recently demonstrated by Dinter et al. who have identified three different T-NET clusters, independent of histology, for better patient stratification and prognostication, according to an integrated evaluation of scored CNI and immunohistochemistry findings (Ki-67, chromogranin A, and EZH2 staining) [209].

As morphology still remains the backbone of NEN classification but molecular profiling is getting increasingly relevant to clinics and Ki-67 plays an indubitable prognostic role, a morpho-molecular approach is useful in clinical practice by attributing relevance to Ki-67 in the decision-making process by increasing cutoff thresholds. We have recently investigated 16 primary carcinoids and 19 corresponding metastases, either synchronous or metachronous, for Ki-67 expression and

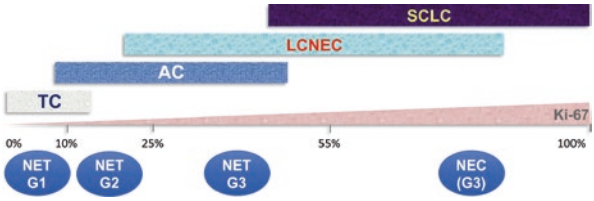

Fig. 9.16 The four different categories of thoracic neuroendocrine neoplasms itemized as NET G1, NET G2, NET G3, and NEC by including Ki-67 proliferation evaluation

morphological definition (TC vs. AC) according to different treatments [somatostatin analogue (SSA), mTOR inhibitor (mTOR-I, everolimus), and platinum and non-platinum chemotherapy)] [212]. Interestingly, survival curves of patients by different treatments paralleled the prediction of survival upon Ki-67 expression (cutoff thresholds 10% and 20%), while histology failed to a large extent, indicating that Ki-67 may have predictive value in lung NENs. By merging Ki-67 and histologic definition of lung NENs according to 2015 WHO classification, we proposed four different categories of tumors with different presentation and treatment options, itemized as NET G1, NET G2, NET G3, and NEC (tautologically G3). This proposal is outlined in Fig. 9.16.

- *Lung NET G1* include indolent behaving NENs with homogeneously low Ki-67 ≤10%, organoid pattern of growth featuring TC or low-mitotic count AC, and with treatment option in the metastatic setting of somatostatin analogues.
- *Lung NET G2* include low to moderate malignant NENs with slightly heterogeneous Ki-67 up to 25%, organoid pattern of growth featuring AC, some TC with "higher" Ki-67 or some carcinoid-like LCNEC, and with treatment options of SSA and/or mTOR-I and/or peptide receptor radionuclide therapy (PRRT) and/or non-platinum CT.
- *Lung NET G3* include moderate to higher malignant NENs with Ki-67 up to 55%; still organoid pattern of growth featuring some AC, carcinoid-like LCNEC, NSCLC-like LCNEC, and some SCLC; and with treatment options of non-platinum CT: alkylating agents, CAPTEM, and gemcitabine.
- *Lung NEC (G3)* include high malignant NENs with quite homogeneously distributed Ki-67 up to 100%; solid to diffuse pattern featuring SCLC, SCLC-like LCNEC, and some NSCLC-LCNEC; and with treatment options of platinum-based CT.

The 55% cutoff to separate, in the lung, NETs characterized by better prognosis but worse response rates to platinum from NECs characterized by worse prognosis but better response rates to platinum is the same as that applied in gastroenteropancreatic NENs to split the previous category of NEC into NET G3 (Ki-67 ranging from 20% to 55%) and NEC (Ki-67 > 55%) [213].

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Neuroendocrine Neoplasms of the Gut

10

Stefano La Rosa, Silvia Uccella, and Guido Rindi

Background

Neuroendocrine neoplasms of the gut are a heterogeneous group of tumors showing different morphological, clinical, prognostic, and molecular features. They were first defined using the term "carcinoid" coined by Siegfried Oberndorfer in 1907 [1]. However, since this term, appropriate in the context of the carcinoid syndrome, did not convey adequately the large clinicopathologic spectrum of this disease, the designation "neuroendocrine tumor" has been successively proposed [2]. Currently, these proliferations are more generally classified as "neuroendocrine neoplasms (NENs)," which include two main entities: well-differentiated neuroendocrine tumor (NET) and poorly differentiated neuroendocrine carcinoma (NEC) [3–6]. It is worth noting that a growing burden of evidence has been accumulating in the last years showing that NET and NEC, despite sharing neuroendocrine differentiation, are, in fact, two different diseases with different morphology, clinical presentation, outcome, and molecular background [5, 7, 8].

In general, digestive NECs show similar morphological and clinical features independently of their localization, while NETs present different clinicopathological characteristics related to their site of insurgence. NETs are graded with a

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Table 10.1 WHO classifications of digestive neuroendocrine neoplasms

	Morphological differentiation	Mitotic count/2 mm^2	Ki67 index
NET G1	Well-differentiated	<2	<3%
NET G2	Well-differentiated	2–20	3–20%
NET G3	Well-differentiated	>20	>20%
NEC	Poorly differentiated	>20	>20%
MiNENs	Well- or poorly differentiated	variable	variable

Modified from Klimstra et al. [6]
NET neuroendocrine tumor, *NEC* neuroendocrine carcinoma, *MiNEN* mixed neuroendocrine/non-neuroendocrine neoplasm

three-tiered system (NET G1, NET G2, NET G3) based on the proliferation assessment, using both mitotic count and Ki67 proliferation index. NECs, which show a poorly differentiated morphology, are high grade by definition (Table 10.1). In addition to pure NENs, mixed epithelial neoplasms composed of a neuroendocrine and a non-neuroendocrine component can be found in the digestive system. When each of the two components is malignant and reaches at least 30% of the tumor mass, these neoplasms are defined as mixed neuroendocrine-non neuroendocrine neoplasms (MiNENs) [6, 9]. The term MiNEN reflects a conceptual neoplastic category rather than a specific entity. Indeed, there are different MiNEN types along the digestive system, which show different morphology and prognosis mainly based on the clinicopathological characteristics of each component [9, 10].

While the global incidence of malignant neoplasms is more or less stable over the last 40 years, the incidence and prevalence of NENs increased importantly over the same period, probably reflecting the effectiveness of modern diagnostic tools with consequent frequent detection of early-stage cases. Due to the early detection and to the use of more effective personalized therapeutic approaches, survival of patients with NETs has been improving over time [11].

Neuroendocrine Neoplasms of the Esophagus

NENs of the esophagus account for about 0.04–1% of all gastroenteropancreatic NENs and include NETs, NECs, and MiNENs, with NETs being extremely rare [11–14].

Esophageal Neuroendocrine Tumor (NET)

Esophageal NETs are very rare and only described as case reports or small series [15]. Some confusion may arise since, over the years, they have been reported in the literature with different denominations, including either NET or carcinoid (both typical and atypical) [15]. However, in line with the current WHO terminology for

the digestive system, they are defined as NETs [5, 6]. They represent about 5% of esophageal NENs and do not show gender predilection, and the average age at diagnosis is 56.8 years [15].

Some patients are asymptomatic and NETs are incidentally discovered during endoscopy [16], while other patients can present unspecific symptoms such as dysphagia or abdominal discomfort [13, 15]. Very rare metastatic NETs associated with carcinoid syndrome have been described as well [12].

NETs present as polypoid, nodular elevated lesions with an overlying smooth surface generally <2 cm in size [13, 17].

Histologically, they show the typical morphological features of a well-differentiated NEN with insular or trabecular pattern of growth. Tumor cells have abundant cytoplasm and a small ovoid nucleus with dispersed chromatin containing small nucleoli. Variable amount of well-vascularized stroma is frequently present. Focal necrosis may be seen. Cells are immunoreactive for neuroendocrine markers (synaptophysin and chromogranin) and cytokeratins (CKs). In addition, some cases can be immunoreactive for vesicular monoamine transporter 2 (VMAT2), serotonin, (entero)glucagon, PP, gastrin, and calcitonin [18]. Grading information is rarely available, especially for cases reported in the older literature, but most cases can be retrospectively graded as G1 or G2.

Esophageal NETs seem to be associated with a favorable prognosis [17]. Tumor stage is the most important prognostic factor, although tumor grade has recently been proposed as a significant prognostic marker as well [13]. A specific treatment algorithm for esophageal NETs is not still standardized. Endoscopic resection has been proposed for NET measuring <1 cm in absence of locoregional lymph node metastases, while surgery, combined or not with adjuvant chemotherapy, remains the treatment of choice for advanced stages [13].

Esophageal Neuroendocrine Carcinoma (NEC)

In a recent 10-year population-based study, NECs represented 1.4% of esophageal tumors and about 90% of all esophageal NENs [19]. They are more frequent in males (male/female ratio of 6:1) in the sixth and seventh decades of life [11–14]. They can occur in any part of the esophagus, and cases arising in the lower part are often associated with Barrett's esophagus [20, 21]. Patients generally present unspecific symptoms related to tumor growth or metastatic dissemination such as dysphagia, pain, weight loss, and asthenia [12, 13]. NECs associated with syndrome of inappropriate secretion of antidiuretic hormone have been reported [22].

Macroscopically, NECs present as large ulcerated neoplasms resembling esophageal cancer [13, 16].

Microscopically, they can be divided into two subtypes: small cell and large cell NECs [3, 6]. Small cell NECs are composed of small cells with scant cytoplasm and hyperchromatic nuclei without nucleoli (Fig. 10.1a). Large cell NECs are composed of large cells with vesicular nuclei showing prominent nucleoli and abundant

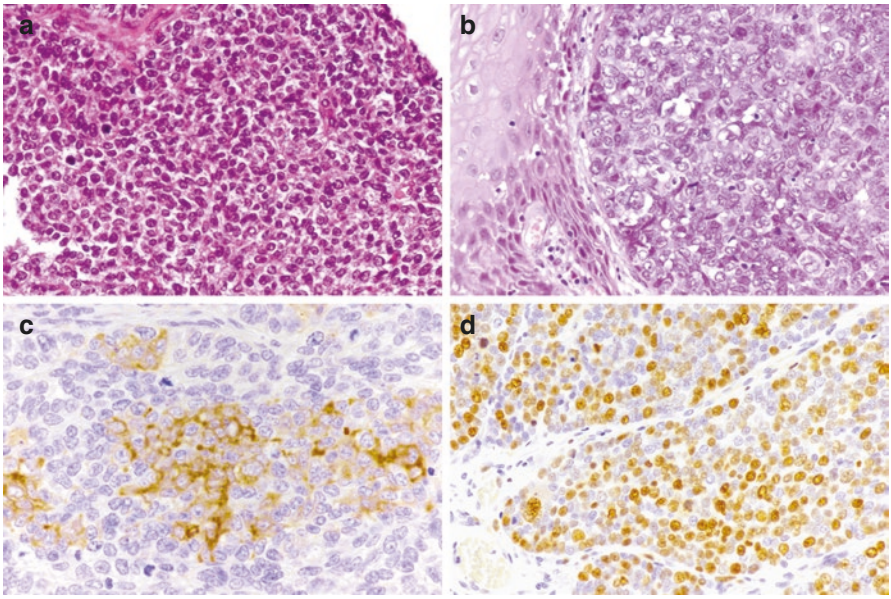

Fig. 10.1 Esophageal NECs: small cell NEC is composed of small cells with scant cytoplasm and hyperchromatic nuclei without nucleoli. In this figure some mitoses are found (**a**). Large cell NEC is composed of large cells with vesicular nuclei and abundant eosinophilic cytoplasm. Normal squamous epithelium is evident at the left (**b**). Tumor cells are positive for synaptophysin (**c**) and the Ki67 index is >20% (**d**)

eosinophilic cytoplasm (Fig. 10.1b). Necrosis and brisk mitotic activity are the rule in both subgroups. Tumor cells are positive for synaptophysin (Fig. 10.1c), while chromogranin A may be absent or focally expressed with a juxta-nuclear dot-like pattern of immunoreactivity [21] Ki67 index is >20% (Fig. 10.1d). TTF1 is positive in about 70% of cases, so this marker is not useful in the differential diagnosis with secondary localizations of pulmonary small cell NECs. Basaloid squamous cell carcinoma is another differential diagnosis, and immunohistochemistry represents a useful diagnostic tool since it is immunoreactive for p40 and CK5/CK6 that are generally negative in NECs [23]. It is worth noting that p63 can be positive in some esophageal NECs so it should not be used alone in the diagnostic workup [21].

Esophageal NECs frequently show mutations in *TP53* and *RB1* suppressor genes. Mutations of *NOTCH1*, *FAT1*, *FBXW7*, *PDE3A*, *PTPRM*, and *CBLN2* have also been reported [24].

Esophageal NECs are frequently metastatic at first diagnosis, and the prognosis is poor with a median overall survival time ranging from 8 to 15 months. As in other sites, there is no statistically significant different survival between small cell and large cell NECs [20].

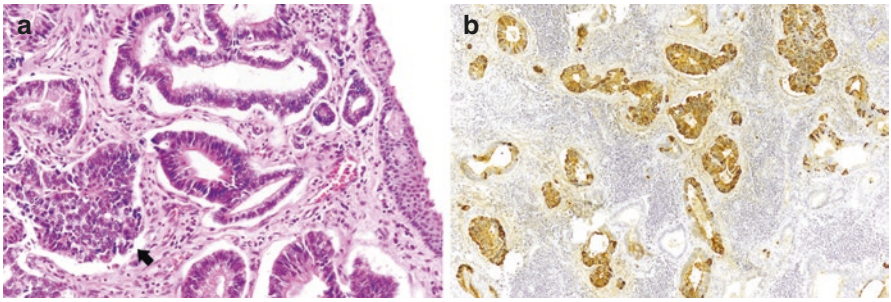

Fig. 10.2 This example of esophageal MiNEN is composed of a glandular component and of a solid component characterized by large cells (arrow). In the right part of the image, the superficial squamous epithelium is well evident (**a**). The solid component is strongly positive for chromogranin, which also labels several cells in the glandular structures. Both components are present in the lymph node metastasis confirming their malignant nature (**b**)

Esophageal Mixed Neuroendocrine-Non-neuroendocrine Neoplasm (MiNEN)

Not infrequently, esophageal NECs can be associated with a non-neuroendocrine component. When each component represents at least 30% of the tumor mass, the neoplasm is defined as MiNEN. Cases composed of adenocarcinoma and NEC can be defined mixed adenoneuroendocrine carcinomas (MANECs) [3, 6]. Extremely rare MiNENs composed of NET and adenocarcinoma have also been reported [17].

Due to their rarity, there are not definitive epidemiological data on this entity. In a recent revision of the literature, they represented 12% of all esophageal NENs [15].

Macroscopically they resemble esophageal NECs. Histologically, the NEC component can be associated with either a squamous cell carcinoma or an adenocarcinoma (Fig. 10.2). In the latter case, neoplasms are generally located in the distal part and are associated with Barrett's esophagus [14, 25].

TP53 mutation, *RB1* deletion or LOH, and *PIK3CA, PTEN, KRAS, SOX2, DVL3, and TP63* amplification have been found in both neuroendocrine and non-neuroendocrine components of esophageal MANECs suggesting their monoclonal origin from a common precursor stem cell [26].

MiNENs seem to have a better prognosis than pure NECs with a median survival time of about 20 months [20]. As for other MiNENs, the Ki67 proliferation index of the NEC component seems to have a prognostic impact [27].

Neuroendocrine Neoplasms of the Stomach

Gastric NENs encompass different categories of neoplastic proliferations showing a spectrum of pathological and clinical features and include both NETs and NECs [28]. Their incidence has increased 15-fold in recent years [11], probably reflecting

the increasing use of endoscopy as diagnostic approach of dyspeptic symptoms. NENs of the stomach represent about 4% of all digestive NENs [29], with a female prevalence and an average age at diagnosis of 64 years. Their annual incidence has been estimated to be 0.4 cases/100,000 persons in both the USA and Europe [11, 28], and survival has progressively improved over the last years [11, 30].

Gastric Neuroendocrine Tumors (NETs)

Several different neuroendocrine cell types are present in the stomach, and they are differently distributed along the gastric mucosa: gastrin-producing G cells and somatostatin-producing D cells are mainly located in the antral mucosa, while histamine-producing ECL cells and ghrelin-producing cells are found in the oxyntic mucosa. Serotonin-producing EC cells are distributed in both the antral and oxyntic mucosa. However, despite these several different cell types, almost all gastric NETs are composed of ECL cells and are located in the corpus-fundus mucosa. Gastric G-cell and D-cell tumors are rare and EC-cell NETs are extremely rare, especially when compared with those observed in the ileum or appendix.

ECL-Cell NETs

ECL-cell NETs, although composed of histamine-producing cells, are a heterogeneous group of neoplastic proliferations, ranging from small, indolent NETs to more aggressive neoplasms, showing different clinical and prognostic features depending on the patient's clinicopathological background (Table 10.2). Based on the morphology of the peritumoral mucosa, gastrin serum levels, presence or absence of antral G-cell hyperplasia, and presence or absence of MEN1 syndrome, Rindi et al. described three different subtypes of gastric ECL-cell NETs: type 1, type 2, and type 3 [31]. Since then, several studies have confirmed that this classification is per se strongly correlated with patient's outcome [32–34]. More recently, a fourth group of ECL-cell NETs which do not fit in one of the previously described categories has been described, and the term type 4 ECL-cell NET has been proposed [28]. In addition to this clinicopathological approach, all gastric NETs are also graded, in analogy with all other digestive NETs [6], according to Ki67 proliferative index and mitotic count, in turn well correlated with prognosis [33, 34]. However, the best prognostic stratification of patients is obtained by combining the Rindi's clinicopathological classification with WHO tumor grade [35].

Morphologically, all ECL-cell NETs show overlapping features and are positive for synaptophysin, chromogranin A, VMAT2, histidine decarboxylase (HDC), and somatostatin receptor 2A (SST_2). Scattered cells immunoreactive for serotonin, ghrelin, somatostatin, and αhCG can also be found. For this reason, the correct subtyping of ECL-cell NETs is achieved by evaluating the peritumoral gastric mucosa and considering the clinical context.

Table 10.2 Clinicopathological features of gastric ECL-cell NETs

	M/F ratio	%	Hypergastrinemia	Acid secretion	Peritumoral mucosa	ECL-cell proliferations	Grading	Metastasis	5-year survival
Type 1	1:2.5	80–90%	Yes	Low or absent	Atrophic gastritis	Yes	-G1 -G2, rare -G3, exceptional	1–3%	about 100%
Type 2	1:1	5–7%	Yes	High	Hypertrophic gastropathy	Yes	-G1 -G2, rare	10–30%	60–90%
Type 3	2.8:1	10–15%	No	Normal	No specific change	No	-G1, rare -G2 -G3, rare	50%	<50%
Type 4	Unknown	Rare	Yes	Low or absent	Parietal cell hypertrophy	Yes	Unknown	Unknown	Unknown

Modified from La Rosa et al. [41]

Type 1 ECL-Cell NET

Type 1 ECL-cell NET is the most common subtype, representing about 80–90% of ECL-cell NETs, and it is more frequently observed in females. It arises in a background of autoimmune chronic atrophic gastritis associated with reduction/lack of acid secretion and consequent antral G-cell hyperplasia and hypergastrinemia. Patients generally have autoantibodies directed against intrinsic factor and/or parietal cells. Due to the impaired absorption of vitamin B12 caused by the lack of availability of intrinsic factor, a subgroup of patients may also present macrocytic anemia.

Tumors are frequently multiple, small (<1 cm), and located in the corpus-fundus. In most of cases, they are limited to the mucosa or submucosa. Only larger tumors (>1 cm) may infiltrate the muscularis propria or beyond. They are composed of well-differentiated cells with monomorphic nuclei, inconspicuous nucleoli, and fairly abundant eosinophilic cytoplasm, arranged in small microlobular and/or trabecular structures (Fig. 10.3). Mitotic activity and necrosis are almost always absent. Most cases are G1, but G2 and exceptional G3 cases have been described [34]. Since the morphological features of type 1 ECL-cell NETs strongly overlap those of type 2 and type 3 ECL-cell NETs, the status of the peritumoral mucosa is a key diagnostic feature. In type 1 tumors, the oxyntic mucosa is atrophic and shows different types of ECL-cell proliferations (Fig. 10.4), including ECL-cell hyperplasia and dysplasia [36]. Only severe ECL-cell hyperplasia and dysplasia (in particular microinvasive lesions) are associated with an increased risk of tumor development [37]. In the antral mucosa, the presence of G-cell hyperplasia is the rule.

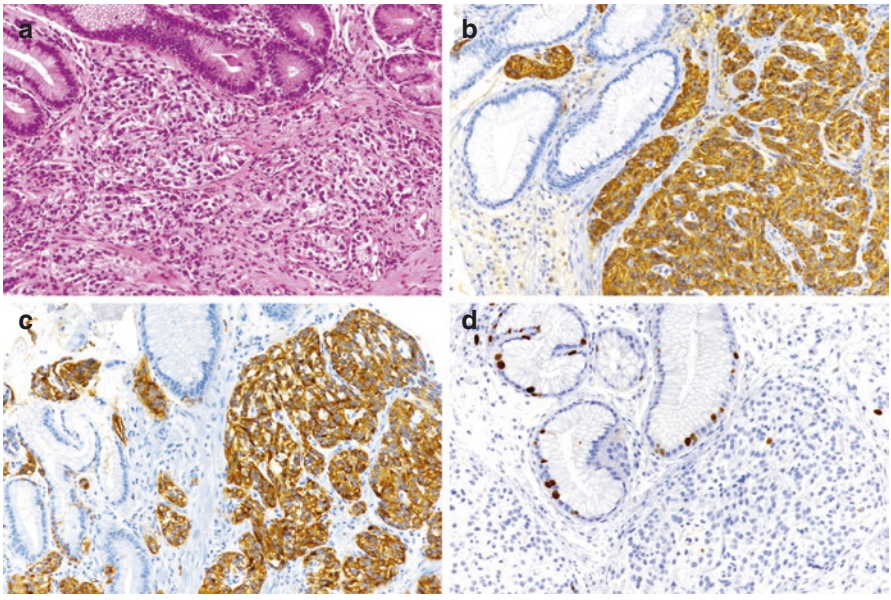

Fig. 10.3 Type 1 ECL-cell NET is composed of well-differentiated cells with monomorphic nuclei, inconspicuous nucleoli, and fairly abundant eosinophilic cytoplasm, arranged in small microlobular and/or trabecular structures. Mitotic activity and necrosis are lacking (**a**). Tumor cells are strongly positive for chromogranin (**b**) and VMAT2 (**c**). The Ki67 index is <3% (**d**)

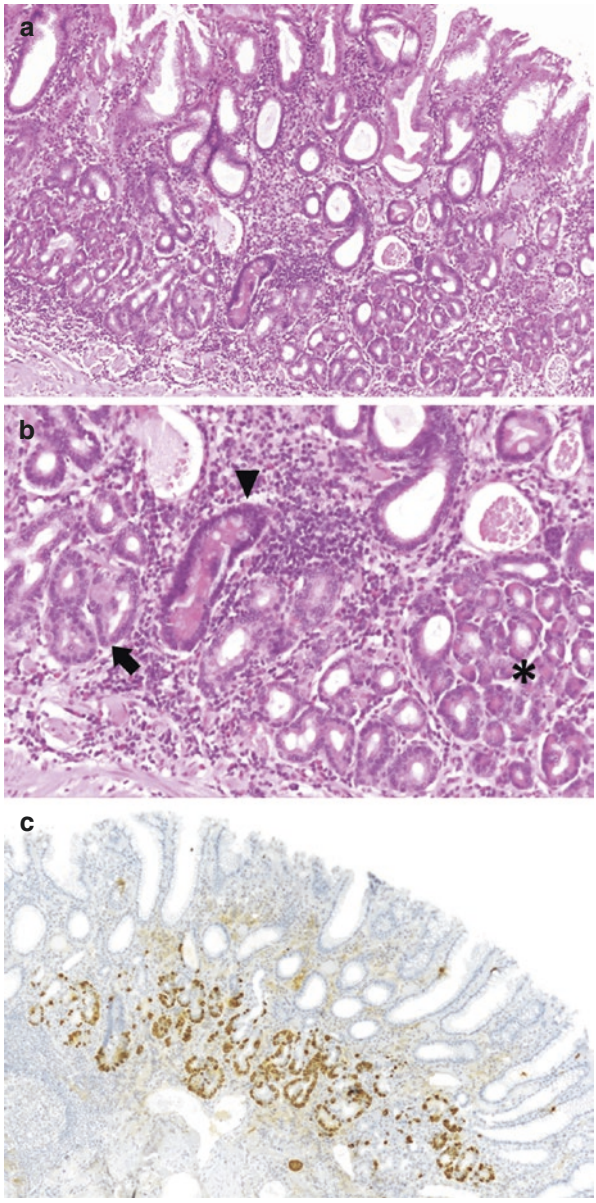

Fig. 10.4 Since the morphological features of type 1 ECL-cell NETs strongly overlap those of type 2 and type 3 ECL-cell NETs, the status of the peritumoral mucosa is a key diagnostic feature. In patients with type 1 ECL-cell NET, the oxyntic mucosa is atrophic without oxyntic gland (**a**), which are substituted by pseudopyloric (**b**, arrow), intestinal (**b**, arrowhead), and pancreatic acinar (**b**, asterisk) metaplasia. In addition, different types of ECL-cell proliferations including ECL-cell linear and micronodular hyperplasia can be identified using chromogranin immunostaining (**c**)

The pathogenesis of type 1 ECL-cell NETs is related to ECL-cell stimulus by gastrin, which acts in cooperation with other growth factors such as TGFα and bFGF [28]. Although alteration of *MEN1* gene is a feature of type 2 ECL-cell NETs, loss of heterozygosity (LOH) for the *MEN1* gene locus has also been identified in 17–73% of type 1 ECL-cell NETs [38].

Patients with type 1 ECL-cell NETs have an excellent prognosis with a 10-year survival rate of more than 90%. Interestingly, among type 1 ECL-cell NETs, no significant difference was found between G1 and G2 cases in terms of gastric

wall invasion, metastases, or patient outcome, suggesting that tumor grade may not be the most important prognostic factor in this subgroup, except for grade 3 cases (Ki67 > 20%). Conversely, the risk for lymph node metastasis correlates with tumor size and infiltration of the muscularis propria [34]. Based on such evidences, a conservative approach is indicated for type 1 ECL-cell NETs of <1 cm in size, while tumor excision, either endoscopic or surgical, should be performed for G1–G2 cases >1 cm in size and infiltrating the muscularis propria and beyond or for G3 cases.

Type 2 ECL-Cell NET

Type 2 ECL-cell NETs account for about 5–7% of ECL-cell NETs and are observed in patients with MEN1 syndrome, without gender predilection.

Tumors are generally multiple, measure <2 cm, and arise in thickened oxyntic mucosa in the context of severe hypertrophic-hypersecretory gastropathy (Fig. 10.5) due to gastric stimulation in presence of high gastrin serum levels. A range of ECL-cell proliferations, including hyperplasia and dysplasia, are observed in the hypertrophic peritumoral mucosa.

While in type 1 ECL-cell NET hypergastrinemia is due to compensatory G-cell hyperplasia, in patients with type 2 ECL-cell NET, it is caused by gastrin hypersecretion by a duodenal or, more rarely, a pancreatic gastrinoma [28]. Interestingly, type 2 ECL-cell NETs are only observed in patients with gastrinoma in the setting of MEN1 syndrome, while patients with sporadic gastrinomas do not develop ECL-cell NETs. This suggests that genetic changes present in MEN1 patients render ECL cells more sensitive to the proliferative effect of gastrin [39, 40].

Type 3 NET

Traditionally, gastric NENs of the oxyntic mucosa and not associated with either chronic atrophic gastritis or gastrinoma have been designed as type 3 ECL-cell NETs. However, since histamine production or typical "ECL-type" secretory granules have not been demonstrated in all cases, the designation *ECL cell* has been

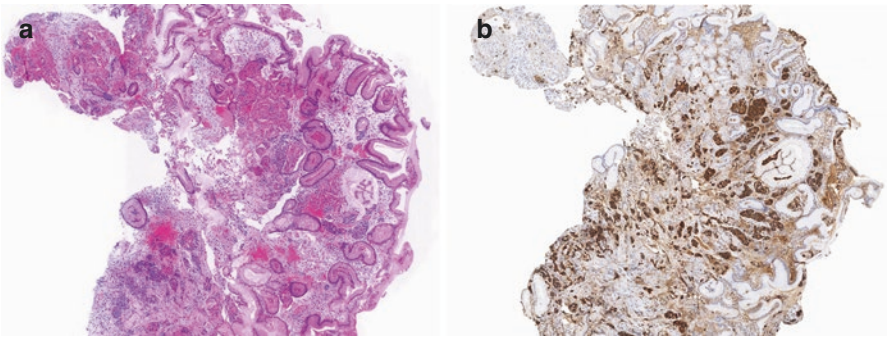

Fig. 10.5 Biopsy of a gastric nodule in a patient with duodenal gastrinoma and MEN1 syndrome. This type 2 ECL-cell NET is composed of well-differentiated cells arranged in small nest and trabecular structures. The gastric mucosa is hyperplastic and lacks atrophy associated with intestinal and pseudopyloric metaplasia (**a**). Tumor cells are strongly immunoreactive for chromogranin (**b**)

removed to identify this specific category of tumors [41]. Type 3 NETs account for about 10–15% of gastric NENs and are more frequent in males than in females. Patients do not show hypergastrinemia, but they may present symptoms related to tumor growth or metastatic dissemination, such as gastric bleeding, abdominal pain, and weight loss. Rarely, patients with extensive metastatic dissemination may present the so-called atypical carcinoid syndrome including cutaneous flushing, facial edema, lacrimation, headache, and bronchoconstriction [29].

Type 3 NETs are usually solitary and large lesions resembling gastric cancer. Composed of well-differentiated cells with neuroendocrine morphology, they frequently infiltrate the muscularis propria and the subserosa (Fig. 10.6). Lymph node and distant metastases are not rare. Most cases are G1 or G2, but G3 NETs have been described as well [33]. Tumor cells are positive for general neuroendocrine markers and SST_2, while HDC may be lost, especially in high-grade cases. Since morphological features overlap those of type 1 and type 2 ECL-cell NETs, the diagnosis on small biopsy may be a challenge and is achieved by evaluating peritumoral mucosa that is general normal or with minimal gastritis.

The pathogenesis of this specific subtype is unknown. LOH of the *MEN1* locus has been observed in 25–50% of cases.

The prognosis of type 3 NETs is worse than that of both type 1 and 2 and depends on both grade and stage with a 10-year disease-specific survival of about 50% [33, 34].

Type 4 ECL-Cell NET

Rare cases of ECL-cell NETs associated with hypergastrinemia, achlorhydria, and parietal cell hyperplasia, but without gastrinoma and MEN1 syndrome, have been described [42] and represent a peculiar entity for which the term type 4 ECL-cell NETs has been proposed [28]. They are multiple and present the same morphological features of type 1 and 2 ECL-cell NETs. However, peritumoral mucosa shows dilated oxyntic glands lined by parietal cells with abundant eosinophilic cytoplasm which can also be vacuolated. Inspissated secretory material is frequently observed in the gland lumens (Fig. 10.7). ECL-cell proliferations are observed as well [42, 43].

The pathogenesis of this subtype seems to be related to an intrinsic inappropriate acid secretion from parietal cells, consequent to a defect/lack of proton pump

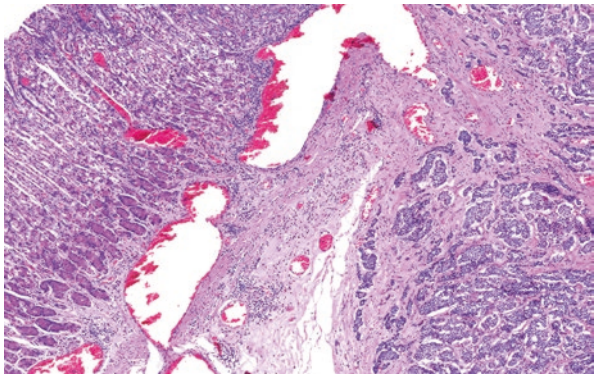

Fig. 10.6 Type 3 NETs are composed of well-differentiated neuroendocrine cells that deeply infiltrate the gastric wall. The peritumoral mucosa is normal (left)

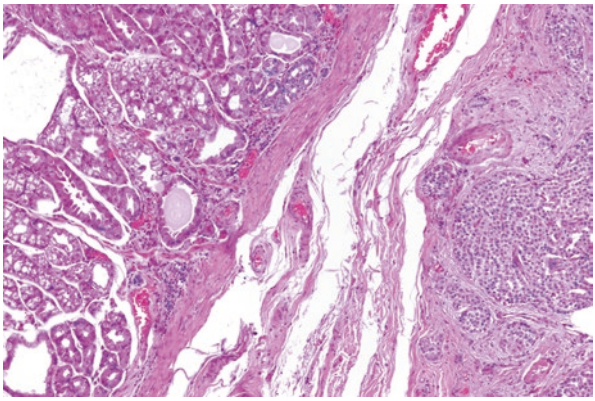

Fig. 10.7 Type 4 ECL-cell NET is composed of well-differentiated cells (right) and shows the same morphological features of type 1 and 2 ECL-cell NETs. However, the peritumoral mucosa (left) shows dilated oxyntic glands lined by parietal cells with abundant eosinophilic cytoplasm frequently vacuolated. Inspissated secretory material can be observed in some gland lumens

function probably determined by an inactivating mutation of the *ATP4A* gene encoding the α-subunit of gastric proton pump [42, 44]. The reduction/lack of acid secretion leads to antral G-cell hyperplasia and hypergastrinemia.

The prognosis is not well defined due to the small number of reported cases, but lymph node metastases have been found in one case [42].

G-Cell and D-Cell NETs

G-cell and D-cell NETs are typically located in the antral mucosa and together represent about 5% of all gastric NENs. They are generally nonfunctioning, though rare cases of G-cell NETs are associated with the Zollinger-Ellison syndrome (ZES) [33]. In this specific clinical context, the term "gastric gastrinoma" is accepted.

Gastrin-producing G-cell NETs are generally small and located in proximity of the pylorus. They are composed of well-differentiated neuroendocrine cells forming thin trabeculae or gyriform structures. They are generally confined to the mucosa or submucosa, while the deep infiltration of the gastric wall is rarer. At immunohistochemistry, neoplastic cells are positive for neuroendocrine markers, SST_2 and gastrin.

Somatostatin-producing D-cell NETs are extremely rare and often incidental. They are composed of well-differentiated monomorphic cells positive for chromogranin A, synaptophysin, and somatostatin.

In general, antral NETs are indolent with excellent prognosis, even when they show muscular infiltration or lymph node metastasis [28, 33].

EC-Cell NET

EC-cell NETs of the stomach are very rare. They are generally nonfunctioning, though they may rarely be associated with the classical carcinoid syndrome. EC-cell NETs can arise in any part of the stomach and are composed of well-differentiated cells with intense eosinophilic cytoplasm forming nests with a peripheral palisading, similarly to the more frequent ileal EC-cell counterparts. Tumor cells are positive for general neuroendocrine markers, serotonin, CDX2, and SST_2 (Fig. 10.8).

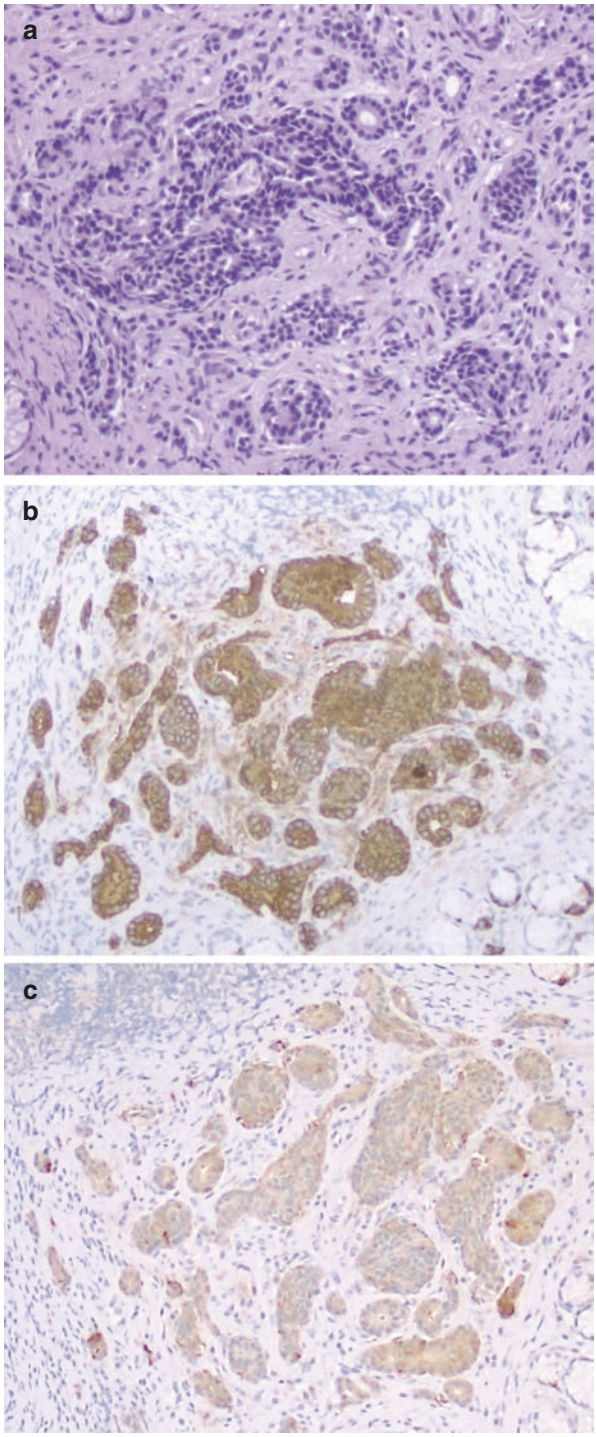

Fig. 10.8 Gastric EC-cell NET is composed of well-differentiated cells forming nests (**a**). Tumor cells are positive for chromogranin (**b**) and serotonin (**c**)

Gastric Neuroendocrine Carcinoma (NEC)

Gastric NECs are aggressive cancers accounting for about 6–21% of gastric NENs and usually arise in the antral or cardial regions. Males are more frequently affected (male/female ratio of 2:1), and the average age at diagnosis is 65 years (range 41–76 years) [28]. Patients generally present nonspecific symptoms including dyspepsia, abdominal pain, gastric bleeding, and weight loss, which are due to tumor growth and/or distant metastases. Primary gastric Merkel cell carcinoma, although extremely rare, can be included in the group of gastric NECs [45].

Morphologically, gastric NECs are divided into small cell and large cell subtypes: small cell NECs are composed of small cells with scant cytoplasm and hyperchromatic nuclei without nucleoli (Fig. 10.9a), while large cell NECs are composed of large cells with vesicular nuclei showing prominent nucleoli and abundant eosinophilic cytoplasm (Fig. 10.9b). Tumor cells are positive for synaptophysin, while chromogranin A may be absent or focally expressed with a juxta-nuclear dot-like pattern of immunoreactivity. NECs may also be positive for TTF1 and CDX2. Immunoreactivity for Merkel cell polyomavirus (MCPyV) has been reported in a primary gastric Merkel cell carcinoma [45]. Mitotic count and Ki67 proliferative index are high (>20 mitoses × 2mm^2 and Ki67 index >20%).

Gastric NECs show multiple chromosomal abnormalities involving cell-cycle regulatory genes such *TP53*, *RB1*, *FHIT*, *DCC*, and *SMAD4* and mutations of *SMAD4*, *PIK3CA*, *KRAS*, TP53, and *RB1* genes [46]. *TP53* and *RB1* mutations can help in the differential diagnosis with NET G3, where they are more frequently wild type [7].

Patients with gastric NEC have a dismal prognosis with survival usually measured in months.

Gastric Mixed Neuroendocrine-Non-neuroendocrine Neoplasm (MiNEN)

MiNENs are defined as epithelial malignancies composed of a neuroendocrine and a non-neuroendocrine component, each representing at least 30% of the tumor burden

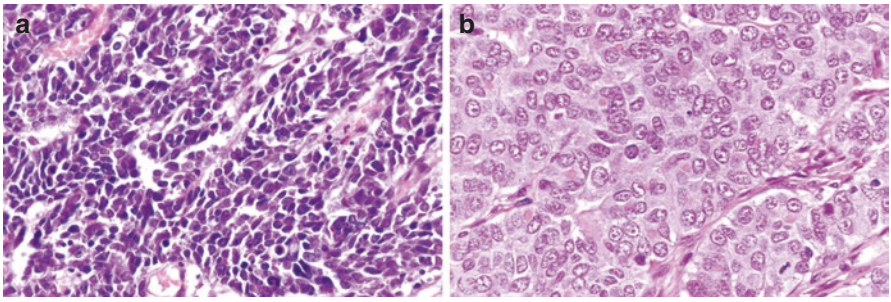

Fig. 10.9 Gastric NECs: small cell NECs are composed of small cells with scant cytoplasm and hyperchromatic nuclei without nucleoli (**a**), while large cell NECs are composed of large cells with vesicular nuclei showing prominent nucleoli and abundant eosinophilic cytoplasm (**b**)

(Fig. 10.10) [9]. The former component is more frequently represented by a NEC (small or large cell subtypes) and only rarely by a NET. The non-neuroendocrine component is generally represented by an adenocarcinoma or, rarely, by a squamous cell carcinoma, especially in cases located in the cardial region. In addition, rare cases of adenoma associated with NET have been described and defined as MANETs [47]. However, since both components of MiNENs are by definition malignant, MANETs do not fall into the MiNEN category [6].

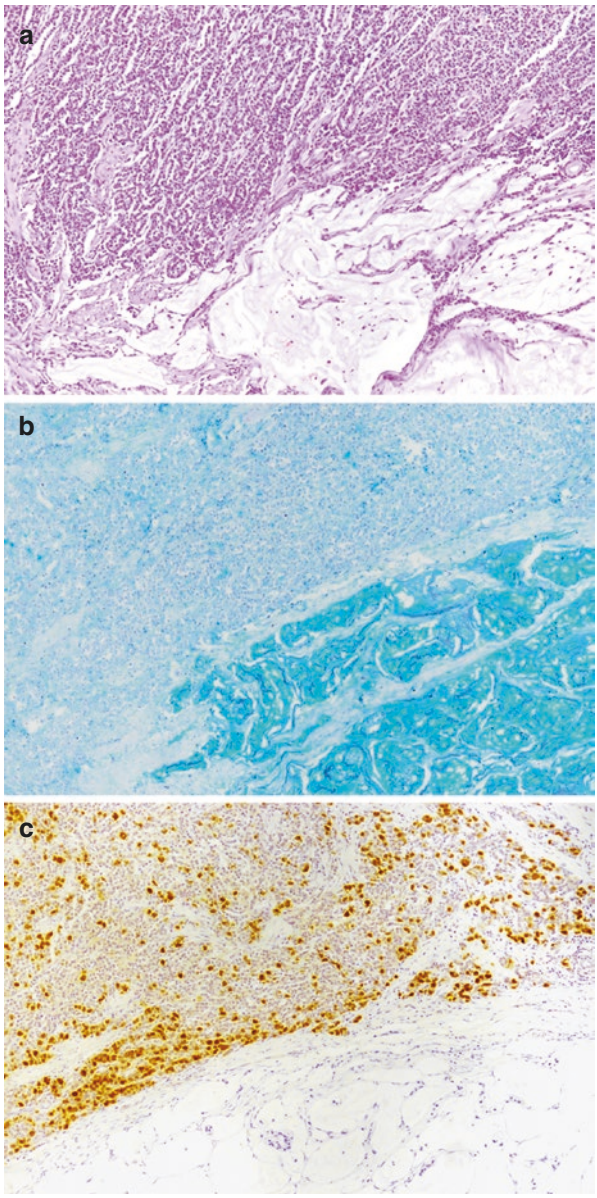

Fig. 10.10 Gastric MiNEN composed of mucinous adenocarcinoma (**a**, bottom) and large cell NEC (**a**, upper). The mucinous component is Alcian blue-positive (**b**). The NEC component of this case is positive for serotonin (**c**)

Epidemiological information on MiNENs composed by adenocarcinoma and NET is lacking due to their rarity. MiNENs composed of adenocarcinoma and NEC (for which the term MANEC is still acceptable) account for about 20% of all digestive MiNENs [27].

Macroscopically, MiNENs may resemble a conventional gastric cancer presenting as polypoid, ulcerating or stenotic large lesion. The morphological features depend on the type of each component. Molecular data suggest a monoclonal origin of the two components of MANECs [48–51].

Gastric MANECs are aggressive and have a poor prognosis, and the Ki67 proliferative index of the NEC component correlates with prognosis [27].

Neuroendocrine Neoplasms of the Duodenum

Several different neuroendocrine cell types are present in the duodenal and ampullary mucosa, and this reflects the spectrum of NEN types that can be found in this organ (Table 10.3). Indeed, duodenal NENs include a heterogeneous spectrum of diseases, with peculiar morphological features, immunohistochemical profile,

Table 10.3 Clinicopathological features of duodenal NENs

	Gastrinoma	NF-NET	Som-NET	GP	NEC	MiNEN[a]
Gender	M = F	M > F	F = M	M > F	M > F	M > F
Mean age (years)	39	64	50	54	66	60
Site						
I portion	79%	76%	3%	0	Rare	Rare
II portion	16%	10%	3%	25%	4%	Rare
Ampulla	5%	11%	94%	67%	85%	>90%
II/IV portion	0	3%	0	8%	11%	Rare
Mean size (cm)	0.75	0.8	2.5	2.5	2.5	Rare
Grade						
G1	85%	82%	65%	92%		
G2	15%	18%	35%	8%		
Vascular invasion	44%	23%	76%	8%	95%	Frequent
LN metastases	50%	18%	54%	25%	79%	32%
Distant metastases	10%	8%	8%	0	52%	Na
Possible associated syndromes	MEN1	MEN1	NF1, MEN1, VHL, Pacak-Zhuang	NF1	None	None
DOD (5 years)	10%	4%	5%	8%	82%	>90%

NF-NET nonfunctioning NET, *Som-NET* somatostatin-producing NET, *GP* gangliocytic paraganglioma, *LN* lymph node, *NF1* neurofibromatosis type 1, *VHL* von Hippel-Lindau, *na* not available, *DOD* died of disease

[a]Data regard MANEC

and distribution. Four main NEN types are characterized and include gastrinoma, somatostatin-producing D-cell NET, nonfunctioning NET, and gangliocytic paraganglioma [18, 52]. Globally, duodenal NENs account for about 6–8% of digestive NENs and their incidence has been increasing in the last decades [53]. NETs of the upper jejunum show overlapping clinicopathological features with duodenal NETs [54].

Duodenal Neuroendocrine Tumors (NETs)

Duodenal NETs include gastrinomas, nonfunctioning somatostatin-producing D-cell NETs, and nonfunctioning NETs [52]. In addition, very rare duodenal insulinomas have been reported [55].

Duodenal Gastrinoma

These gastrin-producing NETs are associated with ZES. They can be either sporadic or associated with the MEN1 syndrome and tend to be diagnosed in younger patients (average age at diagnosis 39 years) than nonfunctioning NETs [52, 56]. They are located in any part of the duodenum, except for the ampullary region. In the setting of MEN1 syndrome, gastrinomas tend to be multiple and smaller than in sporadic cases [57, 58].

Morphologically, gastrinomas are composed of well-differentiated uniform cells with scant eosinophilic cytoplasm forming trabecular-gyriform structures. Tumor cells are positive for neuroendocrine markers, SST_2, gastrin, and CDX2 (Fig. 10.11). They frequently show proliferative grade 1 [52]. Cases developing in the setting of MEN1 syndrome show G-cell hyperplasia in the adjacent mucosa or Brunner glands, a feature not observed in sporadic gastrinomas [58].

Lymph node and distant metastases can be present in about 50% and 10% of cases, respectively; 1 cm seems the best cutoff size to identify gastrinomas with higher risk of lymph node metastases [52]. In a recent series, 10% of patients with duodenal gastrinomas died of disease, but, in general, patients have a good survival with a 10-year disease-specific survival of about 90% [52]. Lymph node metastases seem to have little influence on prognosis [59, 60].

Somatostatin-Producing D-Cell NETs

This tumor type is typically located into the major or minor ampulla [52, 61] being virtually absent in the other portions of the duodenal mucosa. There is not gender predilection, and it is more frequently diagnosed during the fourth or fifth decade of life. Most of the cases are sporadic, but some can arise in the setting of neurofibromatosis type 1 syndrome [52, 62] sometimes in association with duodenal GISTs [63], MEN1, or von Hippel-Lindau syndrome [62]. Somatostatin-producing D-cell NETs are virtually always clinically nonfunctioning, although they can present with obstructive biliary disease, due to their specific site of insurgence.

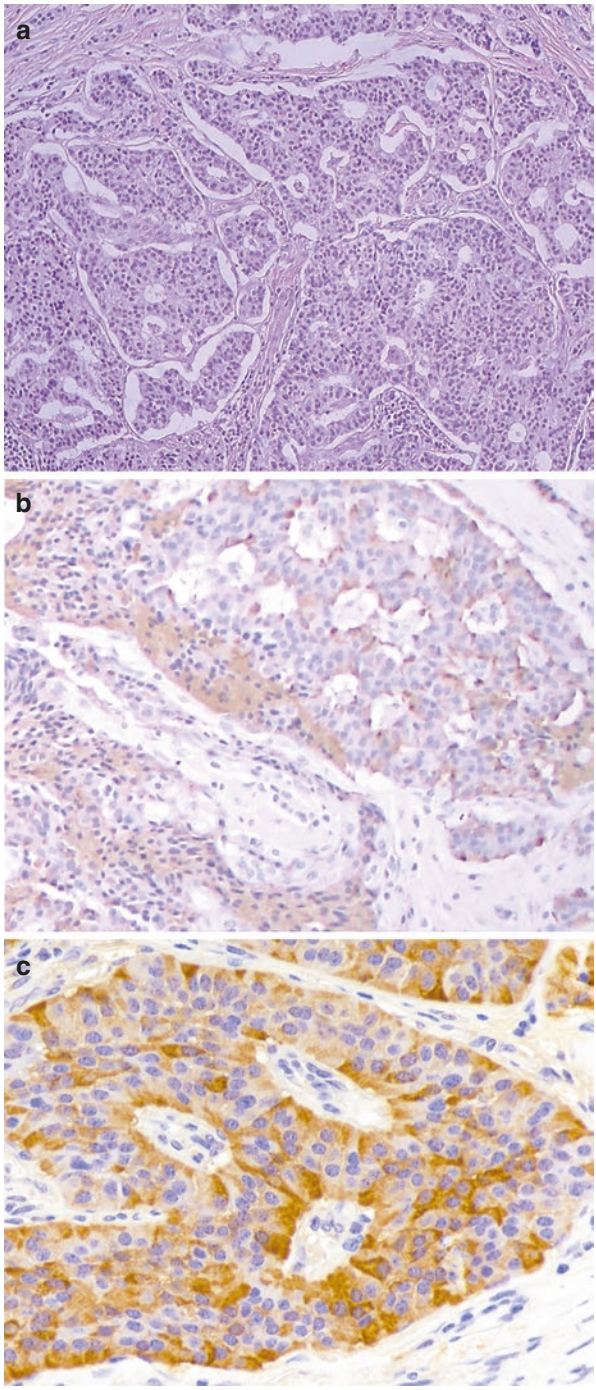

Fig. 10.11 Duodenal gastrinoma is composed of well-differentiated uniform cells forming trabecular-gyriform structures (**a**). Tumor cells are positive for chromogranin (**b**) and gastrin (**c**)

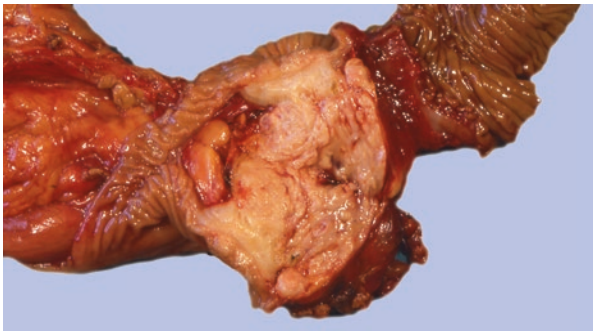

Fig. 10.12 Macroscopic appearance of an ampullary somatostatin-producing D-cell NET, which infiltrates the duodenal wall and the pancreatic head. The pylorus and antrum are on the left. (Reprinted with permission from Piccin Nuova Libraria S.p.a. From: Riva et al. [119], Fig. 29 p 1403)

Somatostatin-producing D-cell NETs are usually large neoplasms with an average size of 2 cm (Fig. 10.12). Histologically, they show a peculiar predominant tubulo-acinar structure with or without intraluminal psammoma bodies (Fig. 10.13a). Minor trabecular or solid areas can also be observed. Tumor cells are large with abundant granular eosinophilic cytoplasm and frequently infiltrate the muscular layer and vessels. Tumor cells are positive for general neuroendocrine markers and somatostatin (Fig. 10.13b, c), CK7, and MUC1. In addition, a minority of cells may be positive for PP, VIP, and gastrin. Usually SST_2 is negative, while SST_5 is widely expressed [52, 61]. The peculiar histological features and immunophenotype may represent a diagnostic challenge, especially on biopsy specimens, and this neoplasm needs to be distinguished from well-differentiated adenocarcinoma. Somatostatin-producing D-cell NETs are generally G1 or G2 and frequently associate with lymph node metastases, especially in cases with a diameter ≥2 cm. Disease-related death has been reported in about 8% of patients after a mean follow-up time of 108 months [52].

Nonfunctioning NETs

Duodenal NETs located out of the ampulla, void of tubulo-acinar morphology and somatostatin immunoreactivity, and in absence of ZES are the majority of duodenal NETs. They are clinically nonfunctioning and arise in every duodenal part, except for the ampullary region. Although tumors can be positive for different hormones including serotonin, PP, and somatostatin, most of the cases are widely positive for gastrin [52]. Etiological factors are almost unknown, but long history of *Helicobacter pylori*-associated gastritis or long-term use of proton pump inhibitors was suggested to be associated with increased risk of developing nonfunctioning G-cell NETs [64]. Nonfunctioning G-cell neoplasms are more frequently located in the duodenal bulb and are often incidentally found during endoscopy or in surgical

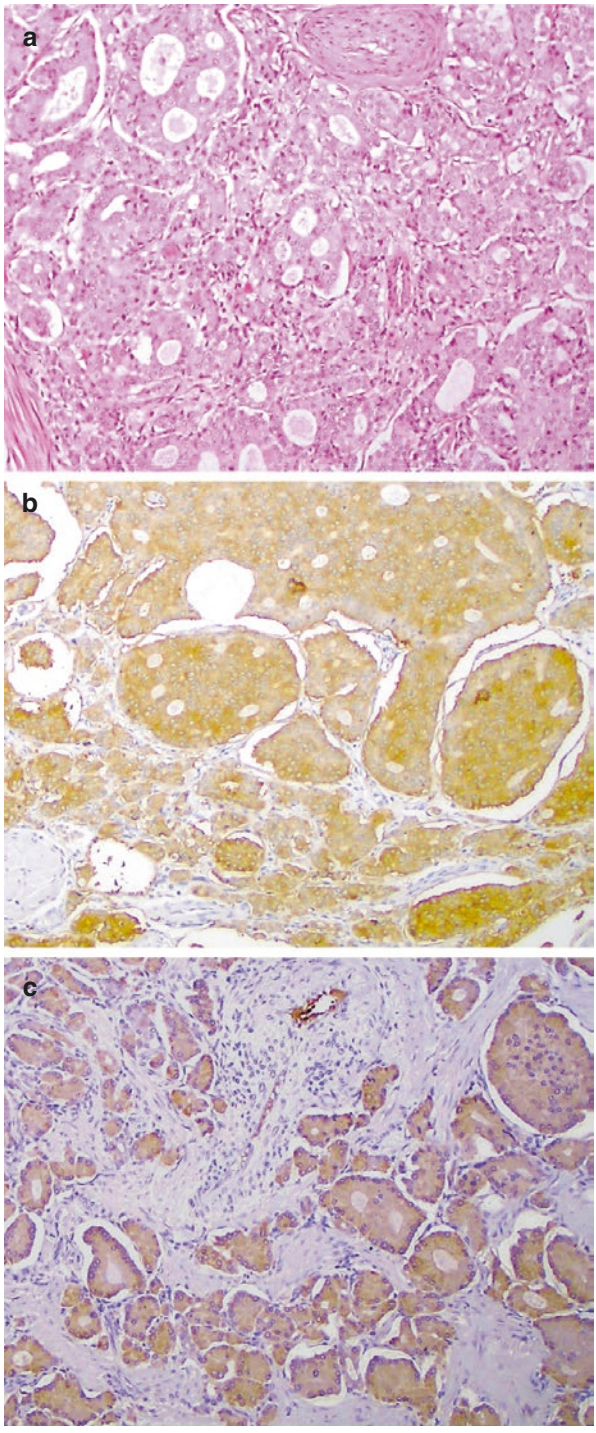

Fig. 10.13 Ampullary D-cell NET shows a peculiar predominant tubulo-acinar structure (type C according to Soga and Tazawa). Tumor cells are large with abundant granular eosinophilic cytoplasm (**a**). They are immunoreactive for synaptophysin (**b**) and somatostatin (**c**)

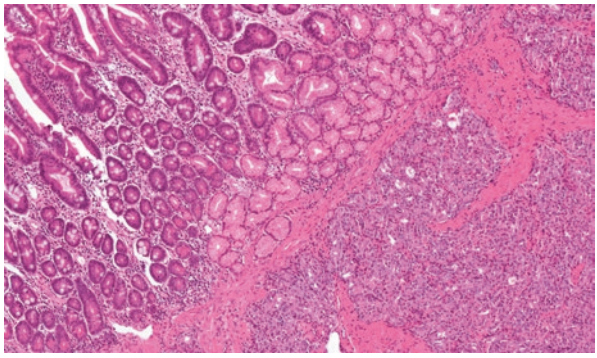

Fig. 10.14 Nonfunctioning NET of the duodenum composed of well-differentiated cells forming pseudoglandular and trabecular structures. This tumor showed some gastrin-immunoreactive cells, although it was not associated with Zollinger-Ellison syndrome

specimens resected for other diseases. They are generally small (mean size 0.8 cm) polypoid lesions, composed of well-differentiated cells forming trabecular-gyriform structures (Fig. 10.14). Infiltration beyond the submucosa is not frequent. Most of the cases of G1 or G2 and lymph node metastases are more frequently observed in cases measuring >0.9 cm in size. Disease-specific death has been observed in about 5% of patients [52].

Gangliocytic Paraganglioma

Gangliocytic paraganglioma (GP) is a rare low-grade neoplasm accounting for about 6% of duodenal NENs [52]. Although traditionally considered as a benign tumor, cases with lymph node and/or distant metastases have been reported as well [65–67]. Males are more frequently affected (male/female ratio of 1.5:1) and the mean age at diagnosis is 54 years. Patients do not present endocrine syndromes, but nonspecific symptoms related to tumor growth such as gastrointestinal bleeding associated or not with anemia, abdominal pain, nausea, weight loss, and jaundice. In about 10% of cases, they are incidentally discovered during endoscopy [68].

Macroscopically, GPs are large (mean size 2.5 cm) and typically located into the major or minor ampulla [52, 68].

Histologically, they present an admixture of epithelial, spindle-shaped, and ganglion-like cells with variable distribution of the three different tumor cell types (Fig. 10.15a). The epithelial component is positive for cytokeratins (Fig. 10.15b), neuroendocrine markers (synaptophysin, chromogranin), PP, somatostatin, and progesterone receptor in most cases. The spindle-shaped cells are S100 positive (Fig. 10.15c) and can also express neurofilaments and vasoactive intestinal peptide (VIP). Ganglion-like cells are CD56 and synaptophysin positive. The majority of GPs are G1 despite their high propensity to be deeply located within the duodenal wall. The most important risk factor for lymph node metastasis is tumor size >3 cm.

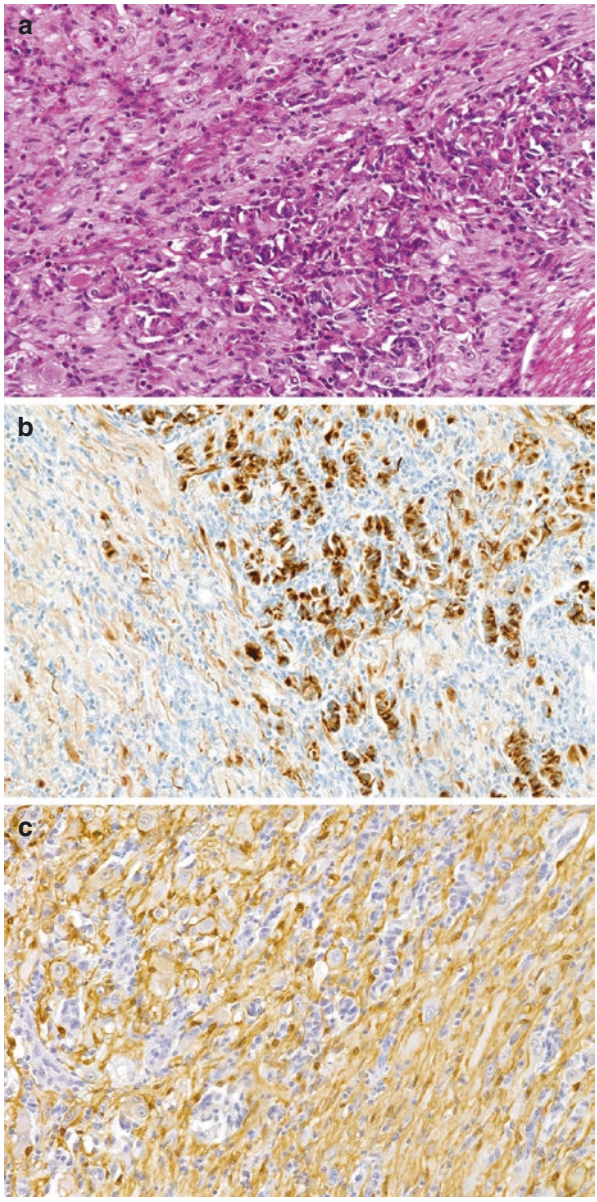

Fig. 10.15 Gangliocytic paraganglioma is constituted by an admixture of epithelial (**a**, right), spindle-shaped, and large ganglion-like cells (**a**, left). The epithelial component is positive for cytokeratins (**b**), while spindle-shaped cells are S100 positive (**c**)

Duodenal Neuroendocrine Carcinoma (NEC)

Duodenal NECs account for about 13% of all duodenal NENs [52] and 4% of digestive NECs [69]. They are mainly located in the ampullary region and are more frequent in males.

Usually they are large and ulcerated neoplasms deeply infiltrating the duodenal wall. They are composed of poorly differentiated either small or large cells forming solid sheets associated with necrosis, vascular and perineural invasion, and high mitotic activity. As in other sites, neoplastic cells are diffusely positive for neuroendocrine markers (chromogranin may be focal expressed or absent) and Ki67 index is >20%.

Prognosis is poor with a median survival of 10 months.

Duodenal Mixed Neuroendocrine-Non-neuroendocrine Neoplasm (MiNEN)

Duodenal MiNENs are rare and more frequently composed of adenocarcinoma and NEC (MANEC) and show overlapping clinicopathological features of duodenal NECs. In addition, rare cases of mixed neoplasms composed of adenoma and NET (MANET) have been described in the duodenum [47], but they are not strictly referred as MiNENs (see sections "Background" and "Gastric Mixed Neuroendocrine-Non-neuroendocrine Neoplasm (MiNEN)").

Neuroendocrine Neoplasms of the Lower Jejunum, Ileum, and Right Colon

NENs arising in the lower jejunum, ileum, and right colon are most frequently NETs and represent a homogenous category of neoplasms, mainly composed of serotonin-producing EC cells. In addition to NETs, NECs and MiNENs have also been described, but they are more frequent in the cecum and right colon than in the lower jejunum and ileum.

Ileal and Lower Jejunal Neuroendocrine Tumors (NETs)

As for other digestive NETs, their incidence and prevalence have been constantly increasing in the last two decades [11, 30, 70]. The age-adjusted incidence per 1,000,000 population has been estimated to be 0.4 for jejunal NETs and 3.2 for ileal NETs [71]. Apart from this light difference in epidemiology, there are no other significant clinicopathological differences between lower jejunal and ileal NETs [54]. They are equally distributed between males and females and more frequently diagnosed in the sixth and seventh decades of life. Patients can be asymptomatic or may present intermittent abdominal pain for several years. Rarely, patients may present with acute symptoms of intestinal obstruction and/or intestinal ischemia. The carcinoid syndrome characterized by cutaneous flushing, diarrhea, and fibrous thickening of the endocardium and valves of the right heart is rare (5–8% of patients) and is observed only in association with liver metastases.

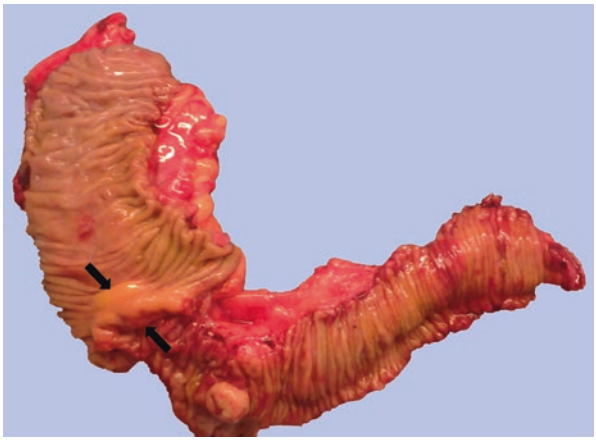

Fig. 10.16 This EC-cell NET, located in the terminal portion of the ileum at about 3 cm from ileocecal valve (arrows), appears as a well-delimited mucosal nodule

Tumors are generally small (usually 1–3 cm in size) and multiple in about 30% of cases [18]. They appear as yellow nodules frequently determining retraction of the serosal surface (Fig. 10.16). In some cases, locoregional mesenteric lymph node metastases are larger than the primary neoplasm and represent the first lesion to be identified at radiological examination.

Histologically, they are characterized by a typical insular architecture (type A according to Soga and Tazawa [72]) with solid and cribriform structures showing peripheral palisading (Fig. 10.17a). An abundant desmoplastic reaction is frequently observed as well as thickened vein and artery walls, due to elastic sclerosis. These vascular lesions may lead to vessel lumen occlusion and ischemic consequences. Tumor cells are well differentiated, with brightly eosinophilic cytoplasm that can also show a fine granular aspect (Fig. 10.17b). Although small in size, the vast majority of cases infiltrate the muscularis propria and the subserosa. Typically, fixation and processing artifacts determine retraction spaces between tumor nests and fibro-muscular stroma that may mimic lympho-vascular invasion (Fig. 10.17c). Pathologists need to be aware about this phenomenon, and the use of CD31 or CD34 immunostaining is strongly recommended to confirm lympho-vascular invasion in difficult cases. Tumor cells are positive for neuroendocrine markers, serotonin, substance P, VMAT1, CDX2, and SST_2 (Fig. 10.18). Most of cases are G1 or G2 NETs but, however, very often metastatic to lymph nodes. This suggests that Ki67 index is not a good predictor of metastatic potential in this specific site [35].

Ileal NETs have a complex genomic landscape, which includes different chromosomal abnormalities, high rate of epigenetic changes, and a low rate of somatic mutations [73–75]. Chromosome 18 deletion is observed in about 60–90% of cases, including non-metastatic cases [76], while gain of chromosome 14 seems to be associated with advanced staged NETs [77]. Promoter gene methylation has recently been demonstrated in several cases, and more than 50% of ileal NETs have been found to show a CpG island methylator phenotype [78]. Among mutations, *CDKN1B* mutations are found in less than 10% of cases [79, 80], while the few other mutations observed cluster within the mTOR, PDGFR, and TGF-β pathways

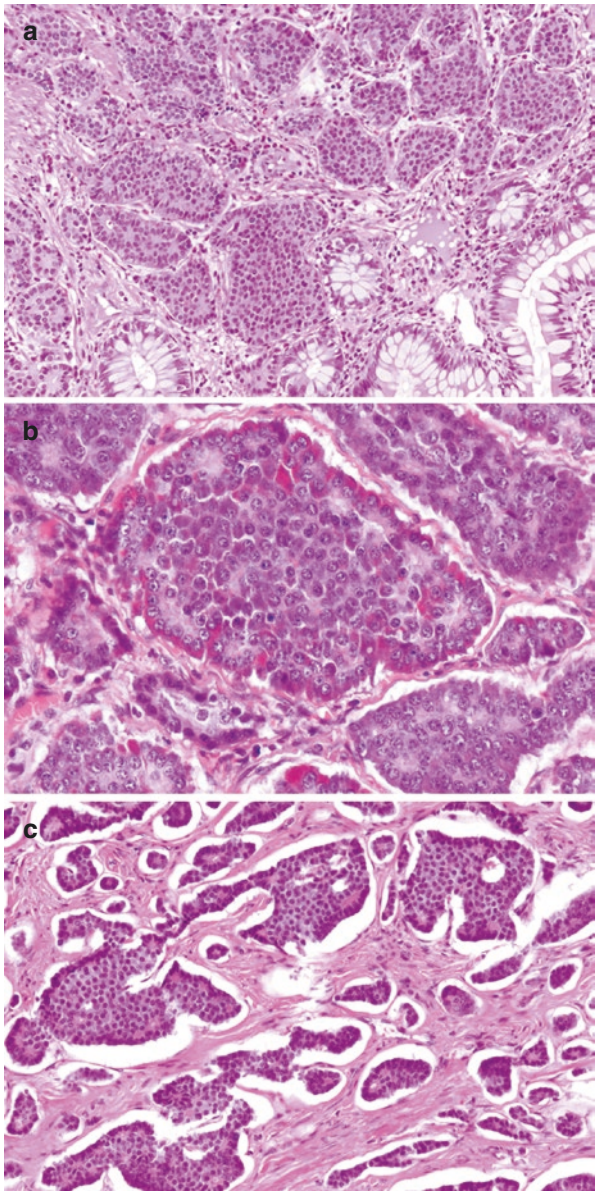

Fig. 10.17 Ileal EC-cell NET is characterized by a typical insular architecture (type A according to Soga and Tazawa) containing rare pseudoglandular structures and showing peripheral palisading (**a**). Tumor cells are well differentiated and can show a brightly eosinophilic cytoplasm (**b**). It is worth noting that fixation and processing artifacts can determine retraction spaces between tumor nests and fibro-muscular stroma mimicking lympho-vascular invasion (**c**)

[74, 81]. The integration of all these different molecular alterations may allow stratifying patients in prognostic categories. Indeed, NETs with chromosome 18 loss, *CDKN1B* mutation, and lacking a CpG island methylator phenotype show a better prognosis than cases without whole-arm copy-number variation and a CpG island methylator phenotype. NETs showing whole-arm copy-number variations seem to be associated with the worst prognosis [82].

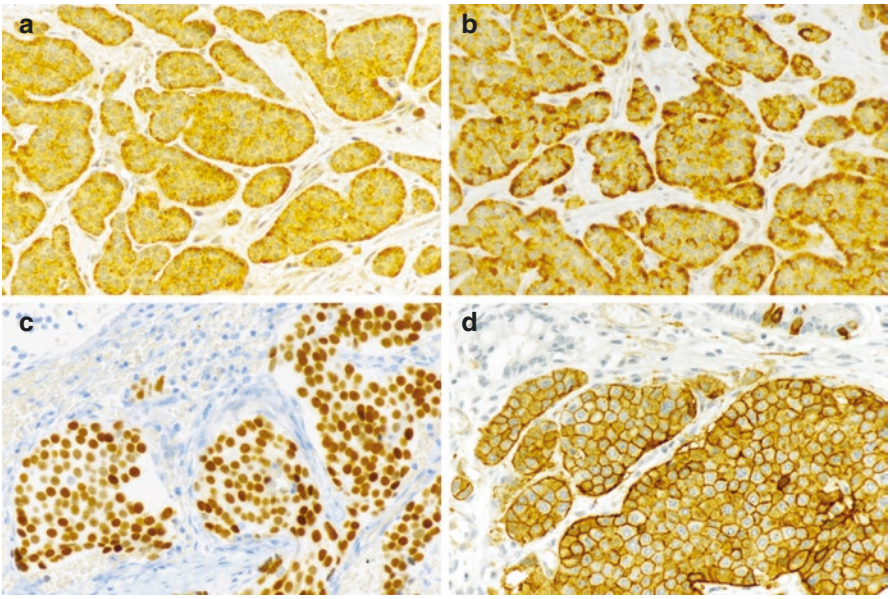

Fig. 10.18 In addition to general neuroendocrine markers, ileal EC-cell NETs are positive for serotonin (**a**), substance P (**b**), CDX2 (**c**), and somatostatin receptor 2A (**d**)

The 5-year and 10-year overall survival has been estimated to be 60% and 43%, respectively. Tumor stage is a prognostic factor; indeed the 5-year and 10-year overall survival is 72% and 60% in patients without metastases, respectively, and decreases at 35% and 15% in those with metastatic disease [3, 83, 84]. Tumor grade mainly based on Ki67 index is also associated with outcome, although it is not a predictor of lymph node metastasis [85]. The best predictive Ki67 cutoff in discriminating G1 versus G2 tumors, with consequent prognostic implication, seems to be 5% and not 3% [86].

Ileal and Lower Jejunal NEC and MiNEN

Jejunum-ileal NECs and MiNENs are rare and show similar clinicopathological features of the same cases observed in the other portion of the gut. However, survival is worse than that of patients with NECs located in the foregut region [69].

Neuroendocrine Neoplasms of the Appendix

Neuroendocrine neoplasms (NENs) of the appendix include NETs, NECs, and MiNENs, with NETs being the more frequent tumor type. The so-called goblet cell carcinoid is not currently considered as a neuroendocrine neoplasm or MiNEN, but

as an adenocarcinoma with amphicrine features. In the last WHO classification of digestive neoplasms, it is defined as goblet cell adenocarcinoma [87].

Appendiceal Neuroendocrine Tumors (NETs)

NETs of the appendix are the fifth most frequent digestive NET [11], found in about 0.2–0.7% appendectomies [88]. The annual incidence has been estimated to be 0.15–0.6 cases per 100,000 persons, with the highest incidence in the second-third decades of life [11, 88]. However, not rarely appendiceal NETs can also be discovered in children [89].

Patients do not show specific symptoms, and in about 80% of cases NETs are found incidentally after surgery for acute appendicitis (Fig. 10.19a) [90]. The association with carcinoid syndrome is extremely rare and is observed in metastatic cases [88].

Appendiceal NETs usually occur in the tip of the appendix, are yellowish, and measure 1–2 cm in size. Only less than 20% of cases are >2 cm.

Microscopically, most of the cases are composed of uniform polygonal serotonin-producing EC cells, which grow forming nests, often with peripheral palisading and pseudoglandular formations (type A according to Soga and Tazawa) (Fig. 10.19b).

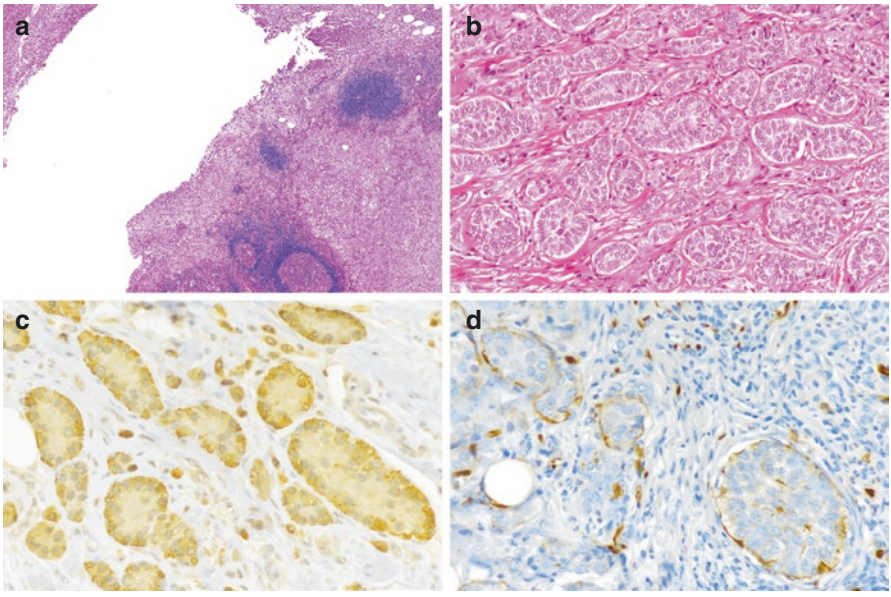

Fig. 10.19 This patient underwent appendectomy for acute appendicitis, and in the inflamed mucosa a NET was incidentally found (**a**, right). Tumor is composed of uniform polygonal well-differentiated cells, which grow forming nests (type A according to Soga and Tazawa) (**b**). Tumor cells are positive for serotonin (**c**). S100 labels sustentacular cells, a typical feature of EC-cell NET of the appendix (**d**)

In some cases, tumor cells may have a clear cytoplasm. Necrosis is absent and mitoses are infrequent or even absent. Despite an indolent behavior, tumor cells generally infiltrate deeply the muscular wall and not rarely the subserosa or the mesoappendix. More rarely tumors are composed of proglucagon-derived peptide-producing L cells, and these neoplasms show a trabecular pattern of growth resembling that of rectal L-cell NETs (Fig. 10.20a).

Tumor cells are positive for neuroendocrine markers and in EC-cell NETs for serotonin (Fig. 10.19c) and substance P. These neoplasms also show S100-positive sustentacular cells (Fig. 10.19d). L cells can be positive for GLP-1, pancreatic polypeptide (PP), glicentin, or other proglucagon-derived peptides (Fig. 10.20b). Most of both EC-cell and L-cell appendiceal NETs diffusely express SST_2 and are G1 or G2 (9–14%) [90, 91]. Although tumor grade 2 seems a risk for lymph node metastases [91], grading does not seem to influence patients' outcome [92].

Molecular data on appendiceal NET are limited: they lack mutations in common cancer-associated genes and only rarely they show chromosome 18 deletions [93].

Appendiceal NETs are generally indolent neoplasms and patients show an excellent prognosis even when NETs are metastatic [94]. The reported 10-year survival rate is >92% [91, 92, 94, 95]. Locoregional lymph node metastases occur in about 5% of cases, and tumor size >15.5mm, grading G2, and presence of lympho-vascular invasion have recently been demonstrated to be independently related to nodal metastases [91]. However, the prognostic role of lymph node metastases is still a matter of debate since no statistically different prognosis has been observed between patients with or without lymph node metastases [89–91, 96]. Liver and other distant metastases are rare and associated with a 5-year survival rate of 34% [97]. Since there are not established prognostic factors, the survival benefit of right hemicolectomy is still unclear [90, 94]. For these reasons, the choice of right hemicolectomy in patients with appendiceal NETs, who are frequently young, is demanded to multidisciplinary tumor boards in expert referral centers.

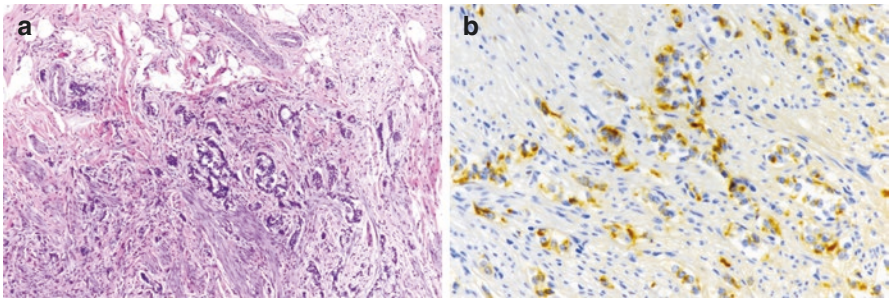

Fig. 10.20 Appendiceal L-cell NET shows a trabecular pattern of growth resembling that of rectal L-cell NETs (**a**). Tumor cells are positive for glicentin (**b**)

Appendiceal NECs and MiNENs

Appendiceal NECs and MiNENs are extremely rare representing about 8% and 4% of appendiceal neoplasms, respectively. NECs are more frequently in females, while MiNENs do not show gender predilection. The average age at diagnosis is 45.6 years for NECs and 59.7 years for MiNENs [98].

Both NECs and MiNENs do not show specific preferential localization and generally present as advanced disease with deep wall invasion and metastases.

Morphologically, NECs resemble their counterparts of other sites either of small cell or of large cell type [92, 99]. MiNENs are generally composed of adenocarcinoma and NECs with overlapping features with this type of MiNENs located elsewhere. For these cases the term mixed adenoneuroendocrine carcinoma (MANEC) can be used.

Prognosis depends on stage. The reported 4-year survival rates range from 92.6% for stage I to 20.4% to stage IV NECs and from >90% stage I to 26.5% stage IV MiNENs [98].

Neuroendocrine Neoplasms of the Rectum

NENs arising in the left colon and rectum include NETs, NECs, and MiNENs. As for NENs located in other sites, rectal NENs have showed an increasing incidence over the last 20 years, and the annual incidence in the USA has been estimated to be 1.2 per 100,000 person-years [11, 100]. They represent about 2% of all rectal neoplasms and are incidentally discovered in about 0.05–0.07% of patients undergoing screening colonoscopy [101].

Rectal Neuroendocrine Tumors (NETs)

Rectal NETs are more frequently diagnosed in the sixth decade of life (median age 56 years). Being generally clinically silent or associated with nonspecific symptoms, they are often discovered incidentally. Very rare metastatic cases associated with the carcinoid syndrome have been described [102].

Macroscopically, rectal NETs present as small submucosal polyps measuring less than 1 cm in more than half of cases [103].

The majority of rectal NETs are composed of mild to moderate atypical L cells showing a trabecular architecture (type B according to Soga and Tazawa) which infiltrate the mucosa and submucosa (Fig. 10.21a). Cells are well differentiated with abundant eosinophilic cytoplasm and monomorphic nuclei with salt-and-pepper chromatin. Mitotic count is low and necrosis is exceptional. Tumor cells are diffusely positive for synaptophysin but often express only focally chromogranin A. They are also immunoreactive for glicentin, GLP-1, GLP-2, pancreatic polypeptide, PYY, and prostatic acid phosphatase (PAP) (Fig. 10.21b–d) [104, 105]. More rarely, rectal NETs are composed of serotonin-producing EC-cell NETs showing

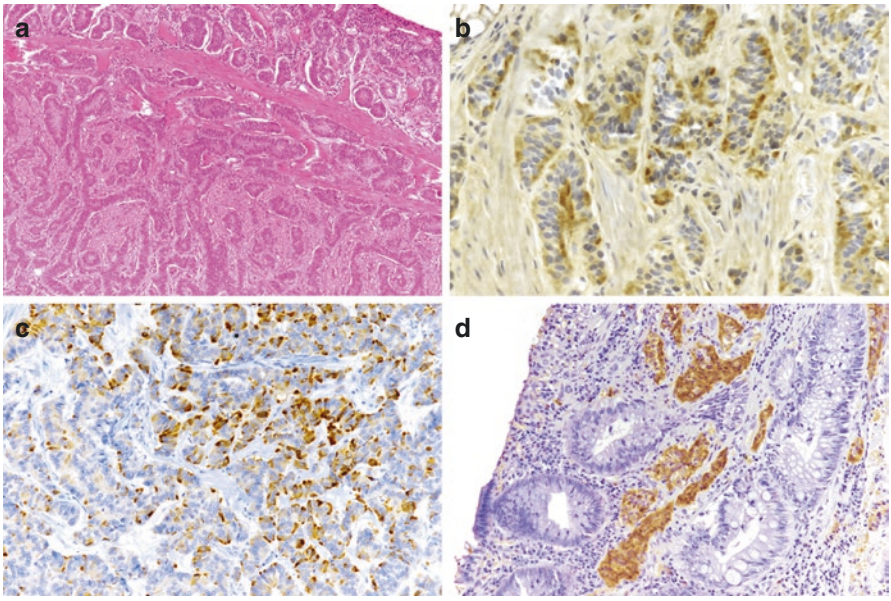

Fig. 10.21 Rectal L-cell NET is composed of mild to moderate atypical L cells showing a trabecular architecture (type B according to Soga and Tazawa) which infiltrate the mucosa and submucosa (**a**). Mitotic count is low and necrosis is exceptional. Tumor cells of this NET are positive for glicentin (**b**) and pancreatic polypeptide (**c**). In addition, they are also immunoreactive for prostatic acid phosphatase (PAP); the epithelium of crypts is PAP-negative (**d**)

a nested architecture (type A) such as jejunoileal EC-cell NETs. These tumors are positive for synaptophysin, chromogranin, serotonin, substance P, and CDX2 [106]. Several cases are also positive for SST_2 [107]. Rectal NETs are usually G1 or G2 and only rarely G3.

The prognosis largely depends on tumor grade and stage, although tumor cell type seems to have a prognostic impact as well [11, 103, 108–110]. The median overall survival time of low-stage NET has been reported to be 30 years [11] with 5-year survival of 93% for localized NETs and 86% overall [101].

Rectal NECs and MiNENs

Rectal NECs represent about 12% of digestive NEC. The media age at diagnosis is 64 years with a slight male predominance [11, 69].

Macroscopically, they are similar to conventional adenocarcinomas.

NECs usually display a solid growth with "geographical chart" necrosis and brisk mitotic activity and deeply infiltrate the rectal wall. NEC cells display severe atypia, and, as in other sites, they can be of small cell or large cell type. Some cases, showing large cell morphology and intra-tumor lymphoid infiltrated, present microsatellite instability, a feature associated with a better prognosis. These cases frequently

present BRAF mutation and are Epstein-Barr negative [111, 112]. A minor non-neuroendocrine component (either adenocarcinoma or squamous cell carcinoma) is frequently observed, but the term MiNEN is only used when it reaches at least 30% of the tumor mass. MiNENs are mostly composed of NEC and adenocarcinoma, but rarely MiNENs with a low-grade NET component have been described as well [9, 10]. In addition, rare NETs that are associated with adenomas have been described [47]. NEC tumor cells and cell of the neuroendocrine component of MiNENs are synaptophysin positive. Chromogranin A may be scant or faint. CDX2, TTF1, and at lesser extent SST_2 may also be positive.

As in other sites, NECs show several genetic abnormalities, usually including mutations in *TP53* and *RB1* tumor suppressor genes. Other abnormalities include mutations in *APC*, *KRAS*, *FHIT*, *DCC* and *SMAD4*, *MEN1*, and *BRAF* [113, 114]. A few studies on MiNEN characterized by a NEC component (MANEC) showed that they have similar mutations observed in pure NECs [50, 115]. In addition, recent data suggest that colorectal MANEC and NEC are genetically related to colorectal adenocarcinomas [116], though possibly representing a rather unique entity [117]. Finally, a potential pathogenetic role for high-risk HPV has been recently put forward especially for lesions of the left colon and rectum [118].

NECs display an ominous outcome, which depends on tumor stage and Ki67 proliferation index. A median overall survival time of 25.4 months and 5.3 months has been observed in NECs showing a Ki67 index <55% or >55%, respectively [69]. Similarly, the prognosis of MiNEN with a NEC component depends on stage and the Ki-67 proliferation index of the NEC component. The median overall survival time of patients with colorectal MANEC was 12.2 months [27].

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Pancreatic Neuroendocrine Neoplasms

11

Sylvia L. Asa and David S. Klimstra

Historical Background

The history of the endocrine pancreas dates back to 1867 when Langerhans identified groups of distinct cells scattered throughout the gland [1]; these islets that bear his name are the normal neuroendocrine elements that were subsequently implicated by Laguesse as the source of internal secretions that regulated blood sugar, which was known to be attributed to the pancreas by Mering and Minkowski [2, 3]. In 1992, Banting and Best derived the term "insulin" based on the insular structures that were responsible for the synthesis and secretion of this important hormone [4].

Neuroendocrine tumors of the pancreas were initially called *islet cell tumors* as they were thought to arise from these structures. Subsequent studies showed that, in fact, many pancreatic neuroendocrine tumors may arise from the pancreatic ducts where neuroendocrine cells are also found and where they often proliferate in the phenomenon known as *nesidioblastosis*; this apparent neogenesis of islet cells from ducts is also mimicked by the aggregation of islets in any situation where there is ductular obstruction and exocrine atrophy [5]. In fact, both sources of neuroendocrine cells are likely capable of giving rise to tumors; in patients with germline mutations that predispose to the development of pancreatic neuroendocrine tumors, there is frequently a proliferation of islets that become atypical, or *dysplastic*, and likely progress to microtumors that then grow into clinical neoplasms [6, 7]. For these reasons and because these tumors do not always faithfully recapitulate the

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morphology of mature islet cells, the term "islet cell tumor" fell out of favor. The use of *pancreatic endocrine tumors* is technically correct in pancreas, as it distinguishes these neoplasms from non-endocrine tumors. However the WHO/IARC has proposed a consistent terminology at all body sites, including others where there may be other variants of endocrine tumors that are not neuroendocrine (e.g., thyroid, ovary, and tests); therefore, the term "neuroendocrine" is applied. In the overarching approach to classifying *neuroendocrine neoplasms*, the WHO has proposed restricting the term *neuroendocrine carcinoma* to high-grade poorly differentiated neoplasms which usually have mutations that are found in adenocarcinomas and applying *neuroendocrine tumor* to all well-differentiated tumors that generally have a specific set of genetic and epigenetic alterations that differ from those in non-neuroendocrine carcinomas [8]. The term used for pancreatic NETs is PanNETs.

Epidemiology

While it is often difficult to separate PanNETs from the wider group of gastroenteropancreatic neuroendocrine tumors (GEP-NETs), the epidemiology of pancreatic neuroendocrine neoplasia has shown a general increase in incidence and prevalence over time. Data from the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) registry identified a steady increase and improved survival between 1973 and 2007 [9]. The age-adjusted incidence of GEP-NETs increased over the same four decades, 3.65-fold in the USA and 3.8- to 4.8-fold in the UK [10]. In Germany as well, the incidence of GEP-NETs increased between 1976 and 2006, going from 0.31 to 2.27 per 100.000 inhabitants per year for men and from 0.57 to 2.38 for women [11]. In a population study from Iceland, mean annual incidence was 3.65/100,000, with a slight increase over time from 3.39/100,000 during 1985–1999 to 3.85/100,000 during 2000–2014 [12], and in that study, pancreas had the least favorable 5-year survival of all GEP-NETs at 31% [12].

Data on pancreas alone are difficult to extract, but several reports show an increase in the USA from 0.17 in 1973–1977 to 0.43 in 2003–2007 [9, 13–15]; only one study showed a reduction [16] that may be due to coding and terminology issues. Only a portion of the increased incidence can be attributed to the increased detection due to greater use of cross-sectional imaging studies. The peak incidence is between 30 and 60 years of age. In Norway, pancreatic NETs doubled from 0.15 in the mid-1990s to 0.3 in the early 2000s [14]. In most countries there is a male predominance except in Italy where it is more common in females [10]. In the US SEER population, pancreatic NETs were more common in Whites and African Americans than in Asian Americans and American Indians [17].

Tumor Classification and Morphology

The islets of Langerhans are a complex organ composed of four different hormone-secreting neuroendocrine cell types that work in a coordinated endocrine and paracrine system that controls the uptake, storage, and use of glucose and fatty acids to maintain metabolism and energy homeostasis.

The four cell types are classified as A or α cells, B or β cells, D or δ cells, and PP cells. The A cells produce glucagon that increases the concentration of glucose and fatty acids in the bloodstream; B cells produce insulin that reduces glucose, fats, and proteins in the circulation by stimulating liver, muscle, and fat absorption of glucose. D cells produce somatostatin that was named for its ability to inhibit growth hormone in the pituitary but is actually a systemic inhibitor of many peptide hormones, including insulin and glucagon. PP cells produce pancreatic polypeptide (PP) that regulates exocrine as well as endocrine secretions of the stomach and pancreas and is a member of a family of homologous peptides that include peptide YY (PYY) and neuropeptide Y (NPY). The four pancreatic endocrine cell types have a characteristic distribution (Fig. 11.1). In the pancreatic body, tail, and superior head that all derive from the dorsal primordium, they are clustered in compact islets that have an organized pattern of convoluted cords, with glucagon-containing

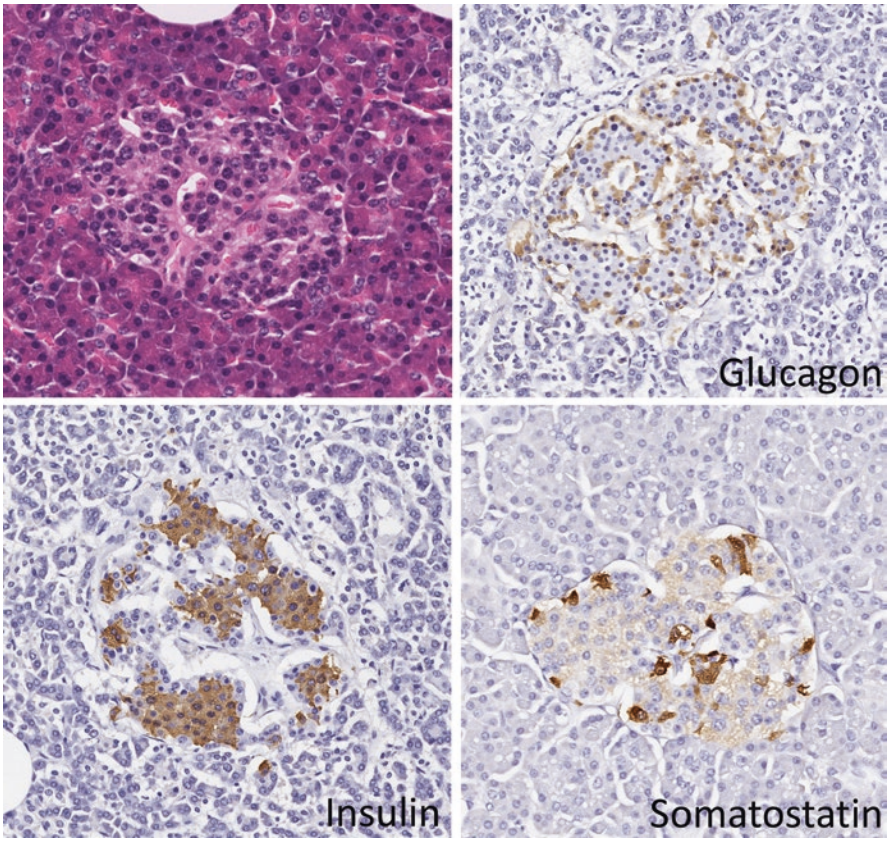


Fig. 11.1 The normal pancreatic islet. The islet of Langerhans is composed of several endocrine cell types organized in a specific fashion. The glucagon-containing A cells (top right) form the periphery of cords that are composed mainly of insulin-expressing B cells (bottom left) and interspersed between them are scattered somatostatin-reactive D cells (bottom right)

A cells at the periphery, insulin-containing B cells filling the cords, and somatostatin-containing B cells randomly scattered between the A and B cells. PP cells are scant and relatively random in those portions of the pancreas, but are numerous in the diffuse islets of the inferior head and uncinate process, which derive from the ventral anlage and are arranged in less circumscribed ribbons that interdigitate among surrounding acinar cells.

Pancreatic endocrine cell development is driven by transcription factors [18] including pancreatic and duodenal homeobox 1 (PDX1), proneural basic helix-loop-helix (NeuroD1/BETA2), neurogenin 3 (NGN3), insulin gene enhancer 1 (ISL1, also known as islet 1), insulinoma-associated 1 (INSM1), the paired box gene PAX6, and NKX2.2; expression of some of these transcription factors is preserved in PanNETs and can help identify their pancreatic origin (see below). The B and D cells are determined by PAX4 and NKX6.1; musculoaponeurotic fibrosarcoma oncogene homolog (MAF) A (MAFA) is specific for B cells. A cells rely on ARX and MAFB [19]. These factors can also serve as biomarkers of pancreatic NETs, but they are not in widespread use.

PanNETs are well-differentiated tumors of neuroendocrine cells that are clinically classified as functioning and nonfunctioning. Functioning tumors are defined based on their associated hormonal syndromes and can secrete insulin, glucagon, somatostatin, and/or PP, but they can also secrete gastrin, VIP, or serotonin. Some produce ectopic hormones such as ACTH, GHRH, GH, vasopressin, and/or parathyroid hormone-related peptide causing Cushing's syndrome, acromegaly, SIAD, and hypercalcemia [20–29]; ectopic hormone production is usually a feature of aggressive tumors. Nonfunctioning tumors are more common and are usually diagnosed because of mass effects or the presence of metastases; however, it is likely that a significant number synthesize and secrete hormones that are not measured, and the vague symptoms associated with these lesions are not confirmed to be relevant.

The morphology of PanNETs is highly variable – more so than NETs of almost any other anatomic site. They are usually well delineated but locally infiltrative (Fig. 11.2). They may be composed of solid nests, they may form trabeculae, or they may even form gland-like structures (Fig. 11.3). The tumor cell cytoplasm can be eosinophilic, amphophilic, oncocytic [30], clear [30], or rhabdoid [31] (Fig. 11.4); the nuclei tend to be bland with the characteristic "salt and pepper" morphology, although nuclear pleomorphism can occasionally be pronounced. The stroma is vascular, and there may be peliosis (Fig. 11.5). Some tumors have a dense fibrous stroma; this is usually found in the rare serotonin-producing pancreatic tumors that arise adjacent to large ducts [32]. Occasional lesions have amyloid deposition that is attributed to the production of islet amyloid polypeptide, a feature that is characteristically found in insulinomas. Calcification may be seen as a dystrophic phenomenon; psammoma bodies are found in somatostatinomas, although more commonly in duodenal than pancreatic primaries [33]. Features that portend aggressive behavior include a high proliferative rate (see grading, below), advanced stage, invasion of lymphatics and blood vessels, and perineural invasion [34].

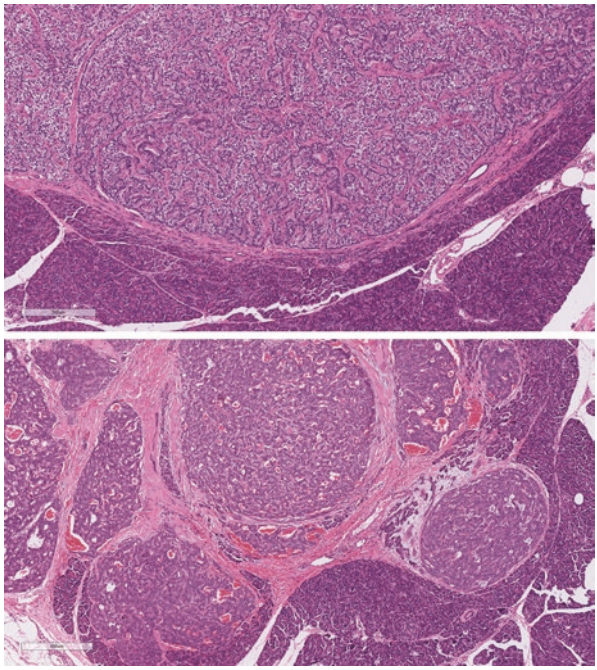


Fig. 11.2 Well-differentiated pancreatic neuroendocrine tumors (PanNETs). These tumors are composed of nests, cords, and glands of epithelial cells in a vascular stroma. They are usually well delineated (top) but locally infiltrative (bottom)

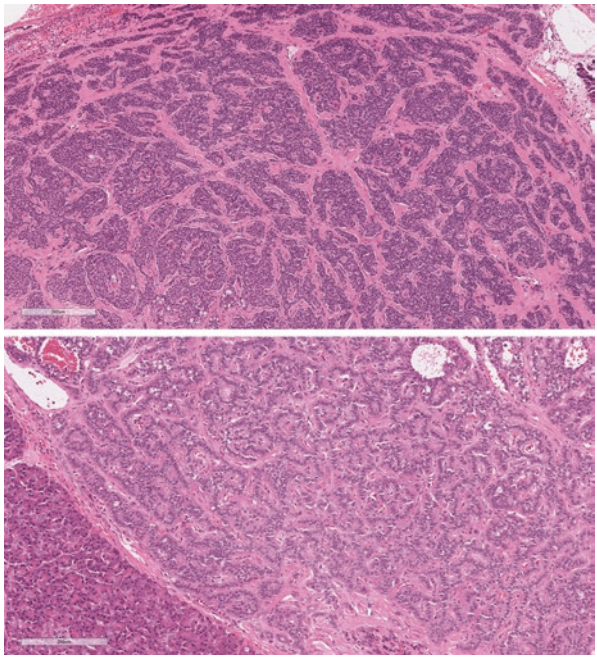
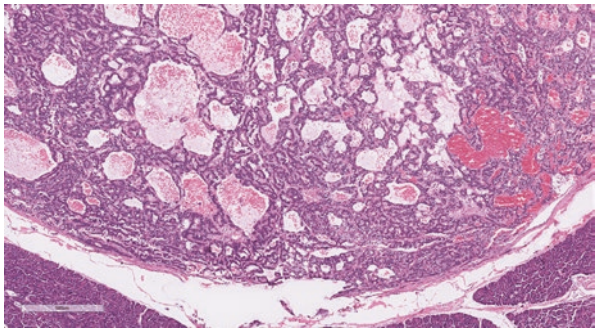


Fig. 11.3 Well-differentiated pancreatic neuroendocrine tumors (PanNETs). The tumor cells may form large solid nesting patterns (top) or a trabecular and glandular morphology may predominate (middle). The stroma may be fibrotic (top and middle) or highly vascular with pools of blood and stromal edema (bottom) yielding a cystic appearance

Fig. 11.3 (continued)



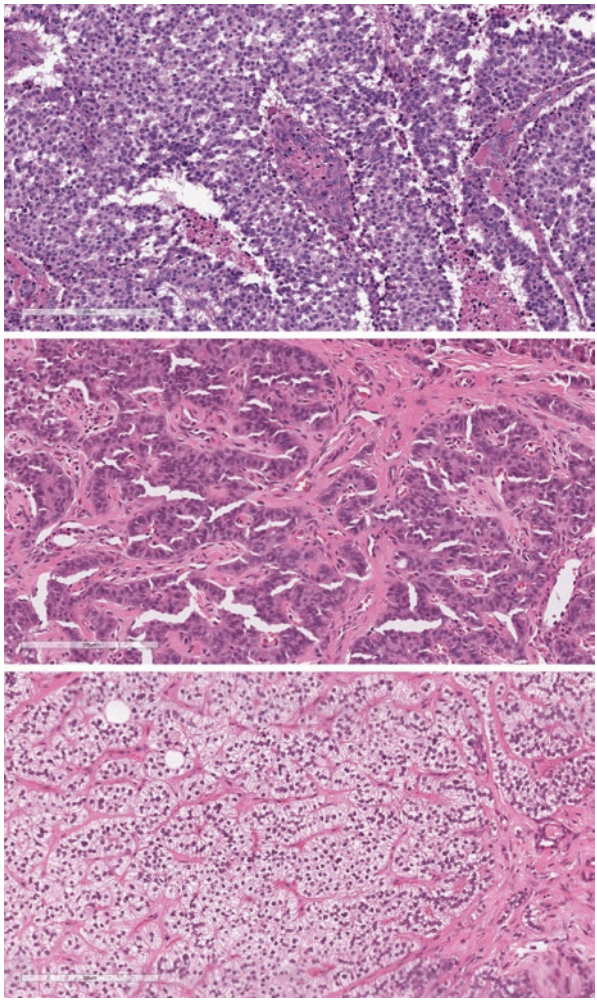


Fig. 11.4 Well-differentiated pancreatic neuroendocrine tumors (PanNETs). The tumor cell cytoplasm can be amphophilic (top), oncocytic (middle), or clear (bottom); nuclear pleomorphism is usually not evident

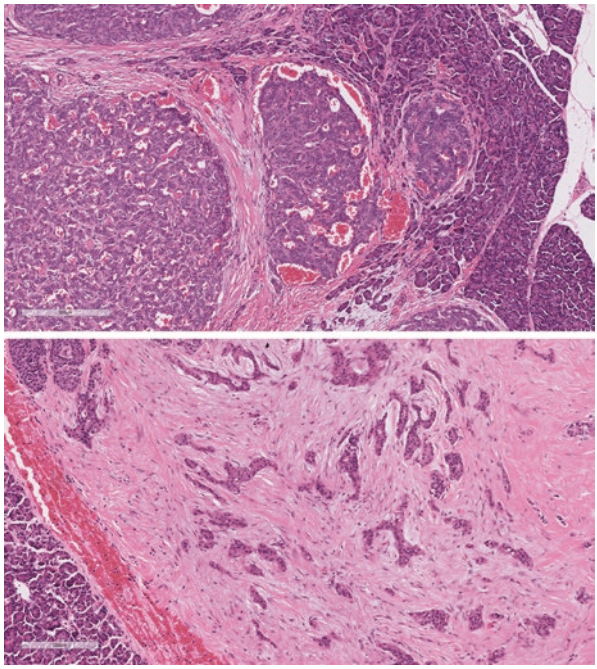


Fig. 11.5 Well-differentiated pancreatic neuroendocrine tumors (PanNETs). The stroma tends to be highly vascular, and there may be peliosis (top). Some tumors have a dense fibrous stroma (bottom)

Immunohistochemistry identifies biomarkers of neuroendocrine differentiation, synaptophysin and chromogranin (Fig. 11.6); more recently, INSM1 has been added to the armamentarium of NET biomarkers. Most pathologists do not pursue hormone immunohistochemistry, since it is not an independent prognostic feature, and only do so when asked to confirm a clinical picture of hormone excess. However, in cases where there is no clinical evidence of a syndrome such as glucagonoma, insulinoma, or gastrinoma, immunolocalization of hormones can assist clinicians in uncovering signs and symptoms or explaining vague symptoms. This also provides a rational approach to surveillance using appropriate biomarkers. In patients with multifocal disease in the pancreas, hormone profiling can indicate metastasis or suggest instead multiple primary tumors. In the case of patients with metastatic disease of unknown primary site, the identification of pancreatic transcription factors (ISL1 in particular) is useful to indicate the primary site. There is some evidence that these biomarkers can assist in determining cell differentiation: A cells express ISL1 but are negative for PDX1, NGN3, and CDX2; B cells express ISL1 and PDX1; D cells express ISL1, PDX1, and NGN3; and G cells express PDX1, ISL1, and NGN3 as well as CDX2 [35]. Staining for keratins should be performed to ensure that one does not miss a rare primary pancreatic paraganglioma; this can be performed with either a pankeratin marker such as AE1/AE3 or with the CAM 5.2 antibody. If the tumor is negative for keratins as well as pancreatic transcription factors, paraganglioma can be confirmed by staining for GATA3 and tyrosine hydroxylase [36]. In tumors with

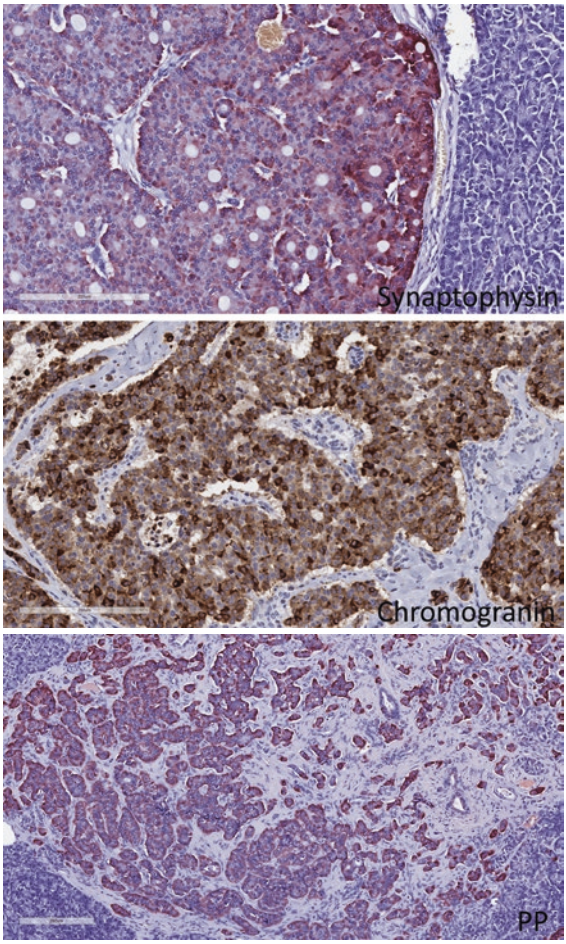


Fig. 11.6 Immunohistochemistry of pancreatic neuroendocrine tumors (PanNETs). These tumors have diffuse cytoplasmic positivity for synaptophysin (top) and chromogranin (middle). Staining for hormones identifies the cytodifferentiation of these tumors and can provide biomarkers for surveillance, as in this tumor producing pancreatic polypeptide (PP, bottom)

rhabdoid cytology, CAM5.2 identifies juxtanuclear aggregates of keratins that account for the cytoplasmic morphology [37]. Cytokeratin 19 positivity has been identified in more aggressive tumors [38] and therefore may be of prognostic value.

Tumor grade is based on mitotic counts and Ki67 labeling indices (Fig. 11.7). Grade 1 tumors are defined as a Ki67 labeling index <3% and mitoses <2/2 mm^2, grade 2 tumors are defined as a Ki67 labeling index between 3% and 20% or mitoses 2–20/2 mm^2, and grade 3 tumors are defined as a Ki67 labeling index >20% or mitoses >20/2 mm^2 [39]. In cases where the two proliferation indices indicate different grades, the higher grade is assigned. Immunolocalization of pHH3 is a helpful tool for mitotic counting. Because tumor heterogeneity is a well-recognized phenomenon, multiple areas of a tumor should be examined to determine the areas of highest proliferation, known as the proliferative "hot spot." Clinical progression

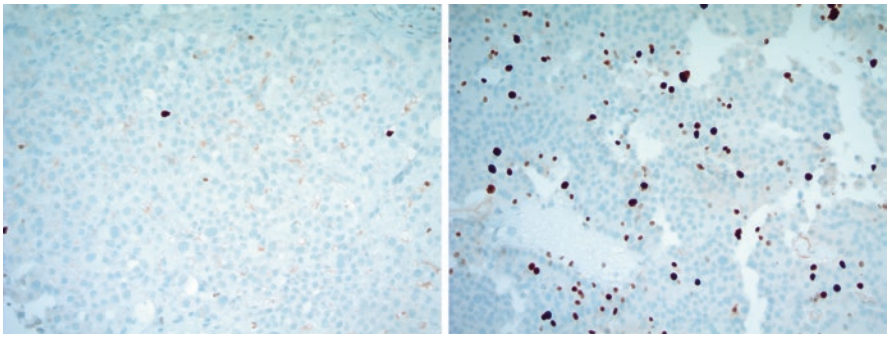


Fig. 11.7 Grading of pancreatic neuroendocrine tumors (PanNETs) using Ki67 labeling index. These neuroendocrine tumors are graded based on proliferation that is quantified by Ki67 labeling. These two examples show a grade 1 (G1) tumor with a Ki67 labeling index <3% (left) and a grade 2 (G2) tumor with a Ki67 of 12% (right)

may be associated with changes in proliferation, and metastatic foci should be regraded to explore the possibility of grade progression.

Careful assessment of the surrounding pancreas is an important component of the workup of a PanNET. These tumors can be multifocal and may be associated with hyperplasia as well as other changes in islets and ducts that are characteristic of specific germline alterations that predispose to PanNETs (Fig. 11.8). In contrast, the identification of an adenocarcinoma obstructing a duct, a stone, or chronic pancreatitis accounts for *pseudo-neoplastic aggregation of islet cells* (Fig. 11.9) that can mimic a PanNET, especially on biopsy. These reactive lesions due to ductal obstruction and acinar atrophy are distinguished from neoplasia by their immunoprofile; tumors are usually monohormonal or express unusual hormones, whereas foci of islet aggregation contain multiple discrete cell types that resemble normal islets but with unusual distributions of the four hormones and with a predominance of PP in the distal pancreas, where it is usually scant.

Poorly differentiated NECs are high-grade tumors (Fig. 11.10) that have a distinct biology and require a different therapeutic approach. They can be subclassified as small cell or large cell neuroendocrine carcinomas and share morphologic features with similarly termed carcinomas of the lung and other organs. They are usually diagnosed at a higher stage and progress much more rapidly [40]. Their different genetic backgrounds provide tools to confirm the diagnosis. Well-differentiated NETs, even those with high proliferation (grade 3), generally have loss of menin, DAXX, and/or ATRX, whereas NECs tend to exhibit loss of *TP53, RB1*, and/or *SMAD4* [41–45]. Expression of SSTR2 is another helpful biomarker of NET that is usually negative in NEC [43].

Mixed tumors with neuroendocrine and non-neuroendocrine components occur in the pancreas (Fig. 11.11). The old term MANEC (mixed adeno-neuroendocrine carcinoma) has been replaced by MiNEN (mixed neuroendocrine and non-neuroendocrine neoplasms) for this conceptual category of neoplasms [39], since it

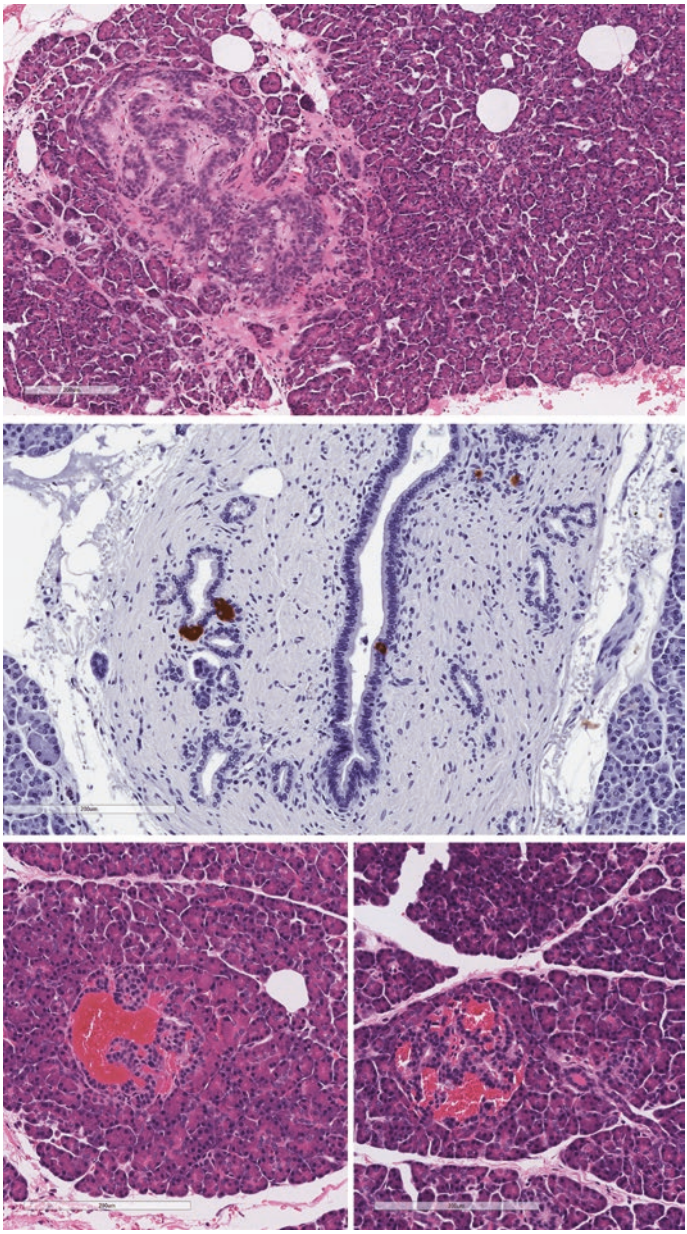


Fig. 11.8 Nontumorous islet pathology identifies genetic predisposition to PanNETs. Patients with MEN1 or MEN4, VHL, or other predisposition syndromes may be identified by the recognition of pathology in the nontumorous pancreas. They may have nesidiodysplasia and abnormal morphology of islets (top), abnormal proliferation of endocrine elements arising from pancreatic ducts and ductules (also called "tubulo-insular complexes" or "nesidioblastosis") as seen with this insulin stain (middle) and peliosis of the islets (bottom)

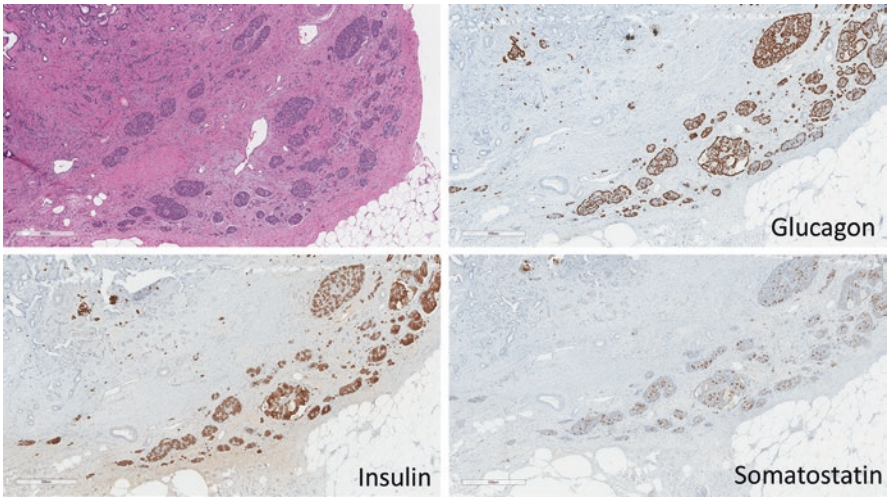


Fig. 11.9 Pseudo-neoplastic aggregation of islet cells. Patients with pancreatic ductal obstruction develop pancreatitis that results in loss of exocrine pancreatic parenchyma and fibrosis, but the endocrine elements remain and resemble a neuroendocrine cell proliferation. In this example of a patient with a PanNET that caused obstruction, the distal pancreas contains numerous islets that have variable size and morphology but retain a relatively normal distribution of glucagon-positive A cells (top right), insulin-reactive B cells (bottom left), and scattered somatostatin-containing D cells (bottom right)

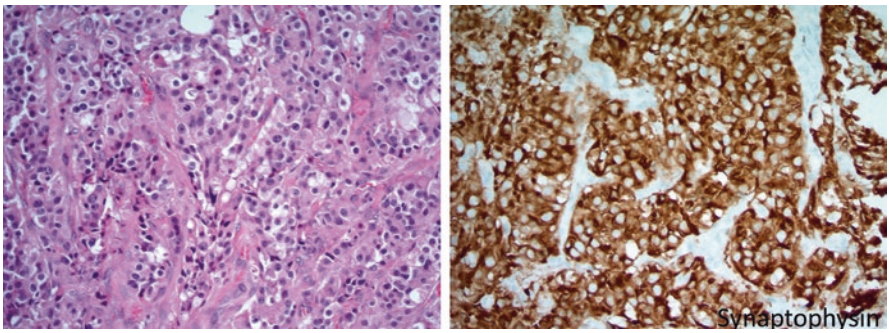


Fig. 11.10 Poorly differentiated pancreatic neuroendocrine carcinoma (NEC). These high-grade tumors are usually composed of large cells that show evidence of neuroendocrine differentiation including expression of synaptophysin (top right) and chromogranin (middle left), but the latter is often weak and focal. The Ki67 labeling index is usually high (50%) as seen here (middle right), but this alone cannot distinguish NEC from NET. Because there tumors commonly have mutations in TP53 and RB1, loss of these tumor suppressors (see p53, bottom right) with positive internal controls in stroma can provide evidence for this diagnosis; in contrast to NETs, stains for ATRX (bottom right), DAXX, and menin as well as somatostatin receptors (not shown) are usually intact

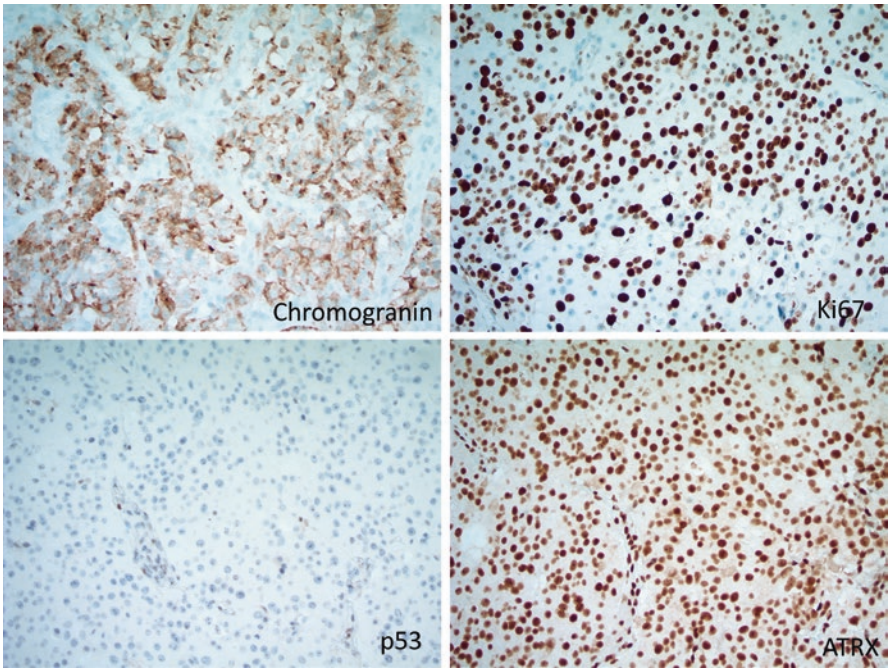


Fig. 11.10 (continued)

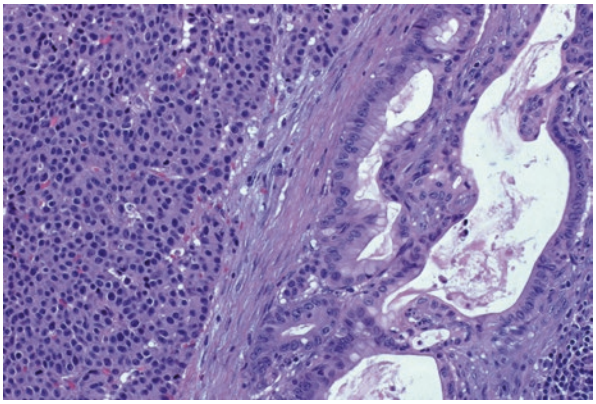


Fig. 11.11 Mixed tumor with neuroendocrine and non-neuroendocrine components (MiNEN). These tumors are composite lesions most often composed of a poorly differentiated neuroendocrine carcinoma (large cell neuroendocrine carcinoma in this example) and a ductal type adenocarcinoma. Combinations involving well-differentiated neuroendocrine elements are rare

has become clear that these composite lesions may contain different types of non-neuroendocrine neoplasms, including adenocarcinomas or squamous cell carcinomas, along with various grades of NET or (more commonly) with poorly differentiated NEC.

Molecular Pathogenesis

The genetic basis of PanNETS has been clarified significantly in the last decade. For many years, these tumors were known to be a component of the syndrome of multiple endocrine neoplasia type 1; the identification of the *MEN1* gene and its protein product menin [46] allowed studies of the incidence of sporadic menin alterations in nonfamilial tumors [47]. Additional familial predisposition syndrome similarly shed light on the molecular alterations in these tumors, including the *VHL* gene [48] and the gene encoding tuberin that is responsible for tuberous sclerosis [49]. In 2011, a landmark paper reported the landscape of mutations in sporadic pancreatic NETs that provided information about two discrete pathways, one involving chromatin remodeling (*MEN1*, a component of a histone methyltransferase complex, *DAXX* (death-domain-associated protein), and *ATRX* (alpha thalassemia/mental retardation syndrome X-linked)) and the other involving genes in the *mTOR* (mammalian target of rapamycin) signaling pathway [41]. A subsequent and larger study expanded this to four major pathways: chromatin remodeling, DNA damage repair, activation of mTOR signaling (including novel fusions), and telomere maintenance with a subgroup showing evidence of hypoxia and HIF signaling [42]; that study also showed that about 17% of clinically sporadic tumors have a significant proportion of germline events, including mutations in the DNA repair genes *MUTYH*, *CHEK2,* and *BRCA2*. Young patients who develop a PanNET should have an assessment for familial tumor syndromes such as Lynch syndrome [50] and SDH-related disease [51]; immunohistochemistry for mismatch repair (MMR) proteins and SDHB screening can be helpful. Other germline mutations implicated in multineoplasia syndromes include *PALB2*, *APC,* and *NTHL1* [52].

Patients with genetic predisposition to the development of PanNETs often have unusual features in the pancreas that allow identification of this predisposition (Fig. 11.6). In florid cases, there may be multiple tumors, but more subtle findings include (i) *ductulo-insular complexes* also known as *nesidioblastosis* and characterized by the formation of new islets budding off from ducts; (ii) *nesidiodysplasia* or dysplasia of islets, identified by irregular outlines, enlargement, and an abnormal distribution of the islet cell types; and (iii) *peliosis* of islets [7]. With the exception of peliosis, these changes can be seen on H&E staining but are best diagnosed with immunohistochemistry for glucagon, insulin, somatostatin, and pancreatic polypeptide. These features are well recognized to occur in patients with MEN types 1 or 4 and in those with von Hippel-Lindau (VHL) disease who also develop serous microcystic proliferations [30, 53–55]. Molecular immunohistochemistry can confirm loss of menin in MEN1, loss of p27 in MEN4 and positivity for inhibin [56], and

carbonic anhydrase IX (CAIX) in VHL, which is also distinguished by the more common presence of oncocytic or clear cell changes in the NETs.

Two additional familial syndromes exemplify the endocrine nature of genetic predisposition to PanNETs. *Mahvash disease* is an autosomal recessive disorder of A cell hyperplasia and neoplasia due to biallelic germline inactivating mutations of the glucagon receptor gene (GCGR) [57]; elevated circulating levels of glucagon have no clinical manifestations because of lack of receptor signaling. *Familial insulinomatosis* with B cell hyperplasia and neoplasia is due to *MAFA* germline mutations [58].

The molecular basis of *poorly differentiated NECs* is markedly distinct from that of NETs and more similar to that of pancreatic ductal adenocarcinomas; they tend to have mutations in *TP53, RB1*, and/or *SMAD4* [41–45].

Prognosis

The prognosis of patients with pancreatic neuroendocrine neoplasms depends on multiple factors, including tumor classification, size and grade, tumor extent and stage, and patient variables. NETs tend to be more indolent with long and slowly metastatic progression over many years, whereas NECs are highly aggressive and rapidly progressive.

When surgical resection is complete, the outcome for PanNETs is relatively good; however, it is not unusual for patients to develop delayed metastases. There has been little work on prognostic factors other than grade; while it is known that B cell tumors associated with clinical manifestations do well, this may be because of early detection. In contrast, tumors that produce gastrin tend to be aggressive, and other ectopic hormones also portend a worse clinical course.

A number of treatment options are available for these tumors. Surgery is the mainstay of therapy and usually the first approach for NETs, except in cases where there is widespread metastatic disease at diagnosis [29]. Patients with isolated liver metastases may benefit from liver directed therapies, whereas those with more widespread metastases require medical therapy with somatostatin analogues, biological agents such as everolimus and sunitinib, and peptide receptor radiotherapy (PRRT) with radiolabelled somatostatin analogues. More aggressive disease warrants therapy with capecitabine and temozolomide (CAP-TEM). In contrast to these approaches for well-differentiated NETs, the management of NECs utilizes the more conventional chemotherapeutic agents that target highly aggressive proliferative malignancies, and platinum-containing regimens are generally favored. Recent studies of immunotherapy have shown promise mainly for NECs.

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Paragangliomas and Pheochromocytomas

12

Ozgur Mete, Arthur S. Tischler, and Sylvia L. Asa

Historical Background

The concept of paraganglia was first proposed by Alfred Kohn in 1903 [1]. Earlier studies by Bertholdus Werner in 1857 had shown that the adrenal medulla developed brown coloration in the presence of chromate salts [2], but it was Kohn who subsequently coined the terms "chromaffin reaction" for the chemical reaction and "chromaffin cells" for the reactive cells. Using the chromaffin reaction, Kohn identified similar tissue associated with sympathetic nerves and ganglia in extra-adrenal locations in the abdomen and retroperitoneum, and he confirmed that cells in the carotid body also exhibited a chromaffin reaction as previously reported by Stilling [2, 3]. Kohn's unifying hypothesis was that these various cells were ganglion-like, derived from sympathetic ganglion precursors, innervated by sympathetic axons, and analogous to neurons, but not neuronal, hence the term "paraganglia" [1]. Subsequent studies disputed Kohn's proposals on the basis that the carotid body usually did not demonstrate a chromaffin reaction in the hands of other investigators

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and that it was innervated by the glossopharyngeal nerve. Over time, paraganglia were divided into chromaffin and nonchromaffin categories and some of mixed type. When it was discovered that the carotid bodies were chemoreceptors, the nomenclature became more confused, with the proposed terms "chemodecton" (from the Greek *dechesthai*, to receive) and "chemodectoma," for parasympathetic paraganglia and their tumors.

Modern medicine has largely reverted to Kohn's initial concept, recognizing that differences between the paraganglia associated with sympathetic and parasympathetic nerves and the many stimuli and chemical pathways that control their functions are less distinct than previously thought. In particular, the ability to sense changes in oxygen concentration, which is a major function of the adult carotid body, is performed by abdominal paraganglia and developing adrenal chromaffin cells during fetal life [4, 5]. The difference in this respect is based on anatomic context. Catecholamine-secreting cells in the carotid body function in circuits that relay information to the central nervous system, while abdominal paraganglia release catecholamines directly into the circulation before neural circuits are established.

The first report of a patient with a pheochromocytoma, including clinical findings and a gross description of bilateral adrenal tumors, was probably by Charles Sugrue in 1800 [6]. The first histologic report was in a paper by Felix Fraenkel who provided a description of the classical signs and symptoms in a young woman named Minna Roll with bilateral adrenal tumors. On her sudden death, Professor Rudolf Maier performed the autopsy and Professor Max Schottelius performed the histological investigation. He described the brown appearance of the adrenal tumors after exposure to chromate-containing Mueller's fixative. Fraenkel postulated that the tumors secreted a chemical substance that was responsible for the patients hypertension and myocardial infarction [7]. Also of historical interest is the case of Mother Mary Joachim, a Catholic nun who had the first successful resection of a pheochromocytoma at the Mayo Clinic in 1927 [8]. The disease may have had historical relevance, since it was likely the source of erratic blood pressure in President Eisenhower [9], and it may have been responsible for the acrimony of the McCoy family that was responsible for the 30-year Hatfield-McCoy feud [10].

Anatomy and Embryology

The association between the distribution of normal paraganglia during development and paragangliomas in adult life was elegantly documented by Professor Rex Coupland in his classic 1965 book, *The Natural History of the Chromaffin Cell*, and his classic diagram remains essentially correct [11] (Fig. 12.1). During fetal development, paraganglia are associated with paraxial nerve fibers from almost all ganglia of the sympathetic nervous system (Fig. 12.2) and predominantly with branches of the vagus and glossopharyngeal nerves in the head and neck. Sympathetic paraganglia are most common in the vicinity of the preaortic nerve plexuses [11]. Most of these normal paraganglia either involute or are not readily found in adults because of their microscopic size. However, paragangliomas arise anywhere that normal

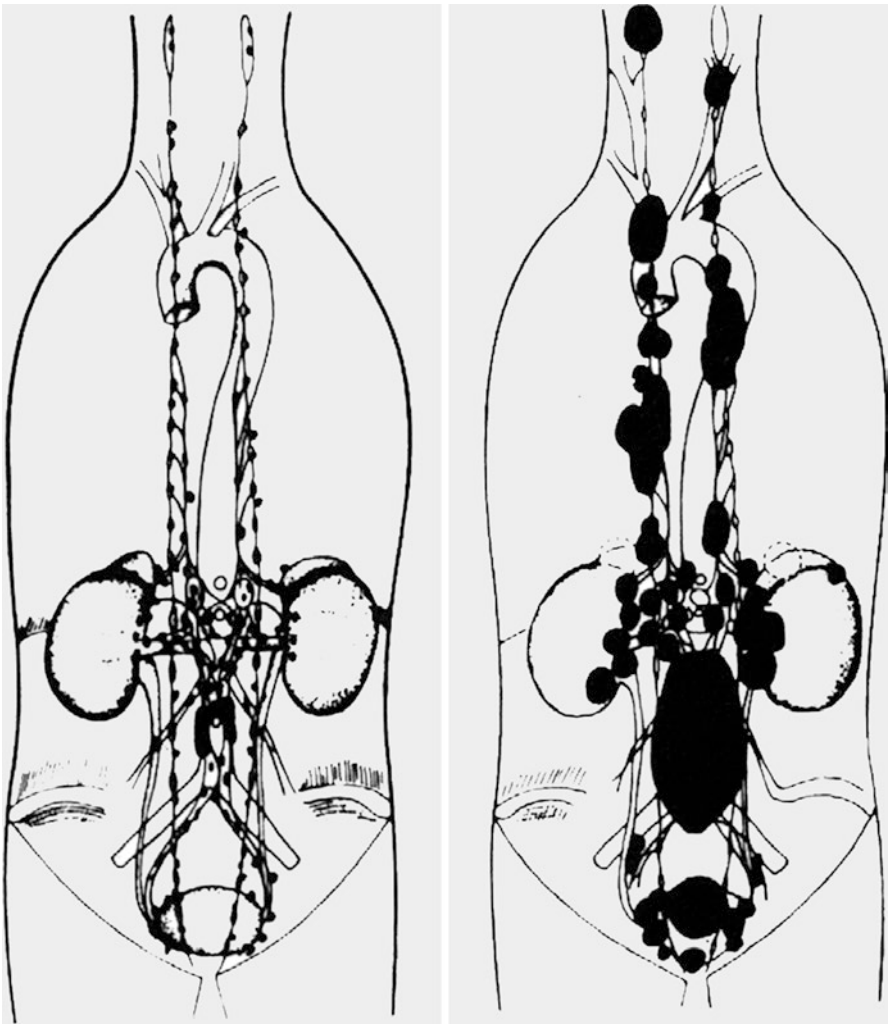

Fig. 12.1 Anatomy and embryology of paragangliomas. This classic illustration correlates the distribution of paragangliomas (right) with the mapped distribution of chromaffin tissue in early development (left). The figure, based on cases reported prior to 1960, is still largely correct. (From Coupland [11])

paraganglia have been identified during development and are most frequent in areas with the densest mapped distribution. They may be found in many locations throughout the entire torso as well as the head and neck and are most common near the inferior and superior mesenteric arteries and celiac plexus [12–16]. The largest single fetal paraganglion, and the only one that is not microscopic, is the organ of Zuckerkandl, at the origin of the inferior mesenteric artery. This structure, that was initially identified by Emil Zuckerkandl and now bears his name, was initially

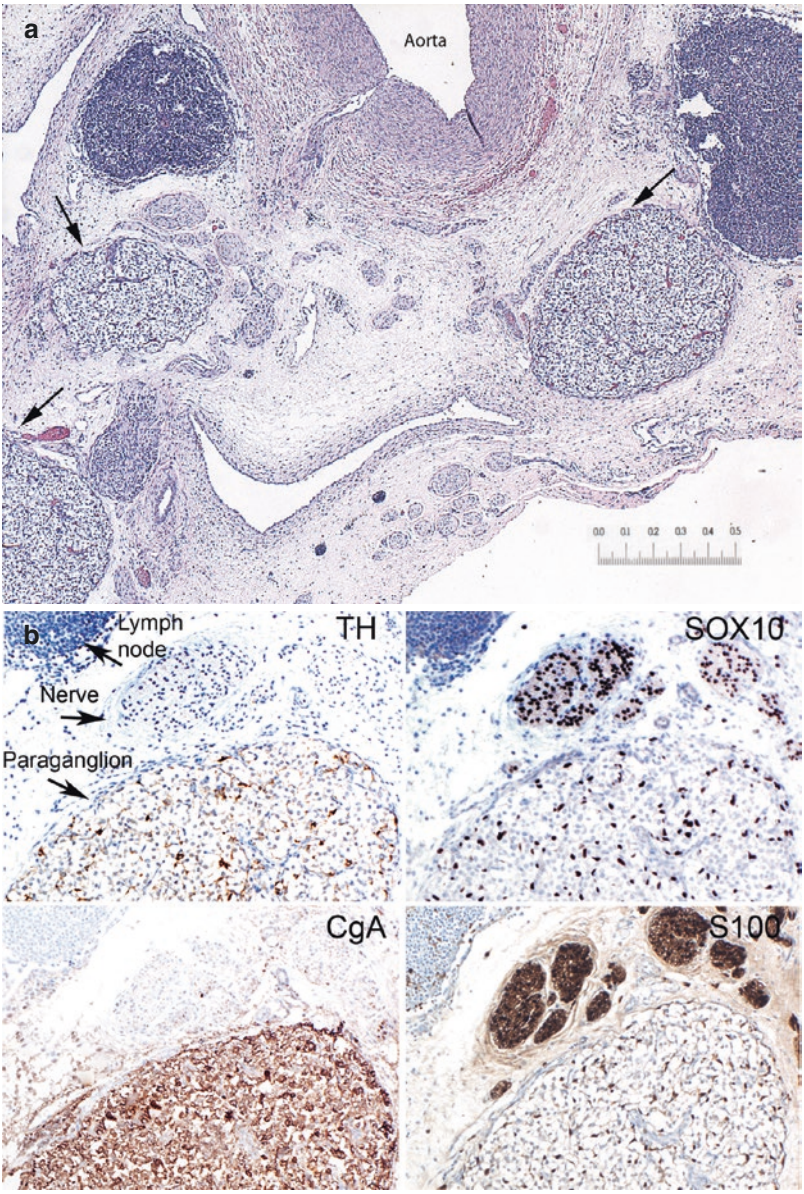

Fig. 12.2 Fetal paraganglia. (**a**) Multiple normal preaortic paraganglia (arrows) from a 19-week human fetus. Note the close proximity of paraganglia to developing lymph nodes. Bar = 200 microns. (**b**) Immunohistochemical markers of chromaffin cells and sustentacular cells in the paraganglia from the same fetus shown in (a). TH, tyrosine hydroxylase, CgA, chromogranin A. At this stage CgA is expressed in all of the chromaffin cells, while tyrosine hydroxylase is strongly expressed in a subset and is beginning in the rest. SOX10 and S100 are markers of Schwann cells in the nerve and of sustentacular cells in the paraganglion. (**c**) A small paraganglion adjacent to the fetal adrenal (same specimen as Figs. 12.2 and 12.3). The extra-adrenal paraganglia are fully formed before the adrenal medulla exists. The adrenal in this section shows only provisional (fetal) cortex and a small focus of primitive sympathetic cells (near top). (Reproduced with permission from: deKrijger [110])

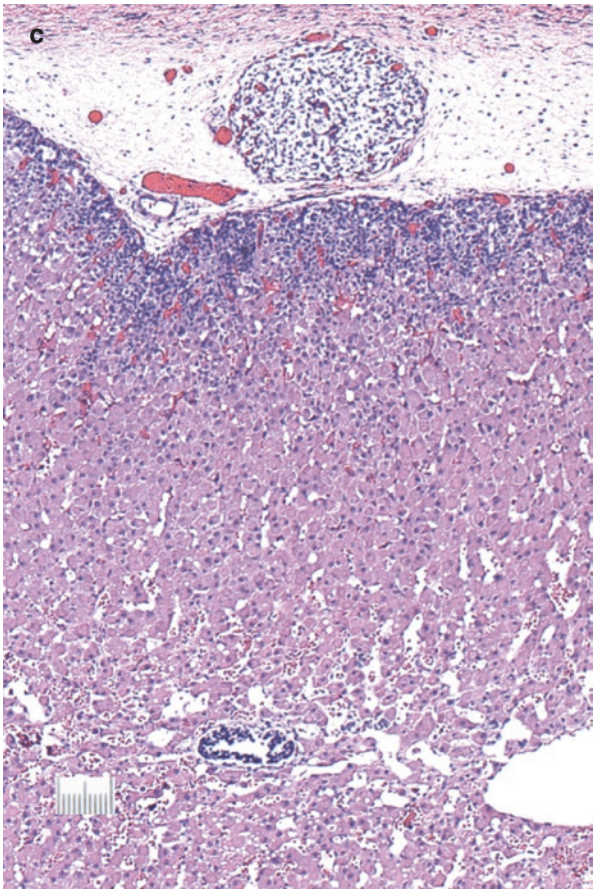

Fig. 12.2 (continued)

thought to be an unusual lymph node [17]. This early mistaken identity evokes a current clinical problem: since abdominal and pelvic paraganglia are found in the same prevertebral and paravertebral locations as lymph nodes, paragangliomas are often mistaken for lymphadenopathy on imaging to this day.

While paragangliomas have been reported in the extremities [12, 18], paraganglia are not normally identified in the limbs. The histogenesis of such tumors is enigmatic, and some reports may be based on incorrect diagnoses.

Paraganglionic neuroendocrine cells are derived from neural precursors, although recent studies indicate that the origin of those precursors is more complicated than previously believed. While their lineage ultimately traces back to the neural crest, it does so for the most part by an indirect route. According to the current concept, late neural crest progenitors that first form in the dorsal root ganglia migrate along sensory axons to join with preganglionic sympathetic axons emerging from the spinal cord and then reach the developing paraganglia with guidance from those axons [19–21]. The neuroendocrine cells in paraganglia all express neuroendocrine markers including synaptophysin and chromogranins [14, 22], and they produce

catecholamines as their principal hormonal product. Unlike other neuroendocrine cells that are epithelial, they do not express keratins.

By convention, tumors of these cells that arise in the adrenal medulla are called "pheochromocytoma" (from the Greek *phaios*, dusky + *chroma*, color), referring to the color change induced during the chromaffin reaction, whereas the functionally similar extra-adrenal tumors are classified as paragangliomas [23]. This distinction was initially an arbitrary convention introduced in the 1950 Armed Forces Institute of Pathology *Atlas of Tumor Pathology: Tumors of the Adrenal* as a way to impose order on increasingly diffuse nomenclature [24]. While it is still argued whether the divided nomenclature was a good decision [25], it is generally accepted that the adrenal medulla has distinct biological features. These include late development (Fig. 12.2) and the exclusive capacity to produce epinephrine. It is also the almost exclusive site of pheochromocytoma/paraganglioma development in patients with MEN2 syndromes.

Epidemiology

Pheochromocytomas and paragangliomas occur in about 2–8 per million persons per year; they are more common in hypertensive patients, where they are detected in about 0.1% of patients [26]. About 3% of these tumors are thought to occur in the head and neck [27], but this is not substantiated in all series [28]; they are thought to represent less than 1% of head and neck cancers [27]. It should be noted that while the terms "parasympathetic paragangliomas" and "head and neck paragangliomas" are often used interchangeably, this usage is not strictly correct. Paragangliomas can arise in association with the cervical sympathetic chain and superior cervical ganglion. The ciliary ganglion, which is postulated to be the origin of rare orbital paragangliomas [29], has mixed sympathetic and motor components.

Parasympathetic paraganglia arise along the glossopharyngeal and vagus nerves [13]. More than half of head and neck paragangliomas are carotid body tumors [27] that occur just superior to the bifurcation of the carotid arteries [12, 14, 15, 30]; the incidence of carotid body tumors is higher in populations living at high altitudes (higher than 2000 meters above sea level) [31]. The second most common site is in the middle ear, where they arise from multiple jugular and tympanic paraganglia known as the "glomus jugulare" and "glomus tympanicum" [12, 22]. Other sites include the larynx and along the path of the vagus nerve as it travels through the subclavian channel to innervate the lungs and heart near the bases of the great vessels or within the lung and in the cardiac septum.

Sympathetic paragangliomas are most frequent in the abdomen [13]. The most common site is in the adrenal medulla, which is the largest sympathetic paraganglion. The second most common site is the vicinity of the organ of Zuckerkandl, which itself involutes in the first few years of life.

Paragangliomas also occur in unusual locations where they are often misdiagnosed [12]. They can occur in the sellar region where they mimic pituitary neuroendocrine tumors, in the paranasal sinuses where they are misdiagnosed as olfactory neuroblastoma, and in the thyroid and parathyroid glands, where they can mimic

medullary carcinoma or parathyroid tumors. They occur in the mediastinum, within the heart and in the lungs, where they are often misdiagnosed as metastatic neuroendocrine tumors. Conversely, in the larynx, primary epithelial neuroendocrine tumors have been misdiagnosed as paragangliomas, probably accounting for the unusually aggressive behavior ascribed to paragangliomas in that location [32]. Rare examples in the gallbladder, liver, gut, and pancreas and mesentery are also often mistaken for primary or metastatic epithelial neuroendocrine tumors.

Tumor Classification and Morphology

The clinical features of paragangliomas usually are attributable to catecholamine excess when they are sympathetic type [33]; parasympathetic paragangliomas are considered to be clinically nonfunctioning; however, they may secrete dopamine or its metabolite, methoxytyramine [34, 35]. Catecholamine excess can result in paroxysmal tachycardia, hypertension, pallor, headache, and anxiety. An unusual presentation is micturition-induced paroxysms caused by urinary bladder paragangliomas. Paragangliomas may also present as a mass. In that situation, biopsy is not recommended, since the procedure can elicit a hypertensive crisis that can result in stroke and/or death. It is recommended that any patient with an adrenal mass or a mass lesion that is suspected to be a paraganglioma should undergo screening by measurement of urinary and plasma metanephrines and imaging with functional ligands such as ^{123}I-MIBG or labeled somatostatin analogs [36].

Pheochromocytomas vary in size and weight and have a distinctive pink-tan or hemorrhagic gross appearance (Fig. 12.3). Large tumors compress and attenuate the surrounding adrenal cortex. Rarely these tumors can be bright yellow and resemble a cortical adenoma; these unusual tumors have been reported in patients with von Hippel-Lindau syndrome who can have lipid degeneration in their paragangliomas

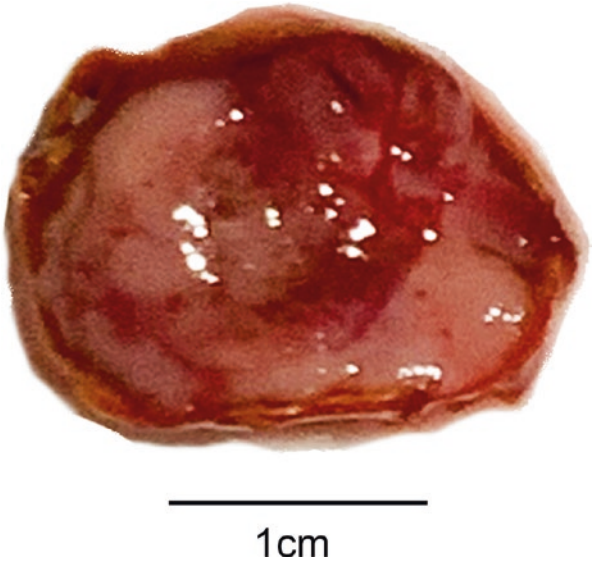

Fig. 12.3 Gross appearance of pheochromocytoma. The tumor has a dusky appearance compared with the surrounding yellow adrenal cortex

[37]. Paragangliomas vary from small tumors in the jugulotympanic region to large retroperitoneal and bladder masses (Fig. 12.4).

Microscopically, pheochromocytomas and paragangliomas are composed of nests known as "Zellballen" within a fibrovascular stroma (Fig. 12.5). The nests

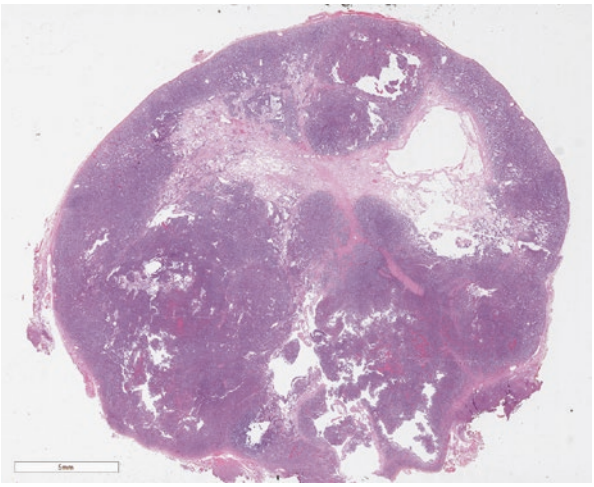

Fig. 12.4 Whole mount appearance of para-aortic paraganglioma. The tumor is well circumscribed and solid with focal stromal edema and fibrosis

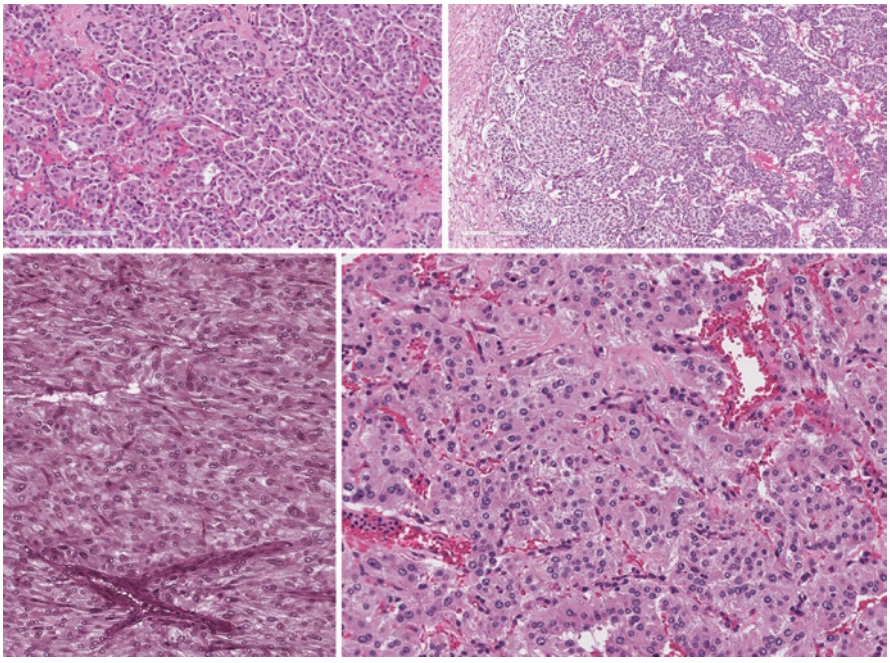

Fig. 12.5 Histology of paraganglioma/pheochromocytoma. A carotid body tumor (top left) is composed of nests of large epithelioid cells. A pheochromocytoma (top right) has characteristic zellballen architecture. Some pheochromocytomas have more spindle cell morphology (bottom left). Patients with SDHx have tumors with abundant eosinophilic cytoplasm (bottom right)

contain tumor cells that vary from large polygonal neuroid cells with abundant granular basophilic cytoplasm to round eosinophilic, chromophobic, or amphophilic epithelioid cells. Rarely, they can be elongated and spindle-shaped. The tumor cell nuclei are usually round with prominent nucleoli; some tumors have nuclear pleomorphism including bizarre large nuclei and/or multinucleate cells. Mitoses are usually inconspicuous and necrosis is not usually found. Hyaline droplets and amyloid stroma have been reported in some tumors [38], and occasional pheochromocytomas may have a focal inflammatory infiltrate.

Immunohistochemistry confirms neuroendocrine differentiation with cytoplasmic positivity for chromogranin (Fig. 12.6) and synaptophysin. Unlike epithelial neuroendocrine tumors (NETs), paragangliomas are usually negative for keratins. They express the nuclear transcription factor GATA3 [39–44] (Fig. 12.6). Immunolocalization of catecholamines, dopamine, adrenaline, and noradrenaline

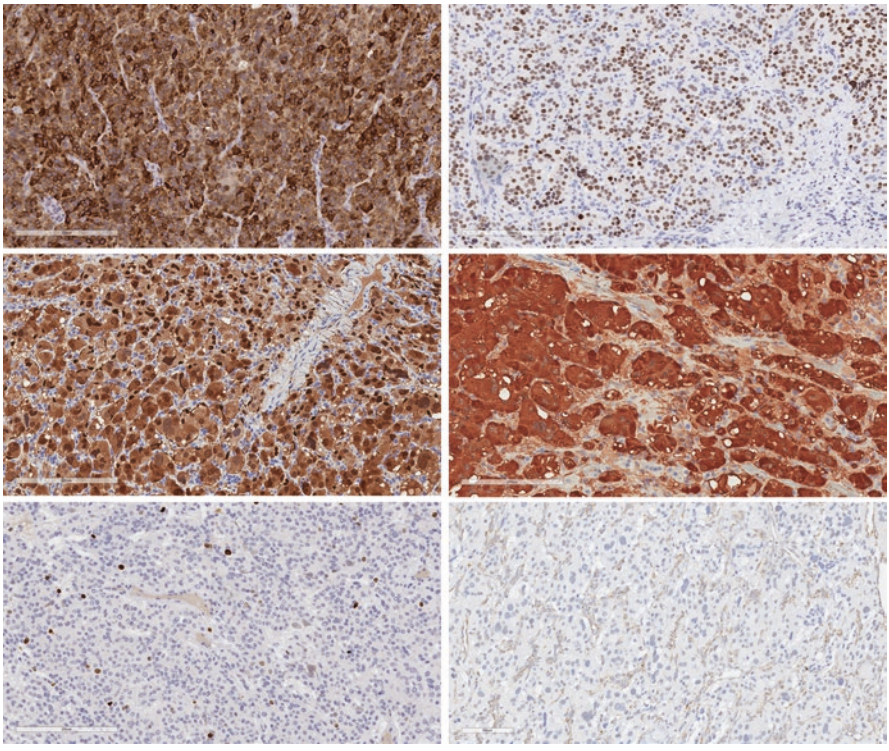

Fig. 12.6 Immunohistochemistry of pheochromocytoma and paraganglioma. The tumor cells have strong cytoplasmic expression of chromogranin (top left) and nuclear reactivity for GATA3 (top right). S100 decorates nuclei and cytoplasm of tumor cells and emphasizes sustentacular cells that are more intensely positive (middle left). Tyrosine hydroxylase is positive in the cytoplasm (middle right). The Ki67 labeling index is highly variable (bottom left). Loss of cytoplasmic SDBH is indicative of *SDHx*-related disease (bottom right); note granular positivity in endothelial cells

[45] is not in general use due to lack of reproducibility; however, the enzymes involved in catecholamine synthesis can be localized as functional markers [15, 33, 36, 46]. The initial step in the biosynthetic pathway is driven by tyrosine hydroxylase, the enzyme that converts L-tyrosine to L-DOPA. Therefore tyrosine hydroxylase immunolocalization (Fig. 12.6) has been implemented as a valuable tool for confirmation of the diagnosis [47]. While sympathetic paragangliomas are usually strongly positive for this enzyme, parasympathetic paraganglia, especially those of the head and neck, may be only focally positive or completely negative. In a 2015 study, 32% of head and neck paragangliomas showed immunoreactivity for tyrosine hydroxylase, even though only 1% of patients had biochemically detectable catecholamine hypersecretion [48]. The enzyme phenylethanolamine N-methyltransferase (PNMT), which converts norepinephrine to epinephrine, can be used to distinguish tumors that make epinephrine.

S100 protein decorates sustentacular stromal cells (Fig. 12.6); there may be positivity in tumor cells but it is usually weaker. The transcription factor SOX10 is a nuclear stain that will also recognize sustentacular cells [49]. Although the presence of sustentacular cells has been used as a diagnostic feature of paragangliomas, it should be noted that epithelial NETs also can have sustentacular cells [50, 51]; therefore, this is not reliable alone and must be considered in context with morphology and other markers. Currently, immunostaining for SDHB is recommended (Fig. 12.6) because of the high incidence of *SDHx*-related disease as discussed below in the section on molecular pathogenesis.

In patients with genetic predisposition to these tumors, they may be multifocal. Patients with MEN2 frequently have adrenal medullary hyperplasia (Fig. 12.7) either as a precursor lesion or associated with frank neoplasia [52], and this phenomenon has also been reported in patients with *SDHB, MAX*, and *TMEM127* germline mutations [53–56]. Tumors associated with von Hippel-Lindau (VHL) disease often have distinct morphological features including a thick fibrous capsule, stromal edema, and prominent clear cytoplasm (Fig. 12.8) [57]; occasional *VHL*-related pheochromocytomas exhibit massive lipid degeneration that causes them to resemble cortical lesions [37]. They also express membranous carbonic anhydrase IX [58]. Tumors associated with SDHx disease may have granular eosinophilic

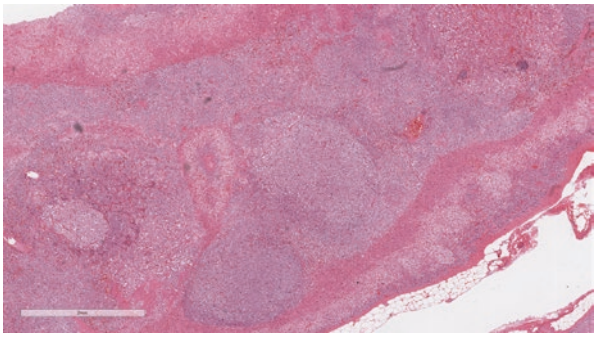

Fig. 12.7 Adrenal medullary hyperplasia in MEN2. The adrenal medulla is diffuse and nodular. The nodules represent micro-pheochromocytomas arising in the background of adrenal medullary hyperplasia

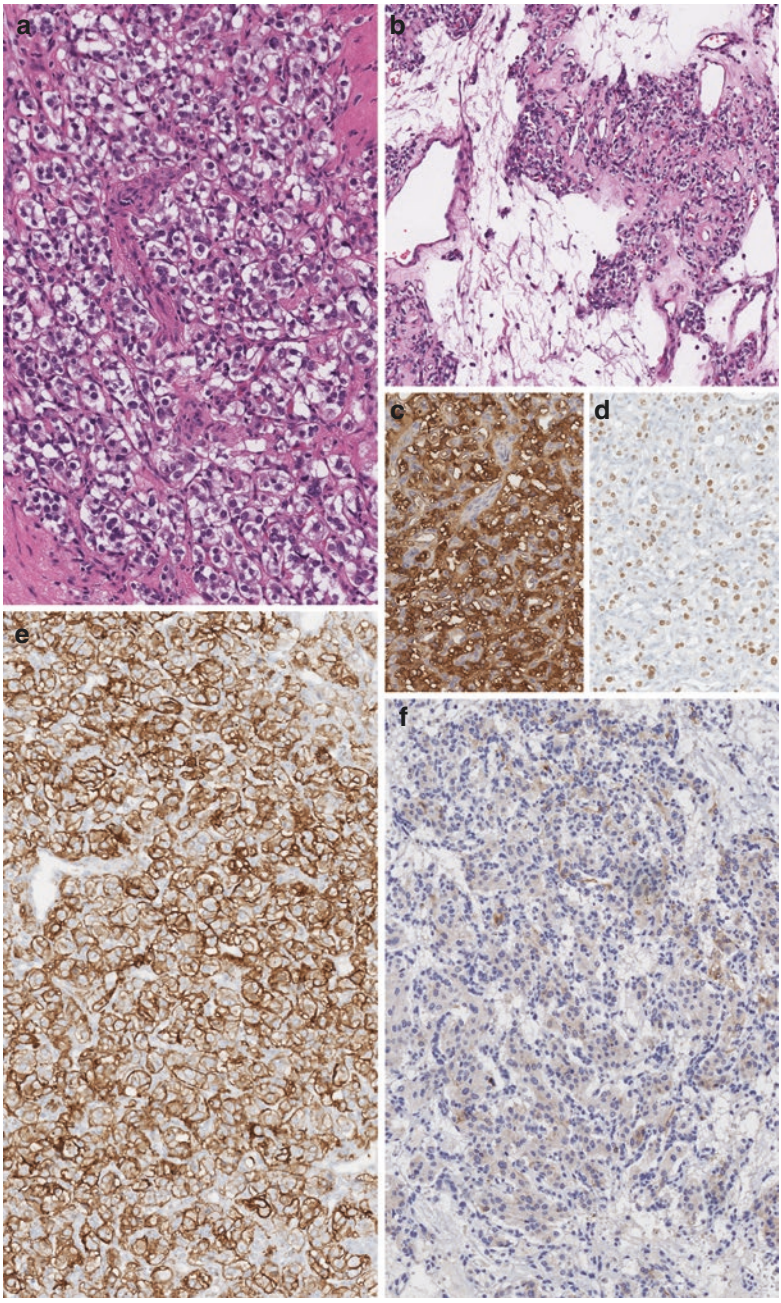

Fig. 12.8 VHL-related pheochromocytoma. In patients with VHL disease, pheochromocytomas often display variable clear cell change (**a**) and vascular stroma with myxoid (**b**) and/or hyaline change. Prominent clear cell change can be mistaken for an adrenal cortical proliferation in some cases. Staining for chromogranin A, tyrosine hydroxylase (**c**), and GATA3 (**d**) distinguishes these tumors from adrenal cortical proliferations as well as other clear cell neuroendocrine tumors that can be seen in affected patients. Membranous carbonic anhydrase IX (**e**) is also another feature of *VHL*-driven pathogenesis. Variable alpha-inhibin expression is seen in some tumors (**f**)

cytoplasm (Figs. 12.5 and 12.9), show loss of SDHB, and express alpha-inhibin [59] but generally do not express carbonic anhydrase IX.

Other immunohistochemical studies depend on clinical features, such as possible ectopic expression of other peptide hormones such as serotonin that can give rise to carcinoid syndrome, ACTH, and/or CRH that can cause Cushing's syndrome [60, 61], GHRH that can cause acromegaly [62], and VIP that may result in watery diarrhea [63].

The classification of primary pheochromocytomas and paragangliomas as benign or malignant has been a controversial subject. Prior to 2017, the WHO definition of malignancy was based on the presence of metastases. However, prediction of metastatic behavior was and remains an issue. The Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) was developed to define malignancy prior to the new

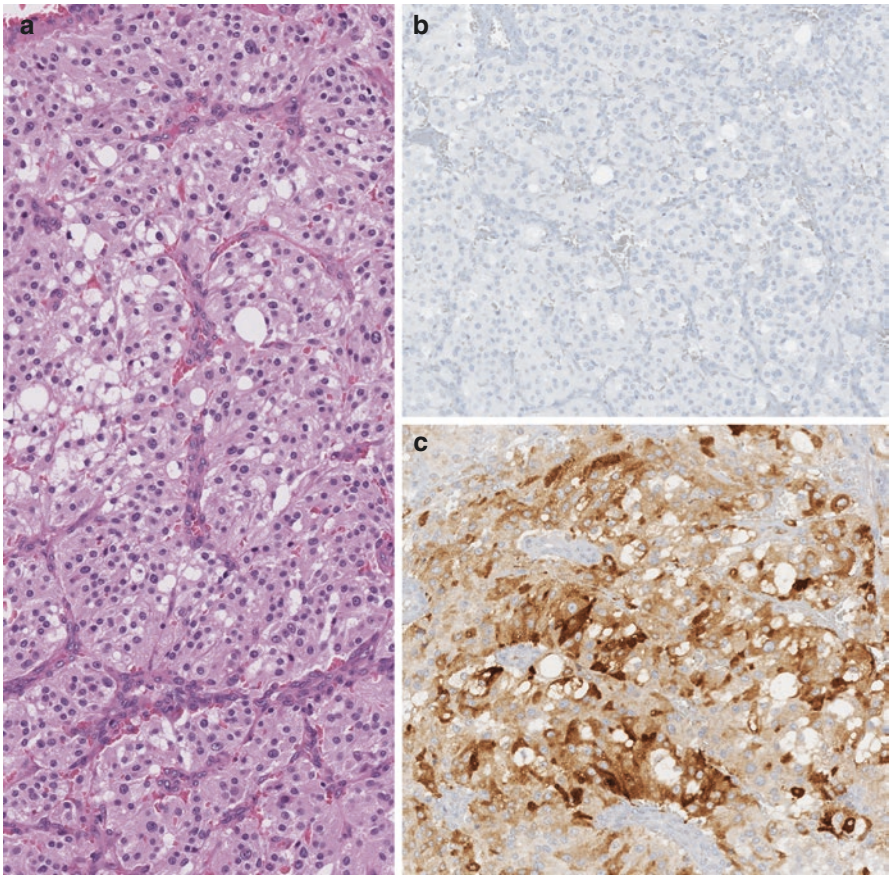

Fig. 12.9 Succinate dehydrogenase (SDH)-immunodeficient paraganglioma. This para-aortic paraganglioma arising in a patient with germline *SDHB* mutation has a characteristic histologic appearance with abundant granular eosinophilic cytoplasm and variable intracytoplasmic vacuoles (**a**). The tumor cells are negative for carbonic anhydrase IX (**b**) but express alpha-inhibin (**c**)

classification [64]. Scoring is based on the presence of invasion of vessels (score = 1), tumor capsule (score = 1), and/or periadrenal adipose tissue (score = 2), the presence of large nests or diffuse growth (score = 2), high cellularity (score = 2), monotonous cytology (score = 2), spindle cell morphology (score = 2), marked nuclear pleomorphism (score = 1), nuclear hyperchromasia (score = 1), tumor necrosis (score = 2), mitotic figures (>3/10 high-power fields; score = 2), and atypical mitoses (score = 2). A score ≥4 identifies an aggressive tumor. However the subjectivity of these features resulted in lack of concordance between expert pathologists [65]. The Grading system for Adrenal Pheochromocytoma and Paraganglioma (GAPP) was designed to address the point that PASS was designed only for adrenal tumors and also incorporated the Ki67 proliferation index as well as the profile of catecholamine production [66]. This system achieves a score from 0 to 10; tumors are classified as well differentiated (0–2 points), moderately differentiated (3–6 points), and poorly differentiated (7–10 points). However concordance between pathologists has not been tested. Critiques of the GAPP system note practical and conceptual difficulties posed by the way biochemical data are incorporated into GAPP grade and also question whether differentiation is truly measured by the GAPP parameters [67]. A 2019 meta-analysis of multiple papers concludes that a low score with either PASS or GAPP is a strong predictor of low metastatic risk but that high scores have little predictive value in the absence of adjunctive markers [68]. Neither system takes into account molecular data that may be prognostic; for example, adrenal tumors with *MAML3* fusions are thought to be more aggressive [69], and germline *SDHB* mutation predisposes to metastatic behavior [70]. Early studies suggested that GAPP can provide prognostic information for patients with *SDHB* mutations [71], and a third proposal added SDHB immunohistochemistry to the criteria proposed in the PASS and GAPP systems [72]. The Ki67 proliferation index (Fig. 12.6), which is one element in the GAPP system, may have predictive value alone [69, 73], but this remains to be proven and these tumors are not classified into grades based on proliferation like the epithelial well-differentiated neuroendocrine tumors [74]. New immunohistochemical markers such as ATRX and TERT may have independent prognostic value [68].

In 2017, the WHO classification finally abandoned the binary classification, considering that all pheochromocytomas and paragangliomas have the potential to metastasize. This new paradigm requires an approach based on risk stratification, although the parameters for stratifying risk are still in flux. In addition, the term "malignant" is replaced by "metastatic," eliminating the potential for competing definitions. The WHO criteria state, "metastatic deposits should only be considered as such at sites where normal chromaffin tissue is not present, in order to avoid the misclassification of multicentric primary tumors as metastases" [75]. However, even the diagnosis of a paraganglioma as metastatic is complicated and controversial. Confirmation of metastatic spread can be almost impossible in some patients with germline predisposition to these tumors who can develop multifocal primary lesions even in unusual sites such as the lung or the hilum of the liver and in paravertebral regions that radiologically mimic lymph nodes. Despite the classic distribution of paraganglia and paragangliomas, it must be remembered that normal and

neoplastic paraganglia do occasionally occur in outlier sites. The only paraxial sites where there are no native paraganglia are the brain and bone and within lymph nodes. Lack of sustentacular cells or a reduced number of these cells has been reported as a feature of metastases [76].

Rare and unusual tumors classified as paragangliomas may stain for keratins [77–80]. These curious lesions fall into two categories. The so-called gangliocytic paragangliomas are triphasic neoplasms with a keratin-positive epithelial cell population, a neural component, and stroma; they have no firm evidence of paraganglionic differentiation and the term is therefore likely to be a misnomer. They occur most frequently in the duodenum, but also have been reported in the nasopharynx, esophagus, appendix, and lung [81]. Tumors that occur in the region of the cauda equina include both typical keratin-negative paragangliomas [13] and occasional tumors that express keratins; the latter may be due to aberrant ependymal differentiation or may represent examples of epithelial neuroendocrine tumors in an unusual location.

Composite pheochromocytomas and paragangliomas are rare neoplasms of the paraganglia (Figs. 12.10 and 12.11) [15, 75]. The term "composite" is applied to a paraganglial neoplasm combining features of pheochromocytoma or paraganglioma

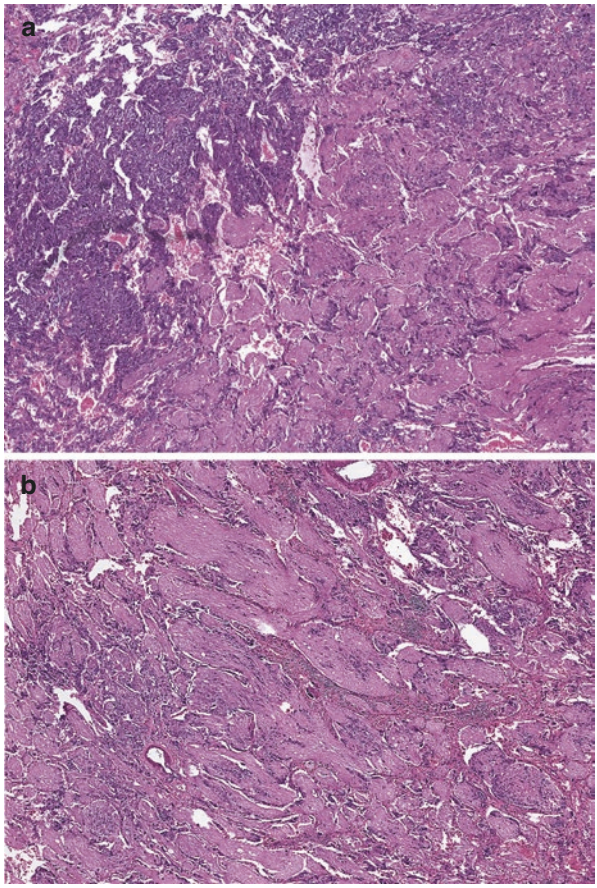

Fig. 12.10 Composite pheochromocytoma. This photomicrograph illustrates a composite tumor with features of pheochromocytoma (**a**) and a schwannian stroma-poor, differentiating neuroblastoma (**b**) with low mitotic-karyorrhectic index (**a**, **b**)

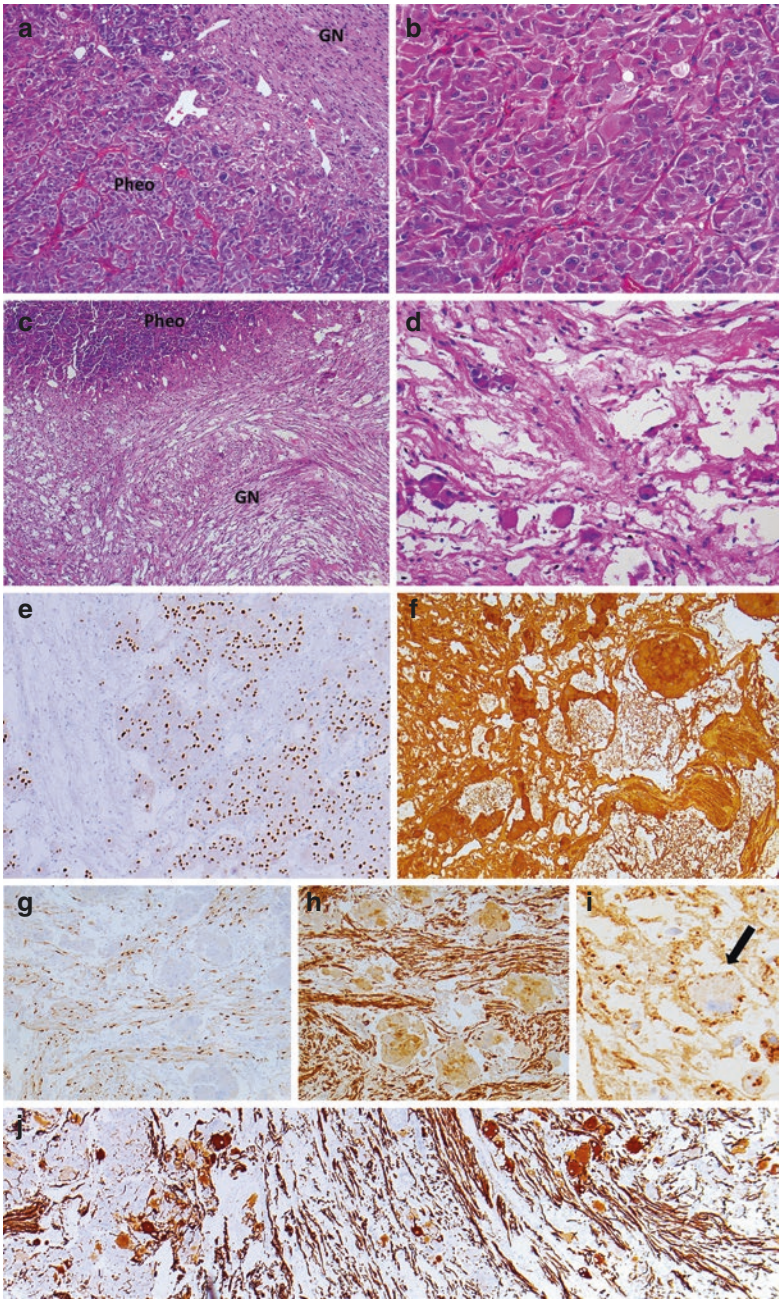

Fig. 12.11 Composite pheochromocytoma. This tumor is composed of a pheochromocytoma (**a**–**c**) with elements of ganglioneuroma (**a**, **c**, **d**) (Abbreviations: GN ganglioneuroma and Pheo pheochromocytoma). GATA3 is negative in the schwannian stroma, whereas chromaffin cells are diffusely positive (**e**). Tyrosine hydroxylase stains all components of this composite tumor (**f**). SOX10 (**g**) and S100 (**h**) highlight schwannian stroma. While chromogranin A is diffusely positive in chromaffin cells, ganglion cells (arrow) are either negative or weakly positive (**i**). Neurofilament (NF) expression is illustrated in the ganglioneuroma component (**j**)

with those of neuroblastoma, ganglioneuroblastoma, ganglioneuroma, and malignant peripheral nerve sheath tumor. The composite elements are further assessed based on the amount of schwannian stroma and the presence or absence of neuroblastic and ganglionic differentiation within the mass. Therefore, adequate sampling is essential in the accurate subtyping of these tumors.

Molecular Pathogenesis

The pathogenesis of pheochromocytomas and paragangliomas has seen extensive study in the last few decades, and it has become clear that they have an exceedingly high incidence of hereditary predisposition. The old dogma stating that 10% of pheochromocytomas were familial has been replaced by evidence that more than 40% of patients with any paraganglioma carry germline mutations [69, 82–84]. These include the classic *RET* mutations in MEN2, mutations in *VHL* in syndromic von Hippel-Lindau disease, and mutations of *NF1* in neurofibromatosis type 1. However the most common mutations result in destabilization of the succinate dehydrogenase (SDH) enzyme complex; this multiprotein complex is composed of four subunits, SDHA, SDHB, SDHC, SDHD, and two assembly factors, SDHAF1 and SDHAF2. Pathogenic mutations occurring in any of the genes encoding these components have been reported, but the most common are in *SDHB*. Rare mutations occur in *TMEM127 (t*ransmembrane protein 127), *FH* (fumarate hydratase), *MAX* (MYC-associated factor X), *HIF2A (*hypoxia-inducible factor 2 alpha) or *EPAS1*, *PHD1* (prolyl hydroxylase) (also known as *EGLN2*), *EGLN1* (formerly known as PHD2), *BAP1* (BRCA1-associated protein-1), *KIF1Bβ* (kinesin-like protein), *KMT2D* (histone-lysine N-methyltransferase 2D; also known as MLL2), and *DNMT3A* (DNA cytosine-5-methyltransferase 3A) [84–89]. The diagnosis of genetic predisposition is important, as it can prevent complications in affected family members [90, 91]. It also indicates that new lesions are more likely to be distinct primary tumors rather than metastatic foci, unlike in sporadic disease where metastasis is more likely.

The various genetic alterations correlate with clinical and biochemical features that can be classified in specific clusters [15, 33, 84, 92]. Cluster 1 disease is associated with alterations in the "pseudohypoxic pathway" involving *SDHx, VHL, HIF2a, FH, MDH2, PHD1/EGLN2,* and *PHD2/EGLN1;* patients have predominant dopaminergic and/or noradrenergic secretory profiles. Cluster 2 disease due to activated kinase receptor signaling from mutations in *RET, TMEM127, MAX, NF1,* or *KIF1Bβ* is characterized by adrenergic or mixed noradrenergic and adrenergic secretion. A small group of sporadic pheochromocytomas with *MAML3* fusions causing activated WNT signaling also have mixed noradrenergic and adrenergic secretory profiles and fall into cluster 2 disease [69, 93].

The presence of adrenal medullary hyperplasia alone or associated with a pheochromocytoma has been recognized to be of clinical significance in that it may predict a pathogenetic germline mutation. This feature has been reported in patients

with *RET* mutations and MEN2 [52] and also in patients with *SDHB, MAX*, and *TMEM127* germline mutations [53–56].

Immunohistochemistry is a useful and inexpensive tool to assist in the diagnosis of genetic predisposition in patients with paragangliomas. Any *SDHx*-related disease results in destabilization of the multiprotein SDH complex; for this reason, loss of immunoreactivity for SDHB is now accepted as a standard screening tool [94]; when negative, additional stains for SDHA or SDHD can be added to specify *SDHA*- or *SDHD*-related disease, respectively [95–97]. Other more rare genetic alterations can also be identified by immunohistochemistry; loss of FH or MAX characterizes disease due to mutations in these genes in at least some cases [83, 94, 98]. Staining for carbonic anhydrase IX (CAIX) has been associated with *VHL*-related paragangliomas [58], and recent data suggest that alpha-inhibin expression is found in patients with this or other genetic alterations affecting the hypoxia pathway [59].

Prognosis

The prognosis of pheochromocytomas and paragangliomas is extremely variable and depends on the location and size of the tumor, the pathogenetic mutation involved, and the presence or absence of germline predisposition [70, 99]. These tumors are no longer classified as benign and malignant [23, 100]. Multifocal or progressive disease even without metastasis can cause significant morbidity and even mortality.

In patients with sporadic tumors that are small and located in a surgically accessible site, complete resection is usually curative. However, some sporadic tumors are locally aggressive, and when invasive of structures that are not amenable to resection, they may require more aggressive therapies. In patients with genetic predisposition, multifocal disease can mimic metastases, and in some patients, the extent of multifocal primary lesions precludes surgical resection. Patients with bilateral adrenal disease may require cortical-sparing bilateral adrenalectomy [101].

The diagnosis of metastatic disease must be made with great caution. Because paraganglia exist at almost any site within the head, neck, and torso, the presence of multifocal disease even in an unusual location such as the lung or liver must be given due consideration as multifocal primary tumors in patients with known germline predisposition [12, 75]. Only bone, brain, and lymph node deposits qualify as bone fide metastases in this situation. In a patient with a solitary aggressive lesion, liver and/or lung deposits may be considered evidence of metastasis in the absence of an underlying germline mutation. Metastatic behavior is most common in sporadic pheochromocytomas with *MAML3* fusions, in *SDHB*-associated disease, and in tumors with *ATRX* and *CSDE1* mutations [13, 69, 70, 84, 99]. TNM staging was introduced in the most recent AJCC guidelines (eighth edition) [102]; however, the clinical relevance of this algorithm remains to be validated.

Several of these genetic alterations also predispose to other neoplasms. Patients with *SDHx* disease are at risk of developing gastrointestinal stromal tumor and renal cell carcinoma. Pituitary neuroendocrine tumors have also been reported, but they are not always SDH-deficient and may be coincidental [103–106]; this may also be the case for thyroid and adrenal cortical carcinoma [106]. It has also been questioned whether a pancreatic neuroendocrine tumor reported in this setting may have been a paraganglioma [106]. Immunohistochemistry for SDHB plays a critical role in discriminating between chance tumor associations and true manifestations of SDHx-related disease, while other immunohistochemical studies are required to distinguish paragangliomas from epithelial neuroendocrine tumors. Patients with von Hippel-Lindau disease may develop retinal and central nervous system (CNS) hemangioblastomas, serous cysts in the pancreas, and renal cysts as well as renal cell carcinomas [107] as well as multifocal endocrine proliferations and neoplasms in the pancreas [108, 109].

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Neuroendocrine Neoplasms of the Breast

13

Silvia Uccella, Giovanna Finzi, Stefano La Rosa, and Fausto Sessa

Background

Neuroendocrine differentiation in breast neoplasms has been a matter of discussion since the first description of a neuroendocrine neoplasm (NEN) in this site [1, 2]. In fact, NENs of the breast (Br-NENs) represent a less well-defined group of neoplasms than analogous entities in other organs, such as the lung and the gastroenteropancreatic (GEP) tract. Pure neuroendocrine phenotype is extremely rare, both for the well- and for the poorly differentiated morphology. In contrast, the expression of neuroendocrine markers in otherwise typical breast carcinomas, both of special and of non-special type, without morphologically evident neuroendocrine differentiation is more common. Consequently, the diagnostic criteria and the classification scheme for Br-NENs have been continuously changing over time and real consensus on this topic is still lacking. In this chapter, we recapitulate the evolution of the concept of Br-NEN; review the available knowledge on their morphological, molecular, and clinical features; and critically discuss the current classification scheme.

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The History of Br-NEN Concept: An Unclosed Circle

The presence of neuroendocrine differentiation in breast neoplasms, i.e., the observation of histopathological, ultrastructural, histochemical, and/or immunohistochemical features that define neuroendocrine cells has been first suggested in 1963, when two cases of breast carcinoma with a "carcinoid growth pattern" were described by Feyrter and Hartmann [1]. Those cases were identified in a series of mucinous carcinomas of the breast using an argyrophilic reaction [1]. Several years later, Cubilia and Woodruff published a series of eight "carcinoids" of the breast, reporting their ultrastructural and immunohistochemical features [2]. Unfortunately, despite claiming that the "carcinoid" nature of the proliferation should have been histologically suspected, they neither specified the morphological criteria to recognize the neuroendocrine nature of the proliferation nor gave clues for the qualitative or quantitative diagnostic evaluation of the histochemical, immunohistochemical, and ultrastructural aspects of neoplastic cells. Subsequent ultrastructural studies helped to better characterize the morphology of neuroendocrine granules in breast neoplasms, which are described as small (150–300 nm diameter) membrane-bound secretory granules, showing a dense homogenous core surrounded by a narrow clear halo [3–5] (Fig. 13.1). In 1990, Capella and coworkers were able to recognize five types of neuroendocrine granules, showing distinct shape and size, as well as synaptic-like vesicles in a series of 24 cases of breast carcinoma with recognized histochemical,and immunocytochemical neuroendocrine differentiation [6] (Fig. 13.2). The advent of immunohistochemistry allowed to confirm the neuroendocrine phenotype of a subset of breast carcinomas, by showing their immunoreactivity to chromogranin A [7] and demonstrating the expression of several hormones, such as ACTH, bombesin, serotonin, hCG, prolactin, gastrin, VIP, leu-enkephalin, pancreatic polypeptide, beta-endorphin, Sub-P [8], and neurotensin [9]. In a few

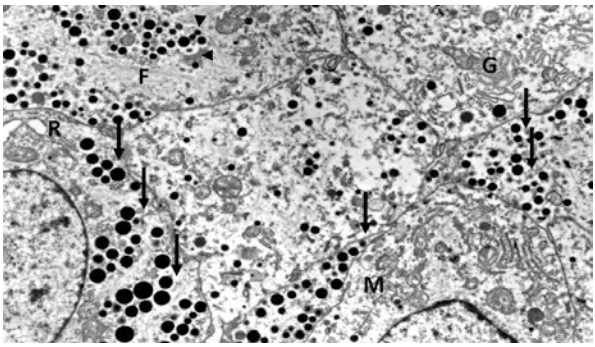

Fig. 13.1 The ultrastructural hallmark of endocrine differentiation in breast neoplasms is the presence of small (150–300 nm diameter) membrane-bound secretory granules, showing a dense homogenous core surrounded by a narrow clear halo (arrows). Neoplastic breast cells also contain abundant endoplasmic reticulum (R) and Golgi apparatus (G), numerous mitochondria (M), and abundant cytoplasmic filaments (F), also arranged in paranuclear whorls

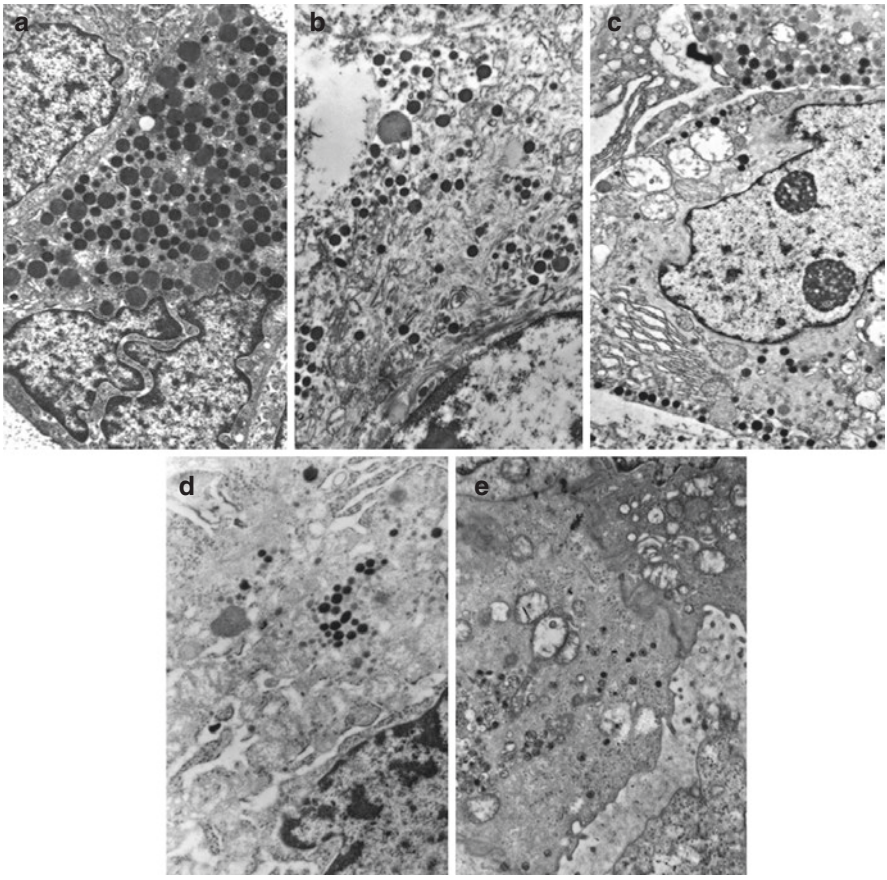

Fig. 13.2 Five types of secretory granules (**a**, type I; **b**, type II; **c**, type III; **d**, type IV, **e**, type V) have been described in neuroendocrine carcinoma of the breast, showing different sizes, contents, and shapes

years, it became evident that ultrastructural, histochemical, and immunohistochemical features reminiscent of the neuroendocrine phenotype were present in a wide morphological spectrum of breast carcinomas, spanning from "carcinoid-like" tumors, through small cell neuroendocrine carcinoma, to morphologically ordinary carcinomas of no special as well as of special types [8, 10].

In fact, the morphological features of Br-NEN have never been univocally characterized, leading to the concept that neuroendocrine differentiation, rather than defining specific entities, was in most cases to be regarded as a variant aspect of other non-neuroendocrine tumors [11]. Nevertheless, the WHO classification of tumors of the breast and of female genital organs, published in 2003, included, for the first time, the category of neuroendocrine tumors of the breast, stating that *"Primary neuroendocrine (NE) carcinomas of the breast are a group, which exhibit morphological features similar to those of NE tumors of both gastrointestinal tract*

and lung. They express neuroendocrine markers in more than 50% of the cell population" [12]. The reference to the morphology of pulmonary and digestive NENs evokes well-known organoid structures (nests, trabeculae, pseudoglands, etc.), for well-differentiated neoplasms, or small and large cell patterns, for poorly differentiated ones. Actually, the poorly differentiated neuroendocrine carcinoma of the breast, which is exceedingly rare, was described in analogy with its more common pulmonary counterpart. In contrast, the putative well-differentiated tumors were less well defined and explicitly included mucinous (claimed to represent 26% of the total) and solid papillary carcinomas, as well as other subtypes of various *"cell types, grade, and degree of differentiation"* with neuroendocrine marker expression [12]. As for prognostic and predictive factors, except for poorly differentiated neuroendocrine carcinomas, for which an ominous prognosis was reported in any case, the WHO extensors stated that neuroendocrine neoplasms of the breast should be graded, subtyped, and treated just as non-neuroendocrine carcinomas [12]. Almost 10 years later, the fourth edition fascicle of the classification of tumors of the breast changed the nomenclature to *carcinomas with neuroendocrine features* and deleted the 50% threshold for the neuroendocrine marker-positive neoplastic cells [13]. The definition confirmed the "*morphological features similar to those of NE tumors of both gastrointestinal tract and lung,"* but also admitted that *"other invasive breast carcinomas of no special type, and some special variants, may show neuroendocrine differentiation."* In addition, three subtypes where recognized: well differentiated, neuroendocrine tumor; neuroendocrine carcinoma, small cell/poorly differentiated carcinoma; and invasive breast carcinoma with neuroendocrine differentiation [13]. While small cell/poorly differentiated carcinoma was still described as morphologically undistinguishable from its counterpart in the lung, for neuroendocrine tumors, well differentiated, it was stated that *"the classic features of carcinoid tumors of the gastrointestinal tract or lung […] are not features of neuroendocrine carcinomas of the breast,"* and this was in some contrast with the definition of the entity itself [13]. Invasive breast carcinoma with neuroendocrine differentiation included mucinous and solid papillary carcinomas, as well as no special and special types of breast carcinomas. In the following years, this classification proved to be poorly efficient in delineating real entities, with well-defined clinicopathological features. Indeed, neither the epidemiological frequency, nor the molecular pathways, nor even the prognosis of breast carcinomas with neuroendocrine differentiation has ever been clearly defined, except, in part, for poorly differentiated neuroendocrine carcinomas [14].

The second decade of this century assisted to an increasing awareness, of both pathologists and oncologists, of the importance of the careful and reproducible classification of NENs of the various organs. In the light of the increasing clinical and diagnostic experience and of new knowledge provided by high-throughput technologies used in genomics, the classification of gastroenteropancreatic (GEP) tumors was revised in order to identify specific clinicopathological entities, each with a defined morphological, immunophenotypical, genetic, and clinical profile [15, 16]. At the same time, both from the pathologists' and from the oncologists' point of view, the need was felt for a common framework for the nomenclature and

classification of neoplasms arising in extra-GEP organs but showing overlapping morphological features with GEP NENs. Based on these starting points, in 2018 a group of expert endocrine pathologists, with competences in the various fields of organ pathology, under the aegis of WHO and IARC, published a consensus proposal for the homogenous classification of NENs of all sites [17]. As for breast NENs, the panel recognized that, except for poorly differentiated NEC, NENs of the mammary gland were a poorly defined category and the neuroendocrine phenotype did not have clinical significance [17]. The last (for the moment) episode of breast NENs story has been written in the fifth edition of the WHO classification on breast tumors, in which the chapter *Neuroendocrine neoplasms* has been introduced, including only neuroendocrine tumor and neuroendocrine carcinoma. Invasive and in situ carcinomas of the breast with neuroendocrine differentiation, also including mucinous carcinomas and solid papillary carcinomas, have been removed from this heading and replaced in the appropriate no special or special subtypes [18]. Again, this classification emphasizes that NEC of the mammary gland is a well-defined, with specific morphological, immunophenotypical, and clinical, feature, which is similar to those of small cell lung neuroendocrine carcinoma, whereas neuroendocrine tumor has far less well-distinct borders, from both a morphological and a clinical point of view [19]. In the end, many unanswered questions (in morphological, pathogenetic, and clinical terms) remain about the definition of well-differentiated neuroendocrine tumors of the breast, even the one about their existence itself.

Br-NENs: Who's Who?

In this section, we will try to recapitulate the entities that, along time, have been identified as Br-NEN and to discuss their being entitled to belong to such category.

Neuroendocrine Carcinoma of the Breast (Br-NEC)

Poorly differentiated Br-NENs are very rare, as they represent less than 0.1% of all breast malignancies and a very small fraction of extrapulmonary NECs. Only case reports and small series are reported in literature [19–21]. Most frequently, their morphology is of the small cell type and closely resembles small cell NEC of the lung. Compared to the latter, their age of insurgence is younger (mean < 60 years), whereas it parallels that of patients with other types of breast cancer [20]. Despite being a very aggressive neoplasm, with a higher rate of advanced disease than non-neuroendocrine carcinomas of the breast, the rate of metastatic disease at presentation is lower than that of its pulmonary counterpart (42% vs 65%) [20].

A significant number of Br-NECs are associated with invasive or in situ carcinoma of the breast, configuring a neuroendocrine/non-neuroendocrine neoplasm (MiNEN) [22]. The etiopathogenesis of Br-NEC is still largely unknown; however, its frequent association with a non-neuroendocrine component suggests that, akin to

NEC of the intestine and lung, they may share the early step of cancerogenesis with autochthonous carcinomas, especially carcinoma of no special type (NST). Indeed, besides showing alterations of *TP53* and *RB* genes, similar to NECs of other sites, Br-NEC, like non-endocrine breast carcinoma, harbors *PIK3CA* mutations in a third of the cases, which is not present in pulmonary NEC [20]. In addition, Br-NECs express high levels of ER and AR in about 30% and 15% of the cases, respectively, which are almost absent in lung NEC [20, 22].

General neuroendocrine markers synaptophysin and chromogranin A are expressed with the same pattern seen in other pulmonary and extrapulmonary NECs, and Ki67 proliferation index is very high [19]. Interestingly, hyperexpression and amplification of HER2 gene are not reported in Br-NEC [20, 23]. The differential diagnosis of Br-NEC is mainly represented by metastatic NEC of other sites. In this context, the use of site-specific immunohistochemical markers is not useful, as both transcription factors and other markers may be abnormally expressed in NECs [24]. The finding of in situ or invasive carcinoma of the breast is a strong clue to the primary origin of the neoplasm from the mammary gland [19, 22]. In absence of morphological hints, a comprehensive clinical and radiological study of the patient is needed to exclude a metastatic NEC.

The prognosis of patients with Br-NEC is poor, in terms of both overall and disease-free survival, and the poorly differentiated neuroendocrine nature of the neoplasm is an independent prognostic factor when compared with stage- and grade-matched non-endocrine breast carcinomas [25]. Intriguingly, when compared to small cell NEC of the lung, Br-NEC seems to have a better prognosis, especially in localized disease [21]. We can speculate that this may be due either to the intrinsic lesser aggressiveness of Br-NEC or to the more efficient screening programs for breast than for lung cancer. The first choice treatment in localized Br-NEC is surgery, and radiotherapy has a role in controlling the disease [21, 25]. As for advanced disease, some cases have been treated with platin and etoposide-based regimens, in analogy with lung NEC, whereas in others a taxane and anthracycline-based chemotherapy has been attempted, but no consensus on a standard treatment has been reached, due to the small number of cases [26, 27]. Anecdotical cases treated with hormone therapy have been reported [28].

Despite the small number of reported cases, Br-NEC is overall a well-defined entity, showing analogies with pulmonary and extrapulmonary NECs, not only at the morphological and clinical level but also looking at the pathogenetic pathways, which seem to recapitulate, in the early steps, those of the autochthonous non-endocrine carcinoma, as it also happens in other sites [29, 30].

Neuroendocrine Tumor of the Breast (Br-NET)

Br-NET is a less well-defined entity than Br-NEC. Indeed, while the latter may be recognized, as already discussed, as mirroring well-known entities, such as pulmonary and intestinal NECs, Br-NET, as historically recognized by many authors [11, 13, 31], generally does not recapitulate either the morphology or the clinical

characteristics of NETs commonly arising in the GEP tract and in the lung, which are the prototype for this category. In fact, Br-NETs, as defined by the WHO classification, despite the diffuse expression of neuroendocrine markers, only present morphological aspects (that can be focal) vaguely mimicking those of thoracic and digestive NETs. Indeed, a significant fraction of cases has been reported to present hybrid histopathological, ultrastructural (Fig. 13.3), and immunophenotypical features of both ductal and neuroendocrine differentiation [32], more reminiscent of amphicrine than of neuroendocrine neoplasms. Available immunohistochemical and molecular data suggest that they are consistently ER+ and HER2- and belong to luminal A group of breast carcinoma [33]. Moreover, the prognostic and predictive factors, as well as the therapeutic management, are those of non-endocrine breast carcinoma and not of NETs of other sites [31]. For example, the grading system used for so-called Br-NET is the Nottingham system and not the proliferative grade proposed for GEP and pulmonary NETs (either with Ki67 proliferative index or with mitotic index). Again, the therapeutic choices for Br-NET rely on predictive factors used for non-endocrine breast cancer (hormone receptor expression, HER2 hyperexpression and amplification, and proliferative index), rather than on the protocols for GEP NETs. In addition, targeted therapies for NETs of other sites

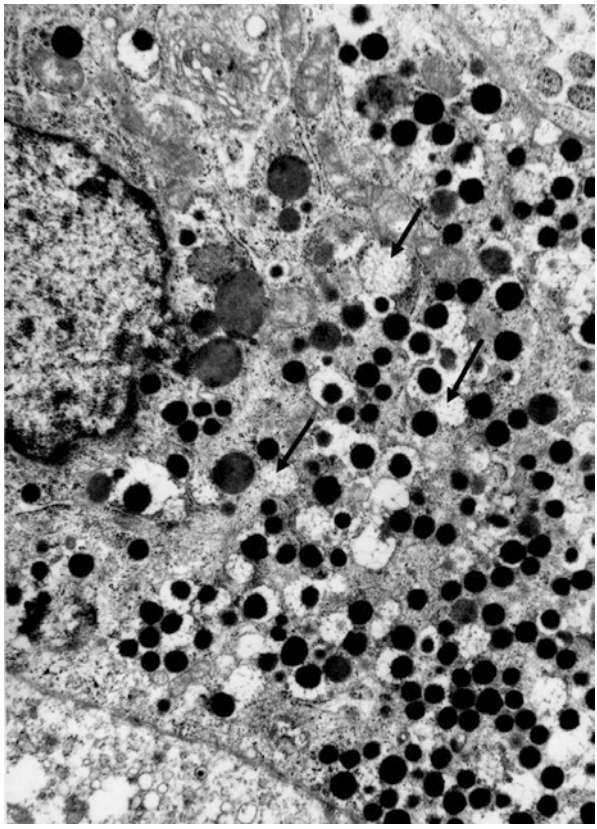

Fig. 13.3 Amphicrine cells can be observed in breast neoplasms, containing both neurosecretory granules and exocrine granules, mainly of mucin type (arrows)

(somatostatin analogues, mTOR inhibitors) have not proved specific efficacy on Br-NETs. Indeed, the expression of somatostatin receptors (SSRs) has been demonstrated in a fraction of Br-NETs [34], but several studies have demonstrated that SSRs, in particular SSR2A, are frequently expressed in breast carcinomas of luminal A type, and this removes any specificity of somatostatin analogues in the management of Br-NET [35, 36]. Finally, Br-NETs seem not to bear a different prognosis, when compared to stage-matched breast carcinomas NST [31].

Overall, so-called Br-NETs are difficult to be precisely framed in a classification scheme, notwithstanding the refinement of their definition, which has deleted from this subtype all carcinomas morphologically amenable to other types of breast carcinomas, such as mucinous and solid papillary carcinomas [18]. It seems that, based on the present evidences, so-called Br-NET, rather than being a specific entity, should be regarded as a variant of breast carcinoma NST (most of which are possibly better called amphicrine), at least until new knowledge accumulates to separate them on clinically meaningful bases.

Other NENs and NEN-Like Neoplasms of the Breast

Metastatic NENs

Metastasis to the breast is uncommon, and little more than 50 cases of NENs metastatic to the breast at diagnosis have been described in literature, to date [37, 38]. Most of the cases have their primary site in the lung or in the ileum (Fig. 13.4), and the vast majority is represented by NETs, whereas metastatic NECs are less frequent [37, 39]. The main problem with metastatic NENs is that they are misdiagnosed, and consequently mistreated, as primary breast carcinoma in about a third of the cases. A detailed workflow for the management of this issue is provided in the Chap. 16; in this context it is worth recalling that a neuroendocrine morphology and immunophenotype in the absence of ER expression and of in situ or invasive non-neuroendocrine carcinoma of the breast should drive the attention to the possibility of a metastasis and prompt the search for the occult primary site.

Carcinomas of the Breast with Expression of General Neuroendocrine Markers

As already stated, general neuroendocrine markers, particularly synaptophysin, may be focally, zonally, or widely expressed in several carcinomas of the breast, both of NST and of special types. Among these neoplasms, solid pseudopapillary carcinomas, both in situ and invasive, and mucinous carcinomas (Fig. 13.5) show the most consistent neuroendocrine differentiation. As a whole, the presence of neuroendocrine immunophenotype does not affect the clinical behavior of the neoplastic proliferation, as well as the morphology remains well recognizable. They do not belong to the category of Br-NENs identified by the WHO classification [18].

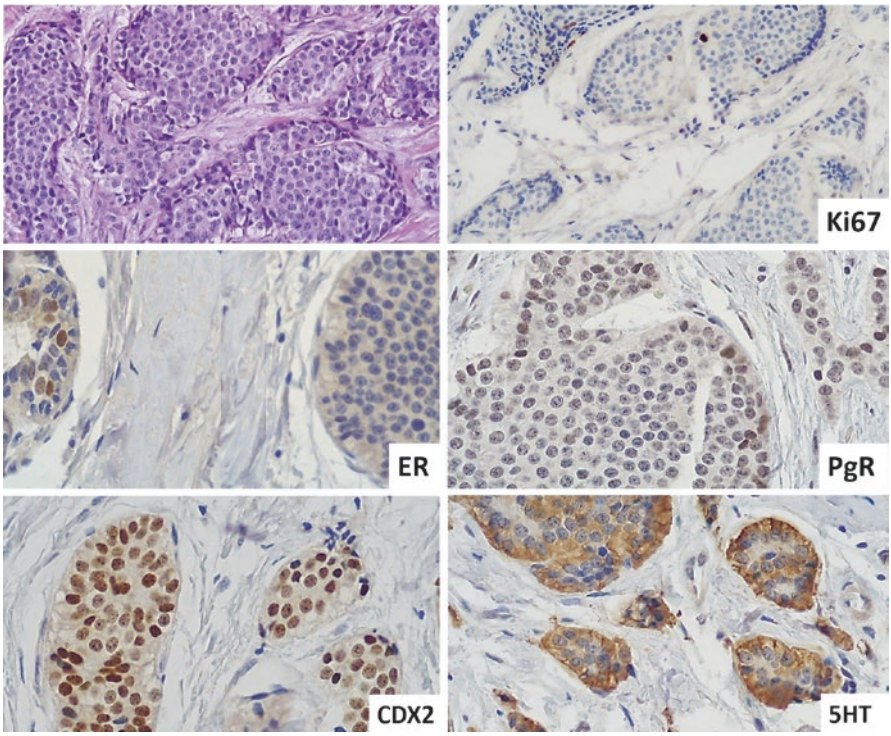

Fig. 13.4 Metastasis of ileal NET G1 in the breast. Ki67 proliferation index is about 1%; estrogen receptors (ER) are negative (positive control in a normal duct); progesterone receptors (PgR) are faintly positive; CDX2 and serotonin (5HT) are intensely expressed

Br-NENs: Just Concepts or Real Entities?

The history of Br-NENs seems to be burdened by an original sin in terms of their own definition. In fact, decades of subsequent studies were not able to define Br-NENs convincingly and univocally as clinicopathological entities, paralleling those arising in the gastroenteropancreatic (GEP) tract or in the lung. Classifications should identify nosologic entities providing affordable criteria for a diagnosis that must be understandable and useful for the clinician in the patients' management. The inclusion of an entity should respond to several requirements, in terms of a univocally recognizable morphology and/or immunophenotype, a defined clinical behavior, and, possibly, a known genetic profile, which can be useful for diagnosis or in predicting prognosis and response to therapy. In this context, GEP and lung NENs are convincing entities and serve as the prototype for similar neoplasms in

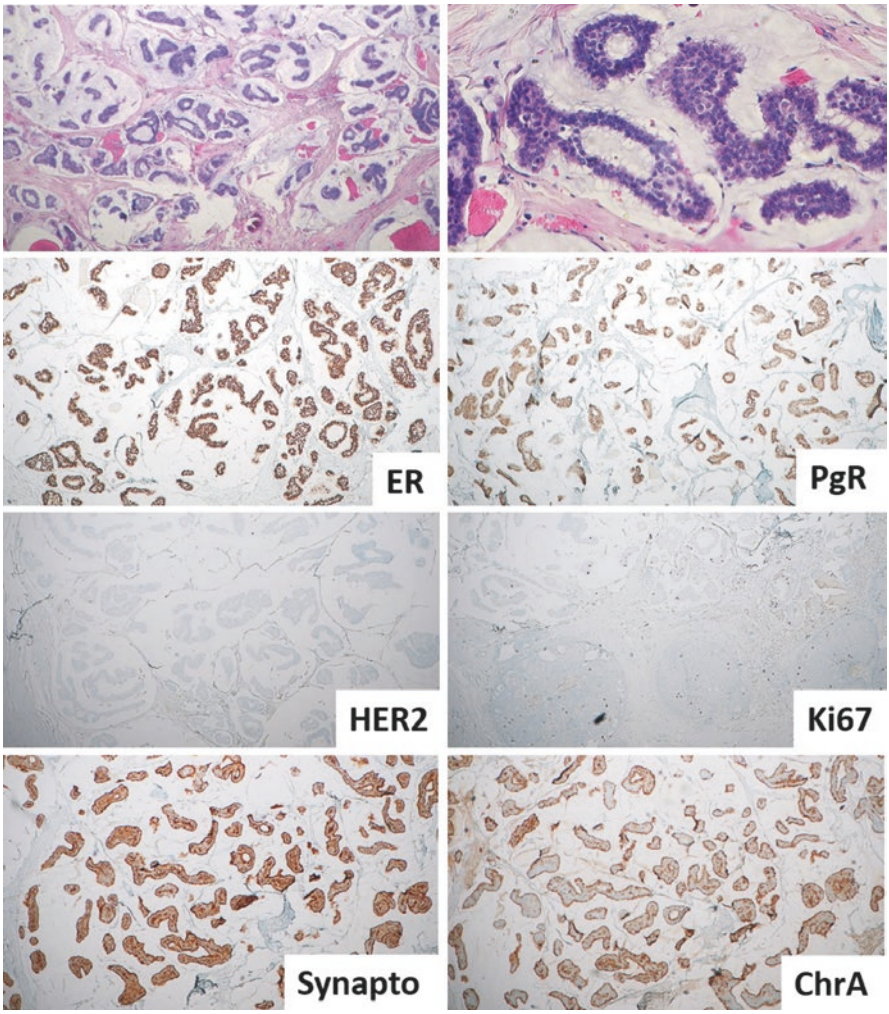

Fig. 13.5 Type A, hypocellular, mucinous carcinoma of the breast with diffuse expression of neuroendocrine markers. Estrogen (ER) and progesterone receptors (PgR) are expressed, HER2 is negative, and Ki67 proliferation index is low. Neoplastic cells are immunoreactive for both synaptophysin (Synapto) and chromogranin A (ChrA)

other sites, such as the head and neck and the urogenital tract (see pertinent chapters). In contrast, except for Br-NEC, Br-NENs are still far to adhere to these criteria. First of all, Br-NET does not present a definite and recognizable morphology, and the diagnosis, in practice, relies on immunostains for synaptophysin and chromogranin A, which is a dangerous criteria as many non-neuroendocrine neoplasms in the body show such immunophenotype without being NENs. Second, the clinical behavior and response to therapy of breast NETs largely overlap those of non-neuroendocrine breast carcinomas. Last, and not least, molecular and genetic analysis

shows that Br-NET does not show similarities with NETs of other sites. On the contrary, it follows in a specific subtype of the molecular classification of breast carcinomas, namely, the luminal A subtype.

In conclusion, if one believes that NENs represent a distinctive category of neoplasms [17], it should be recognized that, apart from expressing neuroendocrine markers, Br-NET, as it is currently defined, only marginally falls in the "neuroendocrine concept," but is not fully entitled to be a part of it.

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Genitourinary Neuroendocrine Neoplasms

14

Silvia Uccella, Xavier Mathias-Guiu, and Stefano La Rosa

Background

Neuroendocrine neoplasms (NENs) of the genitourinary tract are uncommon, but the pathologist has to be prepared to recognize them and to assess the morphophenotypic factors that can drive their treatment and prognosis. NENs have been described in the kidney, urinary bladder, prostate, testes, uterine cervix, uterine corpus, and ovaries. The clinico-pathological features and the nomenclature of these neoplasms may vary from site to site, and this chapter will systematically review the spectrum of genitourinary NENs. In this context, the reader will be provided with a unitary vision of the diagnostic criteria and classification, paralleling the scheme recently proposed by the WHO and the IARC [1]. Non-epithelial NENs (paragangliomas) occur in the bladder and kidney; they are described in Chap. 12 and will not be discussed here but should be included in the differential diagnosis of genitourinary NENs.

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Renal NENs

The kidney is a rare primary site for both well-differentiated neuroendocrine tumor (NET) and poorly differentiated neuroendocrine carcinoma (NEC). In addition, mixed neuroendocrine–nonneuroendocrine neoplasms (MiNENs) have been described.

Renal NET

About 100 renal NETs have been reported in the literature, to date, as case reports or small series. The largest collection of renal NETs was published in 2007 by Hansel and coworkers, who reported 21 cases from five different American Institutions [2]. The review of published cases delineates a clinically indolent entity, almost incidentally diagnosed during imaging procedures for other causes, as local or systemic symptoms are generally absent. Carcinoid syndrome is very uncommon. Males and females are equally affected and the median age at diagnosis is around 50 years, albeit renal NET may arise also in children and in elderly people, as the age range spans from 13 to 78 years. There is still debate on the cell of origin of renal NET, as resident neuroendocrine cells of the kidney have not been identified [3], but their occurrence has been associated with congenital abnormalities of the kidney, such as horseshoe kidney and polycystic kidney disease.

Macroscopically, most renal NETs are unifocal and involve renal parenchyma, whereas renal pelvis is rarely affected. These neoplasms are usually well delineated, with sharp borders separating them from normal kidney, although extra-renal extension can be observed in advanced stages of the disease. The majority of these tumors are larger than 4 cm. The microscopic picture has overlapping features with NETs in other sites, with the most represented pattern of growth being trabecular and ribbon-like. Solid nests and pseudoglandular patterns are less common (Fig. 14.1). Immunohistochemistry is useful in establishing the diagnosis, with synaptophysin being more sensitive but less specific than chromogranin A in detecting neuroendocrine differentiation. Pan-cytokeratin, cytokeratin 20, cytokeratin 7, and Cam 5.2 are expressed by neoplastic cells, confirming the epithelial nature of the tumor. Due to the rarity, a comprehensive profiling of the hormone and transcription factors expression has not been performed and the cells types have not been fully characterized. However, in a series of nine renal NETs, the absence of kidney-related markers, such as PAX2 and PAX8, and the consistent expression of CD99 were reported [4]. In another immunohistochemical study of 11 cases, the absence of pancreatic hormones, as well as TTF-1 and WT-1, was also documented [5]. Proliferation rate is usually low, with most of the cases showing a mitotic index below 2 *per* 2 mm^2 and a Ki67 labeling index lower than 2%. Cases with distant metastases tend to have higher proliferation rates [6]. The expression of somatostatin receptors has been reported in renal NET, representing the rational basis for somatostatin analogue-based diagnostic and therapeutic procedures. From a genetic point of view, loss of heterozygosity (LOH) of 3p21 chromosome region was demonstrated using

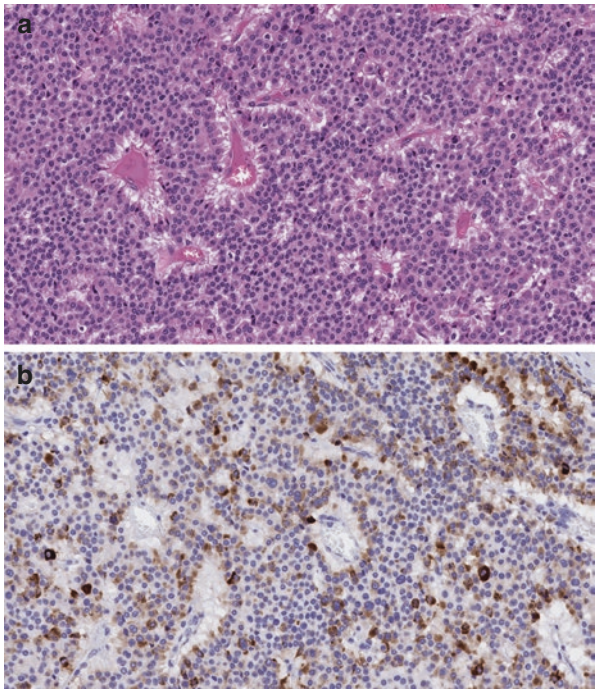

Fig. 14.1 Well-differentiated neuroendocrine tumor (NET) in a horseshoe kidney. Diffuse proliferation with perivascular arrangement of monotonous medium-sized cells with eosinophilic granular cytoplasm and round nucleus showing a salt-and-pepper chromatin pattern (**a**): Neoplastic cells in this case express prostatic specific acid phosphatase (**b**). *(Courtesy of Dr. Sylvia Asa, Case Western Reserve University, Cleveland, USA)*

polymerase chain reaction (PCR), in analogy with clear cell renal carcinomas. No additional chromosome imbalances were shown by comparative genomic hybridization (CGH) [5].

The differential diagnosis of renal NETs mainly includes so called "eosinophilic neoplasms" of the kidney, paragangliomas, and metastases from NETs of other primary sites. Among eosinophilic neoplasms of the kidney, eosinophilic variant of clear cell carcinoma, chromophobe carcinoma, and oncocytoma must be considered. The application of appropriate immunohistochemical panels, including general neuroendocrine markers, is the cornerstone for the assessment of the correct diagnosis. Renal paraganglioma is rare but not exceptional, and its distinction from renal NETs relies on its negativity for cytokeratins and their positivity for GATA 3 and tyrosine hydroxylase [7]. The possibility of a metastatic spread of a NET from another primary site must be taken into account, but, until now, the role of the pathologist is limited in this setting, as site-specific markers for renal primaries have not yet been identified. A careful clinico-radiological study, which may also include Octreoscan or Gallium 68-labeled somatostatin analogues, is mandatory for the optimal management of the patient.

The available data on the clinical behavior and prognosis of renal NETs show that these are a slow-growing neoplasm, usually diagnosed in locally advanced stages. Metastases are detected in about a half of the patients at presentation, both in the lymph nodes and in distant organs, but may also occur several years after initial diagnosis. Increased metastatic potential has been associated with an age

>40 years, tumor size >4 cm, perirenal fat infiltration, and mitotic rates of >1 per 2 mm^2. Patients with renal NET show a better prognosis than stage-matched renal cell carcinoma, even in the presence of widely metastatic disease. Due to the rarity of renal NET, standard therapeutic strategies have not been optimized; however the surgical excision of the primary tumor, combined with chemotherapeutic schemes used for gastroenteropancreatic and pulmonary NETs have been employed [8].

Renal NEC and MiNEN

About 50 cases of poorly differentiated neuroendocrine carcinomas of the kidney (NEC) have been reported in literature since Capella and coworkers described the first case [9]. Data from the Surveillance, Epidemiology, and End Results (SEER) registry of the United States National Cancer Institute report an incidence of 60 new cases in a 30-year period between 1973 and 2013 [10]. These neoplasms equally affect males and females and the patients are older than those with renal NETs, with a mean age at diagnosis of 59 years, although cases in adolescents and young adults have been reported. Renal pelvis is involved in a significant number of cases and, in this sub-site, the coexistence of an urothelial component is a frequent event, supporting the concept that NEC may derive from a common precursor with urothelial carcinoma. NECs arising in the renal parenchyma usually do not show a nonneuroendocrine component [11]. Systemic symptoms such as asthenia and weight loss are common, as well as flank pain and hematuria. One case of syndrome of inappropriate secretion of antidiuresis (SIAD) has been reported [10].

Macroscopically, renal NECs are large masses, measuring up to 23 cm in their greatest diameter, with areas of necrosis and hemorrhage. Microscopically, most of the cases are of the small cell subtype, resembling small cell carcinoma of the lung, with an organoid or, more frequently, diffuse proliferation of round to oval cells, not larger than twice the size of a small lymphocyte, with dark nuclei, inconspicuous nucleoli, and scant cytoplasm. The mitotic index is very high (usually more than 20 per 2 mm^2), and apoptotic bodies are numerous. The large cell subtype is very rare and only single cases have been reported in literature [12]. Immunohistochemical stains for synaptophysin, chromogranin A, and other general neuroendocrine markers are positive and must be included in the panel for the differential diagnosis with other blue cell neoplasms that can affect the kidney, such as lymphomas, medullary carcinoma, and Ewing sarcoma/primitive neuroectodermal tumor of the kidney. While in the renal parenchyma pure NEC is the rule, mixed neuroendocrine-nonneuroendocrine neoplasms (MiNENs) with an urothelial component are common in the renal pelvis. The urothelial differentiation is morphologically recognizable and confirmed with appropriate immunohistochemical stains, including p63 and GATA3, which are negative in the NEC component [13]. In this context it is worth noting that chromophobe renal cell carcinoma may show neuroendocrine-like morphological features, although a complete neuroendocrine phenotype is not expressed, as only synaptophysin, and not chromogranin A, is positive in these

cases and ultrastructural studies have not demonstrated the presence of dense core granules [14].

The genetic landscape of renal NECs has not been extensively examined; however loss of p53 function and MYC amplification, in the context of a complex karyotype, have been described [11].

More than a half of the patients with renal NEC present with advanced stage disease and the prognosis is poor, with a median survival of about 12 months. There is currently no standard of care for these neoplasms, as available data are insufficient to suggest benefit of chemotherapy, radiation therapy, surgical therapy, or any combination of treatment.

NENs of the Urinary Bladder

NENs of the urinary bladder are rare, representing less than 1% of all malignancies in this site. The commonest form is NEC, mainly of the small cell subtype, whereas NET is only anecdotally reported. A significant proportion of NECs of the urinary bladder contains a nonneuroendocrine component, represented by urothelial carcinoma, squamous cell carcinoma, or adenocarcinoma, and can be designated as MiNENs [13].

NET of the Urinary Bladder

About 20 cases of confirmed well-differentiated NENs have been described in the urinary bladder [15]. They are almost always discovered incidentally at cystoscopy and mainly affect elderly men (mean age 59 years). Symptoms may include hematuria and dysuria. Carcinoid syndrome has not been reported in association with these NETs.

Macroscopically, NET of the urinary bladder appears as a small polypoid mass and is located in the trigone or in the bladder neck. The submucosa may be infiltrated, but the muscularis propria is generally spared. The microscopic picture resembles that of NETs of other sites, with well-differentiated neuroendocrine morphology. Mitotic figures are rare (at most 1 per 2 mm^2), and necrosis and hemorrhage are absent. Most of the cases arise in the context of a cystitis cystica, chronic cystitis, and cystitis glandularis and the neoplastic cells are typically arranged in pseudoglandular structures. A peculiar feature of these neoplasms is the presence of subnuclear eosinophilic granules in the neoplastic cells [16]. NETs of the urinary bladder are thought to derive from normal neuroendocrine cells of this site, and it has been postulated that the association with inflammatory conditions is due to neuroendocrine cells hyperplasia in irritated mucosa. Importantly, no case of MiNEN composed of NET and urothelial carcinoma has ever been described in the urinary bladder, supporting the concept that these tumors do not origin from a urothelial precursor. The only well-documented association between a NET and a nonneuroendocrine component in this site was with a mucinous adenocarcinoma with signet

ring cells, but it was interpreted as a collision tumor arising from two different cell types in the cystitis glandularis [16].

Immunohistochemistry demonstrates the expression of chromogranin A and synaptophysin; in addition, positivity for prostatic acid phosphatase has been reported, whereas other prostatic markers are negative [15]. One case of calcitonin-immunoreactive NET of the urinary bladder has been described [17]. Interestingly, in at least one case, TTF-1 immunoreactivity has been described in a fraction of tumor cells, limiting the value of this marker in the differential diagnosis of a primary net of the urinary bladder versus a metastatic localization from a lung NET [18].

The differential diagnosis of NETs of the urinary bladder includes inverted papilloma, invasive urothelial carcinoma, and prostatic adenocarcinoma. Inverted papilloma may represent a misdiagnosis in that it is a polypoid, subepithelial nodule with an overlying benign urothelium. In addition, cystitis cystica et glandularis adjacent to the NET may mimic glandular differentiation often seen in inverted papilloma. However, NET tumors have cribriform and acinar structures with neuroendocrine nuclear features and lack the peripheral palisading and central streaming patterns seen in inverted urothelial papilloma. The subepithelial growth of a NET may also mimic a nested variant of invasive urothelial carcinoma or an invasive urothelial carcinoma with focal glandular differentiation. The presence of neuroendocrine features and the absence of nuclear atypia are in favor of a NET. The pseudoglandular and cribriform architecture of NET of the urinary bladder may lead to confusion with prostatic adenocarcinoma secondarily involving the bladder. The presence of prostate-specific acid phosphatase immunostain may support this misdiagnosis. However, neuroendocrine cells lack prominent nucleoli and are negative for prostate specific antigen (PSA). Finally, the possibility of a metastatic NET from other more common primary sites needs to be excluded on clinical grounds.

The clinical behavior of NETs of the urinary bladder has not been definitely clarified due to the lack of appropriate follow-up data. However, it seems that they behave as indolent neoplasms, which are cured by surgical excision. No progression toward a NEC has been documented.

NEC and MiNEN of the Urinary Bladder

Pure NECs of the urinary bladder are very rare neoplasms, as most of the cases are MiNENs in which the nonneuroendocrine component is represented by either urothelial carcinoma, squamous cell carcinoma, sarcomatoid carcinoma, or adenocarcinoma. The male-to-female ratio is 3:1 and the mean age at diagnosis is in the seventh decade of life. A history of cigarette smoking is present in 80% of the patients, whereas no association with human papilloma virus (HPV) infection has been demonstrated. The clinical presentation is dominated by local symptoms, including hematuria, dysuria, urinary obstruction, and pelvic pain, but signs of systemic disease may be present, such as weight loss, anorexia, and asthenia. In addition, cases of paraneoplastic syndromes with hypercalcemia, hypophosphatemia, or

ectopic secretion of adrenocorticotropic hormone (ACTH) have occasionally reported.

The macroscopic picture of NEC of the urinary bladder is generally represented by a large polypoid mass, often ulcerated and largely necrotic (Fig. 14.2a). The neoplasm usually infiltrates the bladder wall, widely involving the muscularis

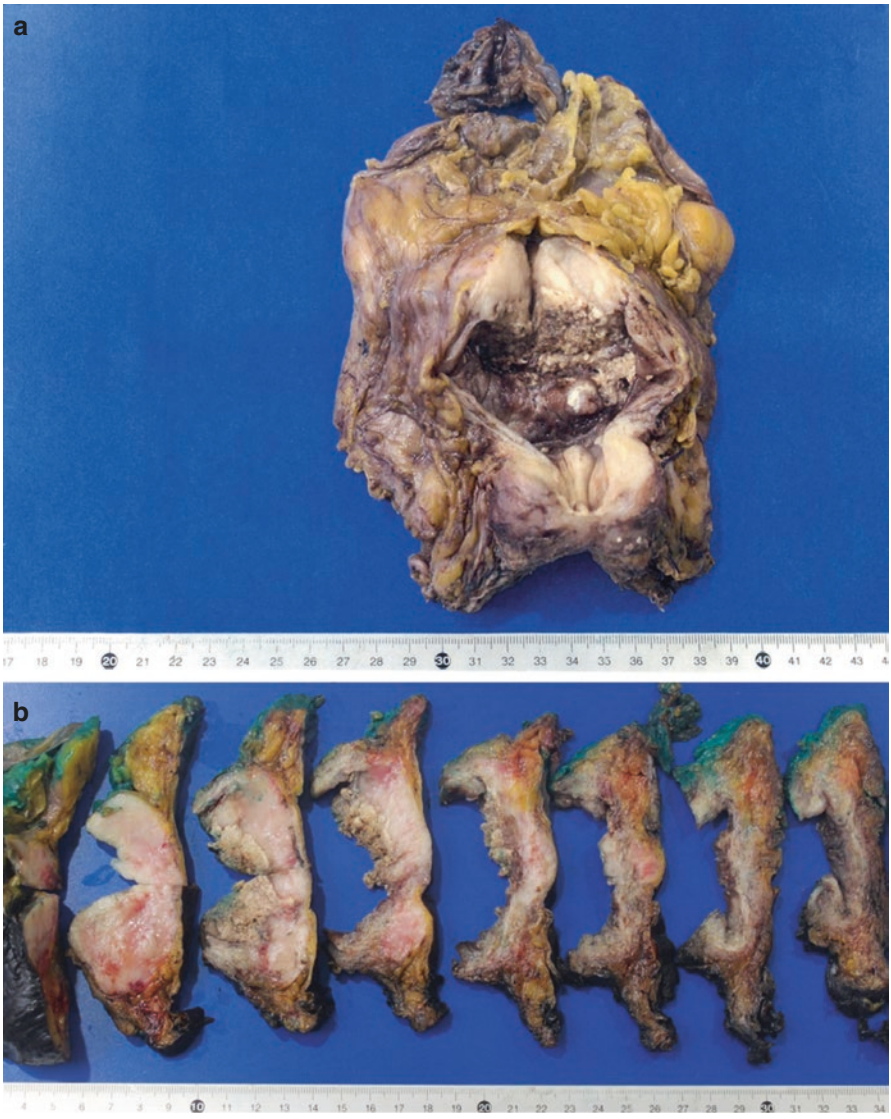

Fig. 14.2 Macroscopic aspect of a neuroendocrine carcinoma (NEC) in the urinary bladder. The dome and the posterior wall of the urinary bladder are occupied by a large ulcerated mass (**a**) with a fleshy cut surface, destroying the muscular wall and infiltrating the perivisceral soft tissues (**b**)

propria and infiltrating the perivisceral fat (Fig. 14.2b). Microscopically, the small cell subtype is the most frequently seen, geographic necrosis is commonly present, the mitotic index is high and vascular invasion, as well as perineural infiltration are the rule. The large cell subtype is exceedingly rare, with fewer than 25 cases reported in the literature. Both the small cell and the large cell types are frequently associated with other carcinomatous components, which can show urothelial, squamous, sarcomatoid, or adenocarcinomatous differentiation (Fig. 14.3a, b). As the poorly

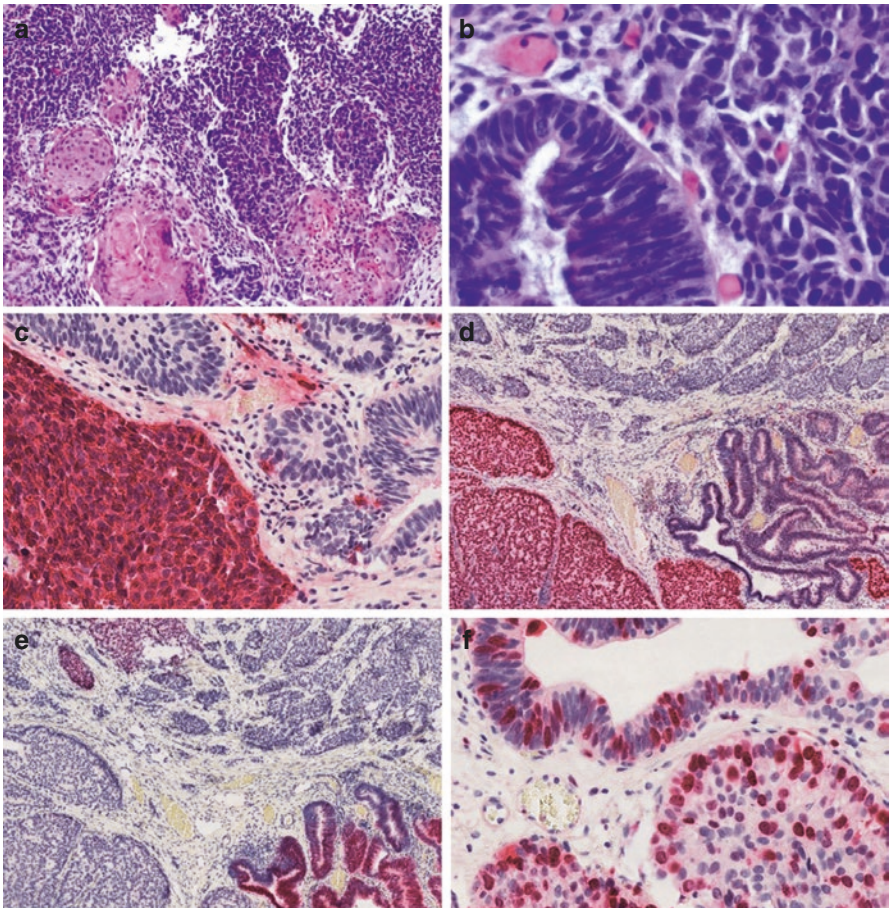

Fig. 14.3 Mixed neuroendocrine–nonneuroendocrine neoplasm (MiNEN) of the urinary bladder composed by a mixture of neuroendocrine carcinoma (NEC), urothelial carcinoma, squamous cell carcinoma, (**a**), and adenocarcinoma (**b**). The NEC component is strongly positive for synaptophysin (**c**), whereas the urothelial component shows nuclear immunostain for GATA 3 (**d**, bottom left), which is negative in the NEC (**d**, top) and in the adenocarcinoma (**d**, bottom right). CDX2 is expressed in the adenocarcinomatous component (**e**, bottom right) and, focally in the NEC (**e**, top), whereas the urothelial carcinoma is negative (**e**, bottom left). Ki67-related proliferation index is higher than 50% in the NEC component (**f**). (Slides provided by Dr. Achim Fleischmann, University of Bern, Switzerland)

differentiated neuroendocrine component is always high grade and drives the prognosis, its proper recognition is important for management. Immunohistochemical stains for synaptophysin and chromogranin A help to confirm the neuroendocrine differentiation of neoplastic cells, but additional markers may be of use in discriminating and quantifying neuroendocrine versus nonneuroendocrine components (Fig. 14.3c–f). It has been demonstrated that NECs of the urinary bladder are consistently p16-positive, CK20-negative and p63-negative, whereas high-grade urothelial carcinomas are likely to show an opposite profile (p16-, p63+, and CK20+) [19]. More recently, the transcription factor GATA3, typically expressed in urothelial carcinomas, has been reported to be negative in NECs of this site, being useful in the differential diagnosis and in discriminating the two components of a MiNEN [20] (Fig. 14.3d). Among immunohistochemical markers useful in programming therapeutic strategies, it is worth recalling that a subset of NECs of the urinary bladder are positive for EGFR and c-Kit (CD117), potential targets for specific monoclonal antibodies and tyrosine kinase inhibitors [21, 22]. In addition, somatostatin receptor 2A has been reported to be expressed in more than half of the cases, representing the rationale for the possible use of somatostatin analogues [23].

The frequent association between neuroendocrine and nonneuroendocrine neoplasms in the urinary bladder supports the hypothesis that NECs in this site may arise from a common multipotential precursor cell. Molecular analyses seem to confirm this theory, as nearly identical patterns of allelic loss have been found in NECs and coexisting urothelial carcinomas, suggesting a common clonal origin [24]. More recently, comprehensive whole-genome and transcriptome sequencing showed that the mutational landscape and signatures of neuroendocrine bladder cancer largely overlap those in conventional urothelial carcinoma, along with typically mixed histologies, supporting a common cellular origin [25]. In particular, it has been showed that urinary bladder NEC and urothelial carcinoma share common driver molecular alterations, such as specific TERT promoter mutations [26], that chronologically precede the crucial loss of function of p53 and Rb, which are the hallmarks of neuroendocrine differentiation in NECs of different sites [27].

The differential diagnosis of NEC of the urinary bladder with high-grade urothelial carcinoma has already been discussed. In addition, NEC must be distinguished from other high-grade epithelial and non-epithelial malignancies, also including non-Hodgkin lymphomas. Moreover, metastatic NECs from other sites may involve the urinary bladder, but, in these cases, immunohistochemistry for site-specific markers is not of help, as already discussed in other sections of this chapter.

More than a half of patients with NEC of the urinary bladder present with advanced disease, most commonly involving pelvic and retroperitoneal lymph nodes (30–50%), liver (24–47%), and bone (24–33%) [28]. Metastases to the central nervous system are less frequent than in small cell lung carcinoma (10% versus 60%). The stage at diagnosis is an important prognostic factor and drives the therapeutic strategies. Early stages can be treated with multimodal therapy, i.e., neoadjuvant chemotherapy followed by radiotherapy or radical cystectomy. Metastatic disease is treated, in analogy with small cell lung carcinoma, with cisplatinum-based regimens [29]. The 5-year disease-specific survival in limited stages treated

with cystectomy and chemotherapy can be as high as 78%, although chemotherapy alone has provided similar results in retrospective studies [15]. Patients with advanced disease have a very poor outcome, with mean overall survival of maximum 15 months.

Prostatic NENs

Prostatic neoplasms with neuroendocrine differentiation encompass a wide spectrum of proliferations, with different morphologic, biologic, and clinical characteristics [30]. Moreover, the acquisition of neuroendocrine phenotype in prostatic cancer is, in most instances, strongly related to tumor progression, transformation to highly aggressive disease, and resistance to androgen deprivation therapy (ADT). It has been demonstrated that an at least focal neuroendocrine differentiation is observed in virtually all prostatic adenocarcinomas and molecular studies have shown that most neuroendocrine carcinomas in the prostate originate by transdifferentiation of adenocarcinomatous cells. In addition, pure NENs have been described in this site. This complex landscape elicits difficulties in defining the nomenclature, classification, and clinical meaning of the different entities. In the last years, the acquisition of molecular data has improved the understanding of the pathogenesis of these neoplasms and has led to the formulation of a classification scheme that is comparable to NENs of other sites. In this section, we will discuss pure prostatic NENs, including NET and NEC, along with MiNEN composed of prostatic adenocarcinoma and NEC. In addition, for a complete discussion of the meaning of neuroendocrine phenotype in prostatic neoplasms, adenocarcinoma with neuroendocrine differentiation will be addressed.

Prostatic Adenocarcinoma with Neuroendocrine Differentiation

Immunoreactivity for General Neuroendocrine Markers in Otherwise Morphologically Typical Prostatic Adenocarcinoma

The great majority of prostatic adenocarcinomas show at least focal immunoreactivity for general neuroendocrine markers (mainly synaptophysin), when tested immunohistochemically at first diagnosis (Fig. 14.4). Although some studies have claimed that the presence of neuroendocrine differentiation is related to high grade and/or stage, its independent value in determining poor prognosis has not been established [31]. The phenomenon of synaptophysin immunoreactivity in adenocarcinomas without histologically recognizable neuroendocrine morphology is observed also in other body sites, including, for instance, large bowel, stomach, breast, and uterus. However, this has never been demonstrated to have a specific prognostic meaning [13]. For this reason, the systematic immunohistochemical study of neuroendocrine differentiation is not advisable in routine pathology and this entity is not to be considered as a real NEN.

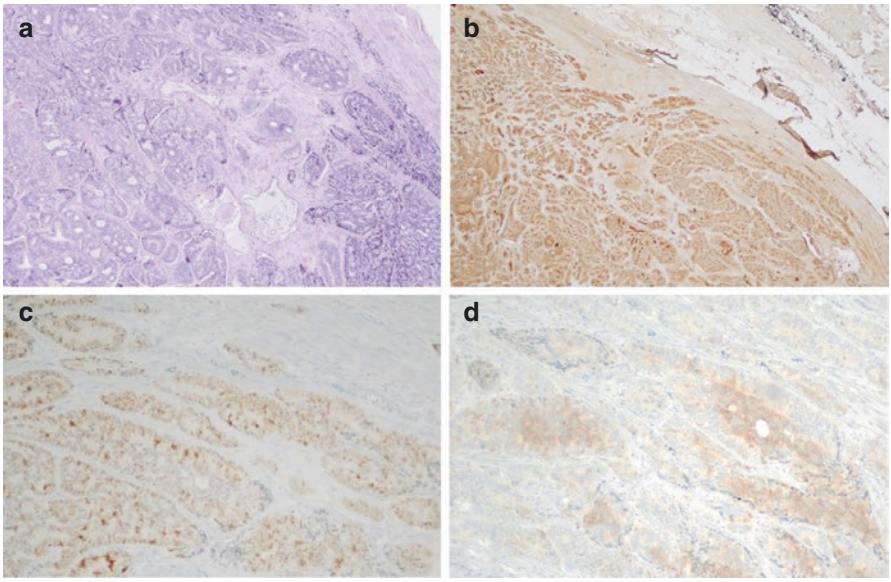

Fig. 14.4 Acinar adenocarcinoma of the prostate with usual microscopic appearance (**a**), widespread expression of PSA (**b**) and concomitant expression of synaptophysin (**c**) and chromogranin A (**d**). This neoplasm should not be considered a neuroendocrine neoplasm

Adenocarcinoma with Well-Differentiated Neuroendocrine Cells

The current WHO classification of prostatic tumors [32] includes an *adenocarcinoma with Paneth cell-like neuroendocrine differentiation*, which is defined as a prostatic adenocarcinoma with morphologically recognizable well-differentiated neuroendocrine morphology, in which neoplastic cells show cytoplasm stippled with brightly eosinophilic granules. The designation "Paneth cell-like" is a misnomer, as these granules do not contain lysozyme, as Paneth cells do, but are rather functionally and morphologically similar to neuroendocrine cells interspersed in the intestinal mucosa. In addition, as discussed below, a subset of these cases do not show the characteristic granules at microscopic observation with haematoxylin and eosin stain. For these reasons, we prefer the terminology *adenocarcinoma with well-differentiated neuroendocrine cells*. In these neoplasms, neuroendocrine cells may be admixed in various proportions with the adenocarcinoma component, being part of the neoplastic glandular structures, or they may proliferate in organoid structures, forming nests or chords (Fig. 14.5). In this latter case, the recognition of the neuroendocrine nature of the proliferation is of crucial importance to avoid overgrading the neoplasm with the Gleason score system. In fact, the absence of glandular structures in these neuroendocrine foci would be graded as Gleason pattern 5, although this well-differentiated neuroendocrine proliferation does not negatively influence prognosis. Consequently, when a well-differentiated neuroendocrine morphology (eosinophilic cytoplasm, nuclei with salt-and-pepper chromatin,

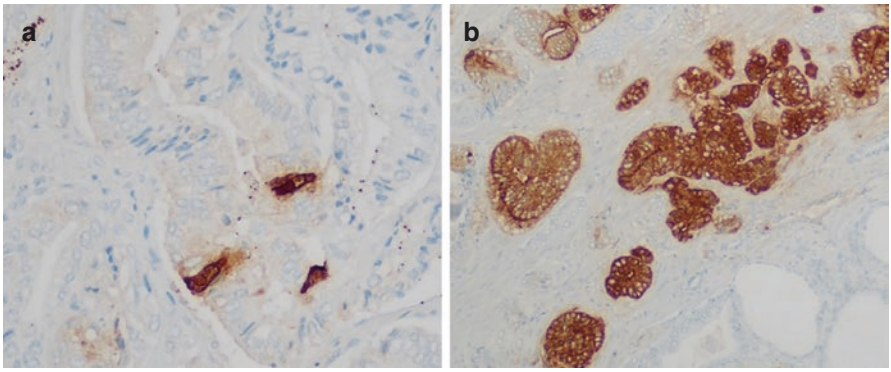

Fig. 14.5 Adenocarcinoma with well-differentiated neuroendocrine cells. Chromogranin A immunostaining highlights scattered neuroendocrine cells in neoplastic glands (**a**) and solid nests in the neoplastic stroma (**b**). In this case, the solid architecture of the neuroendocrine component should not be misinterpreted as Gleason pattern 5

inconspicuous nucleoli, and low proliferation index) is observed in an otherwise typical prostatic adenocarcinoma, Gleason score should be calculated only in the glandular component [33] and the neoplasm can be designed as MiNEN [13]. Noteworthy, it has been reported that some adenocarcinomas with well-differentiated neuroendocrine foci may lack the bright cytoplasmic eosinophilic granules typical of the so-called "Paneth-like cells." In such cases, the careful observation of the organoid pattern of growth and of the nuclear features should prompt the performance of immunohistochemical stains in order to confirm the neuroendocrine differentiation and to prevent the misdiagnosis of a high grade adenocarcinoma [34].

The neuroendocrine cells in these cases are strongly positive for synaptophysin and chromogranin A, and usually lack PSA and AR expression. The Ki67 labelling index is very low. The pathology report should include, in addition to the details of the adenocarcinoma, the presence, the immunophenotype, and the quantification of the neuroendocrine component, which should be clearly indicated as well differentiated, in order to prevent misinterpretation as a NEC component.

The prognosis of adenocarcinoma with intestinal-like neuroendocrine cells is driven by the conventional prognostic factors of prostatic adenocarcinomas, independently of the neuroendocrine differentiation. Moreover, no progression toward a NEC has been described until now. Importantly, cases in which the well-differentiated neuroendocrine proliferation constitutes the majority of the neoplastic mass have been reported to bear a good prognosis [33].

Prostatic Carcinoma with Amphicrine Features

This is a newly described aggressive variant of prostatic carcinoma in which the totality of neoplastic cells present both a neuroendocrine and exocrine phenotype [35]. Amphicrine carcinomas have been described in other anatomic sites, mostly

including the tubular digestive system and the breast. By definition, these tumors show a hybrid histology and immunophenotype that recapitulates both exocrine and neuroendocrine morphology. The pathogenesis and the clinical meaning of these entities are not well established. A recent study reported the clinico-pathological characteristics of a series of five prostatic carcinomas with amphicrine features [35] and the genomic features of an additional case were previously described [36]. Overall, these neoplasms present as extensive or rapidly progressing diseases, with elevated PSA serum levels. Metastatic deposits are found in bones and lymph nodes.

The histological picture is dominated by a homogeneous solid or nested proliferation of cells with atypical nuclei and moderately abundant amphophilic cytoplasm. Glandular differentiation, as well as the typical aspects of NEC of small or large cell types (including necrosis), is completely absent. Mitotic index is high. The immunohistochemical stains for AR and PSA, as well as for chromogranin A and synaptophysin, are intensely positive. The Ki67 labelling index is usually greater than 50%. Ultrastructural examination shows the coexistence of dense core neuroendocrine granules and exocrine secretory vesicles, confirming the amphicrine nature of the neoplastic cells. Next-generation sequencing of one case revealed 15 novel fusion genes in the tumor, encompassing a combination of those related to AR regulation, which are normally expressed in prostatic luminal cells, and genes normally expressed by neuroendocrine cells [36].

Amphicrine carcinoma of the prostate may either arise de novo or appear in the progression of a high-grade adenocarcinoma. In this latter case, it has been suggested that it may represent a transition between the adenocarcinoma and a NEC, especially in patients treated with ADT [37]. However, at least two considerations argue against this hypothesis. First, amphicrine carcinoma also arises in patients not treated with ADT and, second, neither residual adenocarcinoma nor foci of NEC have been observed until now in the reported cases. Further studies are needed to verify the hypothesis that an early precursor of prostatic epithelium may, through clonal selection, give rise to such an ambiguous phenotype.

The correct recognition of amphicrine carcinomas may be of importance both in the differential diagnosis of a prostatic carcinoma and in the identification of the unknown primary of a metastatic lesion. In the former case, careful morphologic examination and the use of a proper immunohistochemical panel may avoid the misdiagnosis of both a high grade conventional adenocarcinoma and a NEC of large cell type. Indeed, high-grade conventional adenocarcinoma would be positive for AR and PSA and negative for chromogranin, whereas NEC would present specific morphological features and would be negative for AR and PSA, but strongly positive for general neuroendocrine markers. As for metastatic lesions, the prostatic primary may be missed and an incorrect diagnosis of NEC may be formulated if only neuroendocrine markers, and not prostate-specific immunostains are performed. In both cases, the patient would experience an improper treatment, as ADT, with or without chemotherapy, has showed good results in amphicrine carcinomas [35].

Prostatic NETs

True prostatic NETs are exceedingly rare, as most of the reported cases are, in fact, prostatic adenocarcinomas with neuroendocrine differentiation. The diagnosis of prostatic NETs must rely on five concurrent criteria: (1) the presence of well-differentiated neuroendocrine morphology; (2) the absence of adenocarcinomatous component; (3) immunohistochemical expression of general neuroendocrine markers; (4) negativity of immunostainings for AR and PSA; (5) exclusion of prostatic metastasis or infiltration from a primary NET of another site. As already mentioned, well-differentiated neuroendocrine phenotype may be extensively present in an otherwise typical adenocarcinoma. For this reason, the diagnosis of prostatic NET on biopsy material should be formulated with great caution, and only confirmed on completely sampled surgical specimens. Indeed, the distinction of NET from prostatic adenocarcinoma with neuroendocrine differentiation is crucial both for the management and for the prognosis of the patient.

Based on the definition given above, only eight cases of true prostatic NET have been reported to date [31, 38–40]. Patients' mean age was significantly younger than that of patients with adenocarcinoma, being about 30 years. Four cases were diagnosed in children or adolescents and were associated with multiple endocrine neoplasia (MEN) type IIB [39, 41, 42]. Intriguingly, a unique case of a prostatic NET arising after an adenocarcinoma treated with ADT was recently reported [38], although no definitive demonstration of its relationship with the previous neoplasm was obtained and it may represent a second primary malignancy.

Histologically, the typical well-differentiated neuroendocrine morphology is observed, with organoid growth (in nests, trabeculae or pseudoglands) of intermediate sized cells with bland nuclear atypia, well-dispersed chromatin, small nucleoli, and moderately abundant eosinophilic cytoplasm. Immunohistochemistry confirms the neuroendocrine nature of neoplastic cells and the absence of AR and PSA expression. Prostatic specific acid phosphatase may be positive, whereas alpha-methylacyl-CoA racemase (AMACR) is consistently negative. No data on the expression of ERG protein in consequence of the prostate cancer-specific *ERG* gene rearrangement are currently available. The Ki67 labelling index is low.

No definitive assessment of the outcome of prostatic NET can be deduced from the scant data available. However, the reported cases, even when locally advanced and metastatic to lymph nodes, have been treated with surgery alone and have shown an indolent course. In view of the possible association of this entity with a MEN IIB, the pathology report may include a sentence to suggest proper follow-up and, conversely, prostate examination may be suggested in patients diagnosed with MEN IIB.

Prostatic NECs

Prostatic NECs are uncommon neoplasms that may present in pure neuroendocrine form or as MiNENs, in association with prostatic adenocarcinoma. About half of cases occur in patients with a previous diagnosis of prostatic adenocarcinoma treated with ADT, which has become castration-resistant, although prostatic NEC may occur de novo. A number of clinical and experimental observations point toward a pivotal role for androgen deprivation in the development of high-grade neuroendocrine differentiation in prostate adenocarcinomas. Indeed, transdifferentiation of adenocarcinoma cells is considered to be the main mechanism by which NEC arises. Nevertheless, the existence of de novo NECs in untreated patients suggests that alternative pathways to high-grade neuroendocrine differentiation may exist in the prostate, possibly directly involving prostatic epithelial stem cells [43]. In this regard, androgen resistance may be, at least in a subset of cases, the effect, and not the cause, of the neuroendocrine phenotype.

Prostatic NECs arise in elderly men in their seventh decade of life. More than 80% of patients present with systemic disease with metastases in lymph node, bones, and visceral organs. Endocrine paraneoplastic syndromes are not common; however, SIAD and Cushing syndrome due to ectopic ACTH and/or CRH secretion have been documented [44, 45].

Microscopically, the most typical pattern is the small cell subtype, with morphological features overlapping those of pulmonary and other extra-pulmonary small cell NECs. The picture is dominated by a diffuse proliferation of lymphocyte-like round or oval hyperchromatic cells exhibiting nuclear molding, hyperchromasia, inconspicuous nucleoli, and scant cytoplasm (Fig. 14.6a). The large cell subtype has been observed in a small number of cases and is characterized by organoid, trabecular, or nested and palisaded growth of large cells with abundant cytoplasm, nuclei with evident nuclear membrane and macronucleoli. Geographic necrosis is common in both subtypes, as well as prominent vascular and perineural invasion. Mitotic figures and apoptotic bodies are frequent findings. Both subtypes may be part of a MiNEN, in which the nonneuroendocrine component is usually represented by acinar adenocarcinoma; however ductal adenocarcinoma and other histotypes may be present [13].

The immunophenotype of prostatic NEC includes the expression of general neuroendocrine markers, like synaptophysin and, less consistently, chromogranin A (Fig. 14.6b). In addition to these traditional ones, the novel marker INSM1 (insulinoma-associated protein 1) has demonstrated very good sensitivity and specificity for prostatic NEC [44]. Other markers for distinguishing NEC from adenocarcinoma of the prostate may include cyclin D1 and CD44, the expression of which has been reported to favour the former diagnosis. As for PSA and AR, they are traditionally considered to be negative in these neoplasms. However,

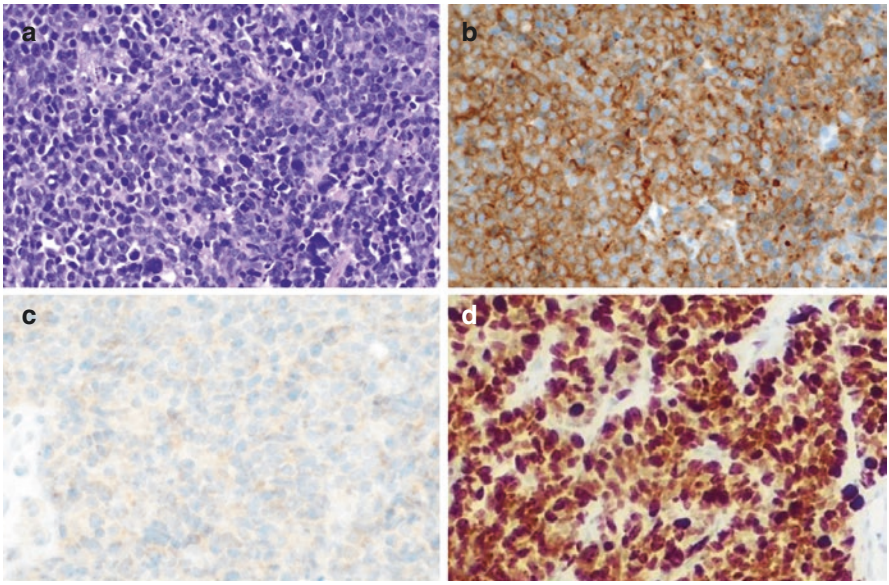

Fig. 14.6 Small cell neuroendocrine carcinoma (NEC) of the prostate. Hematoxylin and eosin stain shows a diffuse growth of neoplastic cells with high nuclear-cytoplasmic ratio, condensed chromatin, inconspicuous nucleoli, and barely visible cytoplasms. Several apoptotic bodies are evident (**a**). Synaptophysin is strongly expressed (**b**), whereas PSA immunostaining is very faint (**c**). Ki67-related proliferation index is higher than 90% (**d**)

there is evidence of focal positivity for these markers in a subset of cases (Fig. 14.6c), which may reflect the above-mentioned transdifferentiation process [46]. The Ki67 labelling index is typically high, usually greater than 50% (Fig. 14.6d). Loss of Rb protein and nuclear expression of p53 are usual features of prostatic NEC, paralleling NECs of other primary sites. Similar to NECs of other primary sites, site-specific transcription factors, like TTF-1, are not useful in detecting the organ of origin.

From a genetic point of view, prostatic NEC is characterized, alike NECs of other sites, by loss of *RB* and *TP53* genes. In addition, the *TMPRSS2-ERG* rearrangement, present in at least 50% of prostatic adenocarcinomas, is also detected in a similar fraction of prostatic NECs. In NEC, however, this rearrangement is not paralleled by positive immunostaining for the ERG protein, as the lack of AR in neoplastic cells prevents the overexpression of this marker [46].

Prostatic NEC bears a poor prognosis, median survival being around 15 months, without significant differences between pure NECs and MiNENs, or small cell and large cell subtypes. Advanced disease is treated with standard polychemotherapy including platinum and etoposide [40].

Testicular NENs

NENs represent less than 1% of all primary testicular neoplasms and less than 130 cases of have been reported in literature. A recent meta-analysis reviewed the clinico-pathological characteristics of testicular NENs published in the literature between 1930 and 2015 [47]. All primary NENs of the testes are well-differentiated neoplasms (NET), and NECs have not been described. Up to 20% of the cases are associated with a teratomatous component and in two cases an intratubular germinal cell neoplasm was seen in the peritumoral parenchyma, suggesting an origin of these neoplasms from germ cells [48].

Testicular NET

Testicular NET arises in young adults, in their third or fourth decade of life (mean age: 39 years), but rare cases in paediatric age and in the elderly have been reported. The presenting symptom is usually testicular enlargement, painful or not, although the finding of a testicular mass may be incidental, during diagnostic procedures for other reasons. Carcinoid syndrome is very rare.

The macroscopic aspect of pure testicular NET is that of a well-defined, but non-encapsulated, solid mass with a tan cut surface. Area of haemorrhage and liquefaction are rarely present. The mean size is of about 4 cm. Bilateral tumours are rare but have been described. Microscopically, a well-differentiated neuroendocrine morphology is evident, with neoplastic cells predominantly arranged in solid nests (Fig. 14.7a, b). Pseudoglandular and trabecular structures are rarer. Most of the cases show low mitotic activity (<2 mitotic figures per 10 HPF) and the absence of necrosis, and can be defined NET G1, whereas in rare neoplasms, mitotic activity is higher and necrosis is present, similarly to NET G2 and G3 NETs in the gastroenteropancreatic tract [49, 50]. Immunohistochemical stains for cytokeratins and general neuroendocrine markers are positive, as well as those for SSR2A (Fig. 14.7c–f), whereas CD117, PLAP, AFP, ACTH, gastrin, glucagon, PP or somatostatin, CDX-2, and TTF-1 are reported to be negative [51]. The Ki67 labelling index is ≤3% in NET G1 and higher in NET G2 [51].

Data on the genetic landscape of testicular NET are poor, due to their rarity; however, in a subset of cases, isochromosome 12p was found, supporting its origin from germ cells [52].

The typical morphologic aspect of testicular NET makes the diagnosis usually straightforward. However, when morphology is atypical, the differential diagnosis may include Sertoli cell tumor, granulosa cell tumor, and paraganglioma. Immunohistochemical stains for general neuroendocrine markers is usually sufficient to solve the problem, although the pathologist should be aware that some

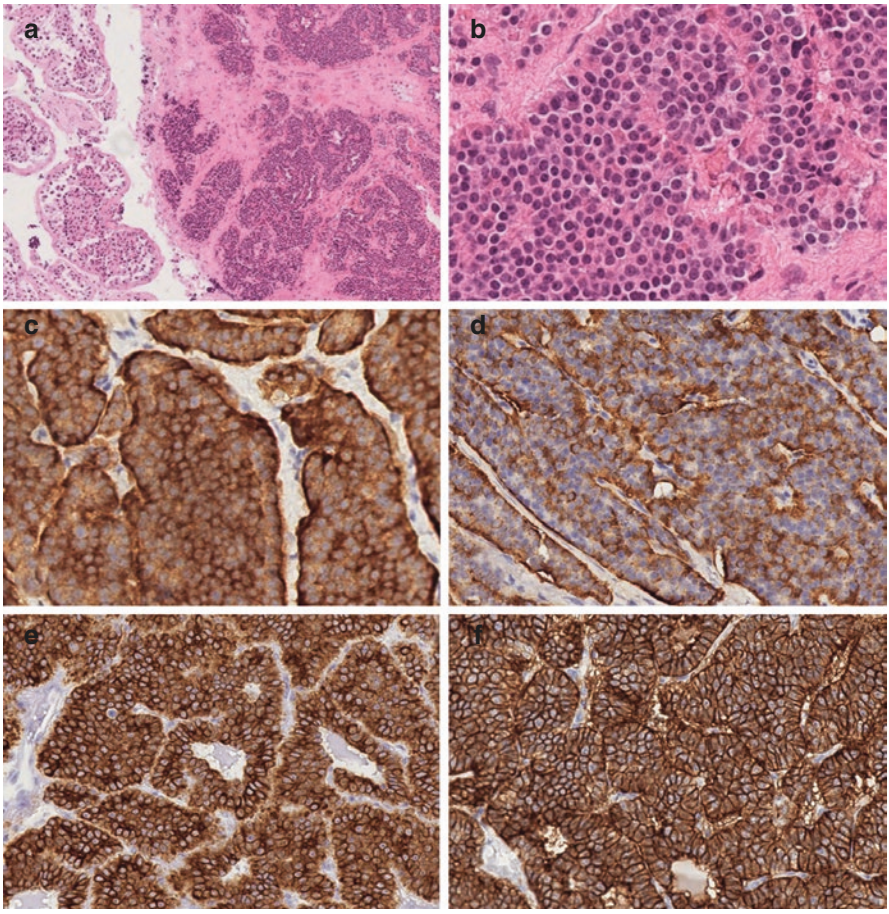

Fig. 14.7 Microscopic aspect of a testicular well-differentiated neuroendocrine tumor (NET). A neoplastic nodule composed of solid nests separated by sclerotic stroma (**a**, right) occupies the testicular parenchyma, in which tubules show no in situ germ cell neoplasia (**a**, left). Neoplastic cells are medium-sized, with round nuclei, small nucleoli, and moderately abundant eosinophilic and granular cytoplasm (**b**). General neuroendocrine markers synaptophysin (**c**) and chromogranin A (**d**) are intensely expressed, as well as cytokeratin AE1/AE3 (**e**), and somatostatin receptor 2A (**f**)

Sertoli cell tumors may be positive for synaptophysin. An additional important differential diagnosis is represented by metastasis from a NET of another site, usually the gastroenteropancreatic tract. In these cases, correlation with clinical and radiological data is mandatory. The prognosis of metastatic NETs to the testes is poor [47].

Overall, the prognosis of primary testicular NET is excellent, with a 5-year overall survival rate of about 80% and a 5-year specific survival rate of about 85%, although lymph node and visceral metastases may be present. Orchiectomy is the treatment of choice and staging retroperitoneal lymphadenectomy may be

performed in patients with an associated teratomatous component. Adjuvant chemotherapy is not indicated in pure primary testicular NET, whereas somatostatin analogues have been administered to a few patients with metastatic disease [47].

Neuroendocrine Neoplasms of the Uterus

NENs of the uterus are very rare and are mainly represented by NECs that can arise both in the cervical mucosa and in endometrium. Several cases are MiNENs, in which the nonneuroendocrine component is frequently a squamous cell carcinoma in the uterine cervix and an endometrioid carcinoma in the endometrium.

NENs of the Uterine Cervix

Cervical NENs are rare, representing less than 2% of all tumors of the uterine cervix [53, 54], and include NETs, NECs, and MiNENs.

NETs of the Uterine Cervix

Cervical NETs are exceedingly rare and are frequently incidentally diagnosed in biopsies or surgical samples removed for other reasons, as they do not present any specific symptom and carcinoid syndrome has not been reported in these cases [55]. About 10 cervical NETs have been reported in the literature to date and the mean age of presentation is 50 years. Histologically the typical well-differentiated neuroendocrine morphology is readily recognizable; the tumors are composed of monotonous cells with a trabecular or organoid arrangement. Immunohistochemistry for general neuroendocrine markers (synaptophysin and Chromogranin A) is mandatory for the diagnosis. Cervical NETs are traditionally subdivided into typical and atypical carcinoids, the latter presenting an increased mitotic rate and focal necrosis. More recently, the terminology NET G1, G2, and G3, based on increasing mitotic and proliferative index, has been preferred, in keeping with the framework for grading and classification of NENs of the gastroenteropancreatic tract [1, 56]. In the few reported series atypical carcinoids (NET G2 and G3) are more frequent than typical ones (NET G1) and demonstrate tendency to metastasize to the liver [57]. An interesting case of a mixed cervical NET and cervical adenocarcinoma, in which microscopic, immunohistochemical, and ultrastructural data pointed toward a common origin of the two components, making this case a unique cervical mixed neuroendocrine-nonneuroendocrine neoplasm (MiNEN) with a well-differentiated NE component [58].

Due to the rarity of these entities, no definitive data about optimal therapy and prognosis are available.

NECs of the Uterine Cervix

Both small cell and large cell subtypes of NEC have been described in the uterine cervix. Small cell NEC of the cervix is the most frequent NEN of the female genital tract, accounting for about 80% of all cases, whereas large cell subtypes are

diagnosed in 12% of the cases [56, 59, 60]. Cervical NEC usually occurs at a younger age than squamous cell carcinoma of the uterine cervix [61]. The most common-presenting symptom is vaginal bleeding. Clinical evidence of hormone hypersecretion is a rare but reported event.

Grossly, the tumor is large and exophytic. There is frequent evidence of deep invasion. Microscopically, small cell NEC shows morphological features similar to small cell carcinoma of the lung. There is a small blue cell appearance at low magnification. Tumor cells are arranged in sheets, cords, and islands. Stroma is generally not abundant. Tumor cells are monotonous, small-to-intermediate size, with scanty cytoplasm. Nuclei are round or oval, with finely dispersed chromatin and inconspicuous nucleoli. Mitotic activity is brisk and apoptotic bodies are easily found, along with foci of necrosis, which can be very large, with geographic necrosis. Spindle-cell morphology is occasionally seen. Crush artifact and Azzopardi phenomena are also frequent, particularly in small biopsies. The large cell subtype is characterized by pleomorphic vesicular nuclei, with central, prominent, and often eosinophilic nucleoli. The tumor cell cytoplasm is abundant and lightly eosinophilic. These tumors have a high mitotic index, apoptosis, and geographic tumor cell necrosis (Fig. 14.8a, b). Cells are arranged in a solid, trabecular, or organoid pattern; and rosette-like arrangements can also be seen. In both subtypes, lymphovascular spaces involvement is a common finding. Cervical NECs may show combined features of small and large cell carcinoma, with small cells with indistinct nucleoli and nuclear molding merging imperceptibly with groups of larger cells with prominent nucleoli. In addition, large and small cell NECs of the uterine cervix may coexist with a variable proportion of nonneuroendocrine cell components, either glandular or squamous. In such cases, the term "mixed neuroendocrine–nonneuroendocrine neoplasm" (MiNEN) is appropriate. Usually, the neuroendocrine carcinoma component is more abundant, and the proportion of each component should be noted in the pathology report, because prognosis usually depends on the neuroendocrine tumor cell component. Interestingly, the presence of a minor adenocarcinoma and squamous cell carcinoma in the form of in situ or early invasive elements is a common finding in what are otherwise pure NECs [62]. This finding supports a common pathogenesis for neuroendocrine and nonneuroendocrine cervical malignancies, as it is also indicated by molecular findings (see below).

Tumor cells show positive immunohistochemical stains for synaptophysin (Fig. 14.8c) and chromogranin A. In addition, insulinoma-associated protein (INSM1) has been demonstrated to be a specific and sensitive marker of neuroendocrine differentiation also in cervical NEC [63]. The diagnosis should be questioned in the absence of positivity for general neuroendocrine markers, and a nonkeratinizing or basaloid subtype of a poorly differentiated squamous cell carcinoma should be suspected. In such cases, p63 and p40 may be of help in confirming the latter diagnosis. TTF-1 is sometimes positive in cervical NEC, and it is not useful in the differential diagnosis of metastasis from a NEC of another primary site.

Due to the rarity of cervical NENs, molecular data on their pathogenesis are poor. Human papillomavirus (HPV) DNA, particularly of type 18 and 16, has been detected in small and large cell neuroendocrine carcinomas of the cervix. A recent meta-analysis confirmed that most of cervical NECs are HPV-related, making this

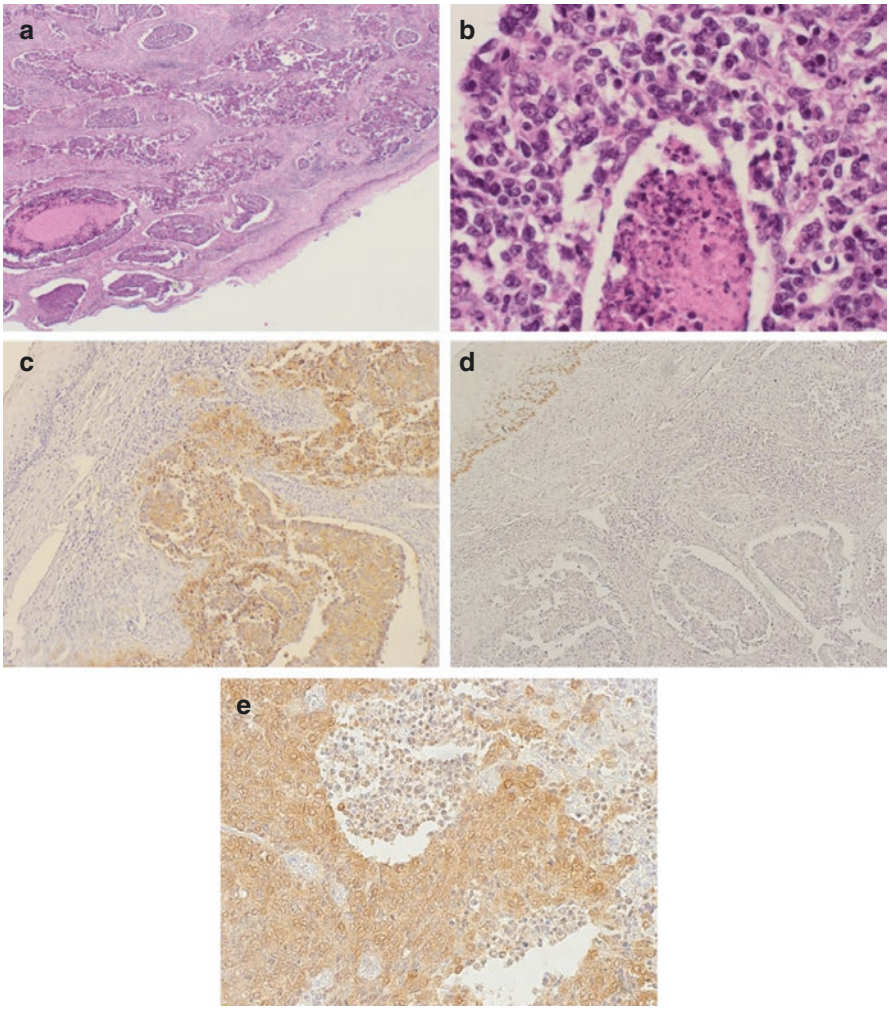

Fig. 14.8 Large cell neuroendocrine carcinoma (NEC) of the uterine cervix. Large and irregular nests of neoplastic cells are seen in the thickness of the cervical wall (**a**). At high magnification, atypical nuclei with irregular chromatin and visible nucleoli are seen, along with moderately abundant light eosinophilic cytoplasms (**b**). Synaptophysin is diffusely expressed in neoplastic cells (**c**), whereas p63 is absent (**d**). In this case, p16 is strongly and diffusely expressed in the majority of cells (**e**)

entity preventable by currently available prophylactic HPV vaccines [64]. Immunoreactivity for p16 is a surrogate marker of HPV infection also in cervical NECs [65] (Fig. 14.8e). Both p16 and HPV DNA are detected in metastatic neuroendocrine carcinomas of the cervix in other anatomic sites and provide a helpful tool to recognize the origin of the primary tumor, as lung tumors do not harbor high-risk HPV [66]. However, HPV infection alone does not explain the acquisition of the neuroendocrine phenotype or of the aggressive behavior of cervical NEC and

additional genetic events have been postulated in the progression of these neoplasms. Targeted next-generation sequencing has been recently applied to a small series of cases and showed recurrent somatic mutations in the MAPK, PI3K/AKT/mTOR, and p53/BRCA pathways, highlighting the possibility of personalized therapy in these aggressive neoplasms [67].

The prognosis of cervical NECs is much worse than that for stage-matched squamous cell carcinoma [68, 69]. The tumor behaves aggressively, with a 5-year survival rate of 36%. A recently systematic review of the published literature on cervical NECs reported that early and later stage disease presentation were evenly distributed (around 50% each). The mean recurrence-free survival was 16 months and the mean overall survival was 40 months. Multimodality treatment with radical surgery and neoadjuvant/adjuvant chemotherapy with cisplatin and etoposide with or without radiotherapy is the mainstay of treatment for early stage disease while chemotherapy with cisplatin and etoposide or topotecan, paclitaxel, and bevacizumab is appropriate for women with locally advanced or recurrent tumors [70]. Immune-checkpoint inhibitors have been administered to patients with recurrent cervical NEC in two case reports, in which nivolumab led to durable remissions [71, 72]. An additional case, bearing KRAS mutation, was treated with the MEK-inhibitor trametinib [73].

NENs of the Endometrium

Endometrial NENs are exceedingly rare, representing about 1% of all endometrial malignancies [74, 75]. Most cases are NECs, of the small or large cell types, whereas NETs are only anecdotally reported.

NETs of the Endometrium

The uterine corpus is an exceptional primary site for NETs, and only four cases have been reported in literature [76–79]. In the described cases, the morphology is very similar to NETs in other sites, but, due to their rarity, they are poorly characterized from a clinical and prognostic point of view. The most important diagnostic difficulty is, once they have been recognized, to distinguish them from metastatic NETs from other primary sites. In such cases, a careful clinical and radiological study is advisable.

NECs of the Endometrium

Albeit rare, endometrial NECs are more common than NETs of the same site. They are most frequently of the small cell type, with almost 100 cases reported to date in the literature, whereas lonely about 30 cases of large cell NECs of the endometrium have been reported [80, 81].

The clinical presentation of endometrial NECs is similar to that of endometrial adenocarcinoma, and abnormal uterine bleeding is the most common sign. Rarely, there is an associated paraneoplastic syndrome such as Cushing's syndrome, retinopathy or glomerulopathy [74]. The mean age of presentation is in the sixth to seventh decades of life and non-white women are more frequently affected than Caucasian [75].

Grossly, NECs of the endometrium do not differ from the most common histotypes of endometrial carcinoma and are represented by endometrium-based irregular polypoid masses with areas of necrosis and hemorrhage. Microscopically, endometrial small cell neuroendocrine carcinomas are similar to pulmonary small cell carcinoma. They are composed of sheets of round or ovoid cells, with dark chromatin and scanty cytoplasm. Nuclear molding is frequently seen. Large cell NECs are composed of round-to-polygonal cells with vesicular nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm. Both types may show a combination of architectural patterns, including solid, trabecular, or insular arrangements. High mitotic rate, geographic necrosis, and apoptosis are the rule. There is usually conspicuous lympho-vascular space invasion. A significant number of cases are represented by MiNENs, mainly composed of small or large cell NEC and endometrioid carcinoma (Fig. 14.9). The combination with serous and clear cell carcinoma is much rarer [80, 82–84].

At immunohistochemical analysis, tumor cells show positive staining for synaptophysin and, less frequently, for chromogranin A. Cytokeratin may show a positive dot-like pattern, as in small cell NECs of other primary sites. PAX8 is inconstantly positive and TTF1 may be expressed, confirming the poor value of transcription factors in identifying the primary site of origin of a NEC [80].

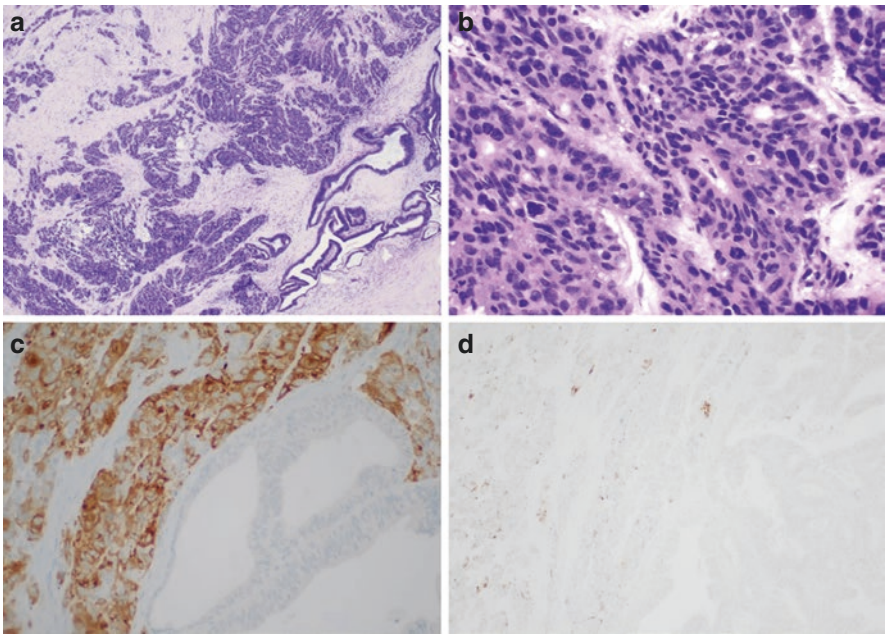

Fig. 14.9 Mixed neuroendocrine–nonneuroendocrine neoplasm (MiNEN) of the endometrium, composed by a large cell neuroendocrine carcinoma (NEC) (**a**, left; **b**) and an endometrioid carcinoma (**a**, bottom right; **b**). The NEC component shows intense immunostain for synaptophysin (**c**, left), which is completely negative in the endometrioid carcinoma (**c**, bottom right), whereas chromogranin A is only focally and faintly expressed in NEC cells (**d**)

There are no systematic studies on the molecular features of endometrial NECs; However, HPV DNA has not been found in tumors cells and p16 is inconsistently expressed. One study showed loss of expression of mismatch repair proteins in nearly a half of the cases, with the most common pattern being loss of MLH1/PMS2, presumably due to epigenetic silencing of MLH1 via promoter methylation [80]. However, no further study has investigated the molecular basis of these alterations.

NECs of the endometrium behave more aggressively than endometrial adenocarcinoma and are generally diagnosed at a more advanced stage. The prognosis for small and large cell NECs is poor, but there is one report that indicates favorable prognosis when the tumor is confined to endometrial polyps [85].

Neuroendocrine Neoplasms of the Ovary

The ovary is the most common site of primary NENs among female genital organs. Neuroendocrine tumors of the ovaries include paragangliomas, well-differentiated neuroendocrine tumors (also called carcinoid tumors, and atypical carcinoid tumors), small cell neuroendocrine carcinoma (pulmonary-type), and large cell neuroendocrine carcinoma. As paragangliomas are treated in a separate chapter, only epithelial NENs will be discussed herein.

Ovarian NETs

Primary ovarian NETs, also called ovarian carcinoids, are classified among monodermal teratomas and somatic-type tumors arising from a dermoid cyst [86]. They are the most common primary NEN in the female genital tract, and almost all arise within teratomas, especially dermoid cysts (mature cystic teratomas). However, microscopic foci of NET are rarely identified in other ovarian neoplasms, such as yolk sac tumor, Brenner tumor, and Sertoli-Leydig cell tumor [74], and some are pure NETs with no germ cell or other associated ovarian tumor. As a whole, ovarian NETs represent less than 1% of ovarian neoplasms [87]. The reported median age of diagnosis is around 50 years and they are generally asymptomatic, with a subset of cases presenting with a carcinoid syndrome, even in the absence of a metastatic disease [88]. Ovarian NETs are nearly always unilateral, and, macroscopically, appear as yellow nodules, frequently in the context of a dermoid cyst; but they can also be incidental microscopic findings. Traditionally, these tumors have been classified into four categories: insular carcinoid, trabecular carcinoid, strumal carcinoid, and mucinous (goblet cell) carcinoid. However, in the view of modern classification frameworks, insular and trabecular carcinoids represent morphologic variants of usual NETs, whereas strumal carcinoids represent a unique form of a mixed tumor, with a well-differentiated neuroendocrine component combined with a thyroid follicular endocrine component, therefore they should be classified as MiNENs (Fig. 14.10). Finally, mucinous (goblet cell) carcinoid is not properly a NEN; like

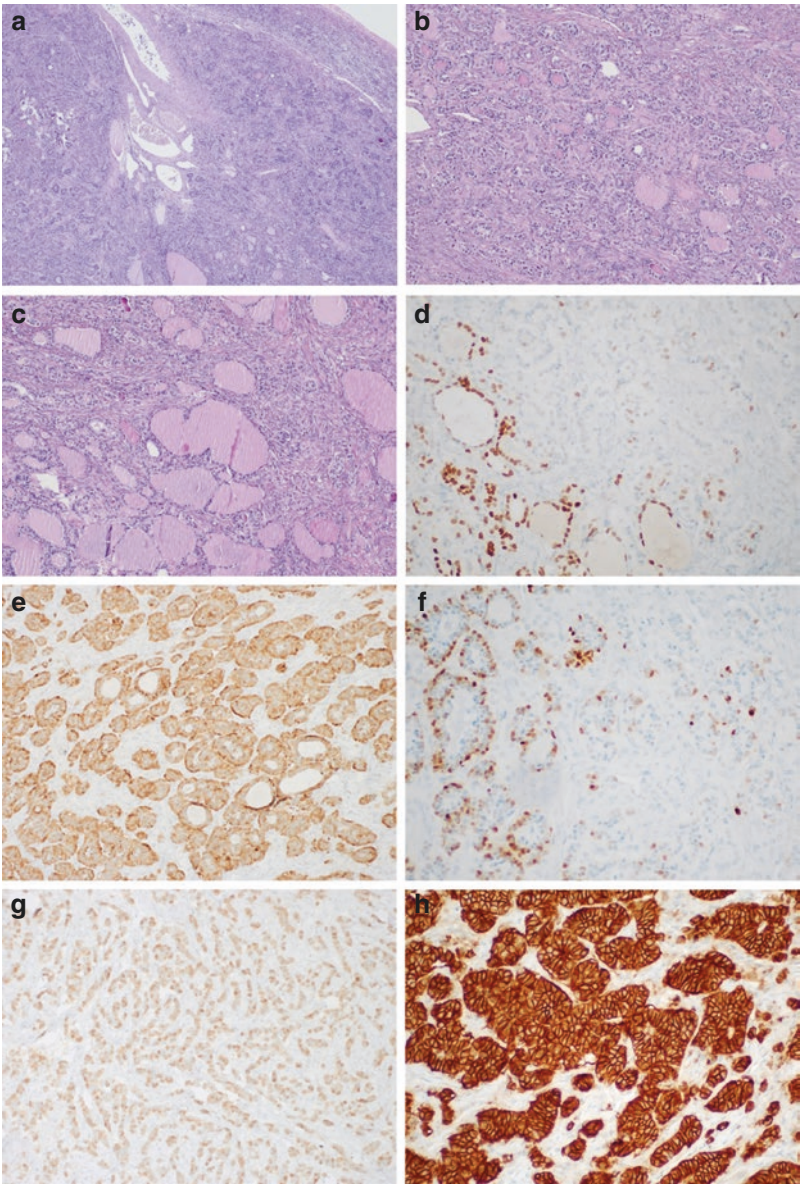

Fig. 14.10 Microscopic aspects of a mixed neuroendocrine–nonneuroendocrine neoplasm (MiNEN) of the ovary (strumal carcinoid) composed of a well-differentiated neuroendocrine tumor (NET) and a benign thyroid follicular proliferation. Low magnification shows a neoplastic nodule (**a**, left and bottom) growing in the ovarian parenchyma (**a**, top right). At higher power, thyroid follicles are visible, admixed with pseudoglandular, nodular, and trabecular proliferation of neuroendocrine cells (**b**, **c**). TTF-1 is expressed in the thyrocytes, whereas the neuroendocrine component is completely negative (**d**). Synaptophysin is intensely expressed in neuroendocrine cells (**e**), whereas chromogranin A immunostaining is limited to pseudoglandular and nodular structures and is absent in the trabecular ones (**f**), composed of L cells, in which glucagon is widely expressed (**g**). Somatostatin receptor 2A is intensely expressed with a membranous pattern in all neuroendocrine cells (**h**)

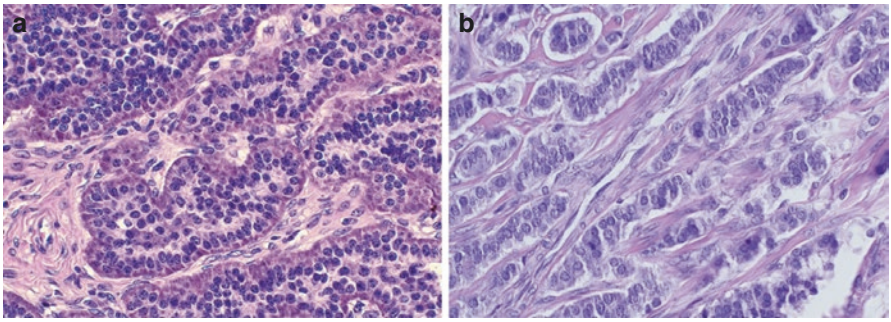

Fig. 14.11 Microscopic architectural patterns in ovarian well-differentiated neuroendocrine tumors (NETs). Solid nests pattern with peripheral palisading of neuroendocrine cells with brightly eosinophilic cytoplasm, overlapping the morphological features of EC-cell midgut NETs (**a**): Trabecular proliferation of elongated neuroendocrine cells resembling L-cell hindgut NETs (**b**)

goblet cell carcinoid of the appendix, as it is composed of amphicrine cells, with both neuroendocrine and mucin-producing features, behaves more aggressively than NETs and can be complicated by the development of a goblet cell carcinoma [89]. For these reasons it will not be discussed here.

Microscopically, ovarian NETs present most frequently an insular pattern, and are very similar to midgut NET, both architecturally and cytologically (Fig. 14.11a). They are predominantly composed of nests of monotonous polygonal cells with round nuclei. They often contain small acini with eosinophilic secretions, typically found at the periphery of the islands. Calcification and psammoma bodies may be found. Immunohistochemical stains show expression of enterochromaffin cell markers, such as CDX2, serotonin, and substance P. A trabecular pattern of growth is less frequent than the insular one and is very similar to that seen in resemble hindgut NETs (Fig. 14.11b). In these cases, tumor cells are positive for PYY and glucagon-like peptides, similarly to L cells. It is worth noting that, like rectal NETs, ovarian NETs with a trabecular pattern of growth may be negative for chromogranin A, whereas they are diffusely positive for synaptophysin. A subset of cases shows a mixed, insular, and trabecular pattern. Mitotic figures are infrequent in both types of carcinoid tumors. Somatostatin receptors are expressed in the majority of tumors. Most of the cases have a low mitotic and proliferative index, resembling NET G1 of the gastroenteropancreatic tract.

The differential diagnosis of ovarian NETs includes Sertoli-Leydig tumors and ovarian metastases of NETs from other primaries. Ovarian NETs are sometimes associated with condensation and luteinization of stromal ovarian cells around nests of neuroendocrine tumor cells, or at the periphery of the tumor. This situation, known as ovarian tumors with functioning stroma, may cause problems in the differential diagnosis; as they resemble sex-cord stromal tumor. Immunohistochemistry can be very helpful in distinguishing NETs, which are positive for general neuroendocrine markers, from the SF-1, calretinin-, and inhibin-positive Sertoli-Leydig tumors. In contrast, the distinction of primary ovarian NETs from metastatic NETs

from ileal and, less frequently, rectal NETs is impossible based on immunohistochemistry alone. The presence of residual teratomatous components of a dermoid cyst strongly supports an ovarian primary, but larger tumors, with no teratomatous aspects, warrant careful analysis of the clinico-pathologic features of the lesion to be correctly framed. In particular, bilaterality, ovarian surface involvement, multinodular lesions in the ovarian parenchyma, angioinvasion, and extra-ovarian involvement have been reported to be clues to a metastatic nature of the lesion [74]. Careful imaging studies of the abdomen are mandatory in these cases.

The so-called "strumal carcinoid" is a rare type of teratoma composed of thyroid tissue and well-differentiated NET in the ovary [90]; it is frequently is associated with a dermoid cyst. The thyroid follicles may be of various sizes and shapes; the neuroendocrine cells form cords, trabeculae, and less frequently nests, of monotonous neuroendocrine cells, with granular, eosinophilic cytoplasm, and salt and pepper nuclear chromatin. The follicular cells show positive expression for thyroglobulin and TTF-1, while the neuroendocrine elements are positive for synaptophysin and chromogranin A.

Primary ovarian NETs are low-grade malignant tumors. The prognosis is very good for patients diagnosed at stage I, which account for most of the cases. Distant metastasis and death occur in less than 5% of patients [88].

Ovarian NECs

NEC of the ovary is very unusual and may be observed as MiNEN, in association with nonneuroendocrine epithelial malignancies, such as serous, mucinous, clear cell, and endometrioid carcinoma, as well as Brenner tumor. In the last WHO classification of ovarian tumors, NECs are classified among miscellaneous tumors, under the heading of "small cell carcinoma, pulmonary type" [86]. Large cell NEC is not mentioned in the classification. Ovarian NEC occurs in a wide range of ages and may be unilateral or bilateral. Most patients present with high-stage disease. Primary small cell NEC is very uncommon, and the most numerous published series reported only 11 cases [91]. Large cell NEC is even rarer and few cases have been reported [92, 93].

Primary ovarian NECs are usually unilateral, solid, and cystic. Occasionally, they occur in association with a dermoid cyst. Microscopically, the neoplastic proliferation is similar to small and large cell NECs of other organs and they occasionally merge with other histologic types, including mucinous, endometrioid and serous carcinoma, or even mucinous borderline tumors.

The differential diagnosis of small cell and large cell ovarian NECs includes metastatic tumors from the lung and other organs. In the presence of a previous diagnosis of NEC in another organ, concordant p53 mutational pattern between the presumed primary and metastatic tumor may be a good tool for confirming the diagnosis and exclude the possibility of a primary ovarian carcinoma [94]. In fact, in a recently reported case, a serous carcinoma of the endometrium metastasized to the ovaries in the form of a high-grade neuroendocrine carcinoma and concordant

mutational profile in both endometrial and ovarian tumors detected by next-generation sequencing confirmed the metastatic nature of the ovarian tumor [95]. The formerly called small cell carcinoma of the ovary of hypercalcemic type, now better known as malignant rhabdoid tumor of the ovary, should not be confused with NEC, as it is not a NEN. It is generally negative for neuroendocrine markers, and recent studies have shown that almost 100% of these neoplasms contain germline or somatic mutation of the *SMARCA4* gene. A negative immunostain for the protein encoded by *SMARCA4*, INI1, may be extremely useful in diagnosing this entity [96].

The prognosis of primary ovarian NEC is very poor, similarly to NECs of other sites [91].

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Skin Neuroendocrine Neoplasms

15

Sofia Asioli

Introduction

The current pathologic classifications of neuroendocrine neoplasms (NENs) across different organ systems use a range of site-specific terminologies and criteria, creating significant confusion among pathologists and treating clinicians. A uniform classification framework for NENs has been recently proposed [1], through a consensus proposal by experts of International Agency for research on cancer (IARC) and of World Health Organization (WHO) in which unification of classification concepts, despite organ-specific differences in classification criteria, has been delineated. The classification suggested intends to allow pathologists and clinicians to manage their patients with NENs consistently, and to facilitate comparisons between the different entities falling into this category of neoplasms.

The new World Health Organization (WHO) 2019 of the skin tumors [2] recognized only two well-established entities of primary cutaneous neuroendocrine neoplasms: Merkel cell carcinoma (MCC) and endocrine mucin-producing sweat gland carcinoma (EMPSGC).

In the skin, some endocrine neoplasms are composed of a mix of endocrine and exocrine cells as reported in a previous review on the spectrum of endocrine tumor of the skin by Foschini MP & Eusebi V [3]. The definition of "pure" neuroendocrine neoplasms is a matter of debate and there is lack of uniform criteria. Besides, a true primary well-differentiated neuroendocrine tumor (NET), although it is exceedingly rare, has been reported [4–10].

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In 2014 Asioli et al. [11] proposed a working formulation of neuroendocrine tumors of the skin to present a list of skin neoplasms showing neuroendocrine differentiation based on morphological, cytological, and immunohistochemical criteria.

To date, based on above proposals and from a practical point of view, here it seems more realistic to subdivide skin NENs into two main groups:

(a) Skin NENs included in the 2019 WHO Classification of Skin Tumours [2] include (1) Merkel cell carcinoma (MCC) and (2) endocrine mucin-producing sweat gland carcinoma (EMPSGC).
(b) Skin NENs not included in 2019 WHO Classification of Skin Tumours [2] are (1) neuroendocrine tumor (NET) and (2) carcinomas with neuroendocrine differentiation.

Skin NEN Included in the 2019 Who Classification of Skin Tumors

Merkel Cell Carcinoma

The 2019 World Health Organization (WHO) classification of skin tumors [2] defined Merkel cell carcinoma (MCC) as synonymous with primary high-grade cutaneous neuroendocrine carcinoma. It was first described by Tocker 1972 [12] and subsequent literature demonstrated the neuroendocrine differentiation [13–15] while the pathogenesis still remains debated. Although MCC is rare with about 2488 cases per year diagnosed in the United States [16], it is an aggressive skin tumor that primarily affects elderly individuals. Its incidence is dramatically increasing [17–25]; and typically, it presents as a rapidly growing pink-red dome-shaped nodule, with a strong preference for sun-exposed areas, and is characterized by a high incidence of local recurrence, regional lymph node, and distant metastasis [26]. On histology, MCC cells are arranged for the most part in small nests with a noncohesive growth pattern and demonstrate vesicular nuclei with well-outlined nuclear membranes. Nucleoli are small and chromatin is dispersed. UV exposure and immunosuppression appear as important risk factors; particularly, immunosuppression may be associated with MCC onset at a younger age [20]. An important study in 2008 demonstrated the clonal viral integration or expression of Merkel cell polyomavirus (MCPyV) oncoproteins in approximately 80% of MCC [27]. As reported for other tumor models, the virus appears to be a cancer promoter. However, since 20% of MCC do not show evidence of MCPyV infection, their pathogenesis remains to be elucidated [28]. MCPyV is a common infection in humans and its role in MCC tumorigenesis is under investigation. Other mechanisms, not involving MCPyV, responsible for tumor onset and progression are under investigation and appear suitable for new possible therapeutic targets.

Molecular Prognostic and Therapeutic Targets

According to COSMIC [https://cancer.sanger.ac.uk/cosmic] (Table 15.1), an online database reporting somatically acquired mutations in human cancer and the major molecular pathways involved in oncogenesis, we report the oncogenes and the tumor suppressor genes that seem involved in the pathogenesis of MCC and we highlight the new possible therapeutic targets for patients affected by MCC.

Table 15.1 Review of the most important results of studies assessing the presence and effects of somatic gene mutations (according to COSMIC [https://cancer.sanger.ac.uk/cosmic]) in MCC*

Author, year	Most important findings
Van Gele et al. 2001 [68]	PTEN gene nonsense mutation identified in one case Homozygous deletion of exon 9 identified in one case
Worda et al. 2005 [108]	No evidence of T1796A BRAF mutation
Houben et al. 2006 [83]	MAP kinase signaling pathway completely silenced in both primary and metastatic tumors Involvement of Ras/Raf/ MEK/ERK signal cascade in tumor pathogenesis
Liu et al. 2007 [89]	No evidence of CTNNB1, APC, and AXIN2 mutation A silent mutation in AXIN1 identified in 3 cases
Swick et al. 2007 [47]	KIT expression in tumors but no activating mutations detected in exons 9, 11, 13, or 17 of KIT
Swick et al. 2008 [50]	Novel single heterozygous base change in exon 10 of the PDGFRA gene, leading to an amino acid substitution at codon 478 identified in 3 out of 9 cases
Kartha et al. 2008 [48]	Identification of silent mutations in exon 17 of KIT and exons 10, 12, and 18 of PDGFRA No correlation between positive immunostaining and activating mutations in KIT and PDGFRA KIT/steam cell factor and PDGFRA/PDGFA co-expression suggest an autocrine/paracrine stimulation
Lassacher et al. 2008 [36]	Methylation of p14ARF promoter DNA in 42% of the cases Methylation of p16INK4a promoter DNA in 5% of the cases
Brunner et al. 2008 [40]	c-kit, Mcl-, Bmi-1, VEGF (A, C, R2), PDGF-α, PDGF-β are possible therapeutic targets No evidence of EGFR and Her-2/Neu mutation No evidence of c-KIT mutation
Andea et al. 2010 [49]	Two silent mutations involving exons 17 and 18 of *KIT* Two mutations involving introns 16–17 of *KIT* Possible correlation between KIT expression and worse prognosis
Waltari et al. 2011 [38]	p53 and KIT expressionin MCPyV-negative tumor Tumor containing MCPyV DNA, without p53 expression, showed the best prognosis
Hafner et al. 2012 [63]	Activation of the PI3K/AKT pathway PI3K/AKT as a potential new therapeutic target
Nardi et al. 2012 [66]	Possible pathogenetic role of the PI3K pathway PIK3CA mutations could be an option for treatment with PI3K pathway inhibitors

(continued)

Table 15.1 (continued)

Author, year	Most important findings
Cimino et al. 2014 [35]	Retinoblastoma gene (RB1) nonsense truncating protein mutations in 3/5 cases; no mutations MCPyV-positive cases
Xie et al. 2014 [57]	Correlation between TERT gene copy number and TERT mRNA expression TERT expression and telomerase activity could be due to a promoter mutation Higher TERT mRNA levels correlate with shorter overall survival and predict poor outcome

Table legend: **MCC* Merkel cell carcinoma, *MCPyV* Merkel cell polyomavirus

P53 and RB1 Pathway Genes

MCPyV DNA integrates into the genome of MCC tumors in a clonal pattern, indicating that viral infection precedes clonal expansion of cancer cells. The *MCPyV* encodes two oncoproteins, Small T (ST) and Large T (LT) antigens. LT binds to RB and p53 and inhibits their function [29]. In particular, even if MCPyV presents a truncated form of LT, it is still able to inactivate p53, and mutations of *TP53* were found in more than 50% of MCCs lacking MCPyV LT expression [29, 30]. Previous studies reported that Simian virus 40 (SV40) large T antigen (LT) probably transforms and immortalizes cells. Particularly, cells lacking the C-terminal of the p53-binding domain remain able to inhibit p53-dependent transcription, and, therefore, SV40 ST could also repress p53 function [31, 32]. In addition, SV40 LT antigen seems to induce neuroendocrine "differentiation," as demonstrated in animal and human cancer models [33, 34].

MCC with no evidence of MCPyV infection presents recurrent mutations in tumor suppressors, including *TP53* and *RB1* [28]. Indeed, Cimino et al. found a high prevalence of truncating, nonsense *RB1* mutations in their series [35]. Two out of the five MCPyV-negative MCCs harbored a *RB1* deletion, while no single-nucleotide variation truncating nonsense mutation was found in MCPyV-positive tumors. The authors proposed the presence of a genetic mechanism leading to RB1 inactivation exclusive for polyomavirus-negative cases, and of RB1 dysregulation in polyomavirus-positive cases. However, no definite conclusions can be drawn due to the small number of cases that have been tested. Moreover, no *N-RAS, H-RAS*, or *K-RAS* mutations were identified in the same MCC series [35].

Previous studies revealed frequent mutation of exons 5 and 9 of the *TP53* gene, especially in MCPyV-negative cases [30, 36]. A DNA sequence analysis of 21 primary MCCs revealed three *TP53* polymorphisms (codon 72, G-C transversion) in 14% of the analyzed tumors, and a polymorphism of p16 protein expression (*INK4a-ARF* mutation) in one MCC [36]. According to these data, p53 mutations seem less commonly involved in MCC pathogenesis compared to other skin tumors. Interestingly, another study identified the presence of methylated DNA at the *p14ARF* promoter, regulating the p14ARF/mdm2/p53 pathway, in 42% of MCC, while *p16INK4* promoter methylation, previously reported as a common epigenetic mechanism of regulation of protein expression, was identified in only 5% of the MCCs tested [37].

However, the involvement of *TP53* mutations in MCC pathogenesis remains controversial. Waltari et al. [38] evaluated *TP53* status and p53 immunohistochemical expression demonstrating a lower copy number of *MCPyV* DNA in p53-positive, in contrast to p53-negative MCCs. Additionally, the percentage of p53-positive nuclei in the tumor was inversely proportional to *MCPyV* DNA copy number. Finally, the best overall and MCC-specific survival was found in patients with tumors that were *MCPyV* DNA positive, without p53 expression. In particular, these tumors also had higher copy numbers of *MCPyV* DNA, in contrast to p53-positive MCCs. Therefore, p53 expression-related molecular mechanisms in MCCs seem to be associated with an adverse outcome.

KIT Receptor Tyrosine Kinase

The expression of the proto-oncogene KIT receptor tyrosine kinase has been frequently detected in MCC [38–41]. Particularly, Waltari et al. [38] postulated an inverse correlation between KIT and p53 expression, and MCPyV expression. KIT and p53 expression were more common in MCPyV-negative MCCs, which showed an unfavorable outcome. However, the real frequency of KIT expression in MCCs still remains to be clarified. Indeed, Feinmesser et al. found KIT expression in 67% of MCCs analyzed, associated with a high mitotic rate and lympho-vascular invasion [41], whereas a more recent study found it in only 7% of the cases [40]. Another series of 21 MCCs demonstrated KIT expression in 95% of cases, but it did not identify any correlation with survival rate [42].

Activating mutations of c-KIT proto-oncogenes are considered the basis of the pathogenesis of gastrointestinal stromal tumors (GISTs) [43], as well as adult mastocytosis and pulmonary small cell carcinoma, in which constitutive activation of the KIT tyrosine kinase has been demonstrated [44, 45] and proposed as a potential target for adjuvant therapies [46]. The association of KIT expression with outcome also remains controversial. A study including less than 30 MCCs did not find any association [38], while in another one [37] KIT expression correlated with S478P substitution in PDGFRA exon10. The activating of KIT and PDGFRA mutations could not be found in other human tumors, for which their pathogenic significance remains undetermined.

Interestingly, MCCs that show p53 or KIT, but are not related to MCPyV, could have different molecular pathogenesis and might respond to treatment with tyrosine kinase inhibitors [47]. Tumors included in this series showed a diffuse KIT expression (88.8%), while none of the most common activating c-KIT mutations (i.e. exons 9, 11, 13 and 17) were identified.

The mutational status of KIT and its expression was also evaluated by Kartha et al. [48] who did not identify any correlation between KIT status, KIT expression, and clinical features, nor activating mutations in its receptor. A rare silent mutation of KIT in exon 17 at codon 798 was reported also in MCC [49]. Kartha et al. [48] analyzed the expression of KIT and PDGFRA and their ligands, stem cell factor (SCF) and PDGFA, respectively. They described weak and diffuse SCF immunohistochemical expression that did not necessarily correlate with KIT staining. In particular, SCF was evaluated to identify if the autocrine/paracrine stimulation of the KIT receptor could

be an alternative pathway of tumor proliferation. Around 15% of MCCs showed a co-expression of KIT and SCF. Therefore, the autocrine stimulation could be responsible for KIT activation in KIT+/SCF+MCCS, as reported in GISTs and small cell lung carcinomas [43, 50]. The authors hypothesized that in the absence of activating mutations in KIT, this paracrine stimulation could be the basis for tumor proliferation in MCCs, even if their analysis of PDGFRA and its ligand suggests also an autocrine mechanism of stimulation for this receptor kinase in the majority of MCCs evaluated [48]. The absence of any significant mutations in these receptor tyrosine kinases suggest a poor response to imatinib mesylate in MCC, due to the strong association with activated KIT and PDGFRA, despite the detection of a novel mutation in exon 10 of the PDGFRA in some cases [51], as demonstrated by tumor progression and poor overall survival in the phase I trial with imatinib mesylate in MCCs [52].

TERT Promoter

Telomerase is an RNA-dependent DNA polymerase responsible for lengthening telomere [53, 54]. It is silent in most of the normal human cells; its activation is an essential step for malignant transformation [55], and its activity has been detected in more than 90% of human malignancies [53–55]. The activation of telomerase is linked to the induction of telomerase reverse transcriptase (TERT) expression [53, 56]. Telomerase activity was detected in all the six MCC cell lines and 11 tumors analyzed by Xie et al. [57]. In particular, mutations of TERT promoter were identified in 4 out of 35 MCCs evaluated and were associated with UV signature. This interesting paper also correlated the increase of TERT gene copy number with its mRNA expression and shorter overall survival. Higher TERT expression could predict poor MCC outcome [57], as per other aggressive cancers [58] since TERT function and mutations have been demonstrated to play a crucial role in cancer development and progression [59]. Another study evaluated TERT mutations in 15 MCCs and found a lower incidence of TERT promoter mutations with respect to other malignancies [60]. Possibly, new cancer treatment targeting telomerase, in combination with conventional therapeutic approaches with telomerase inhibitors, could improve treatment efficacy and survival in MCC [59].

PI3K/AKT

The activation of PI3K/pAKT pathway has been described in several cancers, especially in head and neck squamous cell carcinomas (around 75% of the cases) [61, 62]. The two major hotspots where the mutations were identified are the helical and the kinase domains of PI3K (encoded by *PIK3CA* gene). The mutated PIK3CA proteins could have an active role in tumorigenesis, since PI3K is involved in signaling from receptor tyrosine kinases via the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). In contrast, PTEN reverses this step and induces downstream phosphorylation and activation of the survival kinase AKT1. The PI3K/AKT pathway can be activated by oncogenic mutations. Somatic mutations in the *PIK3CA* gene have previously described in tumors, including skin lesions [63]. Furthermore, mutation in the pleckstrin homology domain of *AKT1* has been found in numerous tumor entities, but less frequent than *PIK3CA* mutations [64]. It has been demonstrated that mutations of *AKT1* and *PIK3CA* are mutually exclusive events [65].

In MCCs, the presence of activating *PIK3CA* mutations is strongly suggestive of an oncogenic role played by the PI3K/pAKT pathway. Firstly, an activating mutation in *PIK3CA* (p110α subunit) was found by Nardi et al. in MCCs [66] and it characterized male patients, MCPyV-negative tumors, with stage II–IV disease. In the majority of cases, the primary tumor locations were head and neck, followed by lower extremities. MCCs with *PIK3CA*-mutation showed distinct histological characteristics, including visible necrosis and pleomorphic or spindle cells. The PI3K/pAKT pathway may also be altered by changes in the *EGFR* and the *HER2/ERBB2* genes encoding receptor tyrosine kinases, which have been extensively evaluated in numerous human malignancies [67, 68]. Nardi et al. [66] evaluated *EGFR* and *HER2* gene amplification by FISH analysis. *EGFR* amplification was not detected in MCC, while only one tumor showed *HER2* gene amplification. In their experience, *MCPyV* integration and *PI3K* activation were not mutually exclusive, suggesting an independent role in the pathogenesis and progression, and the possibility of a combined treatment with anti-viral agents and PI3K-targeted therapies [67–69]. Harms et al. also identified a mutation of PI3K pathway in 5 out of 15 MCCs analyzed [28].

Additionally, the role of the tumor suppressor PTEN was investigated by Van Gele et al. [68], who investigated the loss of heterozygosity. Loss of one allele was observed in 9 out of 21 MCC cases, with predominant involvement of loss of the entire arm of chromosome 10. They concluded that PTEN inactivation does not have a key role in MCC oncogenesis and development [68]. Hafner et al. found *PIK3CA* mutation in only 4% of their cases, but no mutation of exon 4 of *AKT1*. However, considering the high sensitivity to the PI3K inhibitor in MCC cells line in vitro, they suggest a possible therapeutic option [70]. At the same time, this paper demonstrated a significantly higher AKT phosphorylation in MCCs, in contrast with malignant melanoma [71]. The activation and the phosphorylation of AKT of the PI3K/AKT signaling have been extensively investigated [72]. Additionally, in contrast with small cell carcinoma of the lung [73, 74], where PTEN downregulation was described, in MCC it was rarer [75].

However, tyrosine kinase inhibitors could be a possible therapeutic approach to MCC, and multiple ongoing clinical trials have been promoted [76–78]. At the moment, published data of a phase II study of imatinib showed no benefit in patients with advanced MCCs [79].

MAP Kinase

The intracellular signaling cascade of Ras/Raf/MEK/ERK is involved in the controls of cell growth, differentiation, and survival. Its activation could be due to a large variety of extracellular stimuli [80]. This cascade starts with MAP kinase (Raf) phosphorylation, which activates MAP kinase (MEK), followed by MEK phosphorylation, which activates the extracellular signal-regulated kinase (ERK). Three main MAPK pathways have been described: ERK, C-Jun NH2-terminal kinase (JNK), and the P38 pathway [80]. However, only ERK-activation has been involved in human cancer (i.e., small cell lung cancer) [81], even if the other two pathways are involved in carcinogenesis [82]. ERK-pathway is completely inactivated in MCC [83]. Interestingly, Houben et al. [83] found a silent MAP kinase

pathway in almost all samples of MCC evaluated, as well as an increase of Raf kinase inhibitor protein expression in 20 out of 42 MCCs. According to these data, the authors suggested an alternative therapeutic approach based on the inactivation of MAP kinase signal transduction pathway in MCC.

Nocht-1 signaling pathway, which cross-talks with the Ras/Raf/MEK/ERK intracellular signaling, is also involved in cellular proliferation, differentiation, development, and survival. Nocht-1 plays the role of both tumor suppressor and oncogene to regulate cell growth and apoptotic regulation together. Mutations affecting one or more NOTCH genes were described in MCC [84], especially in MCPyV-negative cases, and these were mainly located in EGF or ankyrin repeat regions, consistent with inactivating function [83].

β-catenin

β-catenin is a gene involved in cell adhesion and maintenance of tissue architecture and polarity and is also a transcriptional activator [85, 86]. Mutations in the gene encoding β-catenin, *CTNNB1*, may result in loss of expression or aberrant nuclear accumulation. In neuroendocrine lung cancer and in other human malignancies, β-catenin loss decreases cell adhesion and facilitates metastatic spread [87], and it has been associated with poorer survival. The role of Wnt-pathway has been studied in MCC through the evaluation of the nuclear accumulation of β-catenin, as well as mutations of β-catenin and other related genes. Aberrant or decreased expression of β-catenin has been frequently observed in other tumors (i.e., non-small lung cells lung tumors, in which it also correlated with poorer prognosis [73]), suggesting a possible role in the development of MCC. Interestingly, Tanaka et al. [88] performed an immunohistochemical analysis of adhesion molecules (E-cadherin, α- and β-catenin) in MCC, and reported downregulation of their expression in a significant percentage of cases, suggesting the involvement of β-catenin/Wnt-pathway in MCC pathogenesis. However, subsequent investigations did not confirm these data (β-catenin accumulation was found in only one tumor), suggesting that this pathway is probably not implicated in MCC [69, 89].

Tissue Inhibitors of Metalloproteinases (TIMPs)

Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors of metalloproteinases. Four different isoforms (TIMP-1, 2, 3 and 4) have been described. Their overexpression has been demonstrated in many human malignancies and has been associated with poor prognosis [90].

TIMP-1 and TIMP-2 do not seem to be involved in MCC genesis, while TIMP-3 expression has been associated with poor outcome, probably due to the concomitant mutation or hypermethylation of p53-mediated transcriptional repressor [69]. The downregulation of TIMP-3, associated with the alteration of the Vascular Endothelial Growth Factor (VEGF)/VEGF receptor-2, seems to have a key role in tumor invasion and neo-angiogenesis. TIMP-3 deficiency in the host, but not in the tumor, is responsible for enhancing tumor growth and angiogenesis [91]. More than 90% of MCCs expressed TIMP-3 [92], and this was strongly correlated with a worse prognosis, together with expression of matrix metalloproteinase-1 (MIP-1) and -2 by immunohistochemistry [93, 94]. Moreover, according to other cancer models,

TIMP-3 gene and TIMP-3 protein expression seem to be implicated in tumor progression and in local invasiveness [95]. In MCC, the two isoforms, TIMP-1 and TIM-3, could lead to aggressive tumor behavior and induce local invasion as well as metastasis [92]. TIMP-4 downregulation could also to be related to MCC progression, as described in lung cancer [96], but no data are available at the moment.

PD1/PDL-1

The programmed death 1 (PD-1) immune checkpoint pathway and its ligands PD-L1 and PD-L2, expressed on tumor cells and tumor-infiltrating lymphocytes, mediate the local immune resistance to the tumor [97]. Nowadays, this pathway is a promising new therapeutic option for numerous advanced cancers, currently treated with anti-PD1 and PD-L1 or -L2 monoclonal antibodies [98]. The expression of the PD-1 receptor and its ligand (PD-L1) has been demonstrated in a large number of cancers [97], whereas the immune modulatory activity of PD-1 receptor in T cells only. Nghiem et al. [99] found PD-L1 expression on MCC cells and on infiltrating immune cells. The immunohistochemical expression of PD-L1 correlated with clinical response to pembrolizumab [99]. Particularly, PD-L1 expression was more frequently observed in MCPyV-negative MCCs (71% vs. 25%), without any significant correlation with intra-tumor CD8 T-cell infiltrate. In contrast, Goodman et al. demonstrated that a higher tumor mutation burden could predict a possible favorable outcome in treatment with PD-1/PD-L1 blockade, but not to a combination of anti-PD-1/PD-L1/anti-CTLA4 therapies [100]. PD1 blockade holds the interaction between PD1 expressed on tumor-infiltrating T cells, and PD-L1 expressed on tumor cells. However, PD-L1 expression by MCC tumor cells is not yet a defined biomarker of clinical response [101], and no other specific oncogene or driver mutation correlated with clinical response to PD-1/PD-L1 blockade [102]. Particularly, in MCC the major pathogenetic role is the MCPyV infection, and as already observed in other virus-associated cancer, the expression of viral neoantigens is strongly immunogenic [103]. Lyford-Pike et al. [104] evaluated the expression of PD-1 receptor ligand PD-L1in head and neck squamous cell carcinoma positive and negative for human papilloma virus (HPV), suggesting a role of PD-1/PD-L1 pathway in immune resistance in HPV-related lesions [105]. Recently, PD-1/PD-L1promoter methylation was evaluated in series of 69 MCCs. The status of promoter methylation was associated with higher overall mortality by univariate (log-rank test: $\chi 2 = 5.17$, $p = 0.023$) and multivariate (HR = 2.111, $p = 0.042$) analysis. In addition, the multivariate analysis identified stage III and IV, size >2 cm and MCPyV infection as negative prognostic factors. Moreover, high methylation of the promoter of the immune checkpoint receptor CD279/PD-1/PDCD1 was associated with older age, absence of immunohistochemical expression of PD-L1 on tumor and immune cells, as well as absence of immune cells at the periphery of the tumor [106]. These results support PD-1/PD-L1 blocked agent antibody (Avelumab) as a possible therapeutic approach in advanced MCC, associated with a durable response [107].

BRAF

Finally, BRAF V600E mutation in MCC could be a potential targetable mutation; nevertheless only two different studies are available at the moment. Worda et al.

investigated the role of the T1796A mutation by direct sequencing of polymerase chain reaction (PCR) products and allele-specific PCR in 15 patients affected by MCC but they did not find any mutation [108]. Houben et al., in a cohort of 46 MCC, demonstrated the absence of BRAFV600E mutation [83]. Therefore, despite the frequent similarities between MCC and malignant melanoma, therapies targeting BRAF do not seem useful in the treatment of MCC.

Pathology Report

Pathologist should be experienced in distinguishing MCC from cutaneous simulants and metastatic tumors. Histologically, MCC is a basaloid neoplasm, arranged in sheets, cords, nests, or trabecular arrays, with a non-cohesive growth pattern, characterized by hyperchromatic, vesicular, and large nuclei with well-outlined nuclear membranes, indistinct nucleoli, and scant cytoplasm. An appropriate immunohistochemical panel should preferably include cytokeratin 20 and thyroid transcription factor 1 (TTF1). Immunohistochemestry for CK20 and most low-molecular-weight cytokeratin markers are typically positive with a paranuclear "dot-like" pattern. CK 7 and TTF1 (positive in >80% of small cell lung cancers) are typically negative. Additional immunostains include neuroendocrine markers such as chromogranin A, synaptophysin, CD 56, neuron-specific enolase (NSE), and neurofilament. In some cases, neoplastic cells are positive for p63 (Fig. 15.1), which is directly correlated with patient survival [109, 110].

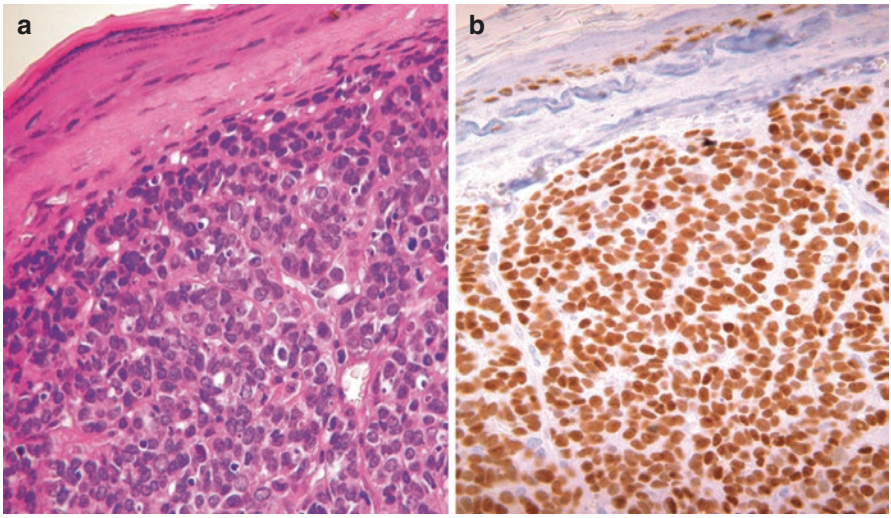

Fig. 15.1 (**a**) At high power, a primary Merkel cell carcinoma of the skin. Histologically it is composed of intradermal proliferation of round cells demonstrating scanty cytoplasm. Cells were noncohesive for the most. The nuclei were vesicular with well-outlined nuclear membranes. Nucleoli were very small and the chromatin appeared to be dispersed. Tumor cell size was equal or smaller than 2 lymphocytes (small cells) in the present case. Mitoses were numerous. (**b**) Neoplastic cells were found to be positive for p63 with variable intensity and percentage. Epidermal basal cells serve as an internal positive control

A synoptic report of MCC diagnosis should be encouraged and minimal elements should be reported as well, including tumor size (cm), peripheral and deep margin status, lymphovascular invasion, and extracutaneous extension (bone, muscle, fascia, cartilage) [111]. The American Joint Committee on Cancer (AJCC) and the College of American Pathologists (CAP) also strongly recommended reporting the following additional clinically relevant factors: depth (Breslow, in mm); mitotic index (mm2 preferred/HPH, or MIB-1 index); tumor-infiltrating lymphocytes (not identified, brisk, non-brisk); tumor growth pattern (nodular or infiltrative); and the presence of a second malignancy within the pathologic specimen (e.g., concurrent squamous cell carcinoma) [112].

Surgery and Systemic Therapy

Surgery is the first-line treatment and requires a wide excision with negative margins [111]. Excision options include wide excision with 1–2 cm margins to investing fascia of muscle or pericranium when clinically feasible [111]. Techniques for more exhaustive histologic margin assessment may be considered such as Mohs micrographic surgery, modified Mohs micrographic surgery, and complete circumferential and peripheral deep margin assessment [111]. They should not interfere with sentinel lymph node biopsy (SLNB) when indicated. SLNB is recommended, regardless of the surgical approach, prior to definitive excision [111, 112]. SLNB evaluation should preferably include an appropriate immunopanel (CK 20 and pancytokeratin AE1/AE3) based on the immunostaining pattern of the primary tumor, particularly if H&E sections are negative as well as tumor burden (% of node), tumor location (sub capsular sinus, parenchyma), and the presence/absence of extracapsular extension [111]. SLNB is an important staging tool and may contribute to regional control but the impact of SLNB on overall survival is unclear [111].

In general, when available and clinically appropriate, enrollment of patients affected by MCC with regional or disseminated diseases, in a clinical trial is recommended [111]. From a practical point of view, in patients with local disease, adjuvant chemotherapy (cisplatin +/− etoposide; carboplatin +/− etoposide) is not recommended; for patients with regional disease, adjuvant chemotherapy is not routinely recommended because survival benefit has not been demonstrated in available retrospective studies, but it could be used on a case-by-case basis if clinical judgment dictates. Disseminated MCC is treated with cytotoxic chemotherapy, although it is scarcely effective since the median progression-free survival is 3 months [113]. Preliminary data from non-randomized trials in patients with MCC demonstrate that rates of durable response are improved with PD-1/PDL1 blockade (Avelumbab; Pembrolizumab; Nivolumab) compared with cytotoxic therapy [106, 107, 111].

Conclusions

MCC is a rare and aggressive neuroendocrine cancer, whose pathogenesis and molecular background remain to be largely determined. Therefore, at the moment, patient age and sex, primary tumor dimensions, site, and thickness, as well as the presence of lymph node involvement or distant metastasis, remain the only useful

prognostic factors for clinician and surgeons [111]. In the last 10 years, the role of MCPyV, UV-exposure, and immunosuppression have been increasingly demonstrated as predisposing factors [37, 111, 112]. In the era of next-generation sequencing, tumor molecular profiles will help in risk stratification and identification of therapeutic options. KIT, p53, PI3KA, TIMP3, and VEGF seem to be associated with poor prognosis [38, 92] but data are still controversial. Finally, PD-L1/PD-1 blockade antibodies seem to be a promising alternative therapy [106, 107], despite the availability of only scanty data. In the near future, therapies targeting MCPyV, as well as p53, PI3KA, VEGF, and multi-targeting tyrosine kinase inhibitors could improve patient outcome [72, 111].

Endocrine Mucin-Producing Sweat Gland Carcinoma

The second skin NEN included in the 2019 WHO Classification of Skin Tumours (Fourth Ed. 2019) [2] is endocrine mucin-producing sweat gland low-grade carcinoma (EMPSGC).

Since the first description by Flieder et al. [114] approximately 70 cases have been reported in total [115–119].

EMPSGC is a rare low-grade neuroendocrine carcinoma which typically affects the eyelids and periorbital skin of elderly (sixth and seventh decades) female patients [2]. Exceptional extra facial sites have been reported [2]. Clinically it is a slow-growing bluish nodule or papule that can mimic a cystic lesion and it can be multifocal [2].

Histologically, it is a well-defined nodule with cystic, solid, papillary, and cribriform growth patterns. It is characterized by neoplastic bland, uniform cells with round-to-oval central, “salt-and-pepper” neuroendocrine nuclei with eosinophilic cytoplasm. Intracellular and extracellular mucin is present at least focally. Tumor necrosis, lymphovascular, and perineural invasion have not been reported and mitotic activity is usually low in EMPSCG. Electron microscopy has detected conspicuous neurosecretory granules in the cytoplasm of EMPSGC [118]. Immunohistochemical expression of at least one neuroendocrine marker such as synaptophysin and chromogranin has been reported even if this expression is often focal [119]. Qin et al. 2018 [119] found synaptophysin to have the highest sensitivity (10 out of 11 cases tested) compared to chromogranin (6 out of 10 cases tested). EMA, estrogen receptor, progesterone receptor, and CK7 are usually positive while CK20 is negative in EMPSCG [119]. EMPSGC commonly lacks a myoepithelial layer [2]. These tumors are probably analogues of solid papillary adenocarcinoma of the breast [2] and it is considered a precursor of mucinous carcinoma [2]. In the literature there are scant molecular data on EMPSGC. Recently, Shon et al. [120] reported overexpression of Wilms tumor 1(WT 1) in EMPSGC supporting the hypothesis that EMPSGC is a precursor lesion of mucinous carcinoma. Qin et al. 2018 [119] performed genome-wide CGH analysis on EMPSGC and found deletion of 6p11.2 to 6q16.1 in 1 of 2 cases, raising the prospect that this region may be important for EMPSGC tumorigenicity. From a practical point of view, if completely surgical removed, EMPSGC has an indolent behavior.

Skin NEN Not Recorded in New Who Classification of Skin Tumors

Neuroendocrine Tumor (NET) of the Skin

Primary skin NET (low or intermediate grade) are rare and heterogeneous; they present a challenging differential diagnosis from: (1) aggressive Merkel cell-small cell neuroendocrine carcinoma, (2) mixed-cell skin carcinomas with NE differentiation, (3) low-grade sweat gland carcinomas with neuroendocrine differentiation (chromogranins, and/or synaptophysin positivity), and (4) cutaneous metastases of visceral carcinoids, especially from gastro-intestinal sites, which herald malignancy and poor prognosis.

To date, after the first cases were reported by Van Dijk and Ten Seldan [4] and by Collina et al. [5], only 12 additional cases of primary cutaneous NET have been reported [6–10].

We also reported [121] a case of 66-year-old North African female presented with a rapidly growing, non-ulcerated, nodule (1.5 cm Ø) in the skin of the nose (Fig. 15.2a). Clinical and imaging techniques revealed no tumors or lesions in the body. Histologically, the lesion was located in the dermis and displayed a typical distinctive "carcinoid" pattern with cord-like trabeculae, sinusoidal/rosette-like features (Fig. 15.2b). Mitotic and apoptotic figures were scanty. At immunohistochemistry, neuroendocrine reactivity was investigated using chromogranin A (Fig. 15.2c), synaptophysin, and NSE, as was also cytokeratin (CAM 5.2), TTF1, CDX2, CK7, and CK20. At electron microscopy (EM), tumor cells were arranged in clusters/rosettes, displayed round/oval nuclei, with marginal and evenly dispersed chromatin and prominent nucleoli. Individual cells were connected by desmosomes, with a few rough ER, polysomes, and dense mitochondria in the cytoplasm. Intermingled with these organelles were fine filaments and keratin clumps. Dense-core (membrane-bound), neurosecretory-type granules were only sparsely distributed throughout the tumor (Fig. 15.3).

Primary cutaneous NETs, first defined in 1988 [5], are unusual rapidly growing primary cutaneous neoplasms that have to be carefully studied to exclude a possible metastatic phenomenon from other sites of the body, of which the gastrointestinal tract is the most frequent candidate [122].

Moreover, some alleged skin NETs may be classified as low-grade sweat gland carcinomas with neuroendocrine differentiation displaying immunoreactivity for chromogranins, and/or synaptophysin or may be sebaceous neoplasms with a carcinoid-like pattern or, finally, may be basal cell carcinomas partly expressing chromogranin A.

Skin NETs are located in the dermis and have well-defined borders, and histologically, they show an organoid pattern that varies from typical insular features [4] to trabecular structures [5]; growth patterns may include sinusoidal, trabecular, cordlike, tubular, rosette, nets, and ribbon-like. Scanty mitotic and apoptotic figures are present. Usually, NETs of the skin have extremely rare lymphovascular invasion. The cases of NET of the skin when tested showed pan-neuroendocrine markers (chromogranin, synaptophysin, NCAM, and NSE) in more than 50% of tumor

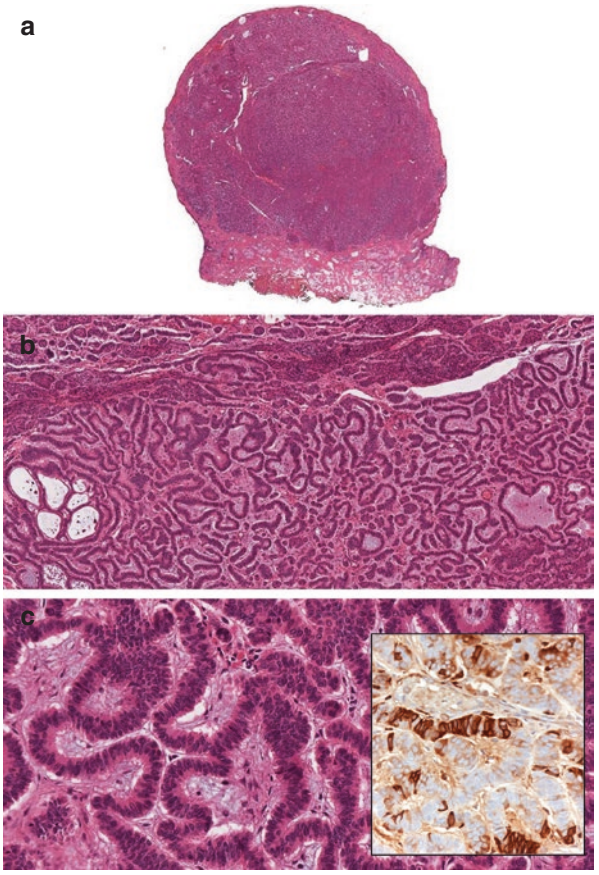

Fig. 15.2 (**a**) At low power, a primary cutaneous trabecular carcinoid of the nose skin, of 66 yr-old female, shows polypoid features, (**b**) histologically, it is composed of long branching trabeculae of cells, and (**c**) at higher power tumor cells are oriented parallel to each other within the trabeculae and are positively immunostained for chromogranin A (**c**, inset). (This figure is previously published in Asioli et al. [11])

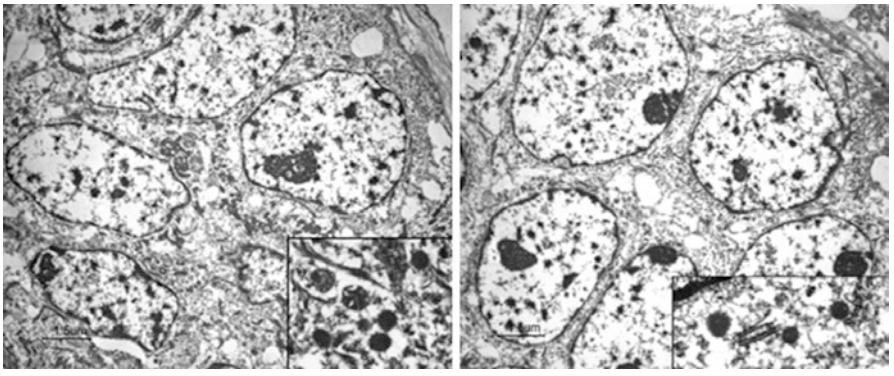

Fig. 15.3 Ultrastructure of tumor cell clusters, displaying oval nuclei, marginally or evenly dispersed chromatin and prominent nucleoli. Individual cells are connected by desmosomes: in the cytoplasm, a few rough ER, polysomes, and dense mitochondria are intermingled with fine filaments and keratin clumps. Inserts: show sparsely distributed dense-core-membrane-bound, neurosecretory-type granules (arrows) (insert1 – near-dense mitochondria) (insert 2 – near RER). (This figure has been previously published in Betts et al. [121])

cells [4–9]. Ultrastructure performed on reported cases usually showed only rare typical NE granules. The definitive diagnosis of a primary skin NET may require prolonged follow-up to exclude a cutaneous metastasis (even when TTF1, CDX2 negative) [122] as the first manifestation preceding the discovery of a visceral neuroendocrine neoplasm, months or years later. Unfortunately, Ki-67 data are not always available. This latter data combined with the rarity of skin NET preclude the possibility to apply a grading system to cutaneous NENs.

Carcinoma with Neuroendocrine Differentiation

An uxepected neuroendocrine cell differentiation in non-neuroendocrine skin neoplasm could be detected and indicate carcinoma with neuroendocrine differentiation. In skin these entities include basal cell carcinoma (BCC) with neuroendocrine (Fig. 15.4a–c) differentiation and trichoblastoma with MCC differentiation. These lesions are exceedingly rare and there is no cutoff of the percentage of neuroendocrine cells to put these lesions into a specific category. Eusebi et al. [123] were the first to report BCCs showing endocrine-like granules in cytoplasm and argyrophilic reaction. Following that, immunohistochemical detection of miscellaneous neuroendocrine markers was reported [124–129]. The prognosis of these entities showing

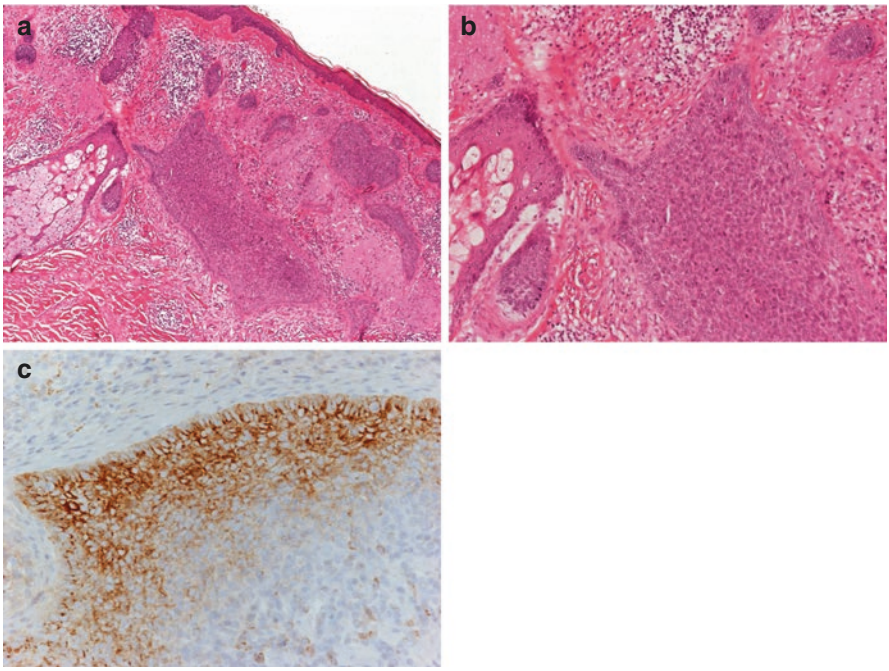

Fig. 15.4 Basal cell carcinoma of the head skin (temporal region) of a 74-year-old-man (**b**) that shows peripheral cell palisading. (**c**) On immunohistochemistry, some of the neoplastic cells are positive for chromogranin A. (This figure has been previously published in Asioli et al. [11])

neuroendocrine differentiation does not seem to differ from the ordinary types of BCC or Trichoblastoma. A case of invasive sweat gland apocrine carcinoma with 10% chromogranin-positive cells was reported by Foschini et al. [3]. During follow-up and after radiation therapy, local recurrences and metastasic deposits in ingunal lynph node showed increasing of percentage of neuroendocrine cells up to 90%, indicating tumor selection after radiation therapy. This seems to be the same occurrence of neuroendocrine differentiation as seen in adenocarcinoma from other organs [3].

Take Home Message

In conclusion, the prototypical primary skin NEN is MCC that it is a high-grade neuroendocrine carcinoma. There are endocrine mucin-producing sweat gland carcinomas and skin neuroendocrine tumors that represent the low-intermediate grade of neuroendocrine skin tumors, but, to date they are exceedingly rare precluding the need for a common classification of skin neuroendocrine neoplasms.

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Metastatic Neuroendocrine Neoplasms of Unknown Primary Site

16

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Virtually all neuroendocrine neoplasms (NENs) have metastatic potential, and up to 20% of the cases present as metastasis from an occult primary [1–3]. The identification of the primary site is an important step toward the correct management of the patient, particularly when dealing with a well-differentiated neuroendocrine tumor (NET), as therapeutic approach may vary depending on the site and cell type. In contrast, poorly differentiated neuroendocrine carcinomas (NECs), independent of the primary site, are currently treated with platinum-based regimens, and the role of the pathologist may be limited to the distinction between a visceral NEC and a Merkel cell carcinoma of the skin, because the latter requires wide local excision, sentinel node biopsy, and, possibly, radiotherapy. In contrast, thorough morphological and immunohistochemical analyses are expected to give important clues to the recognition of the site of origin of a metastatic NET.

Among all NETs, the tendency to metastasize is highest for those of pancreatic origin, followed by small intestinal, colonic, pulmonary, and gastric neoplasms [1]. Irrespective of the primary site, the liver represents the most frequent location of

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metastatic NENs; lymph nodes, peritoneum, bone, and lung represent further usual secondary sites [3]. However, virtually any body organ including those that can give rise to primary NENs may host metastatic NENs, including breast [4], ovary [5], thyroid [6], pancreas [7], and pituitary [8]. Thus, it becomes evident that the diagnosis of a metastatic NEN gives rise to two orders of problems: (i) the identification of the occult primary site and (ii) the distinction from a putative primary NEN of the organ in which the lesion is present. Both challenges are of crucial importance in the management of the patients, and the pathologist should be aware of the diagnostic tools to approach them and of the entities which enter in the differential diagnosis.

This chapter will address systematically the morphological and immunohistochemical markers useful for the identification of the site of origin of NENs. In addition, a review of the most frequent neoplasms that, in the different organs, should be distinguished from a metastatic NEN will be provided.

Morphological Clues to the Primary Site

Pathologists are often exposed to small tissue samples (e.g., core biopsy specimens) during the workup of metastatic NENs. Nevertheless, the cytoarchitectural features of the neoplasm should be carefully considered and can contribute to the diagnostic algorithm assisting the detection of the primary site. This approach may guide the application of appropriate biomarkers including but not limited to transcription factors and site-specific hormones that can help in this task [9]. The discrimination of NET from NEC is often an easy step by taking into consideration the cytomorphology as well as the Ki67 labeling index of the neoplasms. However, in challenging cases, this distinction can be facilitated by using recently defined biomarkers including but not limited to immunohistochemistry for p53, retinoblastoma protein (Rb), and somatostatin receptors (SSTRs) [10–12].

Based on the proposal in 1963 by Williams and Sandler [13], NENs are traditionally classified based on their embryological derivation from the foregut (respiratory tract, thymus, stomach, duodenum, pancreas, and liver), the midgut (jejunum, ileum, appendix, cecum, and right colon), or the hindgut (rectum). However up to 15% of NENs cannot be classified using this scheme, since it does not apply to urogenital NENs, pituitary NENs, and head and neck NENs including thyroid and parathyroid NENs; nevertheless, it still has the merit of separating well-established anatomic sites that, on one hand, have a different morphology and, on the other hand, show expression of different transcription factors related to their molecular cytodifferentiation pathways involved in embryological morphogenesis. Indeed, when comparing the Williams and Sandler's classification with the architectural patterns (A: nested; B: trabecular; C: pseudoglandular; D: diffuse) proposed by Soga and Tazawa in 1971 [14], we recognize that most of the midgut NENs, i.e., jejuno-ileal NETs, belong to pattern A (Fig. 16.1a), whereas pattern B is observed in hindgut NENs (Fig. 16.1b), and pattern C is most frequent in foregut tumors, particularly in D-cell tumors of duodenum (also known as somatostatin-expressing duodenal NENs or duodenal somatostatinomas) (Fig. 16.1c). Pattern D is often seen

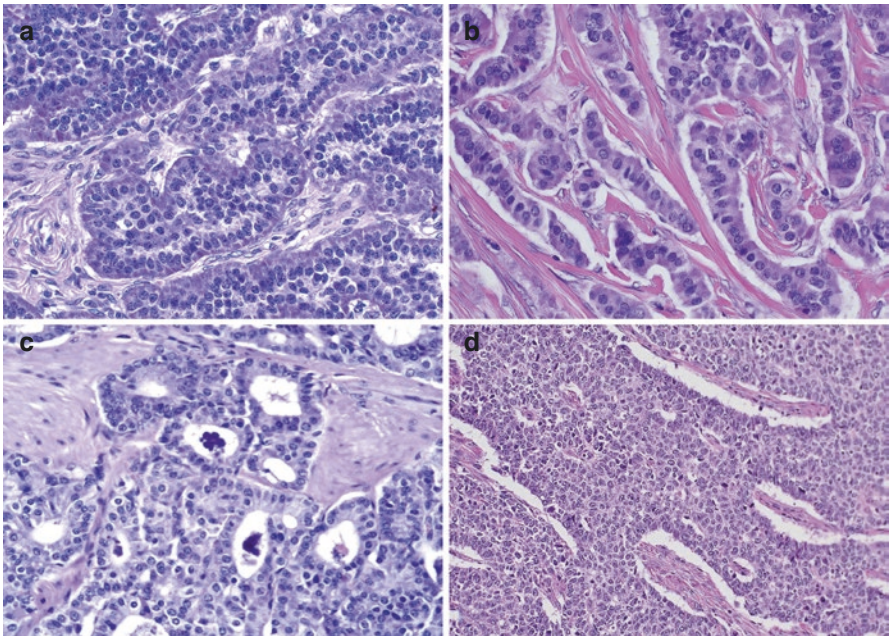

Fig. 16.1 Architectural patterns of NENs according to Soga and Tazawa. (**a**) Ileal NET showing nested architecture with peripheral palisading of cells (pattern A). (**b**) Rectal NET with trabecular growth of elongated cells (pattern B). (**c**) Somatostatin-producing D-cell NET of the duodenum with pseudoglandular pattern (pattern C). (**d**) Large cell NEC of the colon with diffuse proliferation of neoplastic cells (pattern D)

in higher grade neoplasms, both in NET G3 and in NEC (Fig. 16.1d). However in reality, most NENs display a mixed pattern of growth.

Similar to the architectural pattern of a NEN, a detailed assessment of the cytological details, such as the cytoplasmic features (e.g., staining affinity and distribution of granules) and cell shape may provide additional information regarding the origin of a NEN. The diffuse basophilic appearance of secretory granules is a feature of densely granulated corticotroph tumors originating from the pituitary gland, whereas a basophilic to amphophilic granular cell cytoplasm and/or clear to amphophilic granular cytoplasm with loosely cohesive cell borders characterize a medullary thyroid carcinoma (thyroid NEN). The highly granular cell cytoplasm with variable accentuation at the cell membrane is a common feature of serotonin-expressing enterochromaffin (EC)-cell NETs of midgut origin. In contrast, pale-to-clear cytoplasm is more frequently observed in pancreatic and, more generally, in the vast majority of foregut tumors. A spindle cell morphology may also be observed in several NENs including but not limited to pulmonary NENs of the peripheral type, thymic NENs, thyroid, and some pituitary NENs. Similar to architectural features, the cytological features can vary among various sites.

Unlike poorly differentiated NECs, the vast majority of NETs tend to show characteristic anatomic site- and cell-specific biomarker expression profiles that can be

used to determine their origin. For this reason, the use of appropriate immunohistochemical biomarkers is required to determine the potential origin of a metastatic well-differentiated NET. Therefore, the identification of the cell origin requires a biomarker panel approach [9].

Finally, one should always remember that the spectrum of NENs also includes non-epithelial NENs such as paragangliomas and pheochromocytomas (intra-adrenal sympathetic paragangliomas), as well as unusual triphasic NENs such as gangliocytic paragangliomas. Of note, paragangliomas can manifest with multifocal synchronous or asynchronous multifocal disease, especially in the setting of germline predisposition [15]. For instance, the identification of a paraganglioma in the lung or liver does not qualify for metastasis, as these tumors can also occur in any sites where the autonomous nervous system innervation occurs [15]. In a keratin-negative and site-specific transcription factor-negative NEN, a nested growth pattern with sustentacular cells and prominent vascularization ("zellballen" growth) coexisting with cytological aspects, such as low nuclear–cytoplasmic ratio with abundant granular amphophilic to basophilic cytoplasm, should prompt the use of appropriate immunohistochemical biomarkers (e.g., tyrosine hydroxylase and GATA3) to further assess the possibility of a paraganglioma [9, 15].

Immunohistochemical Markers Useful in the Detection of the Occult Primary Site

Immunohistochemistry is the cornerstone of the pathologic workup of NENs of unknown primary site. The assumption that NETs retains markers related to the morphogenesis and to the function of the organs and of the cells from which they are derived is the main starting point to choose the immunohistochemical panel to be applied. Specific antibodies directed against site-related developmental transcription factors and neuroendocrine markers, including hormones and related molecules, represent the most important immunohistochemical tools in this diagnostic setting [9, 16, 17]. While one should never omit the crucial role of cytokeratins in the distinction of epithelial NENs, other markers, such as monoclonal CEA, prostate-specific acid phosphatase (PSAP; also known as prostatic acid phosphatase, PAP or PRAP), and tyrosine hydroxylase, are also helpful. As discussed further, NECs can show unusual transcription factor expression profiles unrelated to their site of origin as dedifferentiated cancer cells lose their lineage-specificity in terms of biomarker expression profiles. For example, TTF1 expression is identified in poorly differentiated NECs of various sites (9; 18).

When looking for the occult primary of a metastatic NET, one should keep in mind that the sensitivity and specificity of immunohistochemical markers, with very few exceptions, is never high enough to allow the use of a single immunostain for the diagnosis. Thus, a panel of immunohistochemical tests should be undertaken, taking into consideration the cytomorphology, anatomic site, and clinical features.

Transcription Factors in NENs

Transcription factors are proteins that bind to specific DNA sequences and modulate the transcription process, in order to regulate gene expression. One of the functions of transcription factors is to selectively direct cell migration, developmental organization, and differentiation of various organs, as well as regulation of normal function or hormone expression of various neuroendocrine cells. Since the expression of these transcription factors, together with other elements, regulates the neuroendocrine cell-specific differentiation within an organ, and most well-differentiated NENs tend to retain characteristics of their normal neuroendocrine cell correlates, this allows the diagnostician to confirm the origin of metastatic NETs. For instance, not all neuroendocrine tumors identified in the pancreas represent primary pancreatic NETs, as metastatic NETs of various sites can morphologically be mistaken for a primary pancreatic NET. Therefore, appropriate biomarkers should always be used to confirm the site of origin of a neuroendocrine neoplasm. In endocrine pathology, several transcription factors have been proposed as site-specific markers. However, despite their unquestionable utility in the diagnostic routine, their use is limited by several considerations. First, their aberrant expression in poorly differentiated NENs of different sites makes them useless in metastatic localization of NECs. Second, with the possible exception of TTF1 and OTP in lung NETs, their specificity for a definite site is frequently low. Third, their sensitivity is far from being high enough to rely on single markers for the diagnosis. For all these reasons, the use of transcription factors should be integrated in a rational, possibly step-wise, algorithm including a panel of other immunohistochemical markers.

The following are the most commonly used transcription factors that have been considered to be useful in the identification of the occult primary site of a metastatic NEN.

Caudal Type Homeobox 2 (CDX2)

CDX2 is a member of the caudal-related homeobox gene family, based on its sequence homology to the caudal gene of Drosophila melanogaster. In vitro and in vivo studies suggest that this transcription factor is important in the early differentiation and maintenance of the intestinal epithelial cell [18].

A systematic immunohistochemical and gene expression profiling study of normal human epithelial and non-epithelial tissues demonstrated that CDX2 is expressed in the epithelial cells of the small and large intestine and of the appendix, as well as in the pancreatic centroacinar cells and in interacinar duct cells of the pancreas [19]. Since CDX2 expression is retained in metaplastic lesions, as well as in epithelial neoplasms with intestinal differentiation, including adenocarcinomas of the intestines, of the biliary tract, of the stomach, of the ovary, of the uterine cervix, of the bladder, and of the nose [20, 21], this biomarker has been extensively used in diagnostic pathology to confirm the intestinal phenotype of neoplasms, both for subtyping adenocarcinomas of a given site and in the setting of a metastatic tumor of unknown primary. Important pitfalls in the use of CDX2 in pathological examination are represented by its, albeit rare, aberrant expression in occasional

carcinomas of the lung, in the columnar cell variant of papillary thyroid carcinoma, and in hepatocellular carcinoma [22–24]. In the context of NENs, CDX2 expression seems to be highly sensitive and fairly specific for midgut NETs. The mouse monoclonal antibody CDX2–88 gives intense immunoreactivity in more than 90% of jejunoileal and appendiceal NETs, whereas a faint and patchy stain has been detected in only about 30% of duodenal and rectal primaries, and 15% of gastric and pancreatic NETs [25–27]. The use of the rabbit monoclonal antibody EPR2764Y seems to further improve the sensitivity and the specificity of the immunostaining for midgut NETs to 100% and 87%, respectively [28]. Noteworthy, a meta-analysis of various studies on CDX2 expression in well-differentiated pulmonary NETs showed that only 7 out of 222 of primary tumors and 1 of 13 metastatic lesions were CDX2-positive with CDX2–88 antibody, whereas no positive case was observed when EPR2764Y antibody was used [1].

Among poorly differentiated NECs, CDX2 is inconstantly expressed, and cannot be reliably used to identify the origin of a metastatic deposit. However, it has been reported that, in the large cell subtype of NEC, CDX2 was useful in distinguishing neoplasms of intestinal origin from pulmonary ones [26, 29]. Similarly, acinar cell carcinomas which are distinguished by their trypsin and BCL10 expression (Fig. 16.2) can simulate a pancreatic NET with variable CDX2 expression, as well

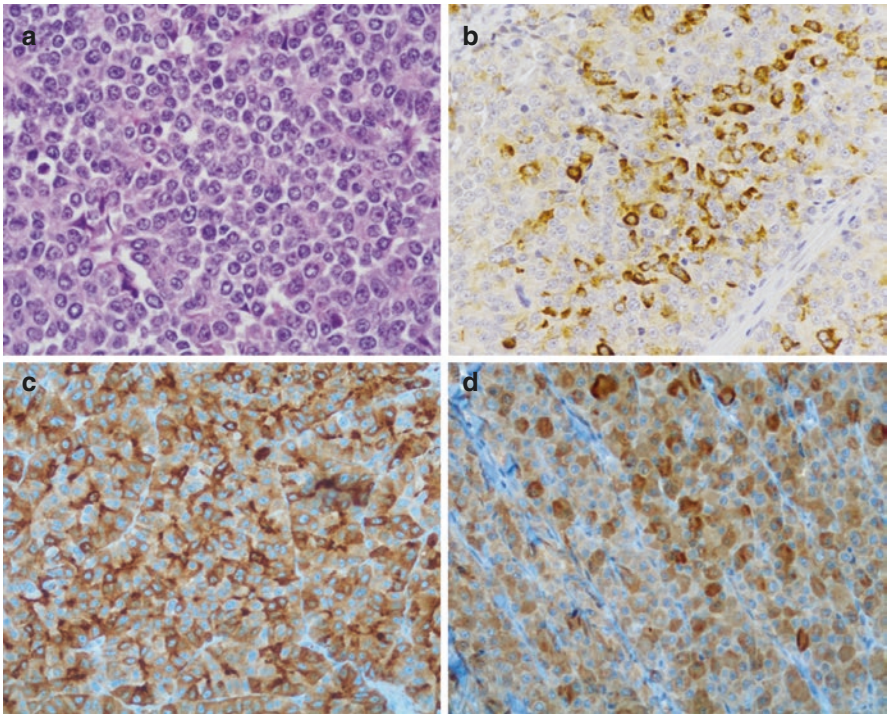

Fig. 16.2 Acinar cell carcinoma (ACC) of the pancreas may show neuroendocrine-like morphology (**a**) and focal expression of general neuroendocrine markers (**b**). However, strong immunostainings for trypsin (**c**) and Bcl10 (**d**) are diagnostic for AAC and exclude a NEN

as variable neuroendocrine biomarker expression [12]. In addition, rare examples of primary high-grade prostatic NENs have been shown to express CDX2 [30].

Thyroid Transcription Factor 1 (TTF1)

TTF1 is a member of the NKx2 family of homeodomain transcription factors and is expressed during the development of the thyroid, lung, and forebrain. It regulates the expression of several genes, including thyroglobulin, thyroperoxidase, sodium-iodide transport protein, calcitonin, and major histocompatibility complex class I genes in the thyroid gland. In the lung, it modulates surfactant proteins A, B, and C, and Clara cell secretory protein genes [31]. As far as normal neuroendocrine cells of the fetal and adult lung are concerned, only a fraction of them has been demonstrated to express TTF1 [32] and, in an elegant colocalization study, Miskovic and co-workers suggested that TTF1 was only detectable in non-terminally differentiated neuroendocrine cells [33].

In the context of well-differentiated NETs, TTF1 expression is commonly identified in medullary thyroid carcinoma as well as in well-differentiated pulmonary NETs. Both pulmonary NETs and medullary thyroid carcinomas can also express similar hormone products including calcitonin and calcitonin gene-related peptide (CGRP). While co-expression of bombesin and serotonin can favor pulmonary origin, rare examples of medullary thyroid carcinoma can also feature this immunoprofile. Therefore, in a TTF1-expressing well-differentiated NET, diffuse positivity for monoclonal CEA is the most useful marker in the distinction of medullary thyroid carcinoma [9, 34] (Fig. 16.3). Among NECs, 90–100% of small cell lung carcinomas (SCLC) show TTF1 expression in a significant fraction of neoplastic cells. However, immunostaining for TTF1 is also present in a non-negligible proportion of extrapulmonary NENs of various sites, except for Merkel cell carcinoma, which is consistently TTF1-negative, whereas it expresses cytokeratin 20 (CK20) and, frequently, Merkel cell polyoma virus (MCPyV), depending on its molecular pathogenetic pathway [35]. For this reason, a panel including TTF1, CK 20, and MCPyV should always be considered in the differential diagnosis of MCC versus a cutaneous localization of a visceral NEC (Fig. 16.4) [12, 29]. In addition, as there is significant immunohistochemical variability and overlap between these tumors, the use of lymphoid markers, such as terminal deoxynucleotidyl transferase (TdT) and B-cell linage markers, which are expressed in Merkel cell carcinomas and not in visceral NECs, may help the differential diagnosis in difficult cases [36]. On the other hand, TTF1 is a specific, but not sensitive marker for the detection of pulmonary NET in the setting of mediastinal mass. Although it has been demonstrated that extrapulmonary well-differentiated NETs, including thymic ones, do not typically express TTF1, only about one-third of pulmonary NETs are immunohistochemically positive in some series [12]. It has been proposed that pulmonary NETs with intermediate grade proliferative features (atypical carcinoid tumors) are more frequently TTF1-positive, due to the fact that, similarly to SCLC, some of them are putatively derived from non-terminally differentiated neuroendocrine neoplastic cells [33]; however, this finding has not been confirmed. Finally, peripherally located pulmonary NETs are more frequently TTF1-immunreactive than central ones [32].

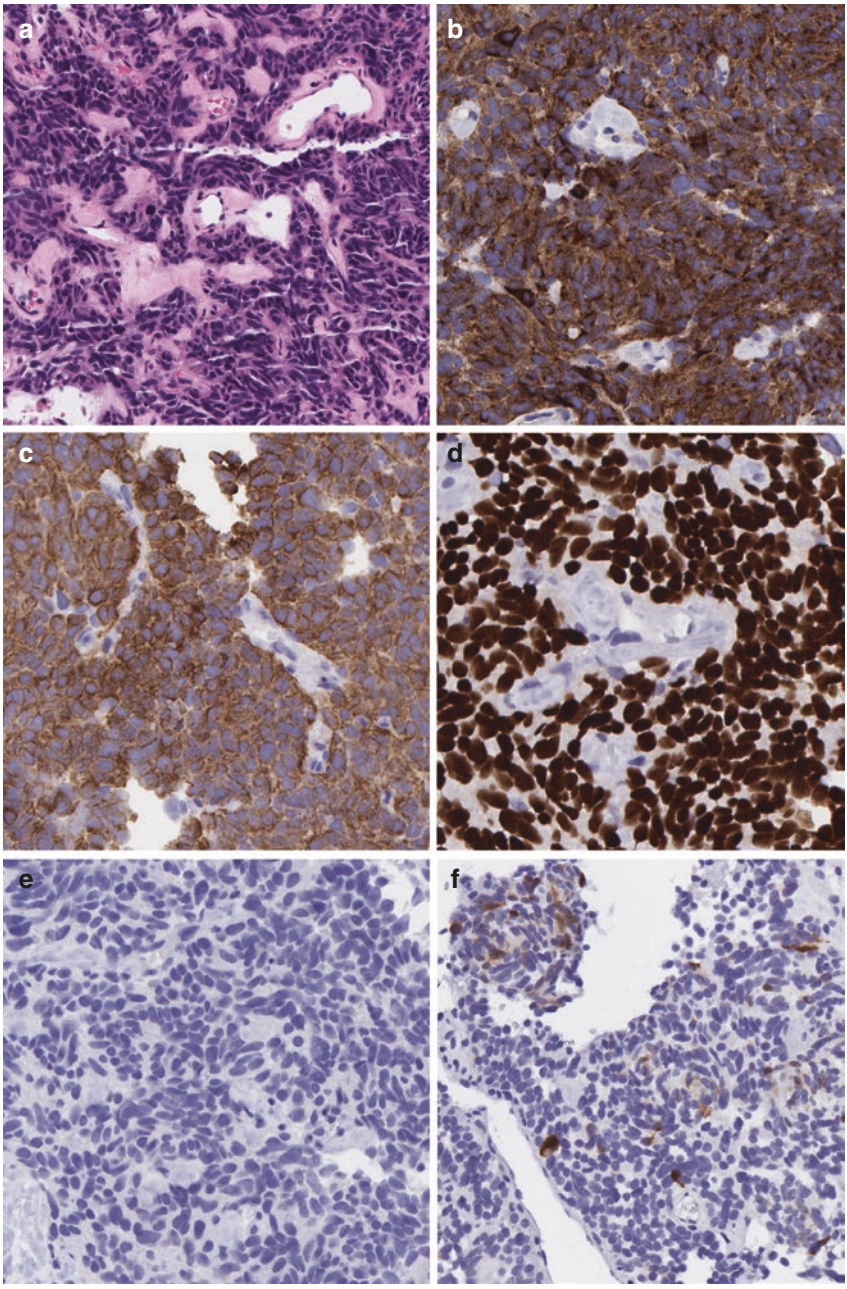

Fig. 16.3 Metastatic well-differentiated pulmonary neuroendocrine tumor in the liver. Hepatic metastasis of pulmonary neuroendocrine tumor is not an uncommon finding (**a**). The tumor cells are positive for chromogranin-A (**b**), synaptophysin (not illustrated herein), CAM5.2 (**c**), and TTF-1 (**d**) and negative for monoclonal CEA (**e**). Not all pulmonary neuroendocrine tumors are positive for pulmonary neuroendocrine hormones. This tumor shows focal reactivity for serotonin (**f**). Absence of diffuse monoclonal CEA and positivity for TTF-1 supports the pulmonary origin in a well-differentiated neuroendocrine tumor

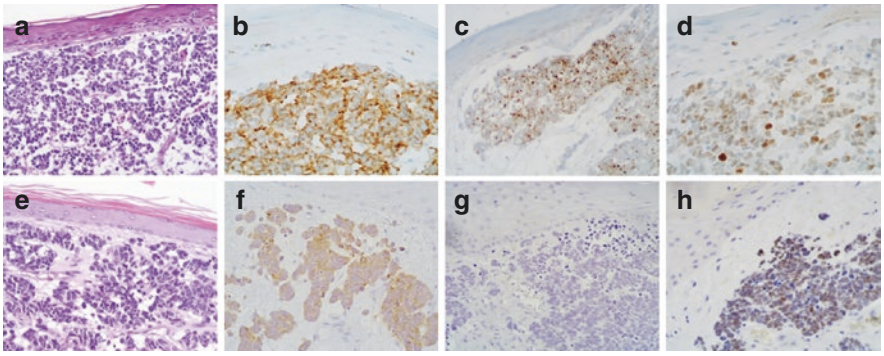

Fig. 16.4 Differential diagnosis between Merkel cell carcinoma (MCC) (**a**,**b**,**c**,**d**) and a cutaneous metastasis of a visceral NEC (**e**,**f**,**g**,**h**). Albeit morphology (**a**,**e**) and general neuroendocrine marker expression (**b**,**f**, synaptophysin) may be overlapping, positive immunostainings for cytokeratin 20, often with a paranuclear dot (**c**), and for Merkel cell carcinoma Polyomavirus (MCPyV) are diagnostic for MCC, whereas they are lacking in NECs (**g**, cytokeratin 20), which, in turn, may express TTF1 (**h**). This latter marker is consistently negative in MCC

TTF1 is also a biomarker of hypothalamic and posterior pituitary cells and is expressed in neurocytomas (hypothalamic NETs) [37] as well as non-neuroendocrine cells of that region.

Importantly, the clone of antibody used to detect TTF1 is an important detail that should be taken into account. Some thymic NETs can show focal/scattered TTF1 expression when the SPT24 clone is applied [38, 39]. In addition, a significant proportion of pulmonary NETs are also positive with the SPT24 clone [40].

Orthopedia Homeobox (OTP)

OTP is a member of the homeodomain family proteins, which are helix-turn-helix transcription factors that play key roles in the specification of cell fates. This protein acts in the neuro-development in the embryo, particularly in the development of the hypothalamus and in the neuroblast differentiation pathway [41]. In recent years, OTP has been proposed as a diagnostic and prognostic marker for pulmonary NETs. On one hand, its expression seems to be a feature of low-grade "typical carcinoid" tumors when compared with moderate-grade "atypical carcinoid" tumors. For this reason, the use of OTP in association with Ki67 has been suggested in small biopsies, where artifacts can preclude accurate assessment of cytomorphological features [42]. Moreover, intense OTP expression seems to be an independent predictor of a better prognosis in locally advanced disease, in combination with CD44 expression and tumor growth pattern [43]. In a large series of neuroendocrine and non-neuroendocrine neoplasms of various primary sites, OTP expression was found to be substantially restricted to well-differentiated pulmonary NETs as well as to pulmonary neuroendocrine cell hyperplasia and precursor neuroendocrine lesions of the lung [44]. The high specificity and sensitivity of this marker for well-differentiated pulmonary NETs has been very recently confirmed in a large study of surgical and cytological samples of neuroendocrine and non-neuroendocrine pulmonary neoplasms, which has confirmed the negativity of squamous cell carcinomas,

adenocarcinomas, and poorly differentiated pulmonary NECs of both large and small cell types [45].

Paired Box Genes (PAX5, PAX6, and PAX8)

The paired-box (PAX) genes encode a family of nine paired-box transcription factors, with important roles in development and disease, and persistent PAX expression characterizes several tumors in a site-specific fashion [46]. Two members of this family, PAX6 and PAX8, have biological and diagnostic relevance in neuroendocrine pathology, and PAX5 has been recently reported to be expressed in NENs. Due to their sequence homology, commercially available antisera for immunohistochemistry show some degree of cross reactivity, but, recently, the use of monoclonal antibodies has increased the specificity of immunostaining [47]. Therefore, published data generated from polyclonal PAX8 antibodies should be interpreted with caution.

PAX5 is known to regulate B lymphocyte differentiation; however, recent evidence also suggested that it is expressed neuroblastic tumors [48] as well as in pulmonary neuroendocrine neoplasms, with increased frequency of expression in small cell NEC of the lung, compared to well-differentiated NETs [48, 49]. It is also expressed in Merkel cell carcinoma.

PAX6 is known to be crucial for pancreatic islet cell differentiation and function through transcriptional control of key genes involved in glucagon and insulin biosynthesis and secretion in A and B cells, respectively. It also plays an essential role in the development and function of endocrine cells in the gastrointestinal tract, particularly in the stomach and duodenum [50]. PAX6 has been reported to have 100% specificity for metastatic NENs of pancreatic origin; however, its sensitivity is much lower (<50%). When NENs were analyzed in various primary sites (not in a metastatic setting), the majority of duodenal and rectal NETs showed PAX6 immunoreactivity, with a potential reduction of the diagnostic value of this marker for pancreatic NETs [50].

PAX8 is involved in the morphogenesis of the thyroid gland, of the kidney, and of Müllerian tract-derived female genital organs and it is expressed in neoplasms arising in these sites [51]. Earlier studies using polyclonal antisera identified that PAX8 is expressed in various NETs including thymic and pancreatic neoplasms [52, 53]. Parathyroid NENs (parathyroid adenomas and carcinomas) also show immunoreactivity using polyclonal PAX8 antisera, but these are negative when tested with C-terminal-specific monoclonal PAX8 antibodies [54]. The application of polyclonal PAX8 antisera also resulted in staining in about 75% of medullary thyroid carcinomas [55]; however, medullary thyroid carcinomas are found to be negative with C-terminus-specific monoclonal PAX8 (clones BC12 and PAX8R1). Although focal staining was noted in 1 of 5 medullary thyroid carcinomas using monoclonal N-terminal specific PAX8 (clone MRQ-50) [56], a subsequent larger series of 45 medullary thyroid carcinomas reported no staining using that clone of monoclonal PAX8 (clone MRQ50) [57]. The diagnostic value of PAX8 immunostaining in NENs has also been recently questioned in a recent study that systematically tested 4 different anti-PAX8 antibodies (1 polyclonal and 3 monoclonal) on a series of

115 NENs including those originating from pancreas, ileum, stomach, duodenum, appendix, rectum, thyroid, parathyroid, thymus, lung, uterine cervix, ovary, and skin. Interestingly, two of the three monoclonal antibodies, directed against a less conserved C-terminal epitope of the protein failed to show immunostaining in any NEN of any sites, whereas with the third monoclonal and the polyclonal reagents identified immunoreaction in a variety of NENs from different sites, except for the lung, the ileum, the ovary and, only for the monoclonal, the stomach [57]. These results support the hypothesis that, in NENs, PAX8 immunoreactivity may be due to cross reaction with other PAX proteins and is dependent on the antibody used. For this reason, the use of PAX8 in the diagnostic workup for the identification of an occult primary is now very limited.

Insulin Gene Enhancer Binding Protein Isl-1 (Islet 1)

Islet 1 (ISL1) is a transcription factor containing two N-terminal LIM domains and one C-terminal homeodomain. The encoded protein plays an important role in the embryogenesis of the pancreas; and, in the adult, it is expressed in the cells of the islets of Langerhans [58]. Initially proposed as a biomarker of well-differentiated NENs of pancreatic origin [28], subsequent studies demonstrated that its sensitivity in detecting pancreatic NENs was not accompanied by an adequate specificity. In fact, ISL1 is consistently expressed in rectal and duodenal NENs, as well as in a fraction of NENs of various sites including the colon and appendix [59]. Moreover, a comprehensive study that also included a series of poorly differentiated NECs showed that ISL1 was also intensely and diffusely expressed in Merkel cell carcinomas, pulmonary small cell NECs, and head and neck NECs, as well as in the poorly differentiated neuroendocrine component of mixed neuroendocrine and non-neuroendocrine neoplasms. In addition, medullary thyroid carcinoma, paraganglioma/ pheochromocytoma, and adrenal neuroblastoma were found to be consistently positive, whereas absent or weak staining was recorded in well-differentiated pulmonary NETs [60].

Pancreatic and Duodenal Homeobox 1 (PDX1)

PDX1, also known as insulin promoter factor 1, is a transcription factor in the ParaHox gene cluster [61]. In vertebrates, PDX1 is necessary for the development of pancreas, including B-cell maturation, and differentiation of the duodenum. During the cytodifferentiation process of pancreas, PDX1-positive epithelial cells of the posterior foregut region give rise to the pancreatic bud, from which pancreatic exocrine, endocrine, and ductal cell populations have origin. For this reason, PDX1 is considered as the earliest marker for pancreatic differentiation, with the fates of the various types of cells of the mature pancreas being controlled by further transcription factors [62]. During adult life, PDX1 is expressed in islet cells, especially in B cells, as well as in some centro-acinar and ductal cells [63]. Although PDX1 immunostaining has been claimed to be useful in recognizing the pancreatic origin of a metastatic NEN [64], the expression of this biomarker is not restricted to pancreatic NETs, as also gastric, duodenal, appendiceal, and rectal NETs may show intense and diffuse immunoreactivity for this marker. However, the absence

of staining in pulmonary and ileal NETs is of interest [65]. Moreover, the sensitivity of PDX1 immunostaining for NETs of pancreatic origin was very low, as it was reported in approximately 30% of cases [65, 66]. Altogether, the available data do not support the usefulness of PDX1 as a reliable biomarker to identify the pancreatic origin of a NEN. However, the application of this biomarker in association with site-specific hormones and other relevant biomarkers may be of interest in the workup of specific cases.

Human Homeobox Proteins NK (Nkx)

Nkx are a phylogenetically ancient family of homeobox domain-containing transcription factors with tissue-specific expression, involved in organogenesis. Increasing evidence indicates that individual Nkx factors are critical regulators of whole organ development. Two members of this family, namely, Nkx2.2 and Nkx6.1, have been demonstrated to be involved in pancreatic development.

Nkx2.2 has been reported to play a crucial role in central nervous system development, oligodendrocyte differentiation, and neuroendocrine differentiation in the central nervous system, gastrointestinal tract and pancreas. During early pancreatic development, Nkx2.2 protein expression is initiated with other transcription factors including PDX1 and PTF1A in the dorsal and ventral pancreatic buds. Successful completion of this step is crucial for the differentiation of B cells in the pancreatic islets. Moreover, Nkx2.2 plays a critical role in the development of intestinal neuroendocrine cells [67]. A recent immunohistochemical study demonstrated that, in adults, Nkx2.2 is expressed in the neuroendocrine cells predominantly located in the deep crypts of the entire tubular GI tract, including stomach, duodenum, jejunum, ileum, appendix, colon, and rectum. In normal pancreas, Nkx2.2 was expressed in the nucleus of islet cells, whereas normal ductal epithelium and acinar were cells completely negative [68]. In the same study, Yang and co-workers showed that the vast majority of pancreatic and intestinal (including jejunal, ileal, appendiceal, colonic, and rectal) well-differentiated NENs were positive for Nkx2.2, whereas only a small fraction of gastric NETs were positive and lung NETs were completely negative. When compared with CDX2 immunoreactivity, Nkx2.2 showed similar immunostaining rate in appendiceal, ileal, and jejunal NETs. In contrast, Nkx2.2 showed a higher sensitivity than CDX2 for pancreatic, duodenal, colonic, and rectal NETs [68].

Human homeobox protein Nkx6.1 is encoded by the *NKX6–1* gene. This is known as a homeobox transcription factor involved in the development, proliferation, and secondary transition of pancreatic B cells. Nkx6.1 expression is modulated by insulinoma-associated protein-1 (INSM1) during pancreatic development, and the experimental over- or under-expression of this protein leads to pathologic conditions in the mouse, respectively enhancing glucose-stimulated insulin secretion and Type 2 diabetes mellitus–like disease [69]. Nkx6.1 is expressed in islet cells of the pancreas and occasionally in the duodenum, whereas it is not expressed in neuroendocrine cells of the lung, stomach, ileum, colon, and rectum. In a series of 26 metastatic NENs of various primaries, Nkx6.1 has been reported to identify the pancreatic origin with a specificity of 100% and a sensitivity of 63% [70].

More recently, however, the specificity of this marker in identifying the pancreatic origin of NENs has been challenged by an another series, in which Nkx6.1-immunoreactivity was found in pulmonary NENs, and was more related to an intermediate or high proliferative grade of the neoplastic proliferation rather than to a specific primary site [71].

GATA Transcription Factors

In humans, the GATA family of transcription factors include six different genes (GATA1 to 6) related by their high degree of amino acid identity throughout the two-zinc-finger DNA-binding domain, which selectively recognize consensus GATA-containing sequences [72]. The expression pattern of each of the GATA family members appears to be highly evolutionarily conserved among vertebrates: GATA1/2/3 are required for differentiation of mesoderm and ectoderm-derived tissues, including the hematopoietic and central nervous system. GATA4/5/6 are implicated in the development and differentiation of endoderm- and mesoderm-derived tissues such as induction of differentiation of embryonic stem cells, cardiovascular embryogenesis, and epithelial cell differentiation in the adult [73].

In diagnostic pathology, the most studied member of the GATA family is GATA3. This transcription factor is essential in T-cell development and differentiation, and it is also required to promote and direct cell proliferation, development, and differentiation in many non-hematopoietic tissues and cell types. In particular, it is expressed during the development of the luminal epithelial cells of the breast, parathyroid gland, kidney, sympathetic nervous system, lens fiber cells of the eye, hair follicles of the skin, and adipose tissue [74]. In adults, GATA3 expression has been reported in T lymphocytes, luminal glandular epithelial cells of the breast, parathyroid glands, urothelium, and the distal renal tubules [75]. In tumor pathology, earlier reports showed that GATA3 expression was restricted to urothelial carcinoma, epithelial breast tumors, parathyroid neoplasms, and a fraction of salivary gland tumors [74]. However, further studies have demonstrated that, among epithelial tumors, also skin and skin adnexal neoplasms may be GATA3 positive. In addition, germ cell tumors, in particular trophoblastic tumors, tumors of the endodermal sinus, as well as mesothelioma, are positive for GATA3 [76]. Importantly, virtually all paragangliomas/pheochromocytomas are GATA3-positive (Fig. 16.5), whereas pulmonary and gastroenteropancreatic NENs are consistently negative, making GATA3 immunostaining an important tool in the differential diagnosis of NENs, also in the metastatic setting, as, despite their rarity, metastatic paraganglioma may occur [15].

GATA3 expression is not restricted to NENs originating from paraganglia and parathyroid. A subpopulation of neuroendocrine cells in the adenohypophysis (thyrotrophs and gonadotrophs) and some pituitary NETs (especially gonadotroph tumors and TSH-expressing tumors) have been shown to express GATA3 [77]. The latter may be explained by high genomic paralogy with the GATA2 transcription factor that is well-known to regulate the cellular differentiation of thyrotrophs and gonadotrophs [77]. However, there is also evidence that a low level of GATA3 is actually expressed in developing and adult pituitaries as well as α-TSH cells and TtT97 thyrotroph tumors [78, 79]. In pituitary pathology, GATA3 positivity should

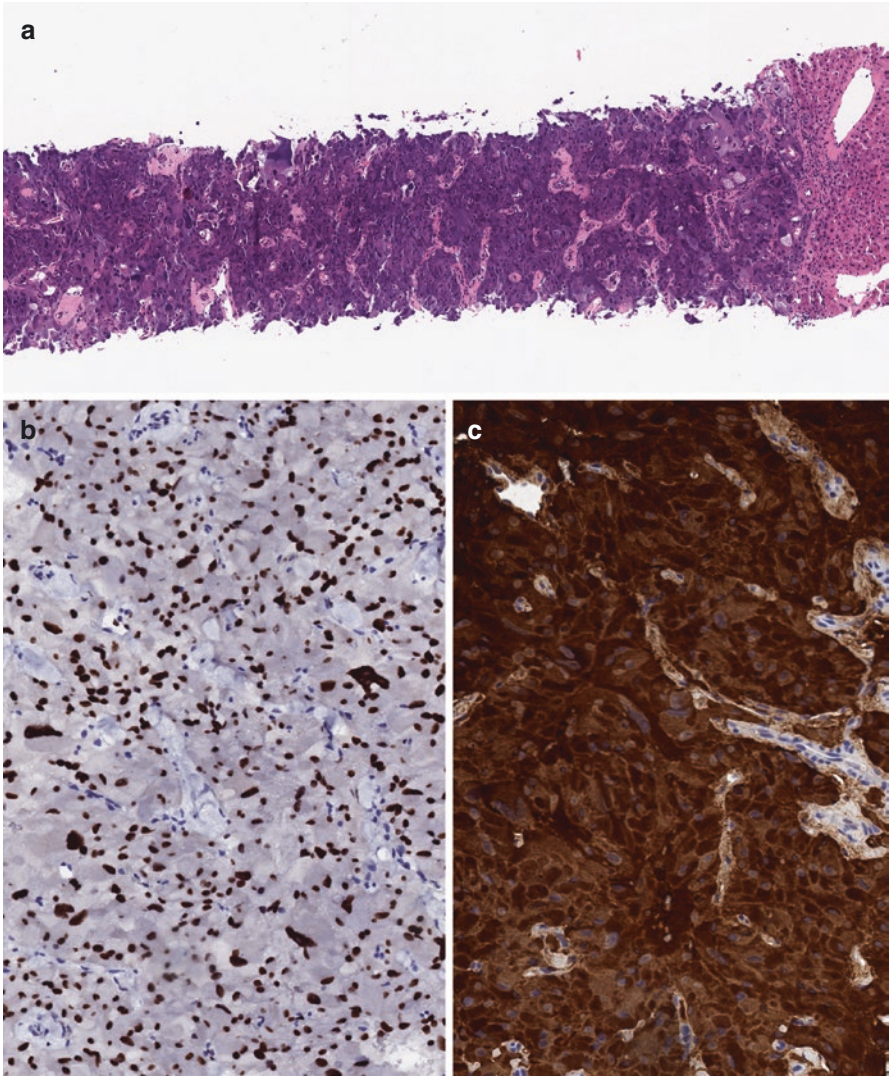

Fig. 16.5 Paraganglioma in the liver. The identification of a neuroendocrine neoplasm in the liver is not always a sign of metastatic disease. Primary paragangliomas can also occur in the liver (**a**). These neoplasms are typically negative for keratins (not illustrated herein) and are positive for GATA3 (**b**) and tyrosine hydroxylase (**c**)

be assessed in association with other adenohypophysial biomarkers [80] as discussed in the chapter on pituitary in this text.

While the link of GATA2/3 in pituitary and GATA3 in parathyroid, pituitary, and paraganglial NENs has been well-established, a number of other GATA transcription factors are known to regulate several cellular processes in various other endocrine organs as well as in neuroendocrine cells [81].

Estrogen and Progesterone Receptors (ER and PR)

ER and PR are nuclear proteins that, upon interaction with their ligands, are able to bind specific DNA consensus sequences and to modulate gene expression. For this reason, they are considered transcription factors. Their expression is tested in diagnostic pathology to predict the response to hormonal therapy in neoplasms of the breast and of the gynecological tract. However, ER and PR are also expressed, both in females and in males, in other tumors, such as meningiomas and carcinomas of various sites, including gallbladder, urinary bladder, and prostate, as well as in NENs [82]. In a series of 71 pulmonary NENs, both ER and PR have been demonstrated to be expressed, without significant differences, in a fraction of both well-differentiated and poorly differentiated neoplasms, independent of the patients' sex. The authors concluded that the sole immunoreactivity for these markers is not sufficient to exclude a lung primary, even in the case of a previous carcinoma of the breast [83]. Among gastroenteropancreatic NENs, PR expression seems to be a feature of pancreatic neoplasms, nearly half of which have been reported to be positive for this marker that was negative in intestinal, gastric, and biliary tumors. By contrast, ER expression does not seem to have a site-specific profile; and, in addition, it appears to be more frequent in females than in males [82, 84]. These results suggest that, in the context of a metastatic NEN of unknown primary, PR might be added to the immunohistochemical panel to support a possible pancreatic origin, whereas ER expression does not have a definite role.

ER-alpha has been used to classify pituitary NENs. ER-alpha is expressed in lactotrophs and mammosomatotrophs in association with PIT1 [80]. ER-alpha is also expressed in gonadotroph tumors which are typically positive for SF1 and GATA3 [80]. While the detection of ER-alpha expression requires optimal tissue fixation, this biomarker when used alone in the distinction of gonadotroph cell lineage origin does not show a better diagnostic performance when compared with SF1 [85].

Pituitary Transcription Factors

The use of pituitary transcription factors (TPIT, PIT1 and SF1) can help diagnosticians to confirm the pituitary origin a NEN when dealing with invasive or ectopic PitNETs [16] as well as in the workup of metastatic NEN of unknown origin [80].

T Box Transcription Factor (TPIT)

TPIT is a nuclear transcription factor that is expressed in proopiomelanocortin (POMC)-producing adenohypophysial cells [80]. Since ACTH expression is not specific to corticotroph tumors and several other NENs can express ACTH [86–88], the application of this biomarker can help diagnosticians distinguish corticotroph carcinoma (pituitary carcinoma of corticotroph cell lineage) from other ACTH-expressing NENs of various organs [89]. Since not all PitNETs can express hormones, the demonstration of TPIT in a hormone-negative PitNET also confirms corticotroph differentiation, allowing the diagnosis of a silent corticotroph tumor [90]. In fact, this is one of the reasons why the 2017 WHO classification of endocrine tumors revisited the definition of null cell tumors and restricted the diagnostic category of null cell tumors to pituitary NETs that are negative for transcription

factors that determine terminally differentiated neuroendocrine cell lineages of the adenohypophysis [91].

Pituitary-Specific Transcription Factor 1 (PIT1)

PIT1 is a transcription factor that regulates the molecular cytodifferentiation pathway of GH-, PRL-, and TSH-expressing cells [80]. PitNETs originating from somatotroph, mammosomatotroph, lactotroph, and thyrotroph cells lineage express PIT1 [80]. Tumors originating from PIT1-lineage stem cells are now classified as poorly differentiated PIT1-lineage pituitary NETs (formerly known as silent subtype 3 pituitary adenomas) and tend to express one or more than one PIT1 cell lineage hormone [91]. The absence of diffuse staining for any of three PIT1-lineage hormones distinguishes these tumors from other PIT1-lineage PitNETs. Since these tumors are biologically aggressive NENs, they can manifest with invasive or metastatic disease and may be misdiagnosed without proper analysis to identify pituitary origin [89].

Steroidogenic Factor 1 (SF1)

SF1 is expressed in all steroidogenic cells that can be identified in various organs including gonads, adrenal cortex, and adrenal rests; however, it is also expressed in neuroendocrine cells of the pituitary gland [80]. SF1 is the most specific biomarker to distinguish gonadotroph tumors (pituitary NETs of gonadotroph origin) from other pituitary NENs [85]. It can help diagnosticians to render the diagnosis of metastatic gonadotroph carcinoma in a visceral organ where there is a metastatic NEN of unknown origin.

Hormonal Markers

Hormone production in neuroendocrine cells is generally site-specific, as it is related to the specific function of the organ in which these cells are located. However poorly differentiated NECs often lack a site-specific hormone expression profile and tend to show aberrant transcription factor and ectopic hormone expression.

The use of hormonal markers in the diagnostic workup of metastatic NENs requires a panel approach that combines hormone immunohistochemistry with transcription factors. Nevertheless, one should recognize the following limitations. First, hormonal production in NENs, particularly in metastatic ones, is not always retained or detectable. Second, ectopic hormone secretion is a well-known phenomenon in NENs and this may be a confounding factor in the search for an occult primary. Examples of this situation are represented by the documented ectopic secretion of serotonin, calcitonin, ACTH, and ghrelin in pancreatic NENs [87, 92–94] and calcitonin and ACTH expression in pulmonary NENs [9, 88]. However, the use of a selected panel of hormonal markers is still useful in the diagnostic workup of NENs, also in the metastatic setting, provided that it is integrated in a wider diagnostic algorithm and, possibly, compared with clinical data [12]. The

knowledge of characteristics of the primary tumors (e.g., transcription factor and hormone immunoexpression status) can be used to rationalize the design of case-specific immunohistochemical panel. Unfortunately, most diagnosticians still fail to recognize how important is the impact of hormone immunohistochemistry when combined with other site-specific features.

Among tubular gut NENs, serotonin and substance P are useful to identify a midgut (jejuno-ileal) primary, whereas hormone products of L cells (PP, peptide-YY, glucagon-like peptide, and glicentin) are commonly expressed in rectal L cell tumors as well as in pancreatic NETs. Antibodies directed against pancreatic and intestinal hormones may help in defining clinico-pathological correlations in NENs. Most clinicians do not measure hormone products in the blood, as a significant proportion of patients manifest with non-specific or vague symptoms that would not prompt the attention of the clinician to measure specific hormones. More importantly, assays for some hormone products are simply not available in all hospital labs. From this perspective, pathologists play an essential role in the clinical management of patients with NENs, since hormones that are immunohistochemically demonstrated in NENs may serve as circulating biomarkers even in patients that are initially considered to have clinically non-functional NENs; it is also important to note that immunohistochemistry to screen for multiple hormones is far less costly than biochemical testing for multiple hormone products.

The panel of commercially available antibodies against hormonal peptides, including serotonin, substance P, calcitonin, gastrin, insulin, glucagon, somatostatin, pancreatic polypeptide, ghrelin, bombesin, gastric inhibitory peptide, motilin, secretin, cholecystokinin, vasoactive intestinal polypeptide, glicentin, and peptide-YY, has significantly advanced the workup of NENs [12]. Commonly accessible hormone stains (e.g., calcitonin, CGRP, serotonin, and bombesin) can be used in combination with TTF1 and monoclonal CEA to confirm the pulmonary origin of a well-differentiated NET; importantly, the lack of diffuse CEA is a critical distinction from medullary thyroid carcinoma. Another example may be represented by a prostatic acid phosphatase-positive NET (see below) in which the expression of L-cell hormones (PP, PYY, glucagon, or glucagon-like peptide) can be used to favor hindgut origin (e.g., rectal L-cell NET) (Fig. 16.6). Similarly, diffuse serotonin expression in a CDX-2-positive NET favors a midgut origin (small bowel EC-cell NET) [9, 17]. Another example would be a PP-expressing NET lacking PAP, glucagon and peptide-YY with PDX1 and CDX-2 (patchy) expression; this phenotype would favor a PP-cell NET originating from pancreas.

Other Markers

Cytokeratins

Cytokeratins are intermediate filaments of the cytoskeleton of epithelial cells and their expression in neoplastic pathology is a marker of epithelial differentiation. As the cytokeratin family includes a number of members differentially expressed in the

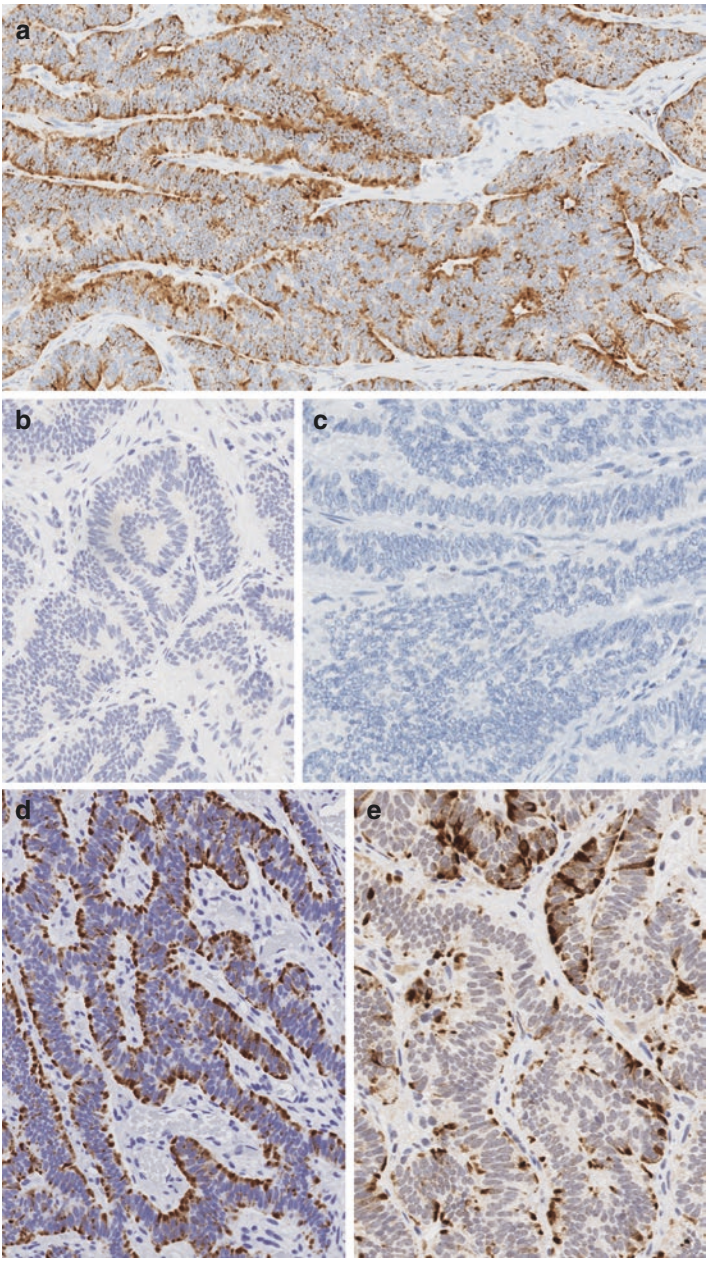

Fig. 16.6 Metastatic well-differentiated L-cell neuroendocrine tumor of the rectum. This composite photomicrograph illustrates immunohistochemical biomarkers related to a metastatic well-differentiated neuroendocrine tumor of hindgut origin. The tumor cells are positive for prostatic acid phosphatase (**a**), synaptophysin (not illustrated herein), and chromogranin-A (not illustrated herein). The tumor is negative for CDX-2 (**b**) and serotonin (**c**), and positive for pancreatic polypeptide (**d**), peptide-YY (**e**), and glucagon (not illustrated herein). The overall morphological and immunohistochemical findings are those of an L-cell neuroendocrine tumor of hindgut origin. Subsequent structrural and functional imaging studies identified a primary tumor in the rectum

epithelial cells of various sites, antibodies specifically directed against single high- or low-molecular-weight cytokeratins are used for the identification of the primary site of metastatic carcinomas.

In the pathology of neuroendocrine neoplasia, immunohistochemistry for cytokeratins is important for several reasons. First, the diagnosis of NENs requires the distinction of non-epithelial paragangliomas from epithelial NENs and neuroblastomas from NECs. Second, the pattern of the immunostain may support, where necessary, the distinction between NETs, in which cytokeratins usually show diffuse and intense cytoplasmic staining, and NECs, in which the staining pattern may be focal, faint or dot-like. Third, in the context of poorly differentiated NENs, positivity for cytokeratin 20 (CK20) is one of the clues to suggest the diagnosis of Merkel cell carcinoma rather than the metastasis of a pulmonary or extrapulmonary NEC. It should, however, be noted that NECs of the uterine cervix have been described to express CK20 [95], as well as cutaneous metastasis of NECs of the urinary bladder [96], and single case reports of CK20-positive colonic and mammary large cell neuroendocrine carcinomas have been reported [97, 98]. In addition, a small fraction of cutaneous Merkel cell carcinomas has been reported not to express CK20 [99]. Fourth, the expression of selected cytokeratins, such as CK7 and CK19, have been proposed as prognostic markers in pancreatic NENs [12].

Prominent fibrous bodies (juxta-nuclear globular keratin accumulation in more than 70% of the tumor) is the diagnostic feature of sparsely granulated somatotroph tumor in a PIT1-expressing PitNET [80]. This feature is a valuable predictor of the response to somatostatin analogue therapy. Similar structures have been identified in pancreatic NETs [100, 101].

Finally, one should also remember that epithelial NENs may sometimes lack cytokeratin expression. One of the most dramatic examples is seen in gonadotroph tumors where around 40% of these tumors lack keratin expression [85]. Therefore, the use of transcription factors related to epithelial NENs and other biomarkers helping in the distinction of paraganglial origin (e.g., tyrosine hydroxylase) should be considered in the appropriate context.

Carcinoembryonic Antigen (CEA)

CEA is a glycoprotein produced during fetal development that acts as an adhesion molecule during organ development. In adult tissues, its expression is very limited, but it can increase in a number of neoplastic diseases, including most adenocarcinomas. As it is expressed on the cell membrane and it can also be secreted, CEA can be detected both in the serum and at tissue level using specific antibodies. Among NENs, diffuse and strong CEA expression, as detected by a monoclonal antibody, is characteristic of medullary thyroid carcinoma and it represent a useful tool, in addition to calcitonin, CGRP, and TTF1, for the diagnosis of this neoplasm [99]. Indeed, with progression and dedifferentiation, medullary thyroid carcinoma may lose expression of calcitonin, but CEA expression is retained, providing an important biomarker for clinical surveillance.

However, monoclonal CEA immunostaining can also be found in pulmonary NETs. Fortunately it is only focal and usually weak and therefore can be useful in the diagnosis of pulmonary NETs, which are TTF1-positive and may express calcitonin and CGRP, but do not show diffuse monoclonal CEA expression [40].

Prostate-Specific Acid Phosphatase (PSAP; Also Known as Prostatic Acid Phosphatase, PAP or PRAP)

PAP is an enzyme produced in the prostatic epithelium and, along with prostatic specific antigen (PSA), is useful in the diagnosis of metastatic or poorly differentiated primary adenocarcinomas of the prostate. Since the early 1980s, PAP expression has been frequently reported in rectal NETs (Fig. 16.6), representing a diagnostic pitfall in small and crushed rectal biopsies, where a misdiagnosis of infiltration by prostatic adenocarcinoma can result in an incorrect management of the patient. Indeed, more than 80% of rectal NETs show immunohistochemical staining for PAP [102], but immunostaining for this marker has also been reported in NENs of other primary sites, although with a lower frequency, such as in gastric, small intestinal, appendiceal tumors, and pancreatic tumors [103]. In addition, some NENs originating from gonads, for instance, ovarian NETs, have been shown to be immunoreactive for PAP [104]. Due to its low specificity, the use of this marker alone in the definition of the primary site of a NEN may be questionable. However, its diagnostic power can be better justified in a panel approach combining site-specific hormones as well as other biomarkers appropriate to the clinical quandary.

Tyrosine Hydroxylase (TH)

TH immunohistochemistry helps diagnosticians to distinguish keratin- and transcription factor-negative NENs from paragangliomas/pheochromocytomas [9]. TH is an enzyme required in the catecholamine synthesis [9]. Rare examples of NENs, such as medullary thyroid carcinomas, can also show focal/variable expression for TH [105] in addition to well-defined characteristic biomarkers such as monoclonal CEA, TTF1, CGRP, and calcitonin. The authors of this chapter find TH immunohistochemistry useful to confirm the diagnosis of paraganglioma, especially when combined with GATA3 [15] (Fig. 16.5). Some experts also combine dopamine hydroxylase (another enzyme involved in the catecholamine synthesis, converting L-DOPA to dopamine) along with tyrosine hydroxylase in the workup of paragangliomas [106].

Somatostatin Receptors (SSTRs)

The cell sensitivity to somatostatin is mediated through members of the SSTRs family, composed of at least 5 subtypes (SSTR1, 2, 3, 4, 5). SSTRs are expressed by NENs, particularly by NETs, and their expression represents the rationale for the diagnostic and therapeutic procedures based on somatostatin analogues. Besides the therapeutic implications, in the setting of a metastatic NEN of unknown origin, performing an immunostaining for SSTRs, and in particular for SSTR2A, may be useful to predict the avidity of the neoplasm for somatostatin-based imaging, which can then be employed to detect the primary tumor. Volante and coworkers have proposed a three-tiered scoring system for the evaluation of SSTR2A immunoreactivity in neuroendocrine tumors, taking into consideration both the subcellular localization and the extent of the staining. Pure cytoplasmic immunoreactivity without

membranous staining corresponded to score 1, whereas score 2 and 3 were attributed to cases with membranous immunoreactivity in less or more than 50% of cells, respectively. Importantly, only membranous immunoreactivity had a good correlation with positivity to somatostatin receptor scintigraphy and a good response to cytostatic therapy with somatostatin analogues [107].

Metastatic NENs in Specific Sites: Differential Diagnosis with Primary Neuroendocrine Neoplasms

As already stated, the liver represents the most frequent metastatic site for NENs of different origins. As primary liver NENs are very rare, the integration of the pathological, clinical, and radiological data may easily drive to the identification of the occult primary site. Based on the above-presented immunohistochemical markers, Table 1.1 in Chap. 1 summarizes the most useful transcription factors, hormones, and other markers for the identification of the main possible primary site of a metastatic NEN. Figure 16.7 provides an algorithmic approach to this dilemma.

In other organs, where metastatic localizations are less frequent and primary NENs are a realistic possibility, a specific diagnostic approach is needed.

Specific Diagnostic Issues in the Lung, Thymus, and Thyroid

The lung is one of the most frequent metastatic sites for all malignant neoplasms, including NENs, and it is also one of the main primary sites for NENs. Most primary

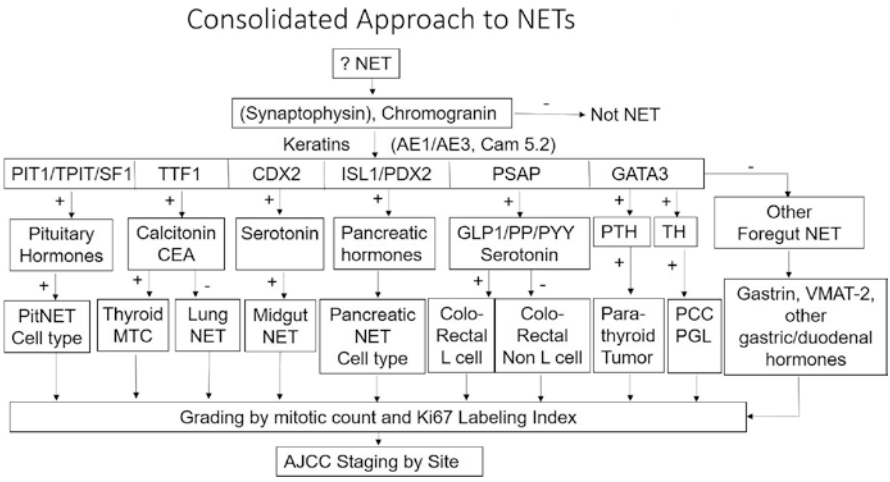

Fig. 16.7 Consolidated approach to metastatic NET. (Adapted from Singh et al. [17])

pulmonary NENs are poorly differentiated NECs of small or large cell type, for which, as already discussed, the definition of the primary site is frequently not crucial for the patient's management. Well-differentiated NETs, in contrast, represent a diagnostic problem. Frequently, the clinical picture and the morphological features are sufficiently straightforward to assess the primary nature of the neoplasm, but there are some situations in which the exclusion of a metastasis represents a challenge.

The identification of a thymic or pulmonary primary site in intra-thoracic NENs may be particularly difficult, and, sometimes, in locally advanced cases involving the mediastinum, the clinical definition of the primary site might not be straightforward, despite a strong impact on the management of the patient. The role of immunohistochemistry in the definition of lung vs thymic origin is limited to a panel of markers including TTF1 and PAX8. Although sensitivity and specificity are far from absolute, in low-to-intermediate forms a TTF1-positive/PAX8-negative profile is more suggestive of lung primary, whereas the opposite profile is more associated with thymic neuroendocrine neoplasms [12].

It must be noted that medullary carcinomas of the thyroid are TTF1-positive and, conversely, pulmonary NETs may be positive for calcitonin. Immunostaining for CEA can be helpful since it is diffusely positive in medullary thyroid carcinoma and often negative or focal in pulmonary NETs. OTP is positive in some pulmonary NETs and negative in medullary thyroid carcinoma and can also help to render a correct diagnosis. Although very rarely, pulmonary NET may metastasize to the thyroid gland and a correct differential diagnosis is important in the subsequent management of the patient. Indeed, a correct preoperative diagnosis can avoid an unnecessary thyroidectomy and, in any case, the prognostic implications of primary medullary thyroid carcinomas and metastatic pulmonary NETs are very different [108, 109].

Specific Diagnostic Issues in the Mammary Gland

Primary pure neuroendocrine neoplasms of the breast are a rare and still not fully clarified entities. Due to the little awareness of practicing pathologist about these tumors, their real incidence is unknown. However, these are considered to represent less than 1% of all breast malignancies. The most recent WHO classification of breast tumors subdivided mammary NENs into NET and NEC, in analogy with digestive NENs, as well as in concordance with the IARC/WHO classification framework for NENs [110]. In contrast to the fourth edition of the WHO classification, invasive breast carcinomas of special type (i.e., mucinous carcinoma of the hypercellular type and solid papillary carcinoma) or no special type that demonstrates neuroendocrine differentiation by immunohistochemistry are not included under the heading of NENs of the breast [111]. Available data on the metastatic potential of mammary NENs show an overall metastasis rate similar to that of invasive carcinomas of no special type [112]. One of the main differential diagnoses of pure neuroendocrine

NENs of the breast are metastatic NENs of other primary sites. Indeed, metastases to the breast are very rare and usually represent secondary localizations of hematological malignancies and contralateral breast carcinomas. Although NENs metastatic to the breast represent only 1–2% of all mammary metastasis, their distinction from primary breast NENs is of paramount importance, due to the critical therapeutic implications. In 2016, Mohanty and co-workers reported a series of 22 NENs metastatic to the breast, 15 of which were NETs and 7 NECs. In 7 cases the primary was unknown at the time of the diagnosis of the breast lesion and three of them were misdiagnosed as primary breast NENs and treated accordingly. Primaries were mainly located in the lung (11 cases), followed by the small bowel (7 cases), the gynecological tract (83 cases) and the colon (1 case). When the immunohistochemical features of the metastatic NENs were compared with those of an equal number of primary breast NENs, the most useful markers to identify primary lesions were ER and GATA3, which were negative in metastases [113].

Specific Diagnostic Issues in the Gonads

Among gonads, the ovary is a relatively frequent metastatic site, most frequently for gastrointestinal and breast malignancies. Metastatic NENs to the ovary are rare, albeit not exceptional, events and must be distinguished from primary ovarian NENs, which include both well and poorly differentiated neoplasms (see Chap. 14 in this text). Macroscopic and microscopic examination of the specimen may give important clues to the differential diagnosis, as unilaterality and the presence of elements suggesting an underlying teratoma strongly favor a primary ovarian origin. Conversely, metastatic NENs are nearly always bilateral, and scattered tumor deposits may be present in the ovarian parenchyma. Immunohistochemistry can be useful in the diagnosis but should be carefully interpreted in the light of morphological aspects. CDX2, for example, is not of great utility, as it is expressed by insular ovarian NET and by mucinous ovarian NETs, as well as by midgut and some pancreatic NETs. Conversely, no CDX2 immunoreactivity is present either in primary ovarian trabecular NETs or in rectal NETs. Pulmonary NETs do not express CDX2, so a positive result would exclude that possibility, whereas TTF1 immunoreactivity is strongly suggestive of a pulmonary origin. In this regard, however, the strumal component of strumal ovarian NET is TTF1-positive. As mentioned earlier, PAP can also be expressed in ovarian NETs, similar to hindgut NETs (e.g., rectal NETs). Positive PAX8 staining may suggest a metastatic origin of the NEN, provided that a non-endocrine primary ovarian tumor has been excluded [114].

Specific Diagnostic Issues in the Sellar Region

Metastases to the sellar region are uncommon, but, probably due to the improved survival of patients with disseminated disease, their incidence has been increasing

in the last decades [115]. Although less than 20 cases of metastatic NEN in the sellar region have been reported, their distinction from pituitary NET has huge therapeutic implications and should not be missed. Metastatic lesions in this site usually do not present with clinical signs of pituitary dysfunction; they are, rather, diagnosed due to mass symptoms or represent incidental findings during radiological workup for other causes. It is evident that the main problem is represented by metastatic well-differentiated NETs, which can morphologically simulate a primary pituitary neoplasm. Secondary localizations of pulmonary NET, ileal NET, medullary thyroid carcinoma, and mammary gland NENs have been reported in the sellar region [116–120]. In unusual cases there may be ectopic expression of hormones that may mimic a primary pituitary NET [8, 121]. The differential diagnosis in this case relies primarily on the use of pituitary transcription factors, including PIT1, SF1, and TPIT, which are able to identify the pituitary origin in nearly all primary pituitary lesions (see chapter x in this text). In this context, it is worth noting that ER-alpha and GATA3 that are expressed in some PitNETs and other NENs of various origin should be interpreted with caution in the background of a comprehensive immunohistochemistry panel.

Conclusion

The workup of a metastatic NEN represents a critical responsibility of pathologists. It does require careful interpretation of clinical, morphologic, and immunohistochemical findings. The use of a panel of approach combining cytokeratins along with anatomic site-related transcription factors, hormones, and other biomarkers can assist identifying the origin of the metastatic NEN. The power of this approach is limited in the setting of poorly differentiated NECs.

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Cytology of Neuroendocrine Neoplasms

17

Massimo Bongiovanni and Anja M. Schmitt

Cytology is a modern, fast, secure, and cost-effective method to render a diagnosis of neoplastic processes throughout the body, including neuroendocrine neoplasms. Fine-needle aspiration and exfoliative cytology specimens show a good sensitivity and specificity in detecting neuroendocrine neoplasms. Increased imaging has led to an increased and earlier detection of neuroendocrine neoplasms. In addition, the easy accessibility of both endoscopic procedures and a fast-diagnostic workup by cytology allow for earlier diagnoses on less invasively obtained material, increasingly in an outpatient setting. Thus, cytology has contributed to the decrease in the mortality of neuroendocrine neoplasms.

This chapter focuses on general and site-specific cytology of neuroendocrine neoplasms to make the reader familiar with the spectrum of the cytology of neuroendocrine neoplasms. In addition, differential diagnoses and the use of ancillary techniques such as immunocytochemistry and immunohistochemistry are discussed. Finally, the widely accepted organ system-based, specific classification systems to facilitate the conversation among members of multidisciplinary endocrine oncology team are discussed.

General Cytomorphology

In general, cytology specimens reflect the histomorphological aspects of neuroendocrine neoplasms on the single cell level. The broad spectrum of epithelial neuroendocrine neoplasms ranges from well-differentiated (neuroendocrine tumor, NET)

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to poorly differentiated neuroendocrine neoplasms (neuroendocrine carcinoma, NEC). Table 17.1 shows features that can be used to distinguish well-differentiated neuroendocrine tumors from poorly differentiated neuroendocrine carcinomas.

NETs usually yield cellular specimens on an either hemorrhagic or clean background (Fig. 17.1). The neoplastic cells are found either as single cells or in small clusters. The single cells are medium sized and have a round nucleus with characteristic "salt and pepper" chromatin and may show a nucleolus. The cytoplasm often is finely granular and eosinophilic but may also be clear. In some cases, the neoplastic cells may exhibit a strikingly plasmacytoid appearance. Nuclear molding is usually not a feature of well-differentiated neuroendocrine neoplasms. If the tumor

Table 17.1 Main cytomorphological features of well-differentiated and poorly differentiated – neuroendocrine neoplasia

	Well differentiated	Poorly differentiated
Cellularity	Moderate to high	High
Arrangement	Cohesive clusters, possible trabecular and rosette-like formations	Mostly isolated cells
Cell Shape	Plasmacytoid, round	Round
Cytoplasm	Abundant, dense, basophilic	Mostly scant
Nucleus	Round to oval, hyperchromatic	Round small or large with marked anysokariosis; molding; hyperchromatic, irregular nuclear membrane
Chromatin	Finely granular, salt and pepper	Salt and pepper
Nucleolus	Single, small	Single, large
Background	Clean (absence of necrosis and inflammation)	Cellular debris, necrosis, hemorrhagic.

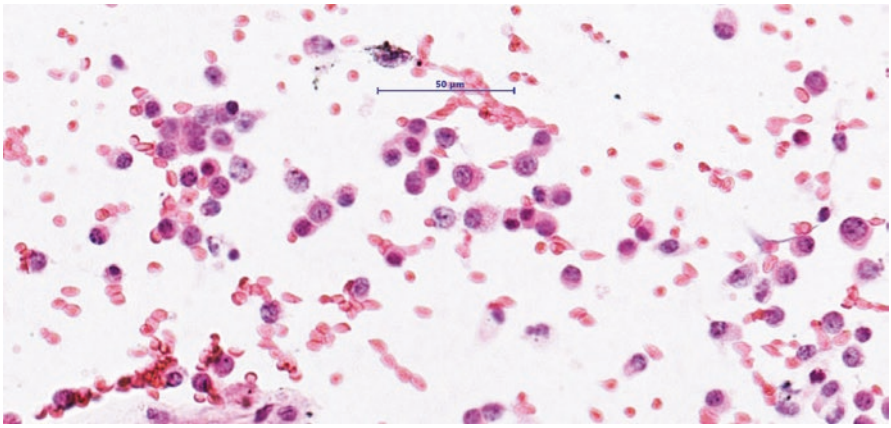

Fig. 17.1 Example of a well-differentiated neuroendocrine tumor. FNAB material of a typical carcinoid of the lung. Note the discohesive pattern and the plasmacytoid aspect of the tumor cells with salt and pepper chromatin. Papanicolaou staining

cells are arranged in clusters, these may appear trabecular, solid, or rosette-like. Mitotic figures, by definition, should be either absent or rare. The presence of necrosis, which confers a "dirty" background, should raise the suspicion of a NEC.

NECs, in analogy to histology, often show either a small or a large cell morphology, which are referred to as small cell and large cell neuroendocrine carcinomas, respectively. Rare examples can combine both cytomorphology. Irrespective of their subtypes, the background often conveys a dirty impression due to tumor necrosis and abundant apoptosis. The classical features of the small cell neuroendocrine carcinoma (formerly called oat cell carcinoma) of the lung are well defined (Fig. 17.2). The nuclei are sometimes small, but despite the tumor designation of small cell carcinoma, more often medium sized and in fact definitely larger than a lymphocyte. Sometimes, the nuclei of small cell neuroendocrine carcinoma can appear unexpectedly large, especially if they are encountered in fluids, for example, in a serous effusion. The nuclei of small cell neuroendocrine carcinomas are hyperchromatic and can show a smudgy aspect. Due to the friability of the chromatin and the nuclear membranes, the nuclei tend to produce smears or so-called crushing artefacts when placed on a slide. There is only scanty, if any cytoplasm at all. Single cell

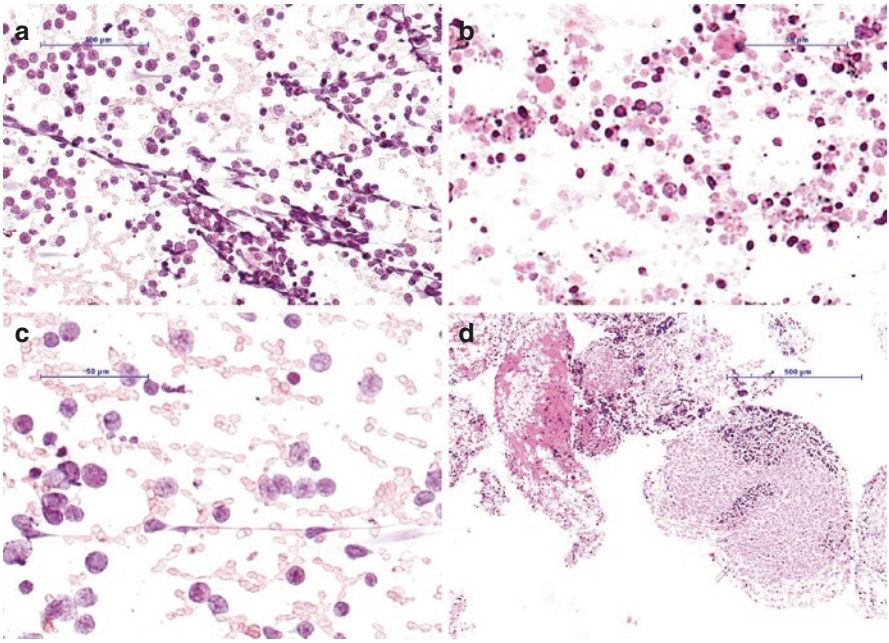

Fig. 17.2 Example of a poorly differentiated neuroendocrine carcinoma with small cell morphology. FNAB material of a SCLC. (**a**) Crushing artifacts are prominent due to the friability of the chromatin. Papanicolaou staining. (**b**) Single cell necrosis or larger areas of necrosis are a typical, yet not specific finding. Papanicolaou staining. (**c**) Note the almost naked nuclei with smudgy chromatin. Tumor cells tend to be arranged in small strands. Nuclear molding is a typical and specific finding. Papanicolaou staining. (**d**) If a cell block is available, geographic necrosis is a finding suggestive of SCLC. H&E

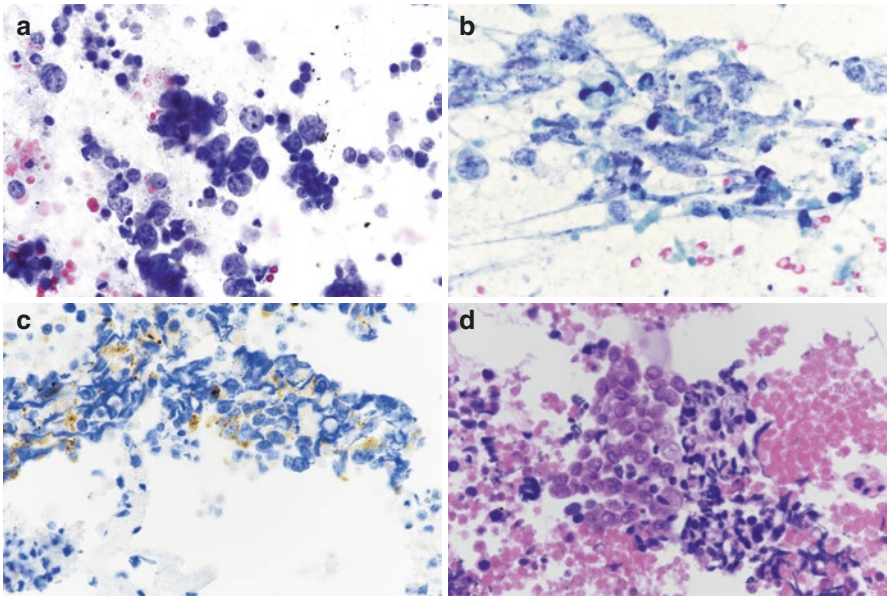

Fig. 17.3 Example of a large cell neuroendocrine carcinoma. (**a**) Cells have larger size than the small cell carcinoma, shows variation in size large hyperchromatic nuclei with multiple nucleoli (smear, PAP staining). (**b**) Chromogranin A staining has a perinuclear dot-like positivity (cell block, Chromogranin A staining), while (**c**) synaptophysin shows a more intense staining in some of the cells (cell block, synaptophysin staining). (**d**) Proliferative index Ki67 is typically high: more than 90% of neoplastic cells are positive (cell block, Mib-1 staining)

necrosis as well as larger areas of necrosis are a typical finding. The tumor cells lie isolated or in loose clusters. If isolated, a typical formation in small strands can be found. If lying in loose clusters, a pathognomonic finding is the so-called nuclear molding, which means that one nucleus is huddled to the other and which is again a consequence of the nuclear friability. In large cell neuroendocrine carcinomas, tumor cells are medium- to large-sized and round or polygonal in shape (Fig. 17.3). In contrast to small cell neuroendocrine carcinomas, a clearly defined cytoplasm can be identified; however, naked nuclei can be observed as well. Nuclei are polymorphic and round, oval, or polygonal with a thin and smooth nuclear membrane. The nuclear chromatin pattern is either finely or coarsely granular. Nucleoli can be inconspicuous; in other cases one or two nucleoli can be observed [1].

General Immunohistochemical and Immunocytochemical Profile

In order to establish the neuroendocrine nature of a neoplastic process, a panel of neuroendocrine biomarkers is recommended as other non-neuroendocrine neoplasms can show an aberrant expression for one or multiple neuroendocrine

markers. If immunostains are planned to be used on smears (immunocytochemistry), it is important that the reactivity is validated for this purpose as the staining protocol can be different from the same antibody on formalin fixed paraffin embedded (FFPE) sections (immunohistochemistry). In our hands, immunostains performed on FFPE sections of a cell block yield the most reliable results.

First of all, it is crucial to confirm the epithelial nature of the cells. For this purpose, broad spectrum keratins like AE1/AE3 is suitable. Especially in small cell cytomorphology, the use of the CAM5.2 antibody is very useful, yielding a typical perinuclear dotlike (Golgi pattern) staining pattern.

Chromogranin A is regarded as the most specific neuroendocrine marker and is used best in combination with the most sensitive marker for neuroendocrine differentiation such as synaptophysin. Both biomarkers yield a cytoplasmic, often slightly granular staining result. CD56 and neuron-specific enolase (NSE) can be used in addition; however, one should be aware of their lack of specificity, meaning that they should never be used on their own to diagnose a neuroendocrine neoplasm.

It is important to realize that the use of transcription factors like TTF-1, CDX-2, and Islet1 only in well-differentiated neuroendocrine neoplasms can help to identify the origin of the tumor. In contrast, in poorly differentiated neuroendocrine carcinomas transcription factors usually are expressed randomly.

If there are enough tumor cells in clusters available (which will most probably be the case in cell block preparations), a Ki67 stain can be performed to give a rough estimation of the proliferation index and to rule in or out a grade 3 neuroendocrine neoplasm. However, the distinction of Grade 1 from a Grade 2 tumor in the lower proliferation range is not encouraged because intratumoral proliferative heterogeneity precludes accurate assessment in cytology specimens.

Pancreas and Biliary Tract

Morphology

The gold standard for the initial evaluation of pancreatic lesions is the endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNAB) with or without rapid on-site evaluation (ROSE). The use of the latter will be especially useful if EUS-FNAB regularly yields unsatisfactory results. It is especially important to establish the diagnosis of a pancreatic neuroendocrine tumor (PanNET) on FNAB as conservative management of tumors <2 cm in size is becoming an increasingly common option. Moreover, the tumor tissue obtained by FNAB has increasingly become the basis for predictive marker studies that help tailor targeted therapies and can be used for molecular testing to understand mechanisms leading to progression and metastatic spread of PanNETs especially in the management of patients with unresectable disease.

Generally, the sonographic aspect of a circumscribed solid and highly vascularized tumor in the pancreas suggests a diagnosis of a PanNET. However, some

tumors can undergo cystic degeneration. FNAB often yields highly cellular smears, often on a hemorrhagic background due to the strong vascularization of PanNETs. Tumor cells are poorly cohesive with a mostly single cell pattern. Occasionally, rosette-like structures can be seen. Tumor cells are uniform, small to medium–sized, and round or polygonal in shape. A finding very suggestive for a PanNET is the presence of plasmacytoid cells with eccentrically placed nuclei. Nuclei are uniform in size and shape, either round or oval, and binucleated cells can be observed occasionally. However, nuclear pleomorphism so-called "endocrine atypia" can be encountered as well. Nucleoli are mostly inconspicuous. Chromatin is evenly distributed and shows the typical "salt and pepper" aspect. From scant to abundant, there is a variable amount of dense and basophilic cytoplasm. Rare variants can show a clear and vacuolated or an oncocytic cytoplasm. Mitotic figures and necrosis are absent to rare. A panel of images of PanNET is shown in Fig. 17.4.

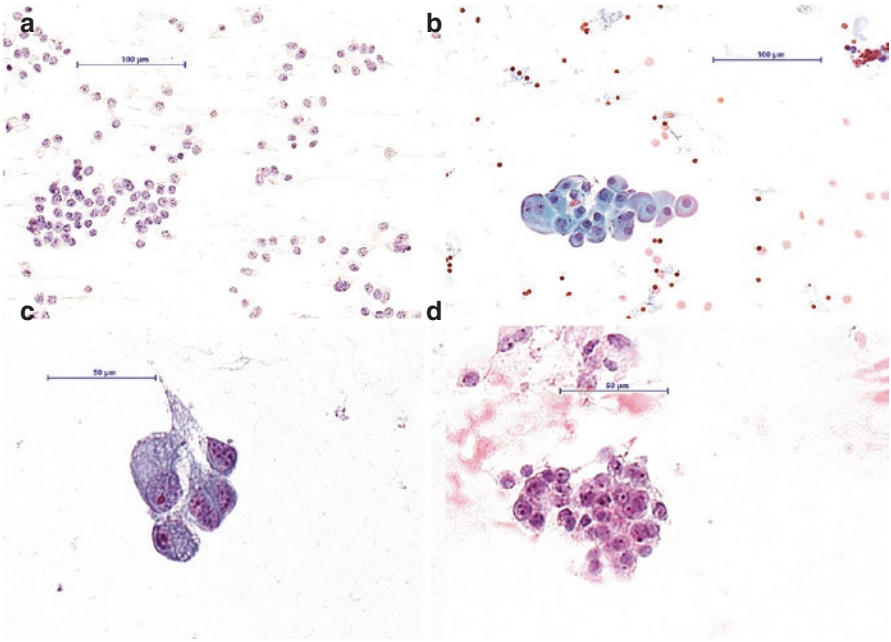

Fig. 17.4 Examples of well-differentiated neuroendocrine tumors of the pancreas and comparison with its most important differential diagnosis, acinic cell carcinoma. (**a**) In this FNA smear of a PanNET the predominantly discohesive pattern of a highly cellular aspirate can be appreciated. Papanicolaou staining. (**b**) In this example of a PanNET the tumor cells lie in loose clusters and show a strikingly plasmacytoid appearance with a normal nuclear-cytoplasmic ratio and small nucleoli. Papanicolaou staining. (**c**) In another case of PanNET the plasmacytoid tumor cells show prominent nucleoli. Papanicolaou staining. (**d**) Example of an acinic cell carcinoma. The tumor cells form more cohesive and three-dimensional clusters. Single-cell morphology is less plasmacytoid, the nuclear-cytoplasmic ratio in this case is increased, nuclei are hyperchromatic and show large nucleoli and the cytoplasm is finely granular and eosinophilic. Papanicolaou staining. Especially comparison between C and D highlights the similarities PanNET and ACC can show, making ACC the most important and challenging differential diagnosis

Ancillary Techniques

For the above-mentioned reasons the diagnosis of a PanNET should be confirmed using immunostains. As discussed earlier, both smears and cell blocks can be used for this purpose. Since antibodies require a specific validation process, when smears are used for immunocytochemistry, the interpretation can be challenging [2]; therefore, the use of immunohistochemistry on cell blocks is often recommended.

After having confirmed the epithelial nature using pancytokeratin (AE1/AE3) and/or CAM5.2 staining, the use of a small panel of neuroendocrine markers consisting of chromogranin A and synaptophysin is recommended [3]. Although less specific, synaptophysin is more sensitive and leads to a strong and diffuse staining, while chromogranin A is more specific but often leads to a patchy and focal staining which can be unsettling when dealing with an FNAB [4].

Grading of PanNETs on FNAB specimens is a controversial issue. Obviously, the WHO grading scheme requires counting of mitoses from 50 high power fields (10 mm^2) in areas of high mitotic density and Ki67 labelling index by assessing tumor nuclei in 2000 cells from hot spots. Thus, the nature of FNAB specimens precludes accurate grading. Although there are studies showing a high correlation between the grading on cell blocks and on histology [5], the final tumor grading is typically done on the resection specimens. If a conservative treatment is favored, the use of Ki67 staining lies in the distinction of Grade 1/Grade 2 disease from Grade 3 neoplasms. If the result of the Ki67 staining is unequivocal in terms of a Grade 3 neuroendocrine tumor and a Grade 3 neuroendocrine carcinoma (NEC), immunohistochemical biomarkers including but not limited to SSTR2, DAXX, ATRX, menin, Rb, and p53 can assist this distinction [6].

Standardized Reporting

In 2015, the Papanicolaou Society of Cytopathology published the first edition of "The Papanicolaou Society of Cytopathology System for Reporting Pancreatobiliary Cytology" [7]. This system comprises six categories: I, non-diagnostic; II, negative for malignancy; III, atypical; IV, neoplastic: benign and neoplastic: other; V, suspicious (for malignancy); VI, positive or malignant. While all neuroendocrine neoplasms are now considered to be malignant with various metastatic potential, unless dealing with a Grade 3 pancreatic neuroendocrine neoplasm, they are classified as category IV, neoplastic: others. This categorization comes the closest to the biological behavior and the long clinical course of these tumors and helps the treating oncologist to pursue a conservative treatment regimen [7].

Differential Diagnosis

An overview of the distinctive morphological and immunohistochemical aspects is given in Table 17.2. For keratin-negative pancreatic neuroendocrine neoplasms, the

Table 17.2 Distinctive cytomorphological and immunohistochemical features in pancreatic neuroendocrine tumors and acinar cell carcinoma

	PanNET	ACC
Arrangement	Increased cellularity; single cells, occasional rosettes	Loose cohesive clusters, vague acini; loss of polarity
Cell Shape	Round, polygonal, plasmacytoid	Cuboidal, columnar, triangular
Cytoplasm	Scant to abundant, dense, basophilic	Scant to abundant, finely granular, eosinophilic
Nucleus	Round/oval: eccentric, uniformity of size and shape, binucleate	Round/oval: uniform size, slightly irregular membrane
Chromatin	Salt and pepper; even distribution	Irregular clumping
Nucleolus	Single, small, inconspicuous	Single, prominent to macro
Background	Clean	Clean
Synaptophysin	+	–/+
Chromogranin A	+	–/+
Trypsin/ Chymotrypsin	–	+
BCL10	–	+
PAS + Diastase	–	+

Modified after Labate et al. [3]

possibility of a *paraganglioma* should be excluded by performing GATA3 and tyrosine hydroxylase stains.

The most challenging differential diagnosis of PanNET is *acinar cell carcinoma (ACC)* as these two entities at first (and sometimes second) sight have a lot in common. The tumor cells of ACC are loosely cohesive clusters and sometimes form vague acinar structures on a clean background. They can be cuboidal, columnar, or triangular and show a loss of polarity. In contrast to PanNET cells, they are virtually never plasmacytoid. The nuclei are uniform in size, round to oval, and have a slightly irregular membrane. The chromatin shows irregular clumping. Nucleoli, in contrast to PanNET, are at least prominent and sometimes correlate to macronucleoli. Similar to PanNETs, the amount of cytoplasm ranges from scant to abundant; however, in contrast to PanNETs the cytoplasm of ACC is finely granular and eosinophilic. In contrast to PanNETs, ACCs can be focally positive for neuroendocrine markers but they are negative for insulinoma-associated protein 1 (INSM1). These tumors are positive for trypsin, chymotrypsin, and bcl10. Moreover, PAS staining with diastase shows positive cytoplasmic granular reactivity in ACC but not in PanNET [2].

Solid pseudopapillary neoplasm (SPN) can be another important differential diagnosis of PanNET. However, while PanNETs consist mainly of single and often plasmacytoid cells, SPNs demonstrate papillary and sometimes branching clusters with fibrovascular cores, fine chromatin, and nuclear grooves. While SPNs are positive for synaptophysin, they lack reactivity for chromogranin A. Nevertheless, β-Catenin is the diagnostic stain and will show a nuclear reaction product in virtually all SPNs [8].

A further differential diagnosis at first sight may be *lymphoma* which can usually be excluded by a thorough morphological examination which will never show any cell clusters and which can definitely be ruled out by positive staining for pancytokeratin or CAM5.2 and ruled in by a positive staining for CD45 or, more specifically, B- and T-cell markers.

Finally, in rare cases, *pancreatic ductal adenocarcinoma (PDAC)* may come into the cytomorphological differential diagnosis. Nuclear and nucleolar pleomorphism, irregular chromatin, vacuolated cytoplasm, and the arrangement in so-called "drunken honeycombs" rather than a predominantly single cell aspect as well as negative staining for neuroendocrine markers are the hallmark of PDACs.

Skin

The primary neuroendocrine carcinoma of the skin is Merkel cell carcinoma. The cytomorphology of both the primary tumor and its metastases is the same as in small cell neuroendocrine carcinoma (as discussed in the section "General Morphology"); however, especially in metastatic disease, it is important to think of the possibility of Merkel cell carcinoma and to perform CK20 and Merkel cell polyoma virus (MCPyV) immunostaining in addition to a pancytokeratin staining and other general neuroendocrine biomarkers.

Upper Respiratory Tract and Lung

The most frequent diagnosis among neuroendocrine neoplasms of the lung is *small cell lung carcinoma* (SCLC) (also known as small cell neuroendocrine carcinoma of the lung), followed by *large cell neuroendocrine carcinoma (LCNEC)* of the lung, which are often sampled by EBUS-TBNA, but can also be detected in sputum, bronchial washings, and brushings. The cytomorphology and immunophenotype of SCLCs and LCNECs are those of their counterparts of various sites as discussed earlier.

Well-differentiated neuroendocrine tumors with low-grade (typical carcinoid) and intermediate-grade features (atypical carcinoids) of the upper respiratory tract and the lung can find their way to cytological examination via different sampling methods. While central carcinoids, which usually present as submucosal spherical masses, can undergo washings, brushings, and FNAB, peripheral carcinoids rather are sampled by a peripheral EBUS-TBNA. Moreover, as both typical and atypical carcinoids have the potential to metastasize, another scenario in which carcinoids can be encountered is EBUS-TBNA of mediastinal lymph nodes. The smears show both single and loosely cohesive tumor cells as well as tissue fragments, sometimes with central capillaries due to the rich vascularity of these tumors. Similar to well-differentiated neuroendocrine tumors of various sites, carcinoids can form trabeculae, anastomosing cords, nests, and rosette-like formations. Tumor cells are uniform, with round to ovoid nuclei

and scant or moderate amounts of cytoplasm. Nuclei display evenly dispersed granular chromatin. Nuclear molding is uncommon (Fig. 17.1). Some carcinoids may exhibit predominantly spindle cell features. The presence of necrosis or increased mitoses are suggestive of atypical carcinoid tumors; however, a definitive distinction between typical and atypical carcinoids on cytological specimens is not often possible; thus, this is typically referred to surgical resection specimens [9].

Pituitary

Pituitary neuroendocrine tumors (PitNETs) can be encountered either as an aspiration cytology specimen of a suprasellar mass or in the setting of frozen section as a smear. As normal pituitary consists of a variety of morphologically different cell types, the most striking and suggestive feature of pituitary tumor is the monotony of the cells. Usually, the smears show a large amount of evenly distributed, small- to medium-sized and round to oval discohesive cells. Histological patterns like solid, trabecular, or papillary structures can be reflected in the smears. Nuclei are generally eccentric, round to oval, with speckled chromatin and occasional small nucleoli, resulting in a characteristic plasmacytoid morphology. If preserved, the cytoplasm demonstrates an oval or round morphology and stains from clear to pale to densely granulated, depending on the type and functional state of the tumor. However, due to the fragility of the cytoplasm many bare nuclei are seen in a granular background of cytoplasmic fragments (Fig. 17.5). The biological behavior cannot be predicted by cytomorphology. Nuclear pleomorphism is uncommon and when identified it does not imply a malignant tumor. Vice versa, PitNETs with a bland cytomorphology may show aggressive biological behavior [10].

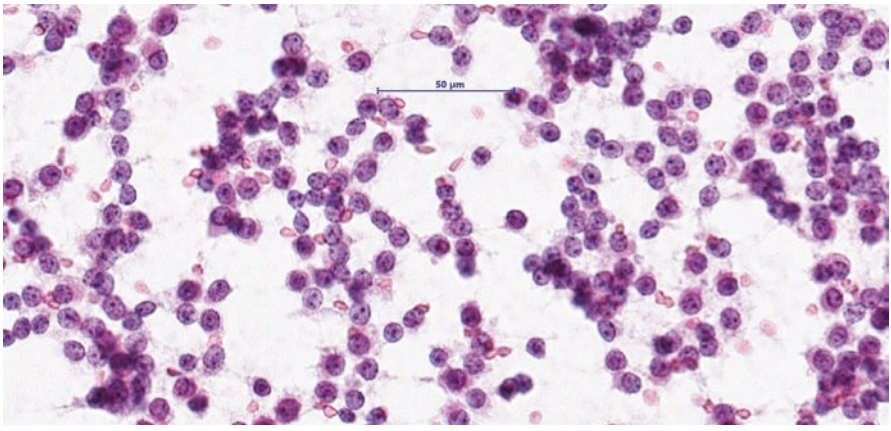

Fig. 17.5 Aspirate of a pituitary neuroendocrine tumor. Note the monotonous appearance of the tumor cells. Tumor cells lie discohesively and show a slightly plasmacytoid morphology. Many bare nuclei in a granular background of cytoplasmic fragments can be appreciated due to the fragility of the cytoplasm. Papanicolaou staining

Thyroid

The thyroid is an endocrine organ devoted to the production of several types of hormones (thyroglobulin, T3, T4, calcitonin). "Strictu senso," the most common primary neuroendocrine tumor arising from the thyroid gland and that shows the general features of neuroendocrine tumors, is medullary thyroid carcinoma (MTC). Rare examples of primary thyroid paragangliomas, intrathyroidal thymic neuroendocrine tumors, and intrathyroidal parathyroid neuroendocrine neoplasms should also be considered in the differential diagnosis (see the chapter on thyroid neuroendocrine neoplasms). The clinical and ultrasound (US) presentation of MTC is similar to that of other most frequent primary thyroid tumors, such as papillary thyroid carcinoma (PTC) or follicular thyroid carcinoma, presenting as a solitary nodule, sometimes with enlarged lymph node and without specific US features. As plasma calcitonin and carcinoembryonic antigen (CEA) measurements are usually not performed routinely, the cytopathologist should always be aware of this possibility in routine daily work.

MTC arises from the parafollicular thyroid cell, also called C cells, and their main role is the production of calcitonin. Recently, there have been new studies about the origin of C cells and a unifying origin from the endoderm has been proposed [11]. MTC is a rare neoplasm (up to 10% of all thyroid malignancies) but the third most frequent thyroid tumor after PTC and FTC. Even if it is quite rare, MTC accounts for more than 14% of thyroid cancer–related deaths. It can present in two forms: familial and sporadic. Both forms show the same neuroendocrine characteristics as well as a large variability in cytomorphological presentation, so MTC is also recognised as a great mimicker, similar to malignant melanoma.

Morphology

Morphologically, MTC cells are polygonal/cylindrical/plasmacytoid with salt and pepper nuclear chromatin. Occasionally, crowded and syncytium-like aggregates are also visible. The stroma has a particularity: it is usually eosinophilic as it contains, in a large amount of cases, amyloid, that is similar to amyloid in any other organs and stains positively for Congo red. Fragments of amyloid can be seen in the background of the slides and should not be confounded with sticky colloid. Morphological cellular and architectural variation comprises cells with small, spindle, clear, oxyphilic (oncocytic), squamoid, giant, binucleated and multinucleated, and pigmented morphology or structures with follicular and papillary architecture. In this eclectic content, the diagnosis is sometimes challenging (Fig. 17.6).

Ancillary Techniques

The use of ancillary techniques is crucial, not only in the presence of bizarre morphology, but also to confirm diagnosis even in cases with classical cytomorphologic details, due to the important clinical implication of this diagnosis. Usually a panel

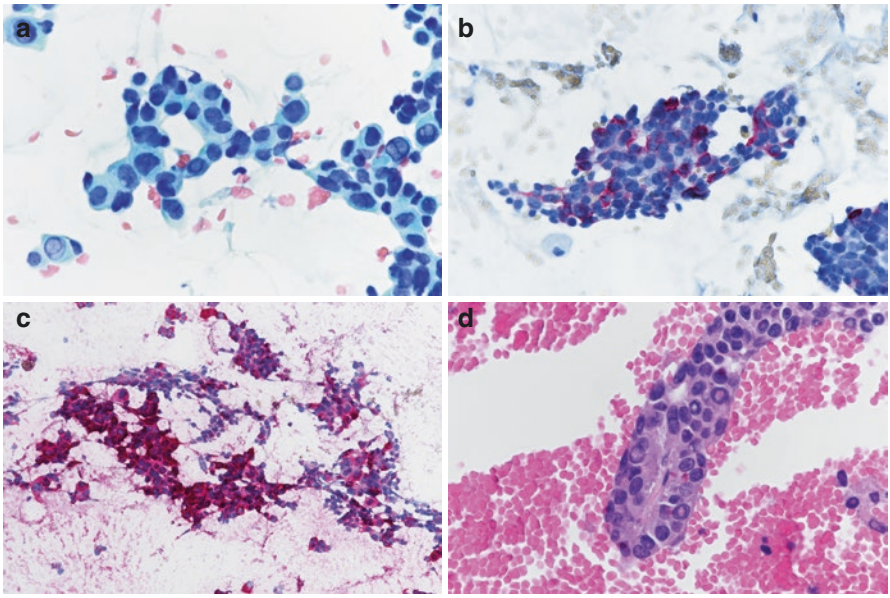

Fig. 17.6 Aspirate of a medullary thyroid carcinoma (MTC). (**a**) Clusters of cells with large and hyperchromatic nuclei and abundant cytoplasm. Note nuclear pseudo-inclusions that raise the differential diagnosis with papillary thyroid carcinoma (smear, PAP staining). (**b**) Chromogranin A staining and (**c**) calcitonin staining are diagnostic of a neuroendocrine tumor consistent with MTC (B, smear, Chromogranin A staining, and C, smear, calcitonin staining). Another immunocytochemical marker that can be useful is CEA. (**d**) Cell block showing pseudo-papillary structures and the same type of cells as the ones present on the smear with evident nuclear inclusions (cell block, H&E staining)

of immunocytochemical markers are used: positivity for calcitonin, calcitonin gene-related peptide, monoclonal CEA, and negativity for thyroglobulin. Among these, staining for monoclonal CEA is the hallmark of MTC. Other markers also show an intense positivity in MTC as almost all neuroendocrine tumours include but not limited to chromogranin A, synaptophysin, NSE, and CD56.

Differential Diagnosis

Many morphological types of MTC have been reported, so the differential diagnosis is quite broad. MTC should be first distinguished from other primary thyroid tumors, such as PTC and FTC. The presence of nuclear inclusions and follicular like structures are the main confounding factors, being both present in MTC. Plasmacytoid/oxyphilic appearance in MTC, in the presence of necrosis, can be misdiagnosed as poorly differentiated thyroid carcinomas and/or oncocytic tumors. Spindle cell forms can be misdiagnosed as anaplastic thyroid carcinomas and/or sarcomas. In the absence of material to perform ancillary studies, correlation with serum level of calcitonin and CEA is helpful. For differential diagnosis, please see Table 17.3.

Table 17.3 Main differential diagnosis of medullary thyroid carcinoma according to cellular morphology and architecture

Cellular morphology/architecture	Differential diagnosis
Nuclear inclusions	PTC, melanoma
Papillae	PTC
Follicles	HN/FTA/FTC
Spindle cells	Sarcomas
Clear cells	Metastatic renal carcinoma, parathyroid tumor
Oncocytic cells	Oncocytic follicular cell neoplasms; oncocytic PTC; HTT
Giant cells	ATC
Small cells	Small cell neuroendocrine carcinoma
Presence of amyloid	Amyloid goiter
Plasmacytoid cells	Carcinoid
Other rare morphologies	
Melanin-producing	Melanoma
Squamous cells	SCC; PTC with squamous differentiation
Paraganglioma-like	Paraganglioma; HTT
Mucoid cells	Adenocarcinoma

PTC papillary thyroid carcinoma, *HN* hyperplastic nodule, *FA* follicular adenoma, *FTC* follicular thyroid carcinoma, *ATC* anaplastic thyroid carcinomas, *HTT* hyalinizing trabecular tumor, *SCC* squamous cell carcinoma

Standardized Reporting

MTC is usually diagnosed in The Bethesda System for Reporting Thyroid Cytopathology Categories V (suspicious for malignancy) and categories VI (malignant).

Parathyroid

Another neuroendocrine organ present in the posterior thyroid region is the parathyroid. Usually it is not aspirated to obtain a diagnosis, but the possibility to encounter parathyroid cells in a thyroid smear is not to be excluded, as parathyroid proliferations can be easily confounded at US with thyroid nodular proliferations. Considerable variations in their position and number exist. Enlargement of the parathyroid glands can be seen in cysts, hyperplasia, adenoma, and carcinoma; and these conditions are subject to be aspirated. Hyperplastic and adenomatous parathyroid cells are almost indistinguishable from thyroid follicular cells. They are arranged in follicular structures or have honeycomb appearance. Colloid is usually absent in the background, but clear and watery material can be seen in cystic forms. Cells are round/cuboidal. Chief cells have less cytoplasm and ill-defined cell borders, whereas oncocytic cells have granular cytoplasm similar to those of Hürthle cells. Nuclei are usually round and uniform. Nuclei tend to be larger and more atypical in carcinoma, and mitoses can be seen. If not suspected clinically due to high plasma parathyroid hormones (PTH) and calcium levels, distinction between parathyroid cells and

follicular cells can be done with immunocytochemistry, both on smears and the cell block. Parathyroid hormone (PTH), chromogranin A, GATA 3, and GCM2 are positive; and TTF1 and thyroglobulin are negative.

Extra-Adrenal Paraganglia and Adrenal Medulla

Tumors originating from the extra-adrenal paraganglia (parasympathetic or sympathetic) are called *paragangliomas,* and those of adrenal medulla (intra-adrenal sympathetic paraganglia) are called *pheochromocytomas*. Both pheochromocytomas and paragangliomas are cytomorphologically identical tumors. These tumors can contain composite tumor elements including neuroblastoma, ganglioneuroblastoma, ganglioneuroma, and malignant peripheral nerve sheath tumor.

The aspiration of adrenal is really a misuse of the technique of cytology with the single exception of confirmation of metastasis in a patient with known malignancy or an infectious etiology. The application for adrenal cortical lesions is virtually useless; moreover, one should not perform any biopsy of a potentially functional pheochromocytoma or paraganglioma as failure to use alpha-blockade results in catecholamine crisis with cardiovascular and cerebrovascular events. When indicated adrenal gland lesions are frequently examined via transcutaneous FNAB under CT or US guidance to exclude a metastasis. Pheochromocytoma is sometimes suspected clinically due to paroxysmal hypertension, or in familial syndromes in association with inherited disease (see the chapter on inherited neuroendocrine neoplasms).

Cytological preparations are usually hypercellular, with isolated (or loosely cohesive) pleomorphic cells with large, irregular, and pleomorphic nuclei (sometimes showing pseudoinclusions) and abundant, elongated cytoplasm (Fig. 17.7). Nuclear polymorphism can be marked, nuclear membrane irregularity is frequent, and chromatin is finely stippled. The pleomorphic aspect of the cells contrasts with the clean background, devoid of necrosis, and without mitotic activity. Due to the presence of pleomorphism also in adrenocortical neoplasms, the differential diagnosis can be difficult, if the clinical presentation does not support any particular type of tumor. Immunocytochemical stains are again mandatory for the correct diagnosis. Positivity for GATA3, tyrosine hydroxylase, and neuroendocrine markers (e.g., chromogranin)

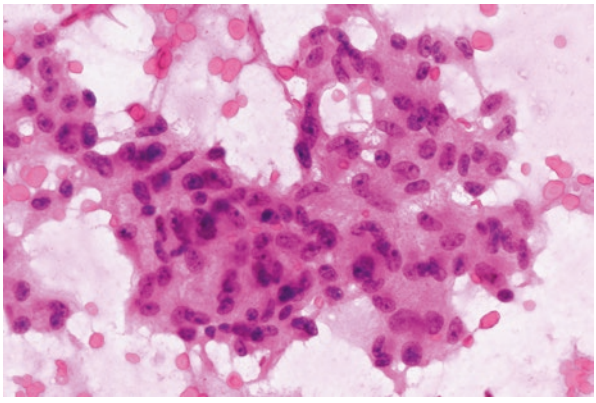

Fig. 17.7 Example of a pheochromocytoma. Loosely cohesive group of pleomorphic cells with large and abundant cytoplasm, irregular and pleomorphic nuclei with finely stippled chromatin. Some cells show a more spindle appearance (touch-imprint cytology, H&E)

distinguish pheochromocytomas from adrenocortical neoplasms that are positive for SF-1, calretinin, Melan-A, alpha-inhibin and synaptophysin.

Among parasympathetic *paragangliomas*, the most common location is the carotid body located at the bifurcation of the common carotid artery. Genetic predisposition predominates paragangliomas and those of the head and neck region more frequently arise in a familial setting. In sporadic forms, their diagnosis is more challenging. Morphologically cells are monotonous, medium sized, with ill-defined cytoplasm. Nuclei are round, uniform, with evenly dispersed chromatin; nuclear anysokariosis can be seen, but usually cells show no atypia, necrosis, or mitotic figures. The cytological aspect of paraganglioma is not specific, but if a cell block is available, one can appreciate the characteristic "Zellballen" pattern with small groups of monotonous cells with round nuclei and a typical salt and pepper chromatin surrounded by sustentacular cells. Immunocytochemistry is mandatory and straightforward for diagnosis. Neuroendocrine cells are positive for GATA3, tyrosine hydroxylase, chromogranin A, and sustentacular cells stain for S100 and S0X10.

Neuroblastoma, ganglioneuroblastoma, and ganglioneuroma are more commonly seen in children and considered as a spectrum of differentiation from the least-differentiated (neuroblastoma) to the most-differentiated (ganglioneuroma). Cytological features for neuroblastoma comprise hyperchromatic small cells with scant cytoplasm with rosette-like formations (Homer Wright rosettes) and fibrillary stroma in a variable amount (more pronounced in less-differentiated forms). Necrosis and mitotic features are easily visible. Cytological features of ganglioneuroma comprise larger cells with abundant cytoplasm (ganglion cells) with large nuclei and prominent nucleoli. The absence of necrosis and mitoses denote the benignity of the lesions. Cytological features of ganglioneuroblastoma are mixed. All the three forms express neuroendocrine markers (chromogranin A and synaptophysin), NB84, and show positivity for S100 (in sustentacular cells). On the contrary, malignant cells do not express CD99, which is positive in all other forms of small round blue cell tumors that enter into the differential diagnosis.

Breast

Neuroendocrine breast carcinomas are rare and can present in pure forms or in combination with a non-neuroendocrine carcinoma (in up to 30% of cases). Neuroendocrine breast carcinomas are subdivided into carcinomas with neuroendocrine features and divided into well-differentiated forms, poorly differentiated forms, and small cell carcinomas. "Carcinoid" tumor, as described in the lung, is virtually absent. Unfortunately, these primary breast NE tumors do not present differently from other neoplastic forms, so the cytopathologist should have in mind this rare possibility. The cytological aspects of primary neuroendocrine breast carcinomas are not site-specific. Well-differentiated tumors consist of tumor cells that are cuboidal to polygonal, with typical granular and abundant eosinophilic cytoplasm, finely stippled chromatin, distinct nucleoli; discrete cell borders and typical absence of mitoses and necrosis (Fig. 17.8). Rosettes or ribbon-like structures can

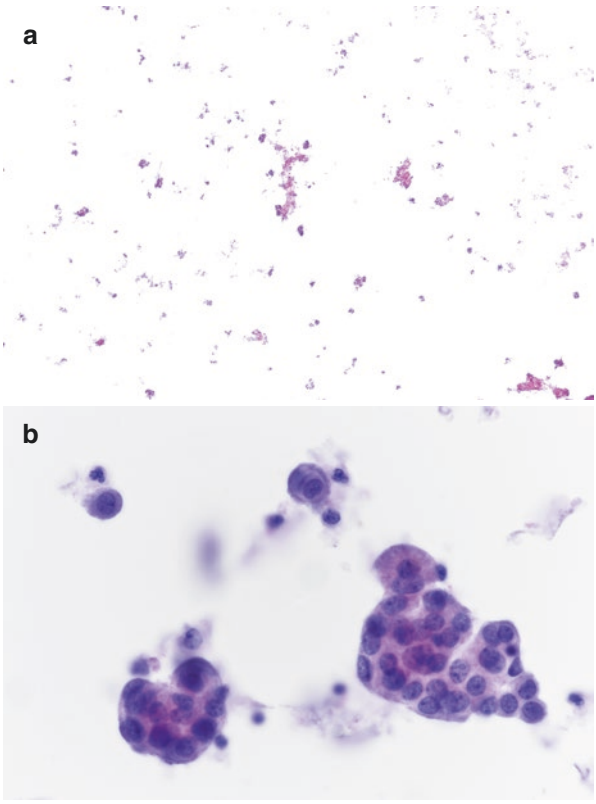

Fig. 17.8 Example of a neuroendocrine breast carcinoma. (**a**) This low power magnification shows a highly cellular smear composed both of cohesive clusters of cells and isolated cells (smear, PAP staining). (**b**) In another field, a small group of monomorphic cells were visible showing a monotonous nuclear appearance with granular chromatin and visible nucleoli. Cytoplasm is finely granular. Positivity for neuroendocrine markers confirmed that the tumor was neuroendocrine in nature (smear, Papanicolaou staining)

be seen. Confirmation of the neuroendocrine nature of such a neoplasm requires the use of chromogranin A and synaptophysin.

Poorly differentiated forms are more difficult to be recognized and should be distinguished from invasive non-neuroendocrine breast carcinomas and metastatic disease. On cytology specimens, neoplastic cells show high-grade features with readily detectable mitoses and necrosis. However, in well-preserved cellular areas, salt and pepper chromatin is suggestive of a neuroendocrine differentiation. Again, immunocytochemistry is essential for the confirmation of the diagnosis. The small cell forms are similar to their counterparts of various organs, with tumor cells that have scant cytoplasm with hyperchromatic nuclei and crush artifacts [11, 12].

Genitourinary

Neuroendocrine tumors have been described virtually in all organs of the genitourinary (GU) tract, in both male and female. These are extremely rare (accounting up to 1–2%) and occasionally discovered either by aspiration (i.e., prostate, kidney), exfoliated cytology (urine) or during cervical cancer screening. The cytological

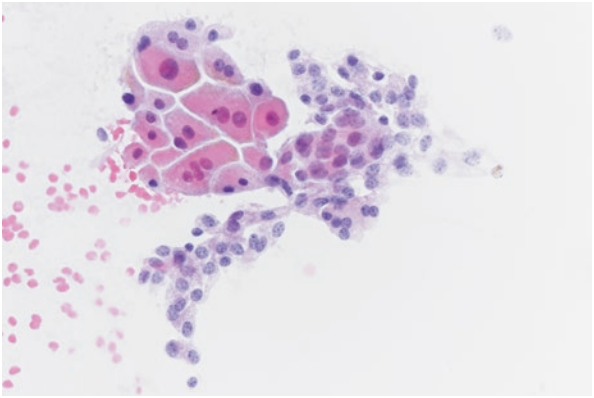

Fig. 17.9 A ultrasound-guided FNA of a liver nodular lesion shows normal hepatocytes on the left and a group of discohesive monotonous cells on the right showing granular chromatin and eosinophilic cytoplasm. The patient had a well-differentiated neuroendocrine tumor of the ileum and the cytological image was consistent with a metastasis of the neuroendocrine tumor (smear, Papanicolaou staining)

aspects are described by a multitude of case reports. In addition, metastatic neuroendocrine carcinomas from other organs can involve the GU tract as well: accurate anamnesis and patient history are helpful in the correct diagnosis. Primary neuroendocrine neoplasms of the GU tract are classified as well-differentiated, poorly differentiated (large and small cell variants), and combined forms and are cytologically indistinguishable from the same classic forms (i.e., lung) [13].

Tubular Gut

FNAB of neuroendocrine proliferations of the tubular gut is quite rare. This is due to accessibility in deep locations of the gut. EUS procedure can be used to investigate esophageal, gastric, or duodenal lesions growing in the wall, and NE proliferations can be an occasional discovery in its pure forms or associated with a non-neuroendocrine component (e.g., MiNEN). The presence of a carcinoid tumor syndrome with chronic diarrhea and flushing (among others) can point the diagnostician to the possibility of a serotonin-producing EC-cell neuroendocrine tumor (especially the ileum). More frequently, it is not unusual to get FNAB specimens of some metastasis that can be the first manifestation of the disease. Especially involved and investigated in this context is the liver (Fig. 17.9). Cytologically, it can be very difficult to determine the primary origin of neuroendocrine neoplasms, as morphology overlaps between the different organs and the material may be insufficient for detailed biomarker studies as discussed in the chapter on metastatic neuroendocrine neoplasms of unknown origin.

Retinoblastoma

Retinoblastoma is a rare neuroendocrine tumor that is diagnosed in young children (<5 years of life) and develops from the retina. Material for cytological analysis can come from the anterior chamber in order to monitor tumor response. Cellularity is usually scant, and the background can be clear or with necrotic debris. Tumor cells are small- to medium-sized with hyperchromatic nuclei, granular chromatin, and scant cytoplasm. Positivity for synaptophysin and NSE confirms the diagnosis and differentiates malignant cells from lymphomas or small round cell tumors [14] (Fig. 17.10).

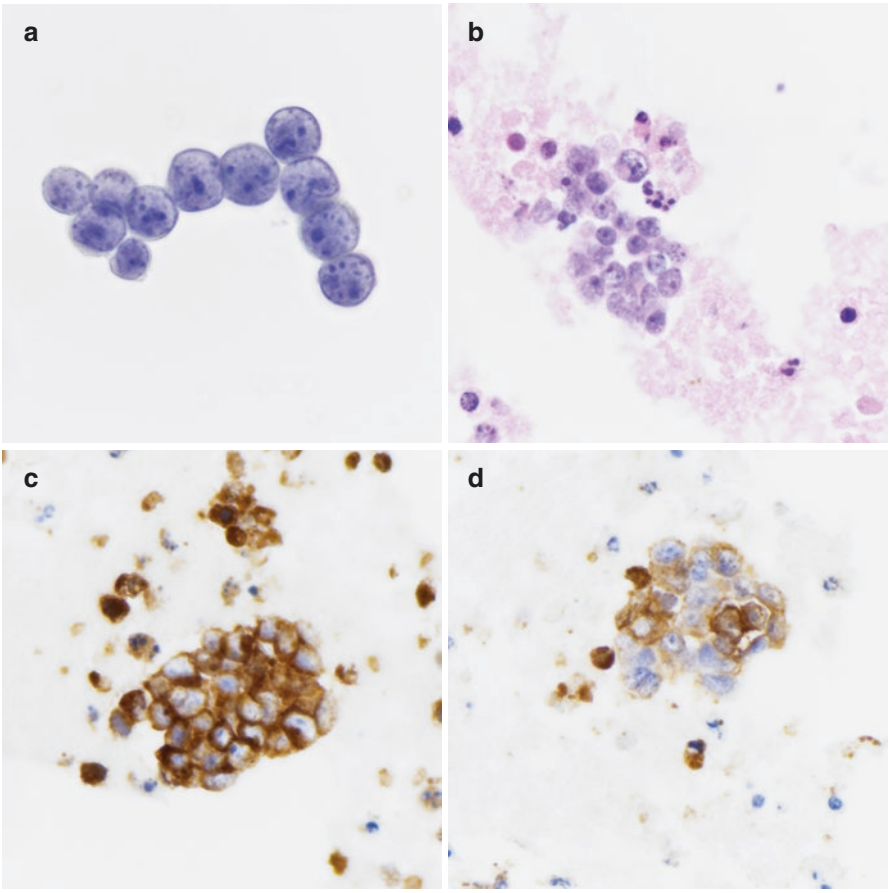

Fig. 17.10 Example of a retinoblastoma. (**a**) In this liquid-based cytology specimen taken from the eye anterior chamber shows small- to medium-sized malignant cells in cords with virtual absence of cytoplasm. The nuclei have granular chromatin with well-visible nucleoli (liquid-based cytology, Papanicolaou staining). (**b**) The same cells in clusters on cell blocks preparation. Note some apoptotic cells and inflammatory cells in the background (cell block, H&E staining). (**c**) Synaptophysin staining was intense and positive in all malignant cells showing high specificity (cell block, synaptophysin staining), while (**d**) NSE was less intensely expressed and not in all cells (cell block, neuron specific enolase staining)

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Inherited Neuroendocrine Neoplasms

18

Ozgur Mete, Fady Hannah-Shmouni, Raymond Kim, and Constantine A. Stratakis

Introduction

The identification of *GNAS1* and *RET* as endocrine-related tumor predisposing genes in the 1980s paved the way for the field of endocrine tumor syndromes and their respective research. The inherited neuroendocrine neoplasms (NENs) represent a heterogeneous group of disorders that often present with subtle clinical or biochemical features and are often missed or misdiagnosed across the patient's lifespan. The well-recognized entities are summarized in Table 18.1; they represent a spectrum of associated neoplasms harboring various degrees of biologic aggressiveness, which often show variable degrees of genotype-phenotype correlations and penetrance [1].

Understanding the framework for common classification schemes and molecular biology of these syndromes has impacted modern strategies on diagnosis, surveillance, and prevention of patients. Recently, the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) provided a modern

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Table 18.1 Familial syndromes manifesting with neuroendocrine neoplasms

Syndrome	Gene	Chr. locus	Inheritance	Endocrine features
Carney complex	CNC1 locus: *PRKAR1A* CNC2 locus: unknown gene	17q22-24 2p16	AD	PPNAD Thyroid carcinoma pitNET
Carney-Stratakis syndrome (Carney dyad)	*SDHA* *SDHB* *SDHC* *SDHD*	5p15.33 1p36 1q21 11q23	AD *SDHD* (paternal imprinting)	PPGL Adrenocortical tumors PBMAH
Carney triad	*SDHC* promoter methylation rare germline variants in *SDHA, SDHB and SDHC*	1q21	Epigenetics AD	PPGL Adrenocortical tumors PBMAH
DICER1	*DICER1*	14q32.13	AD	MNG, DTC
Familial isolated pituitary adenoma (FIPA)	*AIP*	11q13.3	AD	pitNET
Familial PPGL	*SDHA* *SDHB* *SDHC* *SDHD* *SDHAF2*	5p15.33 1p36 1q21 11q23 11q12.2	AD *SDHD* (paternal imprinting)	PPGL RCC pitNET Adrenocortical tumors GIST Pancreatic NET Thyroid cancer
Hyperparathyroidism-jaw tumor syndrome/ familial isolated hyperparathyroidism	*CDC73 (HRPT2)*	1q31.2	AD	PHPT Parathyroid adenoma Parathyroid carcinoma
McCune Albright syndrome	*GNAS1*	20q13.32	N/A	Gonadotropin-independent precocious puberty Leydig and/or Sertoli cell hyperplasia (testis) Neonatal hypercortisolism Adrenal cortical nodular hyperplasia including primary bimorphic adrenocortical disease MNG; papillary adenomas of the thyroid pitNET and pituitary hyperplasia

Table 18.1 (continued)

Syndrome	Gene	Chr. locus	Inheritance	Endocrine features
Multiple endocrine neoplasia type 1	*MEN1*	11q13.1	AD	PHPT with multiglandular parathyroid disease (multiple multiglandular parathyroid adenomas) pitNET GEP-NETs (typically pancreatic, duodenal and gastric) Adrenocortical tumors
Multiple endocrine neoplasia type 2/familial medullary thyroid carcinoma	*RET*	10q11.21	AD	MTC, C-cell hyperplasia PHPT, parathyroid adenoma (frequently uniglandular) PPGL pitNET (rare)
Multiple endocrine neoplasia type 3 (formerly known as MEN2B)	*RET*	10q11.21	AD	MTC, C-cell hyperplasia pitNET (rare)
Multiple endocrine neoplasia type 4	*CDKN1B*	12p13.1	AD	MEN1-like manifestations
Neurofibromatosis type 1	*NF1*	17q11.2	AD	PPGL MNG, thyroid cancer NENs involving the gut and pancreas pitNETs Adrenal cortical tumors
Tuberous sclerosis	*TSC1* *TSC2*	9q34.13 16p13.3	AD	pitNET, pancreatic and intestinal NETs
Von Hippel Lindau disease	*VHL*	3p25.3	AD	Pancreatic NET PPGL Duodenal NET (rare) pitNET (rare)
X-linked acrogigantism (X-LAG)	*GPR101*	Xq26.3	X-linked	piNET
3PA	*SDHA* *SDHB* *SDHD* *SDHAF2* *VHL* *MEN1* *RET* *MAX*	5p15.33 1p36.13 11q23.1 11q12.2 3p25.3 11q13.1 10q11.21 14q23.3	AD *SDHD* (paternal imprinting)	pitNET and PPGL association

Abbreviations: *GIST* gastrointestinal stromal tumor, *PHPT* primary hyperparathyroidism, *DTC* differentiated thyroid carcinoma, *PBMAH* primary bilateral macronodular adrenocortical hyperplasia, *PPNAD* primary pigmented nodular adrenocortical disease, *GEP NETs* gastro-entero-pancreatic neuroendocrine tumors, *RCC* renal cell carcinoma, *MNG* multinodular goiter, *MTC* medullary thyroid carcinoma, *PPGL* pheochromocytoma and paraganglioma, *pitNET* pituitary neuroendocrine tumor, *AD* autosomal dominant

terminology and classification system to reunite the spectrum of epithelial and non-epithelial neoplasms of the neuroendocrine system into NEN [2]. NENs are divided into two distinct groups as epithelial NENs (including well-differentiated neuroendocrine tumors and poorly differentiated neuroendocrine carcinomas) and non-epithelial NENs (neoplasms originating from dispersed neuroendocrine cells of the sympathetic or parasympathetic paraganglia including pheochromocytoma and extra-adrenal paragangliomas). Even this simple distinction provides an invaluable information regarding the risk for germline susceptibility. For instance, with the rate of over 40%, non-epithelial NENs (paraganglioma and pheochromocytoma, hereafter PPGL) have the highest risk of germline susceptibility among all human neoplasms [1]. Unlike non-epithelial NENs, the rate of germline susceptibility ranges from 5% to 25% in epithelial NENs [1, 3, 4]. When compared with poorly differentiated neuroendocrine carcinomas (NECs), well-differentiated neuroendocrine tumors (NETs) are traditionally thought to be more frequently associated with germline susceptibility [1]. Recent evidence also suggests high-grade neuroendocrine neoplasms including NECs and grade 3 NET can also be seen in association with germline susceptibility, especially in the context of pathogenic variants in DNA repair genes [5].

The rate of germline susceptibility in endocrine neoplasms is generally underestimated as most health care providers do not routinely consider the possibility of germline disease in a seemingly sporadic presentation in adults over the age of 50–60 years. Variations in the disease penetrance and de novo pathogenic variants can lead to late-onset manifestations of inherited NENs that can simulate sporadic disease in the absence of family history. Some of these also manifest with a non-syndromic presentation as seen in patients with familial isolated hyperparathyroidism (FIHP), familial isolated pituitary adenoma (FIPA), as well as in a subset of multiple endocrine neoplasia type 2 (MEN2) patients manifesting only with medullary thyroid carcinoma (formerly known as familial isolated medullary thyroid carcinoma syndrome).

The suspicion of an underlying germline susceptibility for NENs should be based on tumor multifocality and morphology including non-tumorous parenchyma, identification of hyperplasia-to-neoplasia progression sequence, early-onset, and co-existence of synchronous or asynchronous endocrinopathies, including endocrine neoplasias [1]. From this perspective, endocrine pathologists play an essential role in the distinction of inherited disease as a significant proportion of these can be predicted by assessing the morphology of the tumor and the non-tumorous parenchyma of the endocrine organs, as well as performing specific immunohistochemical biomarkers that enable molecular immunohistopathology prediction of these disorders [1, 6–12]. In fact, the latter represents an evolved clinical responsibility of diagnosticians in the multidisciplinary management team of patients with NENs. In this chapter, we present a brief overview of germline susceptibility and molecular predictive immunohistopathologic determinant of epithelial NENs originating from various endocrine organs, including pituitary, thyroid, parathyroid, lung, thymus, gut, and pancreas, as well as non-epithelial NENs (PPGL). Additionally, we discuss the role of genetic testing, screening, and counseling of affected and at-risk individuals.

Pituitary Neuroendocrine Neoplasms

The adenohypophysis consists predominantly of hormone-secreting epithelial neuroendocrine cells, aberrations of which can lead to neoplastic (pituitary NET [pitNET] and pituitary carcinoma) or hyperplastic (pituitary hyperplasia) disease, as discussed in the chapter on pituitary NENs. Pituitary NENs are not restricted to epithelial pituitary NENs (pitNET and pituitary carcinoma) but also include paragangliomas of the sellar region. This section of the chapter focuses on inherited epithelial pituitary NENs.

The germline susceptibility for pitNETs is thought to account for approximately 5% of affected individuals. However, this may well be an underestimation given the lack of routine germline screening programs in seemingly sporadic pituitary NENs of adults. A recent Australian series showed that patients with early-onset pitNETs (≤40 years of age) and other personal/family history of endocrine neoplasia were more frequently associated with germline variants in familial pituitary tumor genes [13].

Several genes are responsible for the development of pitNETs (Table 18.1). Epithelial pituitary NEN syndromes have been traditionally linked to multiple endocrine neoplasia (MEN) type 1 (MEN1), MEN2, and MEN4, familial isolated pituitary adenoma (FIPA), Carney complex (CNC), Neurofibromatosis type 1 (NF1), Tuberous sclerosis (TS), and McCune-Albright (MAS) syndrome [13–20]; however, recent progress and observations in the field have expanded the spectrum of inherited pituitary NENs with the inclusion of X-linked acrogigantism (X-LAG) syndrome, as well as extra-colonic manifestations of Lynch syndrome, *SDHx* (succinate dehydrogenase)-, *MAX*-, *CABLES1*-, *USP8*- (ubiquitin-specific peptidase 8), and *IGSF1*-linked disease [20–29]. A distinct diagnostic entity, known as pituitary blastoma, has also been linked to DICER1 syndrome due to germline variants in *DICER1* [30].

MEN1 (*MEN1,* 11q13.1), CNC (CNC1 locus: *PRKAR1A,* 17q24.2; CNC2 locus: gene unknown, 2p16), and MAS (*GNAS1*, 20q13.32)-related pitNETs frequently show other associated defining characteristic features [1, 14–16, 19, 31, 32]. From a histological perspective, growth hormone (GH)- and/or prolactin (PRL)-secreting PIT1 lineage pitNETs predominate pituitary involvement of MAS, MEN1, and MEN4 syndromes, as well as CNC [31–35]. However, other pituitary cell lineages-related pitNETs can variably occur in affected patients. High-risk or aggressive pituitary tumors such as poorly differentiated PIT1 lineage pitNETs (formerly known as silent subtype 3 pituitary adenomas) also expand the pituitary involvement of MEN1 [36] (Fig. 18.1). Trouillas et al. showed that multifocal pitNETs were more frequent in patients with MEN1 [35].

Several inherited syndromes predispose to mammosomatotroph hyperplasia as a prelude to the development of tumors [37–39]. Somatotroph hyperplasia (with or without synchronous pitNET) can rarely be a feature of *MEN1*-related manifestations [35]. Unlike most MEN1 patients, pituitary disease in CNC and MAS shows evidence of hyperplasia-to-neoplasia progression sequence in somatotroph and mammosomatotroph cells, and microscopic tumor multifocality [19, 32,

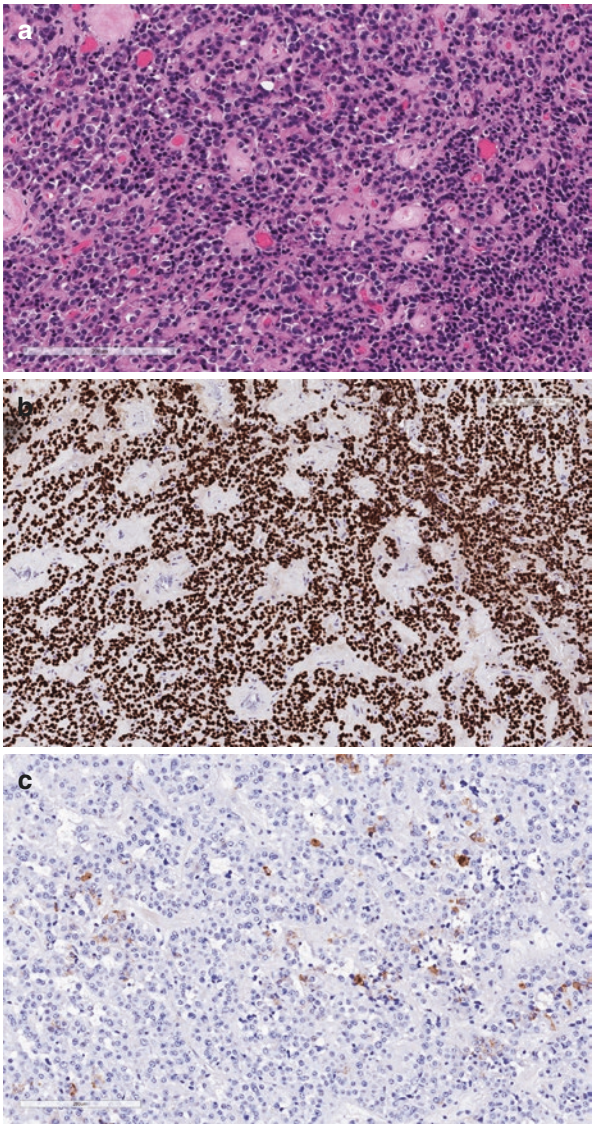

Fig. 18.1 Poorly differentiated PIT1 lineage tumors expand the spectrum of *MEN1*-related Pituitary Neuroendocrine Tumors (pitNETs). These tumors often occur in younger patients and can manifest with prolactin, growth hormone, or thyroid-stimulating hormone excess. These tumors are poorly differentiated with variable stromal fibrosis (**a**) and are diffusely positive for PIT1 (**b**). One or more PIT1 lineage-related hormones are typically present in most cases; however, unlike other terminally differentiated PIT1 lineage pitNETs, hormone expression is often focal and patchy (**c**; growth hormone is illustrated)

38–40]. Patients with NF1 may present with a voluminous pituitary gland, consistent with mammosomatotroph hyperplasia [41, 42]. In the setting of appropriate clinicopathologic features, global loss of menin expression in the pituitary tumor can be used to support *MEN1*-related pathogenesis [1]. Patients presenting with MEN1-like features but lacking pathogenic variants in *MEN1* may harbor a pathogenic variant in *CDKN1B* (12p13.1), which causes MEN4 (MENX in rats affected by the same *CDKN1B*-associated syndrome) [14]. MEN4 predisposes to nonfunctional and functional pitNET, including somatotroph and corticotroph tumors

[20]. Pathologists can facilitate the screening of *CDKN1B*-related manifestations by using p27 immunohistochemistry as a global loss of p27 expression in MEN4 [1]. While corticotroph tumors are uncommon among inherited NEN syndromes (Table 18.1), one should also recognize that sporadic functional corticotroph tumors show frequent inactivation of *CDKN1B* that can result in reduced or even global loss of p27 immunoexpression [43].

FIPA syndrome is considered when two or more kindreds of the family present with pitNETs [44]. Approximately 20–30% of patients with FIPA syndrome harbor pathogenic variants in *AIP* (aryl hydrocarbon receptor interacting protein; 11q13.2). Affected individuals manifest with pituitary tumors across all ages, with incomplete penetrance [45, 46]. Like MEN1 patients, females with FIPA syndrome tend to have functional PRL-producing pitNETs [44, 47]; however, a significantly higher proportion of FIPA patients present with somatotroph tumors when compared with sporadic pitNETs, most of which are sparsely granulated somatotroph tumors (Fig. 18.2) that are frequently hyperintense on T2 MRI-sequence [48–50] and often lack response to somatostatin analogues [51]. While most sporadic sparsely granulated somatotroph tumors have low AIP protein expression [52], young adults

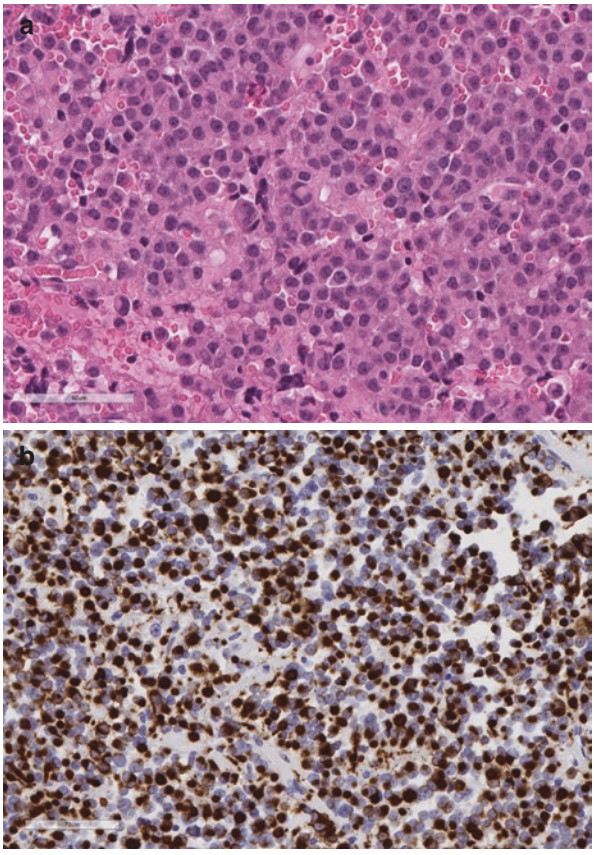

Fig. 18.2 Sparsely granulated somatotroph tumors in young adults may be a harbinger of familial isolated pituitary adenoma (FIPA) syndrome. A significantly higher proportion of FIPA patients present with somatotroph tumors when compared with sporadic pitNETs, most of which are sparsely granulated somatotroph tumors. These tumors are lightly eosinophilic and contain near-diffuse juxta-nuclear fibrous bodies (**a**). Fibrous bodies are best highlighted using low-molecular-weight cytokeratin immunohistochemistry (e.g., CAM5.2) (**b**)

(≤30 years) and pediatric age manifestations of sparsely granulated somatotroph tumors with reduced immunohistochemical AIP expression can be prioritized for germline *AIP* testing [53, 54]. Villa et al. also reported the occurrence of somatotroph hyperplasia-tumor sequence in the background of *AIP* defects [53]. Functional corticotroph tumors and other non-functional pitNETs can also be encountered in FIPA kindreds [44]. Since pitNETs associated with FIPA are often invasive and large, those at high risk may benefit from genetic screening. Evidence suggests that pediatric pitNETs, sporadic large pitNETs diagnosed in patients <30 years, individuals with gigantism, and young adults with sparsely granulated somatotroph tumors can benefit from *AIP* germline screening [44, 54, 55].

Gigantism and/or acromegaly due to excess GH secretion can be inherited in most pediatric or young adult cases. X-linked acrogigantism (X-LAG) represents the most common cause of gigantism in pediatrics, manifesting as early childhood-onset gigantism due to de novo (and rarely familial) germline microduplications on chromosome Xq26.3 (involving *GPR101*) [21, 56]. Similar to MEN1, PRL excess is also a common feature of X-LAG [57]. Affected pituitary glands show pituitary hyperplasia and/or pitNET [21] (Fig. 18.3). Histological correlates suggest the occurrence of mixed GH- and PRL-producing pitNETs; however, detailed reviews of endocrine pathologists in larger series using modern biomarkers are required to further expand subtype correlations of X-LAG-related pituitary disease.

The recently defined 3P association (3PA) represents the co-existence of pitNET and PPGL [26, 58]. 3PA is a heterogenous and rare clinical syndrome caused by pathogenic variants in any of the following genes: *SDHA* (5p15.33), *SDHB* (1p36.13), *SDHD* (11q23.1), *SDHAF2* (11q12.2), *VHL* (3p25.3), *MEN1* (11q13.1), *RET* (10q11.21), and *MAX* (14q23.3) [26, 58]. *SDHx*-related pitNETs are rare and typically composed of tumors cells with intracytoplasmic vacuoles and have been reported more frequently in association with PRL excess [23]. In addition to their cytomorphologic correlates, *SDHx*-related pituitary NENs (Fig. 18.4) can also be

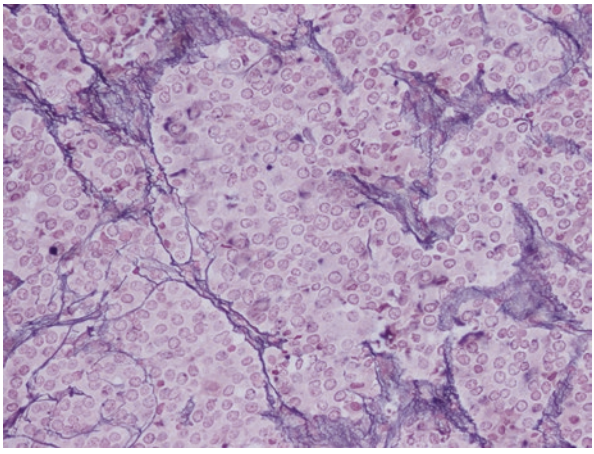

Fig. 18.3 X-linked acrogigantism represents the most common cause of gigantism in pediatrics. Pituitary glands show pituitary hyperplasia and/or pituitary neuroendocrine tumors. The reticulin histochemistry shows an expanded acinar unit with focal breakdown suggestive of an early clonal disease

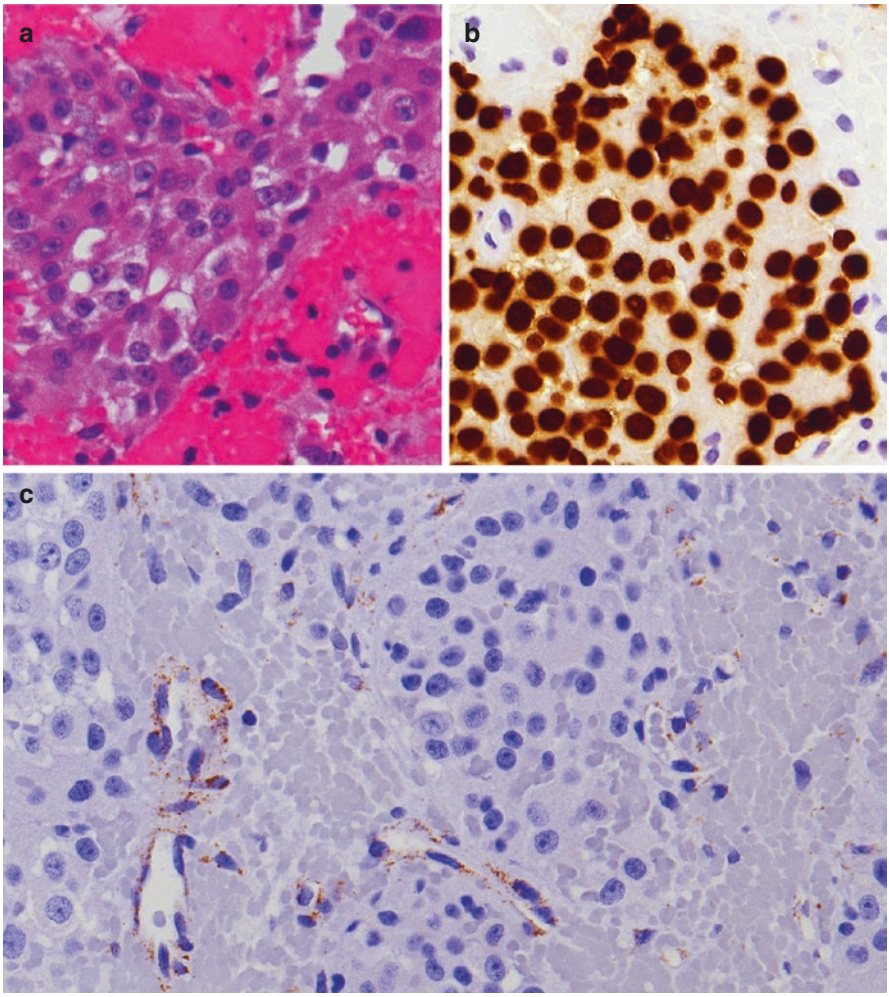

Fig. 18.4 *SDHx*-related pituitary neuroendocrine tumors (pitNETs) are rare. *SDHx*-related pitNETs are typically composed of tumors cells with intracytoplasmic vacuoles (**a**) and have been reported more frequently in association with prolactin excess. The photomicrograph illustrates a pitNET with diffuse PIT1 (**b**) and PRL expression (not shown herein). SDHB immunohistochemistry shows loss of intracytoplasmic granular staining in the tumor cells whereas the endothelial cells (internal control) remain positive for SDHB (**c**)

screened using SDHB immunohistochemistry, where global loss of SDHB expression by immunohistochemistry representing a surrogate biomarker for *SDHx*-related neoplasms [1].

Originally defined as a unique embryonal tumor of the pituitary gland by Scheithauer et al., pituitary blastoma (Fig. 18.5) is a rare triphasic pituitary tumor composed of a combination of three cell types including large secretory

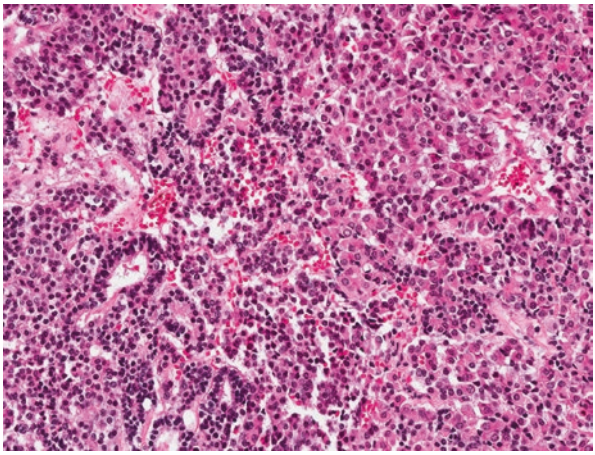

Fig. 18.5 Pituitary blastoma is a hallmark of DICER1 syndrome. This is a rare triphasic pituitary tumor composed of a combination of three cell types including large secretory neuroendocrine cells of adenohypophysis, small immature folliculostellate cells, and gland- or rosette-forming primitive Rathke's cleft epithelial cells. (Case kindly provided by Leanne de Kock, Department of Human Genetics, McGill University, Montréal, Quebec, Canada)

neuroendocrine cells of adenohypophysis, small immature folliculostellate cells, and gland- or rosette-forming primitive Rathke's cleft epithelial cells [59, 60]. These tumors were defined as the hallmark of DICER1 syndrome [30]. DICER1 syndrome is an autosomal dominant condition arising from pathogenic variants in *DICER1* (14q32.13), which encodes the DICER1 protein, a ribonuclease (RNase) III family of proteins [30]. Among endocrine disorders, patients with DICER1 syndrome show features of nodular thyroid disease, which predisposes to well-differentiated thyroid cancers [61, 62], reaching 23% at 20 years and 50–75% by 40 years in females [62].

Several germline pathogenic variants predispose to pediatric corticotroph tumors and Cushing disease, including *MEN1*, *AIP*, *PRKAR1A*, *DICER1*, *CDKN1B* (with or without MEN4), and *CABLES1* [20, 27, 30, 31]. The most frequent (20–60%) genetic defect in sporadic corticotroph tumors and across all ages is pathogenic variants in *USP8* [63–66]. Recently, a new inherited manifestation was described arising from a de novo germline heterozygous variant in *USP8* (c.2155T > C, p.S719P; 15q21.2) and manifesting with a corticotroph tumor leading to severe Cushing disease [28]. Germline pathogenic variants in *CABLES1* were reported in four patients [27]. Among these, 3 patients had Cushing disease whereas 1 patient had a silent corticotroph tumor [27]. Affected patients with *CABLES1* variants were found to have large corticotroph tumors with increased Ki67 labeling indices [27]. The data on the 2017 WHO histological tumor subtype of these tumors was not provided in any of these studies [27, 28].

Isolated reports of pituitary epithelial NENs (pitNETs or pituitary carcinomas) were reported in patients with germline *MAX* [25], Lynch syndrome [22], Von Hippel-Lindau (VHL) disease [67], TS [68], MEN2 [69], and NF1 [70, 71].

Thyroid Neuroendocrine Neoplasms

As discussed in the chapter on thyroid NENs, the spectrum of NENs in the thyroid gland includes medullary thyroid carcinoma (MTC), mixed (composite) MTC, and follicular epithelial-derived thyroid carcinoma, and NENs originating from intrathyroidal thymic remnants (intrathyroidal thymic NENs) as well as thyroid paraganglioma originating from intrathyroidal and perithyroidal dispersed microscopic elements of the laryngeal paraganglia. The distinction of MTC from various forms of thyroid NENs is of clinical significance.

Approximately 25% of MTCs are associated with pathogenic variants in *RET* leading to MEN2 (previously referred to as MEN2A) or MEN3 (previously referred to as MEN2B) [72, 73]. Formerly known as familial isolated MTC syndrome is now recognized as a variant of MEN2 [74, 75]. Studies in MEN2 identified genotype-phenotype correlations and have resulted in risk stratification and disease-specific preventive management strategies in affected families [74, 75]. The latter is beyond the scope of this chapter. However, most MEN2 patients harbor variants in *RET*, codon 634, whereas codon 918 defects are more frequent in patients with MEN3 as well as in the setting of sporadic MTCs due to somatic variants in *RET* [72–75]. Mixed (composite) MTC and follicular epithelial-derived thyroid carcinomas can also be seen in patients with MEN2 syndrome [76].

From a morphological perspective, the identification of multifocal bilateral parafollicular C-cell hyperplasia to neoplasia progression sequence (Fig. 18.6) is a diagnostic feature of germline *RET*-driven pathogenesis [1, 72–74]. For this reason, C-cell mapping on thyroidectomy specimens can facilitate this distinction in sporadic looking C-cell disease from inherited disease. In addition, the use of *NRASQ61R*-specific SP174 immunohistochemistry has been shown to provide an indirect help during the screening process of *RET*-driven pathogenesis [77] since pathogenic variants in *RAS* and *RET* are mutually exclusive in MTCs. The presence of positive reactivity for *NRASQ61R*-specific SP174 antibody would help in excluding the possibility of *RET*-driven tumorigenesis [77].

Although pathogenic variants in *RET* have traditionally been the cause of inherited forms of MTCs, the identification of pathogenic variants in *MET* (p.Arg417Gln; 7q31.2) involving the extracellular Sema domain of the *MET* gene in two siblings with inherited medullary thyroid carcinomas lacking wild-type *RET* has expanded the germline correlates of this disease [78].

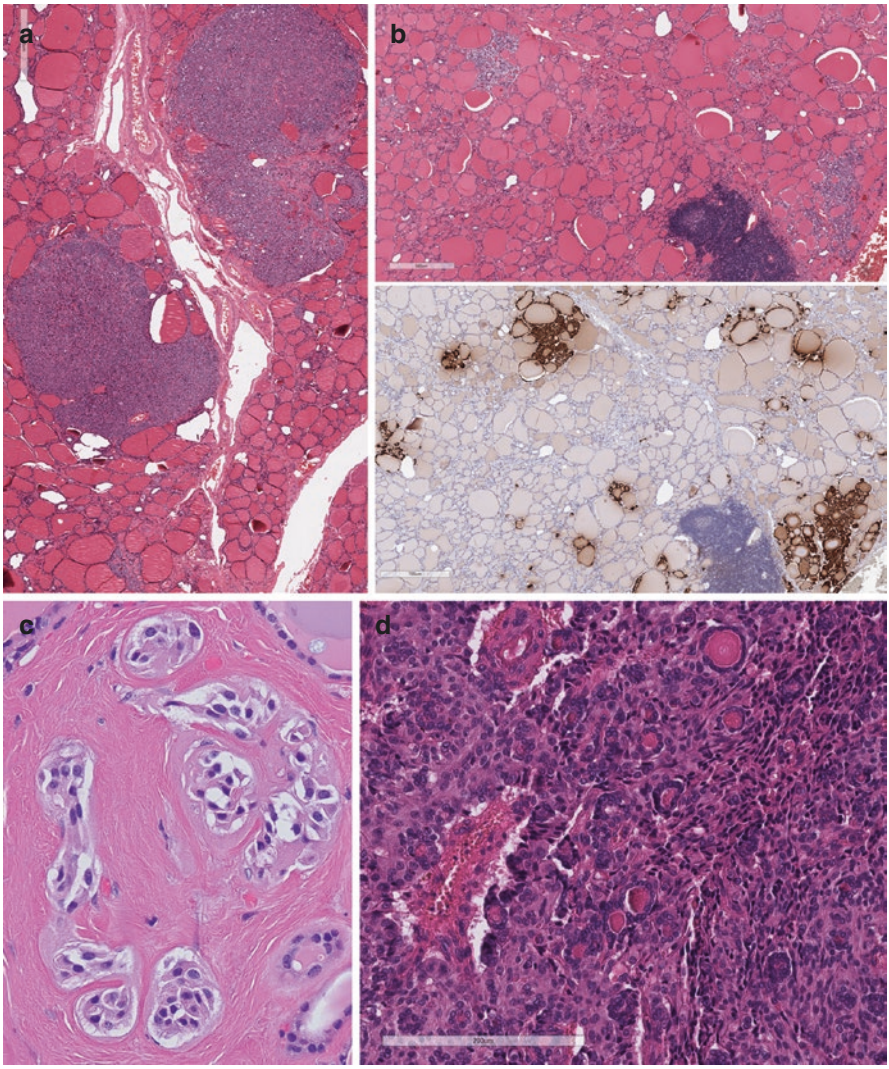

Fig. 18.6 Bilateral multifocal C-cell hyperplasia to neoplasia sequence is a hallmark of *RET*-germline thyroid disease. Patients with germline pathogenic variants in *RET* often manifest with multifocal C-cell disease. Multifocal medullary carcinoma (**a**) arises in the background of C-cell hyperplasia (**b**; upper panel: Hematoxylin and Eosin, lower panel: Calcitonin). It is not uncommon to find medullary microcarcinomas arising in the background of nodular C-cell hyperplasia (**c**). Affected patients can also manifest with mixed (composite) medullary thyroid carcinoma and papillary thyroid carcinoma (**d**)

Parathyroid Neuroendocrine Neoplasms

Epithelial NENs of the parathyroid gland include parathyroid adenoma and carcinoma. Paragangliomas, as a non-epithelial NEN, can also occur in parathyroid glands [79]. Most patients with inherited parathyroid NENs are seen in the setting of primary hyperparathyroidism (PHPT). PHPT refers to the biochemical diagnosis of inappropriately elevated parathyroid hormone in the context of hypercalcemia [80]. Most cases of PHPT are caused by parathyroid adenomas, with <1% of cases from carcinomas, typically presenting with a palpable neck mass and severe hypercalcemia in the 4th–5th decade of life (Table 18.1). These pathologies are traditionally distinguished on histological assessment; invasive growth (e.g., angioinvasion, lymphatic invasion, perineural invasion, local gross malignant invasion into surrounding structures) and/or metastatic disease warrants the diagnosis of parathyroid carcinoma [81]. However, immunohistochemical and molecular biomarkers of parathyroid carcinoma also assist the diagnostic workup of parathyroid neoplasms with worrisome/atypical features [7, 82, 83].

Well-recognized inherited hyperparathyroidism syndromes include hyperparathyroidism–jaw tumor syndrome (HPT-JT), FIHP, MEN1, MEN2, and MEN4 [1, 14, 84–86]. Since over 10% of seemingly sporadic parathyroid neoplasms are caused by germline defects and this can occur even in the absence of a family history, as a general rule, all PHPT patients with any of the following features should be prioritized for genetic screening and counseling [1, 84]: (i) young age of disease onset (<45 years), (ii) family history of PHPT, hypercalcemia, kidney stones, other neuroendocrine tumors, and/or neoplasms that can be seen in familial syndromes, (iii) personal history of a NEN or a neoplasm that can be seen in germline susceptibility syndromes, (iv) persistence or recurrence of PHPT after the surgical removal of an abnormal parathyroid gland, (iv) histologically confirmed multiglandular parathyroid disease, and (v) in the setting of some histological (e.g., multinodular appearance, *CDC73/HRPT2*-related cytomorphology) and immunohistochemical (e.g. loss of expression for menin, p27 or parafibromin) features that may be suggestive of well-defined parathyroid NEN syndromes.

Virtually all MEN1 patients (*MEN1*) develop multiglandular parathyroid disease. While the incidence of MEN4 (*CDKN1B*) is extremely rare, it is generally thought that affected patients tend to manifest with MEN1-like manifestations [1, 14, 86]. One of the characteristics of *MEN1*-related PHPT is the presence of multiglandular parathyroid disease that consists of multiple small parathyroid adenomas in all parathyroid glands [1, 84, 87] (Fig. 18.7). Since the concept of parathyroid hyperplasia is a misnomer in the setting of germline susceptibility-related multiglandular parathyroid disease, most experts have adopted the concept of multiglandular parathyroid disease or multiglandular parathyroid adenomas in this setting [1, 84, 87]. The multinodular appearance of parathyroid glands and loss of nuclear menin (protein

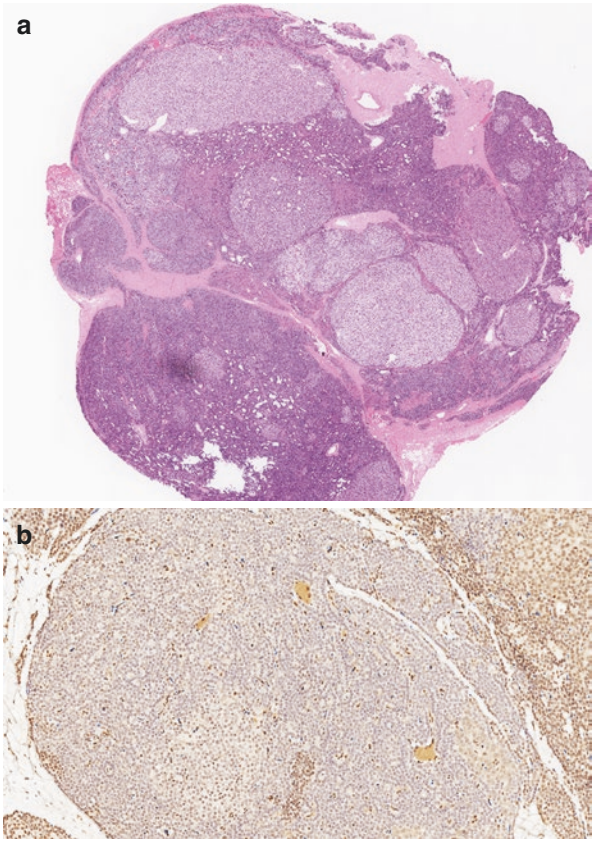

Fig. 18.7 MEN1-related primary hyperparathyroidism is characterized by menin-immunodeficient multiglandular multiple adenomas. The characteristic multinodular appearance of *MEN1*-related parathyroid disease is illustrated in this photomicrograph (**a**). Each nodule within this parathyroid gland represents a clonal (neoplastic) disease (**a**). Menin (protein encoded by *MEN1*) immunohistochemistry shows loss of nuclear reactivity in nodules (**b**; illustrates one of the nodules), whereas remaining background parenchyma and non-tumorous elements (e.g., endothelial cells, inflammatory cells) show no loss of menin expression

encoded by *MEN1*) expression can rationalize germline *MEN1*-testing in the setting of PHPT [1] (Fig. 18.7). The loss of nuclear p27 (protein encoded by *CDKN1B*) in the background of PHPT and multiglandular parathyroid disease would require further germline testing to rule out a *CDKN1B*-driven MEN4 [1] (Fig. 18.8).

MEN2 patients (*RET*) are less frequently associated with multiglandular parathyroid disease and only 20–30% of affected patients manifest with parathyroid disease [84]. Unlike *MEN1*-related PHPT for which multiglandular parathyroid resection along with thymectomy is often considered, the management of MEN2 patients with PHPT is often based on minimally invasive surgery with guidance of intraoperative PTH measurements [88–92]. There are no distinct morphological features suggestive of MEN2-related parathyroid disease.

HPT-JT syndrome is caused by pathogenic variants in *CDC73/HRPT2* (1q31.2), a tumor suppressor gene encoding parafibromin [1, 84, 93, 94]. HPT-JT is also characterized by the development of fibro-osseous jaw tumors (25–50%), renal cysts or tumors (15%) and uterine fibromas (75% of females) [1, 84, 93, 94]. Unlike other germline disorders in association with PHPT, patients with pathogenic variants in *CDC73/HRPT2* are at higher lifetime risk of developing parathyroid carcinoma.

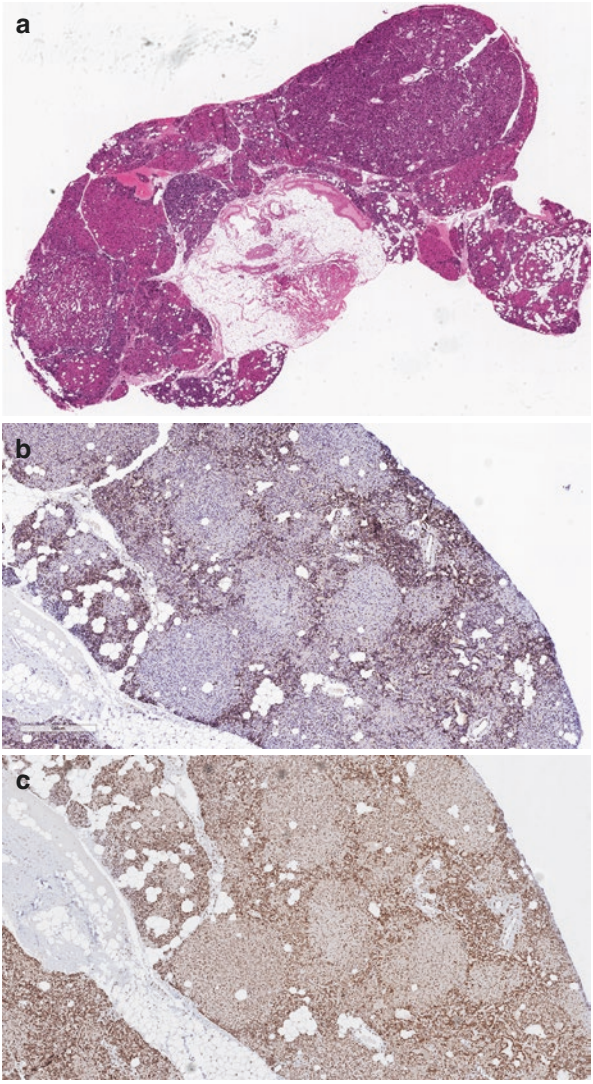

Fig. 18.8 MEN4-related parathyroid disease is characterized by p27-immunodeficient multiglandular multiple adenomas. Patients with MEN4 tend to manifest with multiglandular multiple parathyroid adenomas (**a**). Loss of nuclear p27 (protein encoded by *CDKN1B*) expression is seen in all micro-adenomas (**b**). Menin expression remains positive (**c**)

FIHP can also occur in the setting of *CDC73/HRPT2* as well as various other germline alterations involving *GCM2, MEN1, CASR*, *CDKIs*, and *CDKN1B* [84, 95–100].

Pathologists play an important role in the prediction of *CDC73/HRPT2*-related parathyroid neoplasms by using parafibromin immunohistochemistry [1, 7, 9, 10]. The loss of nuclear parafibromin staining in the tumor cells while nuclear expression remaining intact in non-tumorous cellular component (e.g., endothelial cells) is consistent with *CDC73/HRPT2*-related parathyroid neoplasms [1, 7, 9, 10, 82] (Fig. 18.9). Juhlin et al. also demonstrated that nucleolar loss of parafibromin also correlates with its genetic defects [101]. Subsequent reports also documented nucleolar loss of parafibromin immunostaining in parathyroid carcinomas [102]. Since

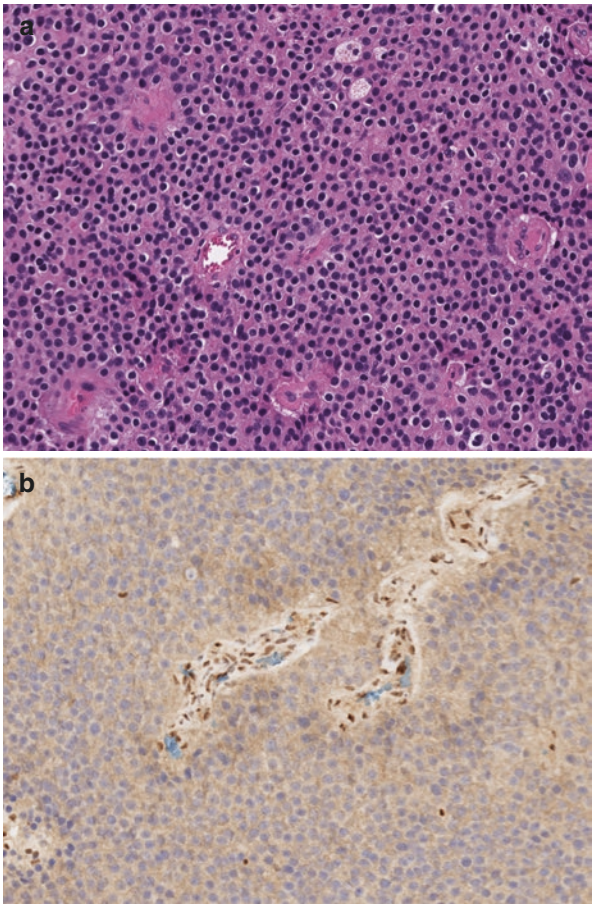

Fig. 18.9 *HRP2/CDC73*-related parathyroid disease displays characteristic cytomorphology and parafibromin immunodeficiency. *CDC73/HRPT2*-mutant parathyroid neoplasms are frequently encapsulated and display diffuse sheet-like growth (**a**). The tumor cells show eosinophilic cytoplasms and nuclear enlargement with coarse chromatin pattern and perinuclear clearing (**a**). The loss of nuclear parafibromin staining in the tumor cells while nuclear expression remaining intact in non-tumorous cellular component (e.g., endothelial cells) is consistent with *CDC73/HRPT2*-related parathyroid neoplasms (**b**)

the vast majority of sporadic parathyroid carcinomas harbor defects in *CDC73/HRTP2*, parafibromin immunodeficiency often supports the diagnosis of malignancy especially in parathyroid neoplasms with atypical features. However, over 10% of patients with apparently sporadic parathyroid carcinoma with parafibromin loss can also harbor defects in *CDC73/HRPT2* [1, 8, 9]. Therefore, it is commonly suggested that all patients with parafibomin-immunodeficient parathyroid carcinoma are tested for *CDC73/HRPT2*.

Since not all patients with parafibromin immunodeficiency manifest with parathyroid carcinomas, this biomarker also provides additional insights into the pathogenesis of *CDC73/HRTP2*-related parathyroid adenomas. From a morphological perspective, there are several cytomorphological features that should prompt the attention of pathologists to the distinction of *CDC73/HRPT2*-related parathyroid disease [103]. Gill et al. reported that *CDC73/HRPT2*-mutant parathyroid neoplasms had frequently thick tumor capsule, diffuse sheet-like growth with arborizing vasculature and micro-cystic changes [103]. In addition, the same series

highlighted that tumor cells had eosinophilic cytoplasms and nuclear enlargement with coarse chromatin pattern and perinuclear clearing [103] (Fig. 18.9). Therefore, the application of parafibromin immunohistochemistry can be justified when similar morphological findings are encountered even in apparently sporadic PHPT.

Lung and Thymic Neuroendocrine Neoplasms

Most bronchopulmonary (hereafter pulmonary) and thymic epithelial NENs manifest with sporadic disease, whereas thoracic paragangliomas (e.g., pulmonary and mediastinal) are frequently associated with defects in *SDHx*. Well-defined germline inheritance in association with pulmonary and thymic epithelial NENs has been traditionally linked to MEN1 [1, 14, 36, 104]. Pulmonary NENs have been reported to occur in about 2–13% of individuals harboring defects in *MEN1* [14, 80, 104–109], whereas thymic NENs have been reported in about 2–8% (with a pool estimate prevalence of 3.7% in a recent meta-analysis) of affected individuals [14, 110, 111]. Well-differentiated NETs predominate thoracic manifestations of MEN1 patients [1, 112]. Among these, thymic NENs have been flagged as an important factor in the MEN1-related disease mortality [110]. While morphological data generated from large series focusing on details of the non-tumorous pulmonary parenchyma is limited, Bartsch et al. reported the occurrence of tumor multifocality and well-differentiated pulmonary neuroendocrine microtumors measuring up to 0.5 cm (tumorlets) in association with underlying pulmonary neuroendocrine cell hyperplasia in MEN1 patients [104] (Fig. 18.10). Although there are no well-documented series of MEN4-related thoracic NENs, patients with MEN4 are also expected to manifest with MEN1-related clinical states [1, 112, 113]. Therefore, the possibility of pathogenic variants in *CDKN1B* should always be considered in patients with negative *MEN1* testing.

As a practical general rule, the young age, tumor multifocality along with neuroendocrine cell hyperplasia, and/or neuroendocrine cell hyperplasia to tumor progression sequence should alter the diagnostician to the possibility of germline disease [1]. The assessment of the non-tumorous pulmonary and thymic parenchyma can provide additional information for diagnosticians [1, 36, 104]. Menin and p27 immunohistochemistry can help facilitate the prioritization of genetic screening for MEN1 and MEN4, respectively [1]. One should also recognize that loss of menin and p27 can also be seen in the setting of somatic/epigenetic inactivation of *MEN1* and *CDKN1B*, which can be featured in sporadic diseases. Therefore, germline testing should always be performed to distinguish a somatic event from a germline alteration.

Recent data on seemingly sporadic thoracic NENs including well differentiated NETs showing high-grade proliferative features (also known as grade 3 NETs) has expanded the role of germline defects involving in the DNA repair genes (e.g., *RAD51C*, 17q22) [5]. A recent series of patients with breast cancer and NETs reported a patient with well-differentiated pulmonary NET with intermediate proliferative features (atypical carcinoid tumor) and breast cancer arising

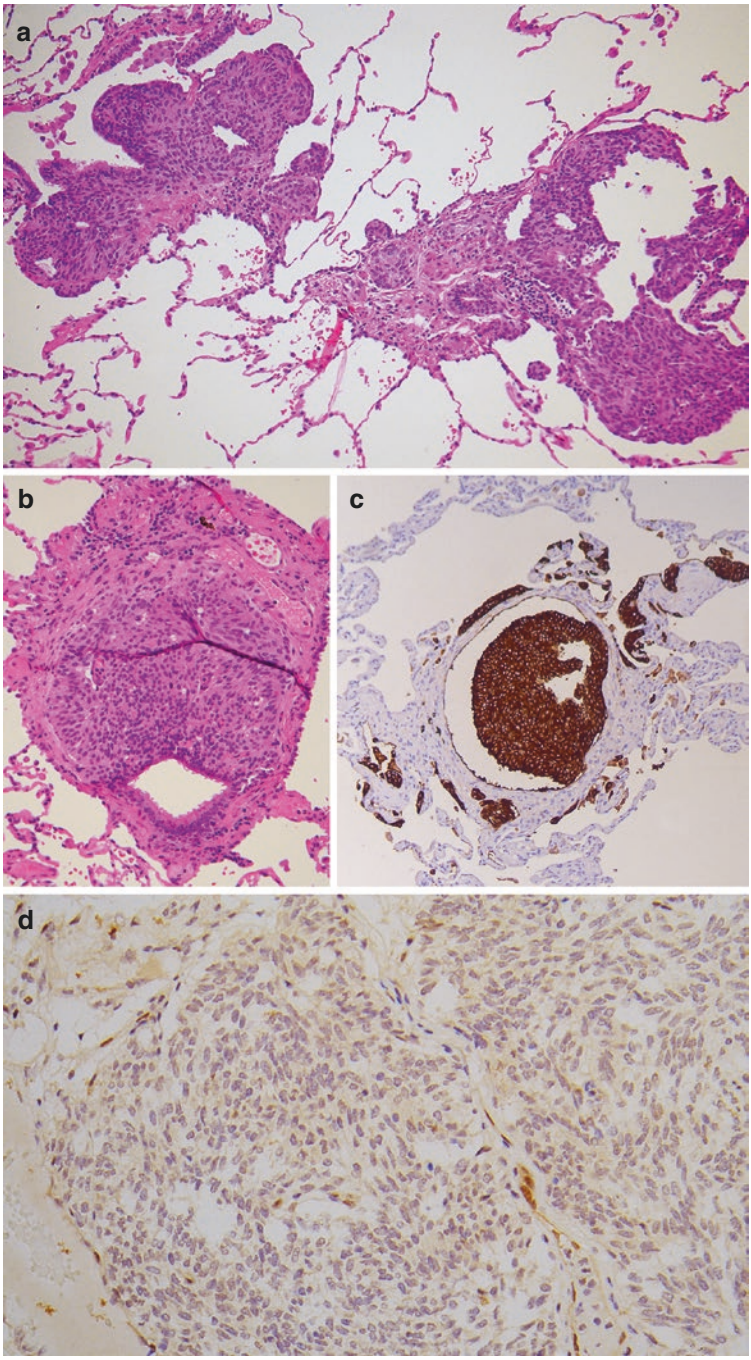

Fig. 18.10 *MEN1*-related pulmonary disease is characterized by multifocal pulmonary neuroendocrine cell proliferation. Affected patients tend to manifest with multifocal pulmonary neuroendocrine tumors (including microtumors that are also known as tumorlets) arising in the background of pulmonary neuroendocrine cell hyperplasia (**a**–**c**; **a**, **b**: Hematoxylin and Eosin; **c**: Chromogranin-A). Loss of nuclear menin expression is seen in *MEN1*-related pulmonary neuroendocrine tumors (**d**)

in the background of a pathogenic variant in *APC* (5q22.2), which was thought to be likely pathogenetic with low penetrance [114]. The same series also highlighted another case of well-differentiated pulmonary NET with intermediate proliferative features (atypical carcinoid tumor) with a variant of unknown significance in *MSH2* (2p21-p16) [114]. These findings not only expand our knowledge, but also underscore the fact that germline susceptibility is often underestimated in the field of thoracic NENs.

Gastric Neuroendocrine Neoplasms

Most gastric NENs are sporadic. Among these, type II enterochromaffin cell-like (ECL) gastric NETs are identified in MEN1 patients with Zollinger-Ellison syndrome due to either duodenal (more frequently) (Fig. 18.11) or pancreatic gastrin-producing NET (G-cell NET; also known as gastrinoma) [1, 36, 115]. This manifestation accounts for approximately 8% of all gastric ECL-cell NETs [115]. Neoplastic hypergastrinemia results in ECL-cell hyperplasia and ECL-cell dysplasia that often progress to ECL-cell NETs in the oxyntic gastric mucosa with features of parietal cell hyperplasia [36, 115] (Fig. 18.11). Most type II ECL-cell gastric NETs are well differentiated. Unlike type I gastric ECL-cell NETs, they lack atrophy of the oxyntic mucosa and are more frequently associated with metastatic disease [115].

Intestinal Neuroendocrine Neoplasms

The germline susceptibility in intestinal NETs is an area of interest since germline disease seems to display site-specific characteristics. Most well-documented intestinal NENs associated with germline disease have been identified in the proximal small bowel, especially in the duodenum. For instance, NF1 patients tend to present with ampullary type D-cell NETs (ampullary type somatostatinoma) (Fig. 18.12), and can co-exist with duodenal gastrinoma [1, 36, 116]. The presence of acinar-glandular structures with psammoma bodies is a characteristic of *NF1*-related ampullary type somatostatinomas [1, 36, 116]. When compared with sporadic forms of duodenal G-cell NETs, duodenal involvement of MEN1 is characterized by multifocal well-differentiated G-cell NETs (Fig. 18.11), which are usually associated with neuroendocrine cell hyperplasia of the non-tumorous mucosa [1, 36, 117]. Linear and micronodular G-cell and/or D-cell hyperplasia in association with a well differentiated D-cell NET can also be identified in association with MEN1 [1, 117].

Although rare, VHL disease patients can also manifest with well-differentiated NETs in the duodenum and ampullary region [118]. It has been an observation of one of the authors of this chapter (O.M) that VHL disease-associated duodenal NETs are typically discovered during the detailed assessment of the duodenal mucosa identified in Whipple resection specimens. Unlike MEN1, underlying neuroendocrine cell hyperplasia has not been a typical feature of duodenal involvement of NF1 and VHL disease [1, 36]. Therefore, the assessment of the non-tumorous

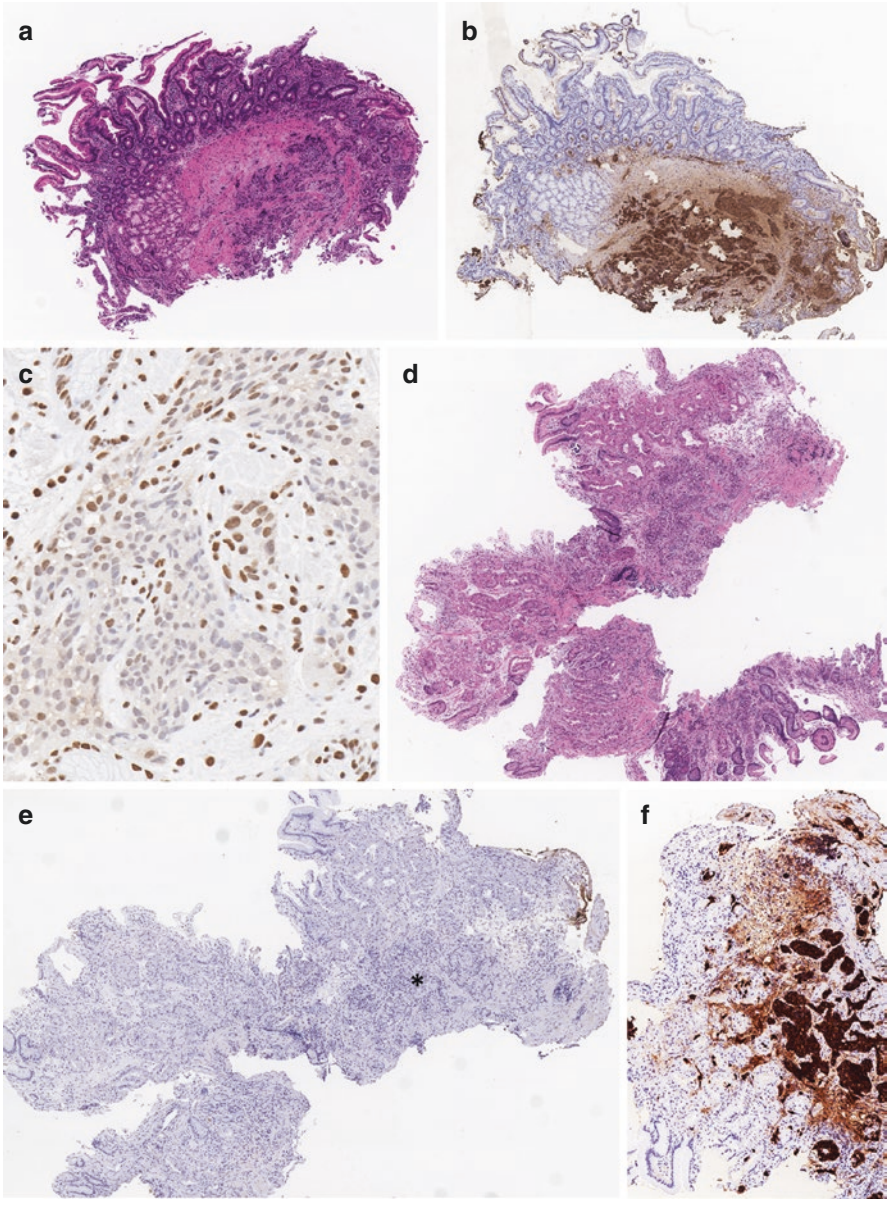

Fig. 18.11 *MEN1*-related duodenal gastrin-producing well-differentiated neuroendocrine tumor (Zollinger-Ellison syndrome) and hypergastrinemia-related ECL-cell gastric neuroendocrine tumor (type 2 gastric neuroendocrine tumor). This composite photomicrograph illustrates *MEN1*-related duodenal gastrinoma (**a**–**c**; **a**: Hematoxylin and Eosin; **b**: gastrin; **c**: menin) and ECL-cell gastric neuroendocrine tumor arising in the background of ECL-cell hyperplasia and hyperplastic changes in the oxyntic gastric mucosa (**d**–**f**; **d**: Hematoxylin and Eosin; **e**: Gastrin; **f**: Chromogranin-A from the region marked with asterisks)

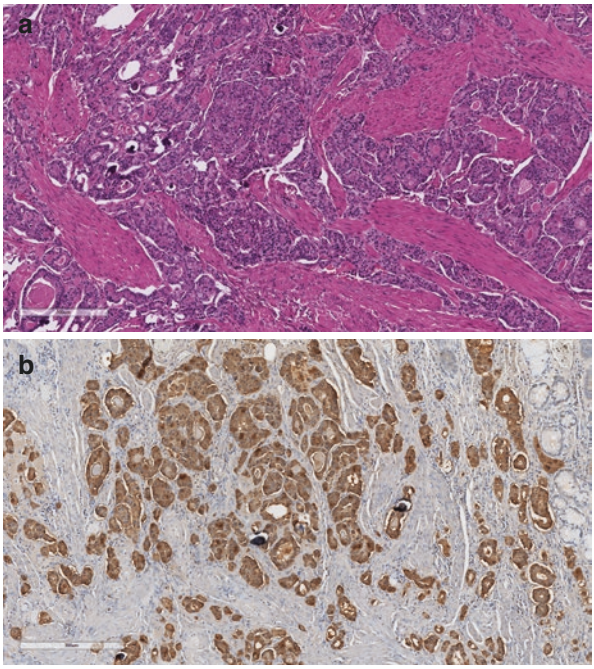

Fig. 18.12 Ampullary D-cell neuroendocrine tumors are characteristic of NF1 syndrome. NF1-related D-cell neuroendocrine tumors display characteristic acinar or glandular architecture with variable psammomatous microcalcification (**a**). Diffuse somatostatin expression confirms D-cell origin (**b**)

mucosa and menin immunohistochemistry can facilitate the distinction of *MEN1*-associated NETs [1]. While the data is being limited with respect to MEN4, one would expect the spectrum of MEN1-associated features in affected patients.

One of the unanswered biological questions is related to distal EC-cell small bowel NETs (e.g., ileal, ileo-jejunal). This particular site tends to frequently host multifocal EC-cell NETs that are unassociated with underlying neuroendocrine cell hyperplasia (Fig. 18.13). Evidence suggests that approximately 1 in 3 patients with small bowel NETs have multifocal synchronous disease [119]. Generally, multifocal neuroendocrine cell proliferation raises the suspicion of a germline susceptibility in NENs [1]. To date, no pathogenic variants in *MEN1* and *VHL* have been identified in association with this particular presentation [120]. The incidence of multifocal small bowel NETs is even higher in patients with a positive family history of small bowel NETs (defined as presence of small bowel NETs in at least two blood relatives) [119, 120], and this has been reported in 57% of the European series [120]. While the molecular alterations leading to familial small bowel EC-cell NETs are poorly understood, there is evidence of an autosomal dominant inheritance pattern in families with sporadic-appearing multifocal EC-cell small bowel NETs [119, 120]. In 2015, a linkage analysis and whole-exome sequencing data identified a novel germline four base pairs deletion in the *IPMK* (Inositol Polyphosphate Multikinase; 10q21.1) gene in a large family with small bowel EC-cell NETs from a prospective series of 33 families with at least 2 small bowel NETs [119]. The *IPMK* defects

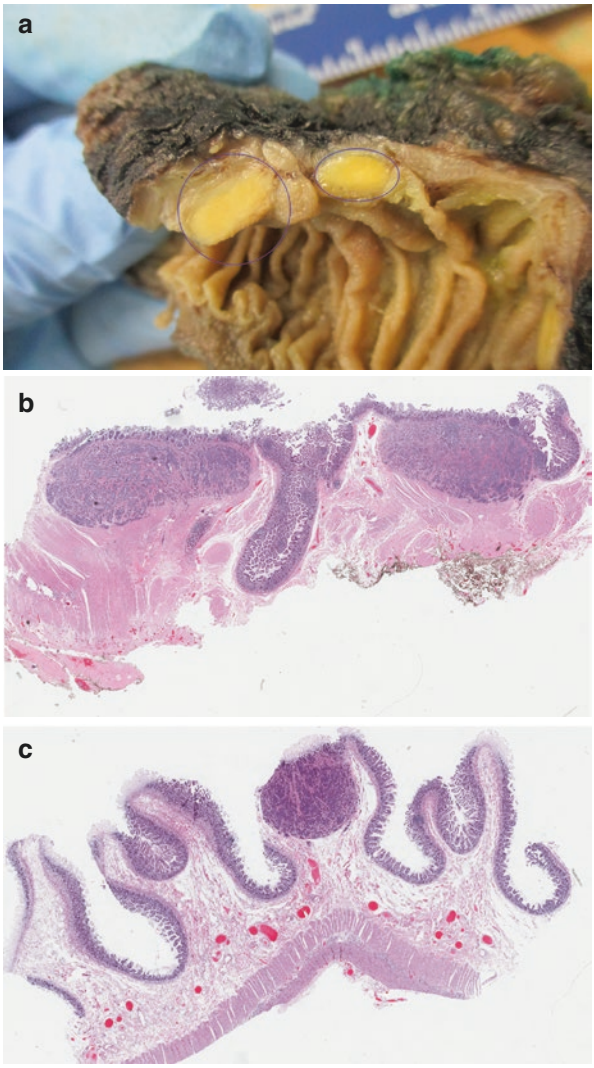

Fig. 18.13 Multifocal distal EC-cell small bowel neuroendocrine tumors (NETs) are uncommon but tend to be more frequent in familial setting. The incidence of multifocal small bowel NETs is higher in patients with a positive family history of small bowel NETs. Tumor multifocality is often detected during gross examination (**a**; circles indicate NETs) and microscopic examination (**b**–**c**; **b** illustrates the grossly identified tumors on photomicrograph **a**)

do not seem to be the only pathogenetic mechanism for this enigmatic manifestation as a recent European series failed to identify pathogenic variants in *IPMK* in a small series of familial small bowel EC-cell NETs; however, the European study reported recurrent chromosome 18 deletions using comparative genomic hybridization [120]. While the molecular correlates of familial small bowel EC-cell NETs remain to be further investigated, it is recommended that patients with a family history of multifocal small bowel NETs can benefit from screening given the increased risk of developing intestinal NETs [119].

Isolated case reports have expanded germline correlations of intestinal NENs including DNA repair genes in pancreaticoduodenal NENs [5]. Interestingly, a well-differentiated small bowel NEN was also identified in a patient with a pathogenic variant in *SMARCB1* (22q11.23) [121], while another patient with germline *BRCA1* (17q21.31) was found to have a small bowel mixed adenocarcinoma and neuroendocrine carcinoma [122]. A patient with TS also developed a rectal well-differentiated L-cell neuroendocrine tumor admixed with perivascular epithelioid cell neoplasms [123]. These occurrences likely represent the tip of the iceberg but have expanded our knowledge on the correlates of inherited intestinal NETs.

Pancreatic Neuroendocrine Neoplasms

Traditionally, inherited pancreatic NEN syndromes have been encountered in MEN1, MEN4, VHL, TS, and NF1 [14, 36, 117, 124–129]. However, progress in the molecular biology of pancreatic neuroendocrine disease has expanded the spectrum of inherited pancreatic NENs with the inclusion of *MAFA*-related familial insulinomatosis [130], *GCGR*-related Mahvash disease (glucagon cell hyperplasia-neoplasia syndrome; also known as glucagon cell adenomatosis) [131–134], germline defects in DNA repair gene identified in seemingly sporadic pancreatic NENs (e.g. *BRCA2, MUTYH, CHECK2, PALB2, NTHL1*) [4, 5, 114], as well as rare reports of *SDHx*- [135] and Lynch syndrome (*MLH1, PMS2, MSH2, MSH6*)-related pancreatic NENs [136, 137]. Unlike the initial data that suggested a germline susceptibility rate of 5–10% [138], discoveries of novel germline susceptibility genes in this field have led to the realization that the inherited pancreatic NENs easily account for approximately 20% of pancreatic NENs [4].

Since the significant proportion of inherited pancreatic NENs present with seemingly sporadic disease, pathologists play a crucial role in the prediction of germline disease in patients with pancreatic NENs. From a histological perspective, the vast majority of inherited pancreatic NENs are well-differentiated NETs [138]. Multifocal pancreatic neuroendocrine proliferation is a characteristic feature of inherited pancreatic neuroendocrine disease encountered in the setting of MEN1/MEN4, VHL disease, familial insulinomatosis, and Mahvash disease [1, 124, 126, 130–134, 138]. The grossly intact-appearing pancreas parenchyma often exhibits islet cell dysplasia-to-neuroendocrine tumor progression sequence with the formation of neuroendocrine microtumors (also referred to pancreatic neuroendocrine microadenomas) [1, 36, 124, 138] (Fig. 18.14). Ductulo-insular complexes (nesidioblastosis) and peliosis of the islets can also be featured in inherited disease [1, 36, 124, 138] (Fig. 18.14).

Underlying cystic epithelial (non-endocrine) disease and the identification of cytoplasmic clearing in NENs often prompts the attention of the diagnostician to

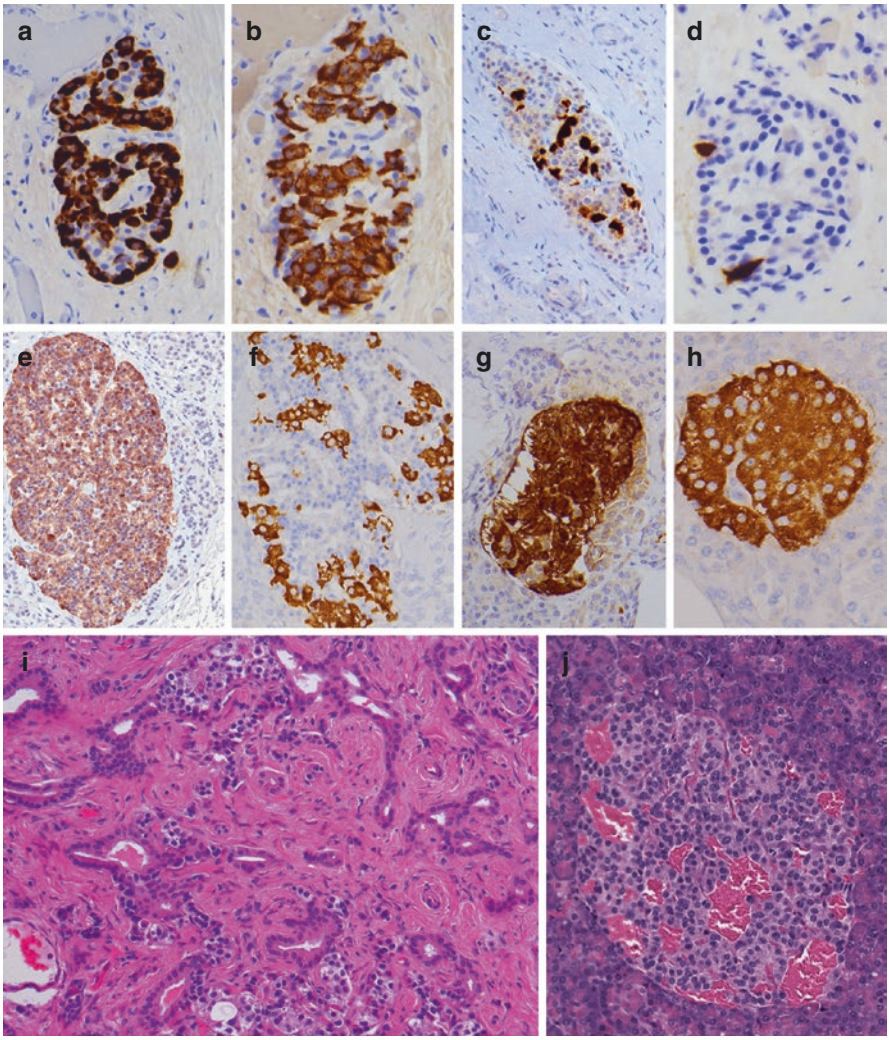

Fig. 18.14 Assessment of the nontumorous endocrine pancreas is the clinical responsibility of the diagnostician. The photomicrographs (**a**), (**b**), (**c**), and (**d**) represent normal quantitative and qualitative distributions of alpha-cells (glucagon), beta-cells (insulin), delta-cells (somatostatin), and gamma-cells (pancreatic polypeptide) in pancreatic islets, respectively. An islet with quantitative and/or qualitative alterations in alpha (**e**), beta (**f**), delta (**g**), and gamma (**h**) cells is referred to as islet dysplasia (**e**–**h**). When a dysplastic islet measures between 0.5–5 mm, the term "pancreatic microadenoma" or "pancreatic neuroendocrine microtumor" is applied. Pancreatic neuroendocrine tumors typically exceed 0.5 cm. The nontumorous pancreas also shows ductulo-insular complexes (**i**) and peliosis of islets (**j**). The identification of islet dysplasia, neuroendocrine microtumors, ductulo-insular complexes, and peliosis warrants further investigations to rule out germline disease. One should also be aware of gamma cell pseudohyperplasia, which is typically seen in the posterior surface of pancreatic head (**k**). The latter is not a sign of dysplasia

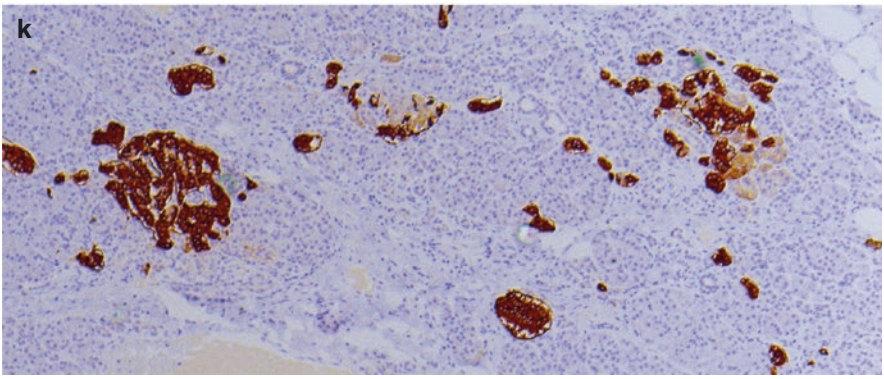

Fig. 18.14 (continued)

the possibility of VHL disease [1, 36, 118, 124, 126, 138, 139]. In *VHL*-related disease clear cell change present with many faces ranging from glassy cytoplasm to vacuolated or even signet ring-like appearance, or in association with variable oncocytic changes [118, 124, 126, 139] (Fig. 18.15). However, clear cell change can sometimes be seen in MEN1-related pancreatic NETs as well as in sporadic disease due to cellular senescence [140]; therefore, presence of multifocal pancreatic neuroendocrine proliferations in association with clear cell cytomorphology requires exclusion of *VHL*-related pathogenesis [1, 118]. Since patients with VHL disease can also develop cystic epithelial proliferations (e.g., serous microcystic adenomas), careful examination of the nontumorous pancreas provides additional insights [124, 126, 138]. Moreover, positivity for biomarkers of hypoxia pathway including HIF1-alpha, alpha-inhibin, and carbonic anhydrase IX (CAIX) enables the distinction of *VHL*-driven multifocal pancreatic NENs [1, 118, 124, 141] (Fig. 18.15).

MEN1-related pancreatic disease is almost always seen in association with microscopic precursor proliferations characterized by multifocal islet cell dysplasia to neuroendocrine neoplasia progression sequence [1, 36, 112, 117] (Fig. 18.16). Unlike other inherited pancreatic NENs, plurihormonality is a hallmark of *MEN1*-related multifocal pancreatic NEN [117] (Fig. 18.16). By immunohistochemistry, *MEN1*-related pancreatic neuroendocrine tumors and micro-tumors often express glucagon followed by pancreatic polypeptide, insulin and somatostatin [117, 124]. The data on MEN4 are limited, but one would expect to see MEN1-related states in affected patients [1, 14, 113].

One cannot emphasize enough the importance of immunohistochemical staining patterns for hormones in inherited pancreatic NETs [1, 124]. Immunoreactivity patterns for glucagon and insulin distinguishes MEN1/MEN4 from Mahvash disease and familial insulinomatosis. Unlike the immunohistochemical plurihormonality of MEN1/MEN4, Mahvash disease is characterized by multifocal alpha-cell hyperplasia/dysplasia to neoplasia sequence leading to neuroendocrine (micro)tumors that

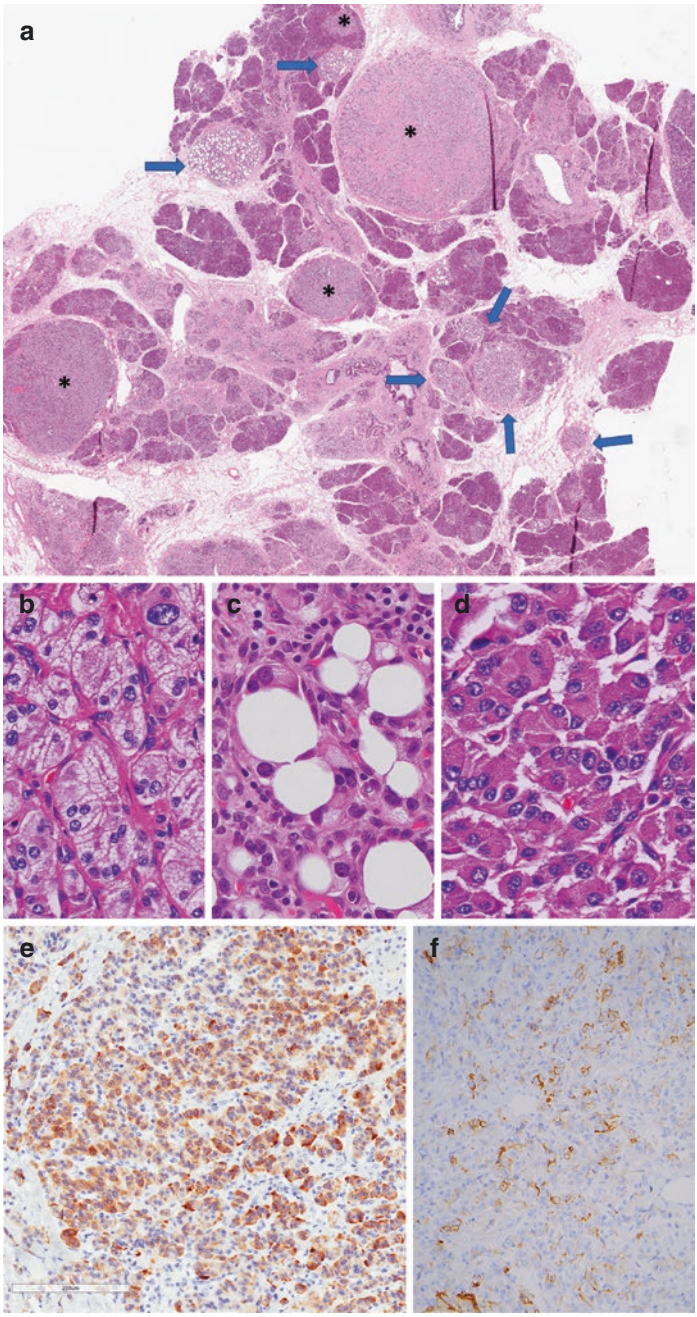

Fig. 18.15 *VHL*-related pancreatic neuroendocrine disease. Affected patients tend to show multifocal pancreatic neuroendocrine (micro)tumors (asterisks in **a**) arising in the background of islet dysplasia (**a**). Variable degree of cystic disease also occurs in most patients (**b**: arrows indicate serous cystic microadenomas). Neuroendocrine tumors display variable clear cell change (**b**, **c**) as well as oncocytic change (**d**). Positivity for alpha-inhibin (**e**) and carbonic anhydrase IX (**f**) in the setting of multifocal pancreatic neuroendocrine proliferation is a characteristic finding of pancreatic VHL disease

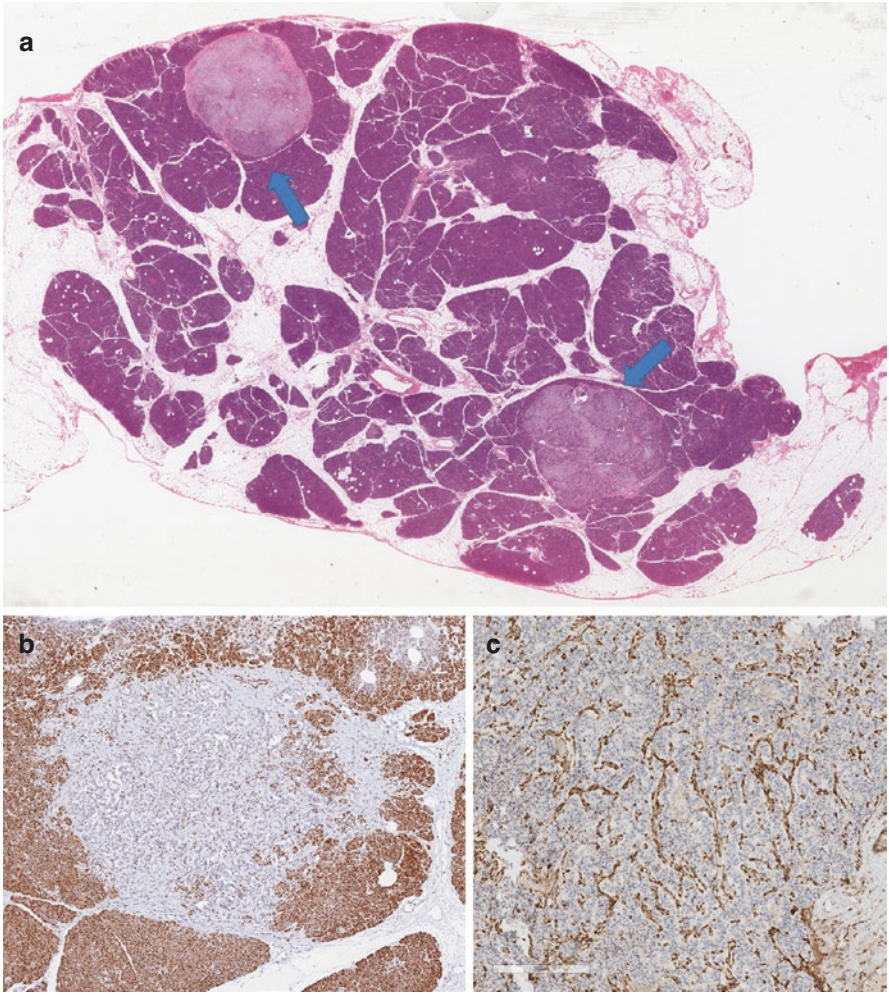

Fig. 18.16 MEN1- and MEN4-related pancreatic neuroendocrine disease. Both MEN1 and MEN4 manifest with multifocal pancreatic neuroendocrine tumors (**a**; arrows indicated tumors) including pancreatic neuroendocrine microtumors arising in the background of islet dysplasia. Ductulo-insular complexes and peliosis of islets can be identified. Loss of menin (**b**) and p27 (**c**) in neuroendocrine (micro)tumors can prioritize genetic testing for *MEN1* and *CDKN1B*

are exclusively positive for glucagon [117, 131–134] (Fig. 18.17). Patients with familial insulinomatosis tend to show beta-cell hyperplasia/dysplasia-to-neoplasia progression sequence leading to neuroendocrine (micro)tumors that are positive for insulin [130] (Fig. 18.18).

Although pancreatic NENs have not been considered as part of the routine screening with respect to neurocutaneous multisystem disease of patients with TS, recent data suggested the occurrence of a solitary pancreatic NEN in approximately 10% of TS patients that underwent routine abdominal imaging screening [142]. The latter underscores the need for the implementation of universal screening protocols for

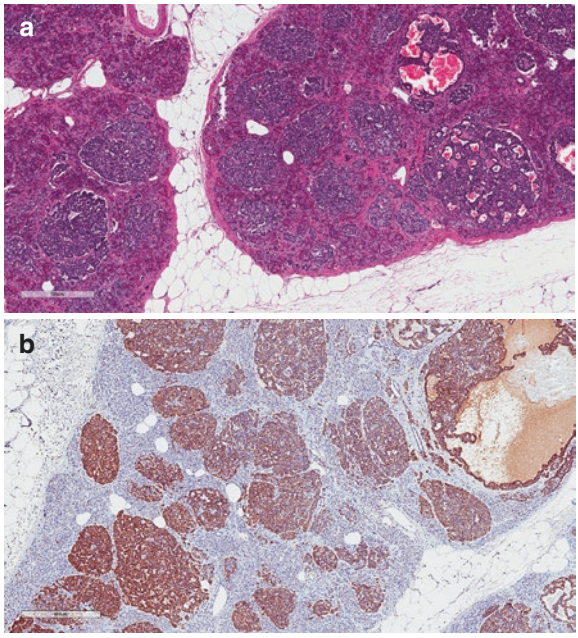

Fig. 18.17 *GCGR*-related pancreatic neuroendocrine disease (Mahvash disease). Affected patients manifest with alpha cell hyperplasia to neoplasia progression sequence characterized by alpha-cell hyperplasia and dysplasia with multifocal alpha-cell neuroendocrine (micro)tumors (**a**). Glucagon immunohistochemistry highlights diffuse staining in neuroendocrine cell proliferations (**b**)

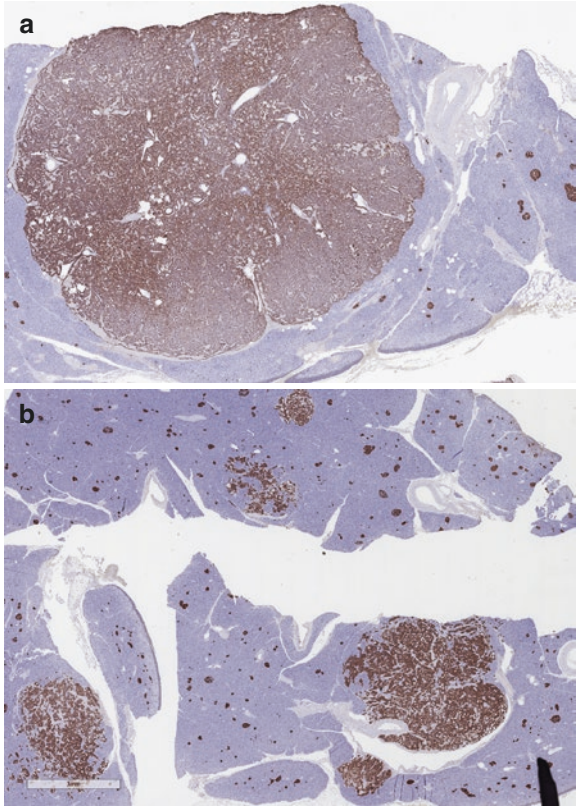

Fig. 18.18 Familial insulinomatosis is a rare germline disease that can be caused by pathogenic variants in *MAFA*. Affected patients tend to manifest with beta-cell hyperplasia to neoplasia sequence leading to multifocal beta-cell neuroendocrine tumors and microtumors (**a**, **b**; insulin)

pancreatic NENs in patients with TS. Nevertheless, little is also known with respect to morphological and immunohistochemical correlates of TS-related pancreatic NENs; however, one third of a TS-related pancreatic NETs were cystic [127]. The mean age at the time of resection was 26 years in that study [127]. Evidence suggests that TS-related pancreatic NETs are not uncommon in pediatric age and young adults [127, 142]. The association of neuroendocrine disease in patients with TS seems to be more frequent in those harboring pathogenic variants in *TSC2* (16p13.3) [123, 127]; however, disease related to pathogenic variants in *TSC1* (9q34.13) has also been reported [143]. Application of antibodies against tuberin (protein encoded by *TSC2* gene) and hamartin (protein encoded by *TSC1* gene) may assist pathologists in triaging patients with potential TS-related pancreatic disease [123].

Given the limited data, the distinction of NF1-related pancreatic NENs from sporadic disease seems to be difficult at the morphological level [124, 138]. Therefore, clinical findings and history for other NF1-related neoplasms can further guide the diagnostician.

In summary, the clinical stigmata, disease multifocality, tumor cytomorphology, assessment of nontumorous pancreas, and application of immunohistochemical biomarkers are integral components of the workup of inherited pancreatic neuroendocrine disease. Similar to the role of menin, p27, alpha-inhibin, CAIX, tuberin and hamartin, application of immunohistochemical biomarkers such as MMR proteins (MLH1, MSH2, MSH6 and PMS2) (Fig. 18.19) and SDHB can assist the screening of rare manifestations driven by the Lynch syndrome genes and *SDHx,* respectively [1, 124]. Pathologists, as a part of their evolved clinical responsibilities, should consider performing routine screening especially in all patients <45 years, those with underlying precursor proliferations (islet dysplasia, ductulo-insular complexes, multifocal pancreatic neuroendocrine tumor and microtumor, peliosis of islets) as well as in those with personal and family history of other endocrine tumors. Patients with other non-neuroendocrine neoplasms (e.g., breast cancer, colon cancer) should also be considered for additional germline screening with respect to defects in DNA repair genes.

Paraganglial Neuroendocrine Neoplasms (Pheochromocytoma and Paraganglioma, PPGL)

The field of inherited diseases is an ever-changing topic in PPGLs as these tumors have the highest frequency of germline susceptibility at a rate of over 40%. To date, several germline alterations including *RET, NF1, TMEM127, MAX, KIF1B-β, VHL, FH, MDH2, HIF2a (EPAS1), SDHx (SDHA, SDHB, SDHC, SDHD, SDHAF2), PHD1 (EGLN2), PHD2 (EGLN1), GOT2, SLC25A11, BAP1, MEN1, KMT2D/MLL2*, and *DNMT3a* have been linked to inherited PPGLs [144–166].

Understanding the clinicopathological correlates of germline susceptibility in PPGLs has led to the development of molecular clusters as follows: (i) cluster 1 disease (HIF or Kreb's cycle related pseudohypoxia-related pathway) and (ii) cluster 2 disease (Kinase signaling pathway) [167, 168]. The TCGA data on PPGLs also introduced the *wnt*-altered pathway, which is also referred to cluster 3 disease

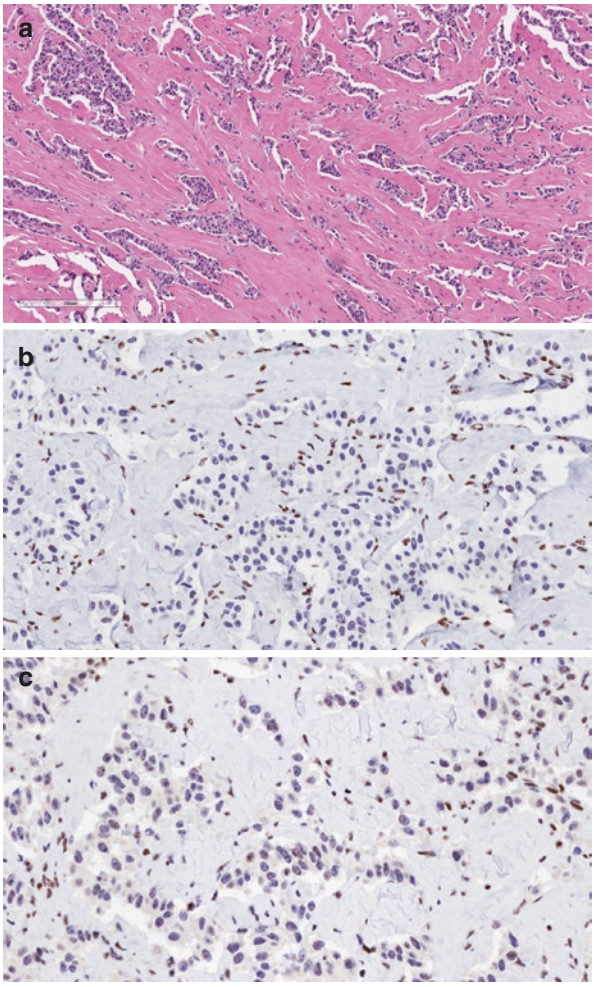

Fig. 18.19 Mismatch repair (MMR) immunodeficient pancreatic neuroendocrine tumors. Pancreatic neuroendocrine tumors can display MMR immunodeficiency. This finding requires further testing to rule out underlying Lynch syndrome-related germline disease, especially in young patients (**a**: Hematoxylin and Eosin; **b**: MLH1; **c**: PMS2)

[169]. To date, cluster 3 disease has been exclusive to sporadic sympathetic PPGLs [169]. As discussed in the chapter on PPGLs, there is a strong genotype (various pathogenic variants or molecular clusters)-phenotype correlation with respect to biochemical catecholamine secretory status, metabolic profiling, anatomic tumor distribution, risk of metastatic disease, optimal functional imaging studies, and potential targeted therapies [167, 168, 170–177].

While multifocal PPGLs and early age at diagnosis often raise the possibility of a germline disease, most inherited PPGLs manifest with sporadic-appearing manifestations that may not prompt the diagnostician to the possibility of a germline disease. For this reason, pathologists often facilitate triaging germline susceptibility by carefully assessing morphological features of the tumor (e.g., multifocal disease, certain cytomorphological features), non-tumorous parenchyma (e.g., adrenal medullary hyperplasia), and by using immunohistochemical biomarkers (e.g., SDHB, SDHA, 2-SC, alpha-inhibin, CAIX, and MAX) [1, 12, 168, 170, 178].

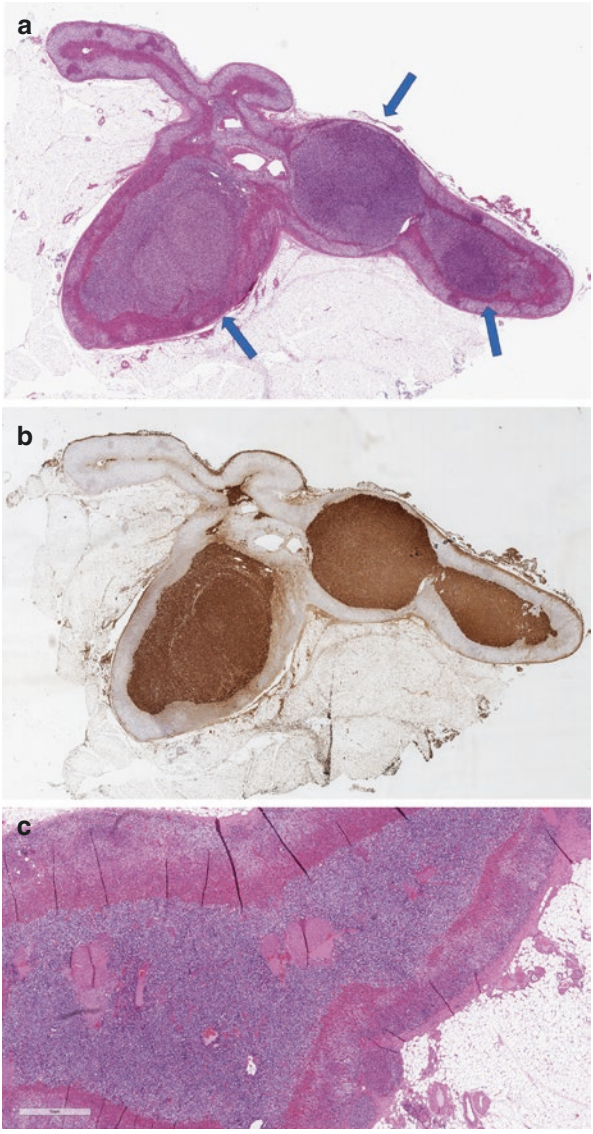

Fig. 18.20 *RET*-related adrenal medullary disease. Germline pathogenic variants in *RET* are typically associated with bilateral multifocal pheochromocytomas arising in the background of adrenal medullary hyperplasia. This composite photomicrograph illustrates multifocal pheochromocytomas (**a**: Hematoxylin and Eosin; arrows indicate multiple small pheochromocytomas) that are positive for tyrosine hydroxylase (**b**). The non-nodular adrenal medulla is expanded (**c**). This finding is consistent with adrenal medullary hyperplasia

Traditionally, adrenal medullary hyperplasia has been considered as a hallmark of *RET*-driven pheochromocytomas (Fig. 18.20); however, recent evidence suggests that adrenal medullary hyperplasia can also be encountered in *TMEM127*- [144], *SDHB*- [179], and *MAX*- [155] related pheochromocytomas.

Since most inherited PPGLs (more frequently in paragangliomas) are linked to *SDHx*-related pathogenesis, application of SDHB immunohistochemistry has become an essential clinical biomarker [1]. Loss of cytoplasmic granular SDHB immunoexpression has been regarded as a surrogate biomarker for any *SDHx* alterations [170, 180, 181] (Fig. 18.21). Since SDHB loss can rarely occur in sporadic

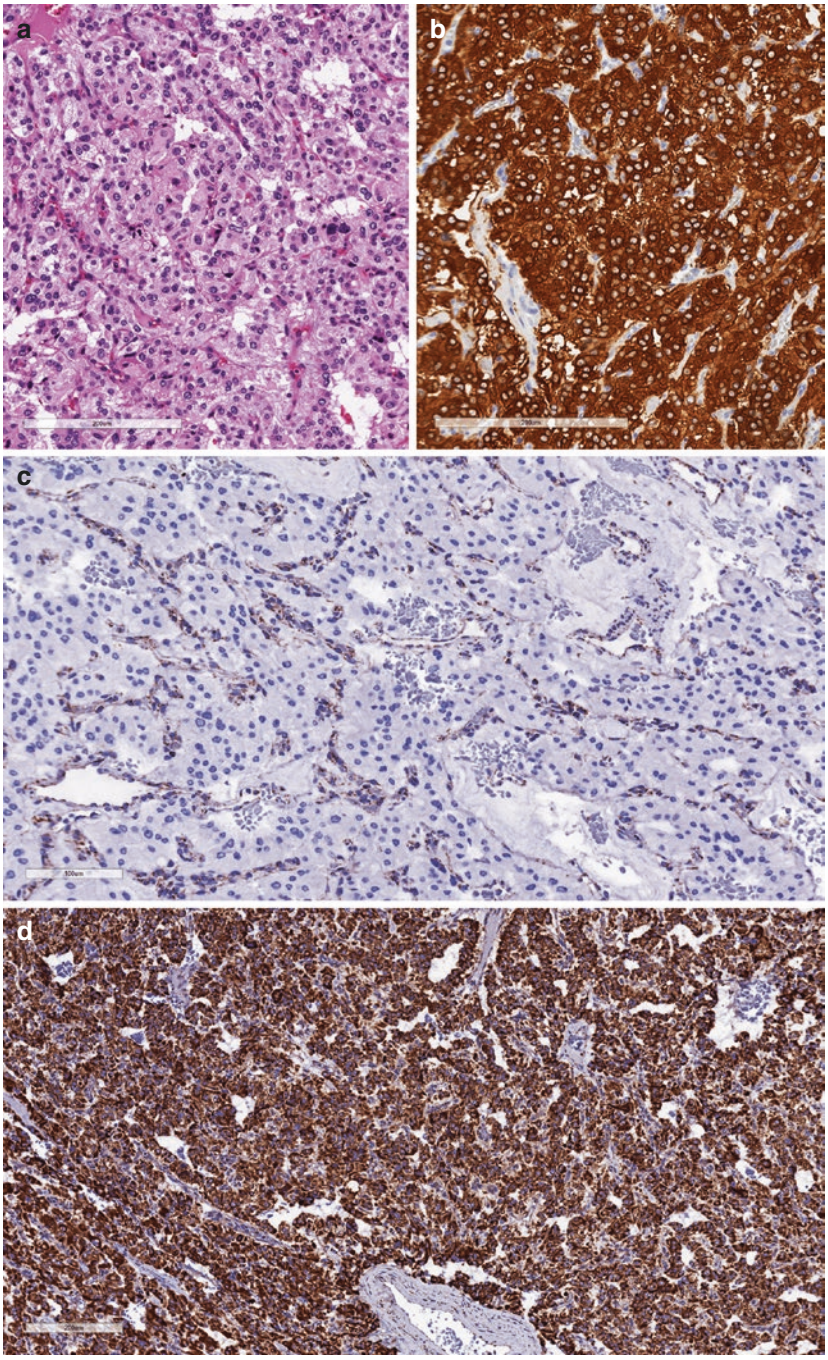

Fig. 18.21 *SDHx*-related paraganglioma. *SDHx*-related paragangliomas tend to display variable intracytoplasmic vacuoles and eosinophilic cytoplasm (**a**). Diffuse positivity for GATA-3 (not shown herein) and tyrosine hydroxylase (**b**) confirms the diagnosis of paraganglioma. Loss of cytoplasmic granular SDHB expression is regarded as a surrogate marker for *SDHx*-related pathogenesis (**c**). In this case, the identification of intact SDHA expression argues against a pathogenic variant in *SDHA* (**d**)

disease due to somatic or epigenetic inactivation of *SDHx* genes [182], germline testing is indicated to confirm the presence of pathogenic variants. Loss of immunoexpression for SDHB and SDHA also suggests *SDHA* alteration [183]. These biomarkers have also offered additional benefits especially when dealing with germline *SDHx* variants of uncertain significance. Loss of SDHB expression is often considered to be a sign of pathogenic alteration in such situations.

The distinction of *SDHx*-related pathogenesis is also of clinical significance since affected patients not only develop synchronous and/or asynchronous PPGLs in the context of familial PGL (FPGL) syndromes (FPGL1 due to *SDHD,* FPGL2 due to *SDHAF2*, FPGL3 due to *SDHC*, FPGL4 due to *SDHB*, and FPGL5 due to *SDHA*) but may also manifest with other endocrine (e.g., pitNETs, pancreatic NETs, thyroid cancer, adrenal cortical tumors) and non-endocrine (e.g., GIST, renal cell cancer) neoplasms [1]. A subset of patients with PPGL and GIST have been referred to have Carney-Stratakis syndrome or Carney dyad (often due to *SDHA, SDHB, SDHC, SDHD*), whereas those manifesting with an additional pulmonary chondroma have been linked to Carney Triad (due to *SDHC* promoter methylation as well as germline variants in *SDHA, SDHB* and SDHC) [1, 184, 185].

VHL-related PPGLs often show characteristics of clear cell change in association with thick vascular capsule and myxoid hyaline stroma rich in microvascular network [1, 186] (Fig. 18.22). When the tumor shows a normal (intact) cytoplasmic granular SDHB staining, one can exclude the possibility of *SDHx*-related pathogenesis. Furthermore, positivity for alpha-inhibin and/or CAIX can further help in the distinction of non-*SDHx*-related cluster 1 disease including *VHL*-related PPGLs [1, 163, 178].

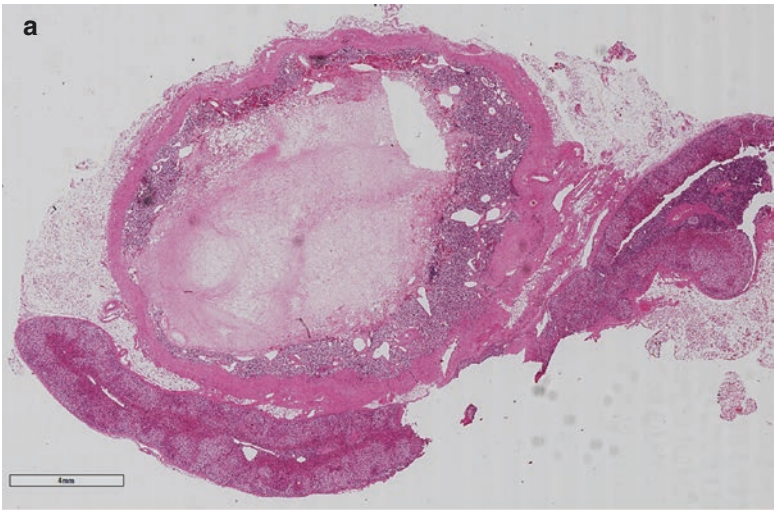

Fig. 18.22 *VHL*-related pheochromocytoma. *VHL*-related pheochromocytomas often show characteristics of clear cell change in association with thick vascular capsule and myxoid hyaline stroma rich in microvascular network (**a**). The tumor cells show variable degree of cytoplasmic clearing (**b**, **c**). Membranous carbonic anhydrase IX can further help in the distinction of VHL disease (**d**)

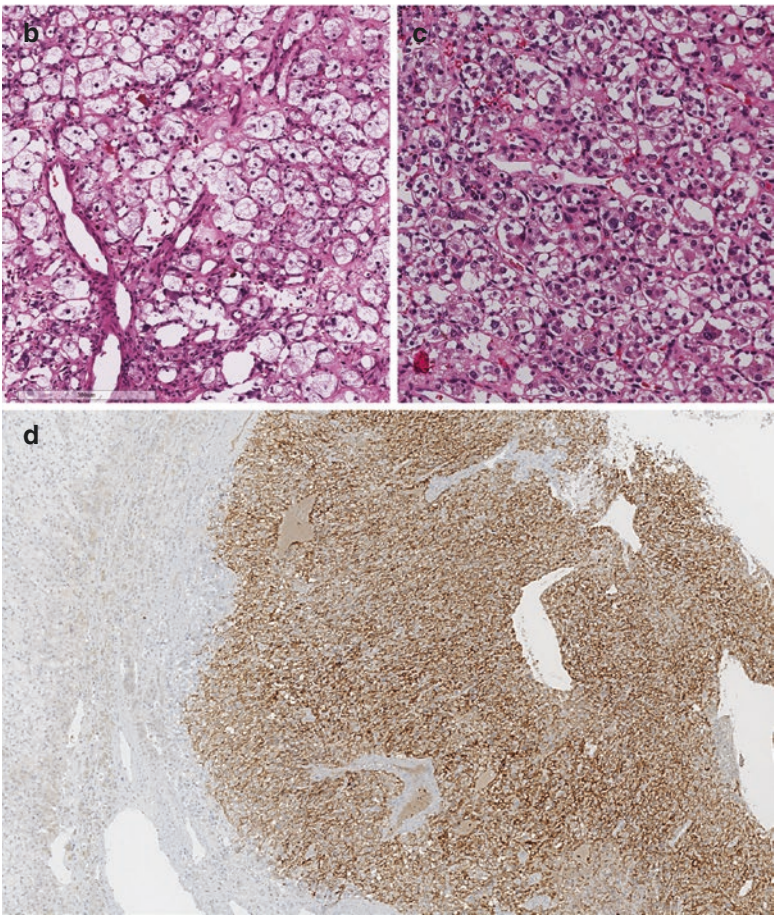

Fig. 18.22 (continued)

Positivity for 2-SC and loss of MAX expression can also facilitate the triaging of *FH*- and *MAX*-related manifestations [1, 181, 187, 188]. *MAX*- and *NF*-related disease can manifest with composite PPGLs (Fig. 18.23).

Whole exome sequencing as a diagnostic and screening tool can be employed for the evaluation of germline susceptibility syndromes, including PPGLs; however, the high cost limits its first-line and widespread use. For this reason, biochemical profiling (catecholamines and metanephrines), detailed morphological assessment, and immunohistochemical biomarker studies have helped in rationalizing genetic screening protocols through targeted single gene or panel testing.

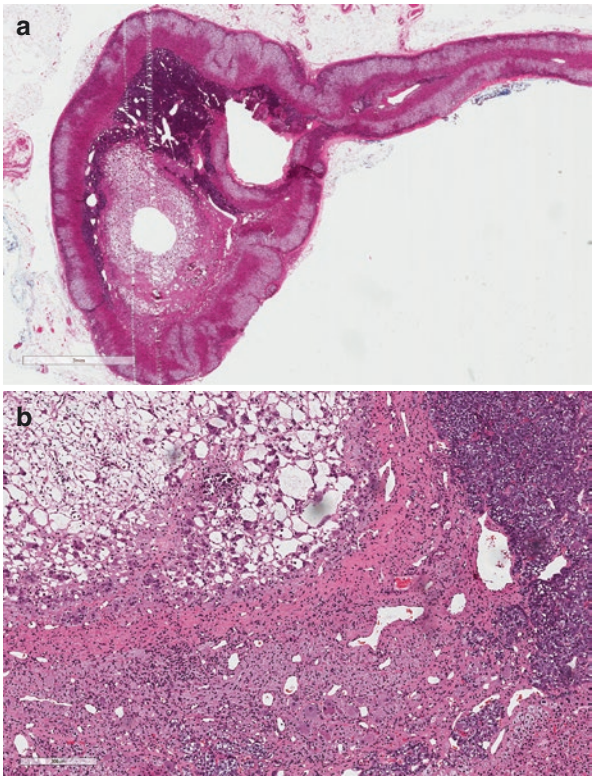

Fig. 18.23 *NF1*-related composite pheochromocytoma. This composite photomicrograph illustrates a composite pheochromocytoma confined to the adrenal gland (**a**, **b**)

Role of Medical Geneticist in the Workup of Inherited Neuroendocrine Neoplasia Syndromes

The delivery of genetic care for hereditary inherited disorders is multifaceted and involves multiple healthcare practitioners. This includes laboratory geneticists, physician geneticists, and genetic counsellors. The inherited NEN syndromes covered in this chapter are considered Mendelian, where a variant in a single gene is responsible for the phenotype [189]. The identification, implementation, and psychosocial implications of such genetic variants for the patient and family are handled by a multi-disciplinary genetics team.

Once a health care provider identifies a patient at risk of an inherited NEN syndrome, they are frequently referred to a genetics clinic with a genetic counsellor or physician geneticist (medical geneticist). During this encounter, the patient's cancer history, family history, and comprehensive pathological data are typically reviewed. The appropriate genetic tests are selected by obtaining the patient's informed consent.

Laboratory geneticists are involved in conducting genetic testing (frequently multi-gene panel testing). In inherited disorders, the most frequent tissue to test is peripheral blood lymphocytes, which represents a patient's germline. Tumor genetic testing is an emerging area of laboratory genetics, where tumors are tested for therapeutic options in cancer patients and can include genes involved in inherited NEN syndromes. It is important to recognize that tumor genetic events include acquired (somatic) genetic mutations and inherited genetic defects. A pathogenic variant detected in the tumor could therefore be an inherited variant, and if in a known Mendelian hereditary cancer syndrome gene, should also have germline genetic testing [190]. Most laboratories are currently using massively parallel sequencing (also known as next-generation sequencing) which is the fraction of the cost of Sanger sequencing and has increased genetic testing sensitivity and throughput. This technology advancement has propelled the field of genetics (the study of a small number of genes) to genomics (the study of the entire human genome, ~20,000 genes exons and intronic) [188].

Genetic counsellors and medical geneticists frequently are involved in assessing a patient's and family's risk of having an inherited etiology of their cancer. This is based on a three-generation family history with clustering of similar types of cancers, reviewing the pathology of tumor and organizing further medical investigations, genetic testing, or analysis of tumors [189]. Immunohistochemical analysis of the proteins (e.g., menin, p27, parafibromin, SDHB, MMR proteins, alpha-inhibin, and CAIX) is often required in patients with NENs [1]. Specific staining patterns (loss of expression in menin, p27, parafibromin, SDHB, and MMR proteins, and positivity for alpha-inhibin and CAIX) of such proteins could be a sign of an inherited NEN as discussed in previous parts of this chapter. If a mutation or pathogenic variant is identified, personalized surveillance recommendations are carried out for other at-risk organs.

Neuroendocrine Neoplasia Inheritance and Genetic Testing Approach

The NEN syndromes are generally inherited in an autosomal dominant fashion, where a pathogenic variant in only one allele is necessary for the manifestation of the disease (this is contrary to autosomal recessive where a variant in both alleles is needed) [191]. Due to this, first-degree relatives (children, siblings, and parents) of patient with a pathogenic variant are at 50% risk of harboring the same variant. Testing family members for an identified gene variant is known as "predictive genetic testing" [192]. Previously, many families faced genetic discrimination based on their genetic testing results, but many jurisdictions now have policies and laws in place to prevent such practices [193]. Among the inherited NENs, the exception to autosomal-dominant inheritance is *SDHD* and X-LAG. *SDHD* families follows a maternally imprinted or paternally inherited pattern. Here, only family members who inherit the mutation from their father are at risk of PPGLs. Epigenetic

factors are thought to be involved in regulating the *SDHD* locus resulting in this unique inheritance pattern [194]. Some cases of X-LAG are familial and follow and X-linked inheritance pattern [20].

The penetrance of Mendelian NEN inherited syndromes is highly variable depending on the gene and syndrome. Penetrance is defined as the proportion of pathogenic variant carriers which demonstrate a manifestation of the disorder (i.e., some carriers never develop any manifestations and remain healthy throughout life). The multiple endocrine neoplasia syndromes are highly penetrant (>95%) [195] while other hereditary syndromes such as *AIP*-related FIPA have a penetrance of ~20% [196].

Depending on the number of genes associated with a given inherited NEN syndrome, different genetic approaches can be used. If a single gene is associated with a disorder, for example, *RET* in medullary thyroid cancer, a single gene can be tested. If several genes are associated with a tumor, e.g., PPGL, a gene panel approach is frequently used. Usually genetic analysis comprises the coding exons of the gene and the adjacent intronic boundaries for variants affecting splicing. Most intronic variants are not analyzed under current genetic testing platforms, and a negative genetic test does not rule out an inherited etiology [188]. Once a specific variant is found in a patient, that specific variant can then be used to conduct predictive genetic testing on at-risk family members.

Nomenclature on Genetic Testing Results

Genetic variants are classified using laboratory standards and guidelines (e.g., the American College of Medical Genetics and Association for Molecular Pathology) which have certain specifications to annotate a given genetic variation as disease causing or benign [197]. Practically speaking, results are classified into three broad categories [192]:

(I) *Pathogenic and likely pathogenic variants* are rare genetic changes which are known to cause the phenotype and upon which medical recommendations can be made and predictive testing can be conducted on family members.

(II) *Benign and likely benign variants* usually do not have any medical significance as they are considered common in healthy individuals and likely do not cause disease.

(III) *Variants of uncertain significance (VUS)* are gene variations which are uncertain as they do not fulfill the criteria for pathogenic or benign. These are challenging to interpret, and often do not change medical management, but need to be taken in the context of the personal and family history. Predictive testing is generally not conducted with VUS. Up to 30% of multi-gene panel testing are variants of uncertain significance. Pathologists by performing immunohistochemical biomarkers can play an essential role in further validating the significance of VUS.

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The Management of Neuro-Endocrine Neoplasms

19

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Introduction

Neuroendocrine neoplasms arise in tissues of the endocrine system, including pituitary, thyroid, parathyroid, pancreas, respiratory system, gastrointestinal tract, and adrenal glands and rarely in other unusual sites as detailed in the various chapters of this book. Surgery is the first-line of treatment in many of these cases, and can include a complete removal of the organ (i.e. total thyroidectomy or adrenalectomy), partial removal (i.e. hemithyroidectomy or distal pancreatectomy), or removal of only the tumor (i.e. pituitary tumor). Medical treatment is frequently used, and while this is usually reserved as adjuvant treatment to control symptoms or tumor growth in cases of residual disease following surgical intervention, it is sometimes used as a treatment of choice (i.e. prolactinoma), sometimes as neoadjuvant before surgery to improve outcomes (i.e. treatment to control growth hormone or cortisol excess before surgery), or as the sole primary treatment in patients who are poor candidates for surgery. Other treatment options can include radiotherapy and peptide receptor radionuclide therapy (PRRT); in some patients, additional therapies are intended for symptom control.

Surgery

Surgically Resectable Disease

In most neuroendocrine tumors, surgery is the only treatment approach that can lead to cure. Furthermore, surgery in many cases can be used to alleviate symptoms that may be secondary to hormone over-secretion, or local mass effect.

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- *Pituitary gland tumors* – Transsphenoidal surgery with the removal of the pituitary neuroendocrine tumor is frequently used for patients with large non-functioning tumors and is recommended by the Endocrine Society guidelines as the first-line of treatment for patients with acromegaly or Cushing disease [1, 2].
- *Thyroid gland tumors* – Total thyroidectomy, with or without cervical lymph node dissection, is recommended in patients with a diagnosis of medullary thyroid cancer (MTC) following fine-needle aspiration, but may also be used as a prophylactic measure in patients who have inherited a mutated *RET* allele, in the evaluation of patients for persistent or recurrent MTC, and for detecting MTC in patients with nodular goiters. When the diagnosis of MTC is made following unilateral hemithyroidectomy, removal of the remaining gland is recommended in patients with hereditary MTC, while limited data is available on the management of patients with sporadic MTC [3].
- *Parathyroid gland tumor* – Surgical intervention to remove a parathyroid adenoma is recommended for patients with symptomatic hyperparathyroidism, or in cases of asymptomatic disease in young patients with significant hypercalcemia levels, kidney stones or hypercalciuria, or in patients with osteoporosis or a history of fragility fracture [4]. For patients with a germline mutation causing parathyroid hyperplasia and hyperparathyroidism, such as multiple endocrine neoplasia (MEN)-1, subtotal parathyroidectomy with the removal of at least 3.5 glands is recommended [5]. In the rare cases of parathyroid carcinoma, surgery is the most important treatment modality, and the recommended approach is en bloc resection [6].
- *Gastro-entero-pancreatic, pulmonary, and thymic neuroendocrine tumors* – Surgical resection of the tumor is recommended wherever technically feasible. A complete surgical resection includes removing the primary tumor and any lymph node metastases [7, 8]. When surgery is indicated, a decision regarding the extent of surgery must be made.

  Exceptions may be due to severe comorbidities or high surgical risk, or widely metastatic disease. In cases of pancreatic neuroendocrine tumors, active surveillance without surgery may be used for very small (<2 cm) and sporadic non-functional pancreatic neuroendocrine tumors. In contrast, as small bowel neuroendocrine tumors have a significant metastatic potential, even at a size of less than 2 cm, surgery is recommended in all cases, when possible [7, 8].
- *Pheochromocytomas and paragangliomas* – Adrenalectomy is the recommended treatment options for adrenal pheochromocytoma, and in most cases minimally invasive adrenalectomy is recommended. Open resection is recommended for large (>6 cm) or invading tumors as well as for most extra-adrenal pheochromocytoma (paraganglioma). In all patients with a functional pheochromocytoma or paraganglioma, preoperative blockade with α-adrenergic receptor blockers is recommended. Unilateral adrenalectomy combined with cortical-sparing adrenalectomy, i.e., selective removal of medullary tissue leaving only cortical tissue of the remaining adrenal, may be considered to preserve cortical function and avoid hypocortisolism in patients with bilateral adrenal disease, such as those with germline predisposition [9].

Surgical resection is the recommended treatment option for solitary paragangliomas. As paragangliomas are more likely to be malignant than pheochromocytomas and in many cases are located in unfavorable areas for laparoscopic resection, the Endocrine Society guidelines suggest open resection for paragangliomas. However, for small and non-invasive tumors in surgically favorable locations, laparoscopic resection may be performed, as this approach is associated with earlier recovery, shorter hospitalization, and less pain [9].

All patients with a hormonally active pheochromocytoma or paraganglioma require perioperative α-adrenergic receptor blockade for 1–2 weeks prior to the surgical treatment (see symptoms-directed therapy, below).

Surgical Treatment for Advanced Disease

Tumor debulking is not curative, and the main advantage of this approach is for symptom control in functional tumors and to alleviate symptoms secondary to local mass effect. For instance, in cases of a pituitary tumor that cannot be completely excised, tumor debulking may decrease the risk of visual disturbances or improve headaches associated with a large tumor; partial removal of a functional pituitary tumor (such as a growth hormone-secreting pituitary tumor) may also improve subsequent response to medical therapy and the rate of biochemical disease control [10, 11]. As for gastro-entero-pancreatic, pulmonary and thymic neuroendocrine tumors, most guidelines agree that the surgical approach will be based on the extent of tumor burden resection. The NCCN guidelines recommend that the management of locoregional advanced disease and/or distant metastases, resection of the primary tumor, and the metastases will be performed when complete resection is possible and also recommend non-curative debulking in selected cases [7]. Partial hepatectomy can be considered in patients with liver metastases.

Medical Therapy

Medical treatment is required in many patients with advanced, recurrent, or metastatic neuroendocrine neoplasms that are not candidates for surgical intervention. Medical treatment may be used to control hormone excess and alleviate symptoms, but can also be used to control tumor growth.

1. *Somatostatin analogues* – Somatostatin inhibits the secretion of a wide range of hormones. As somatostatin receptors are commonly expressed by neuroendocrine tumors, somatostatin analogues will bind to those receptors and inhibit hormone secretion from the tumor cells. Sandostatin long-acting release (LAR) and somatuline autogel may be effective in both controlling tumor growth and controlling the symptoms of functional endocrine tumors, mainly pituitary [12–15] and gastro-entero-pancreatic neuroendocrine tumors [12, 16]. Pasireotide, a somatostatin multireceptor ligand, with a higher affinity for somatostatin receptor subtype 5,

was found to have superior efficacy over first-generation somatostatin analogues (somatuline and sandostatin LAR) for acromegaly [17], but later studies have suggested that the effect of the various somatostatin analogues may be comparable [18, 19]. Pasireotide was also found to be an effective treatment option for Cushing's disease [20]. However, pasireotide was not found to be more effective than first-generation somatostatin analogues for gastrointestinal neuroendocrine tumors [21]. For patients with MTC, somatostatin analogues may improve diarrhea and may be used when anti-motility agents are not effective [3].

2. *Molecularly targeted therapy – Everolimus*, an oral mammalian target of rapamycin (mTOR) inhibitor, may be used for patients with progressive neuroendocrine tumor, where it has been shown to prolong progression-free survival in patients with lung and gastro-entero-pancreatic neuroendocrine tumors [22, 23]. *Sunitinib*, an oral multi-targeted tyrosine kinase inhibitor, improved progression-free survival and overall survival, compared to placebo, in patients with advanced well-differentiated pancreatic neuroendocrine tumors [24]. Sunitinib may be used for malignant pheochromocytoma or paraganglioma, as in the recent SNIPP trial, disease control rate was 83% and median progression-free survival was 13 months [25]. *Vandetanib* is a tyrosine kinase inhibitor targeting RET, EGFR, and VEGFR kinases, while *cabozantinib* targets RET, c-MET, and VEGFR. Both vandetanib and cabozantinib were approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of patients with significant tumor burden and symptomatic or progressive metastatic MTC, as prolonged progression-free survival is possible. There are other possible molecularly targeted treatment options for neuroendocrine tumors, such as *pazopanib*, *sorafenib*, and *axitinib* [26] (Fig. 19.1).
3. *Cytotoxic chemotherapy* – There is no consensus on the best chemotherapeutic regimen, and the possible agents in patients with symptomatic, and/or progressive neuroendocrine neoplasms include 5-fluorouracil, capecitabine, dacarbazine, oxaliplatin, streptozocin, and temozolomide. Frequently, a combination treatment will be preferred. For patients with persistent or recurrent MTC, cytotoxic chemotherapeutic regimen have a low response rate. Pheochromocytoma or paraganglioma may respond to systemic chemotherapy, in addition to symptomatic improvement, and the regimen usually includes various combinations of cyclophosphamide, dacarabazine, vincristine, and doxorubicin. The WHO classification of neuroendocrine neoplasms is based on Ki-67 expression and mitotic counts. Grades 1 and 2 are considered well-differentiated tumors (Ki-67 < 20%), while grade 3 tumors are further classified to well-differentiated tumors or neuroendocrine carcinoma (NEC). For grade 3 neuroendocrine neoplasms that are well-differentiated, cytotoxic chemotherapy may be used; while there is no consensus on the best cytotoxic chemotherapy regimen, the combination of capecitabine and temozolomide (CAPTEM) may improve overall and progression-free survival. For patients with neuroendocrine carcinoma (NEC), which is poorly differentiated and frequently has a rapidly progressive clinical course, combination chemotherapy of cisplatin and etoposide, or its analogs, is recommended.

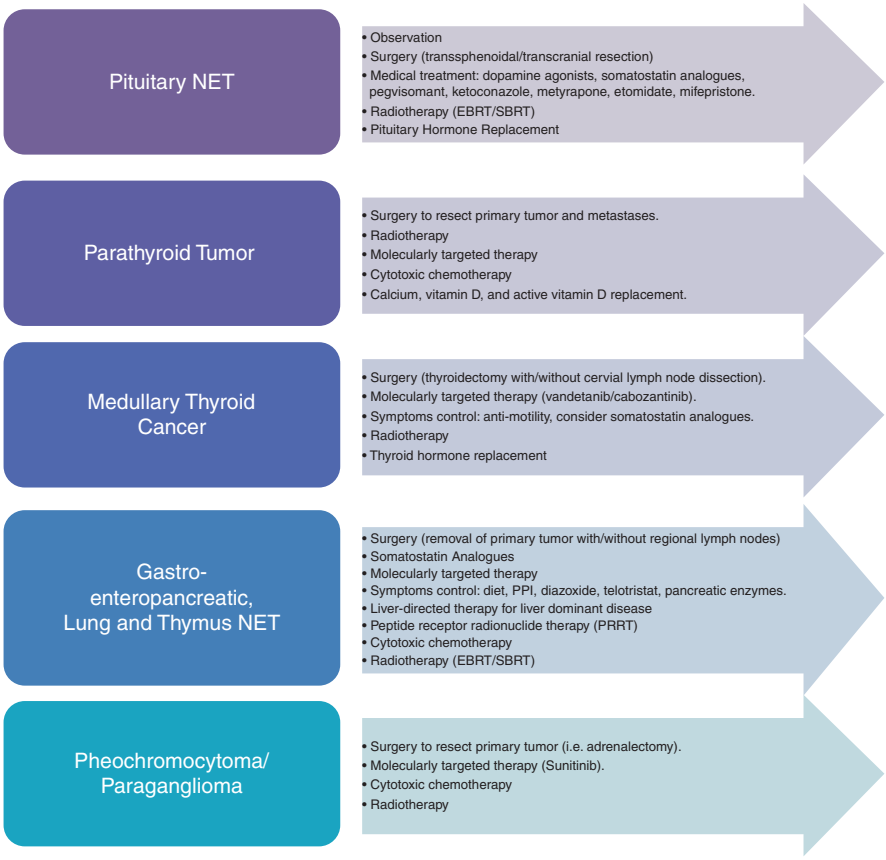

Fig. 19.1 Treatment options. Available treatment options for various neuroendocrine tumors

Targeted Radiation Therapy

1. *Peptide receptor radionuclide therapy (PRRT)* – This treatment modality involves delivery of targeted radiotherapy to malignant neuroendocrine tumor cells that express somatostatin receptors, and was found to improve progression-free survival and overall survival in patients with well-differentiated gastrointestinal neuroendocrine tumors [27]. This treatment option may be used for metastatic pheochromocytoma or paraganglioma as an investigational modality, and should be reserved for tumors with the expression of somatostatin receptors.
2. ^{131}I-metaiodobenzylguanidine (MIBG) – This radioactive iodine (^{131}I) attached to the MIBG molecule may be used for patients with metastatic pheochromocytoma or paraganglioma if ^{123}I-MIBG diagnostic scintigraphy is positive [9].

Symptom-Directed Therapies

Hormonal control is an important treatment goal for patients with unresectable, functional tumor, as hormone excess may cause significant morbidity and mortality.

1. *Diet* – Nutritional recommendations may be particularly important in patients with functional neuroendocrine tumors, such as insulinoma, as nutritional adjustment with frequent small meals may prevent hypoglycemic events, or glucagonoma, which may require parenteral nutrition, including vitamin supplementation.
2. *PPI* – High doses of proton pump inhibitors are required for most patients with gastrinoma with acid hyper-secretion.
3. *Telotristat* – This is a serotonin synthesis inhibitor acting on the rate-limiting enzyme tryptophan hydroxylase, resulting in significantly reduced frequency of bowel movements and urinary 5-hydroxyindole acetic acid in patients with serotonin-producing neuroendocrine tumors [28]. This treatment was approved by the FDA in February 2017 for persistent diarrhea in patients with carcinoid syndrome.
4. *Pancreatic enzymes* – Pancreatic enzyme supplements are particularly important following extensive pancreatectomy and may reduce stool frequency and improve abdominal pain, flatulence, and stool consistency. These should be given before all meals and snacks [29].
5. *Alpha-adrenergic blockers* – α-adrenergic receptor blockade is used to normalize blood pressure and heart rate that may be affected by catecholamine-induced blood volume contraction. This approach is mainly used prior to any intervention that may precipitate sudden release of catecholamines, such as a surgical procedure, but also may be used in situations of severe clinical symptomatology. The treatment of choice includes a high-sodium diet and fluid intake to prevent severe hypotension following surgical removal of the tumor [9].

Additional Treatment Options

1. *Liver-directed Therapy* – Liver-directed therapies may be considered in patients with hepatic-dominant metastatic disease. There are several treatment options, including surgical resection, ablation (radiofrequency ablation, cryoablation, or microwave ablation), hepatic arterial embolization, chemoembolization, or radioembolization.
2. *Hormone replacement* – Different treatment modalities may lead to hormone deficiencies, such as pituitary hormones deficiency following surgery or radiation, or hypothyroidism after treatment for thyroid cancer. It is important to provide adequate hormone replacement therapy, such as corticosteroid or thyroid hormones.
3. *External beam radiation* – Radiation may be indicated for various endocrine tumors but is not used as a first-line treatment option. For pituitary tumors, radia-

tion may be used as a second- or third-line treatment when there is evidence for residual pituitary disease following surgery or when surgery is not possible. External beam radiation or stereotactic beam radiation therapy can also be used in cases of metastatic thyroid or parathyroid cancer or metastatic gastroenteropancreatic neuroendocrine tumors, either directed to the primary tumor site or to metastatic sites.

Future Directions

In recent years there has been a significant increase in the prevalence of neuroendocrine neoplasms, along with marked improvement in our understanding and treatment options. While surgery is frequently the first treatment choice for these tumors, in many cases additional modalities are required, indicating the importance of tailoring more therapeutic paradigms. Additional data on the molecular basis of neuroendocrine neoplasms may lead to providing treatment sequences personalized for each patient, based on the tumor biology, molecular and genetic patterns. Immunotherapy with immune checkpoint inhibitors may prove to be a successful option for selected neuroendocrine neoplasms. Treatment with PRRT is expected to become more popular for patients with these tumors, as more data become available in different clinicopathological settings.

Figure 19.1 summarizes the available treatment options for various neuroendocrine tumors.

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