# 14 Markers and Immunoprofile of Tumors of Endocrine Organs and Neuroendocrine Tumors

## Contents





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## 14.1 Screening Markers of Neuroendocrine Differentiation

Chromogranin, Synaptophysin, Secretogranin, NSE, S100, PGP9.5, CD56, PAX-6, INSM-1, Islet-1, and Somatostatin receptor (SSTR).

Endocrine and neuroendocrine tumors are a heterogeneous group of tumors sharing a common phenotype arising from different cells of endocrine glands or from multipotent neuroendocrine stem cells that migrated from the neural crest to different organs [1, 2].

Neuroendocrine cells and tumors derived from these cells share different common antigens and transcription factors characteristic for neuroendocrine differentiation. The immunohistochemical markers listed in this chapter are used to screen for neuroendocrine differentiation in normal or neoplastic cells; however, none of these markers is a universal marker for neuroendocrine differentiation; consequently, screening for this immunophenotype must include two or more antibodies to neuroendocrine molecules or transcription factors. In routine immunohistochemistry, chromogranin and synaptophysin are the most commonly used markers, and a mixture of both markers gives better results and superior stain intensity. The new neuroendocrine transcription factors, such as INSM-1 and Islet-1, are very helpful to confirm the neuroendocrine differentiation [3].

Neuroendocrine tumors may have epithelial or neuroectodermal histogenesis; accordingly, the absence of cytokeratin expression in tumors does not exclude the diagnosis of neuroendocrine neoplasia.

### 14.1.1 Chromogranin A

| Chromogranin A | | |
|---|---|---|
| **Expression pattern:** cytoplasmic | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Endocrine and neuroendocrine tumors<br>Pituitary adenomas, medullary thyroid carcinoma, parathyroid adenoma/carcinoma, pheochromocytoma, islet cell tumors, Merkel cell carcinoma, small cell carcinoma, carcinoid and neuroendocrine carcinoma | Oligodendroglioma, neuroblastoma, PNET, paraganglioma | Neuroendocrine cells: anterior pituitary gland, C cells of the thyroid gland, parathyroid gland, islet cells of the pancreas, adrenal medulla, gastrointestinal and bronchial endocrine cells, neuronal cells |
| **Positive control:** appendix | | |

**Diagnostic Approach** Chromogranins are glycosylated calcium-binding acidic proteins and members of the Chromogranin/Secretogranin family that includes Chromogranin A, Chromogranin B (known as Secretogranin I), and Chromogranin C (known as Secretogranin II), located in the neurosecretory granules of neuroendocrine cells and synaptic vesicular walls. Chromogranin A is the most commonly used marker in routine immunohistochemistry. Chromogranins are expressed in almost all neuroendocrine cells and neuroendocrine tumors. The intensity of the immunostaining depends on the quantity of neurosecretory granules present in the cytoplasm of targeted cells; an example is small cell carcinoma, which actively synthesizes Chromogranin, but because of the paucity of cytoplasm and scarcity of neurosecretory granules, small cell carcinoma usually shows very weak or negative Chromogranin stain.

**Diagnostic Pitfall** Poorly differentiated neuroendocrine carcinomas can lose the expression of neurosecretory granules and are frequently negative for Chromogranin and Synaptophysin. To exclude the neuroendocrine differentiation, it is recommended to use other neuroendocrine transcription factors such as INSM-1 and Islet-1.

### 14.1.2 Synaptophysin

| Synaptophysin | | |
|---|---|---|
| **Expression pattern:** cytoplasmic | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Neuroendocrine tumors<br>Pituitary adenomas, medullary thyroid carcinoma, parathyroid adenoma/carcinoma, pheochromocytoma, islet cell tumors, small cell carcinoma, carcinoid and neuroendocrine carcinoma | Medulloblastoma, retinoblastoma, neurocytoma, ependymoma, neuroblastoma, adrenocortical tumors, Merkel cell carcinoma | Neuronal and neuroendocrine cells, choroid plexus epithelium, carotid body cells, adrenal cortex and medulla |
| **Positive control:** appendix | | |

**Diagnostic Approach** Synaptophysin is a transmembrane calcium-binding glycoprotein present as a major component of presynaptic vesicles found in all neurons. Synaptophysin is a broad-spectrum marker for neuroendocrine cells and tumors with endocrine and neuroendocrine differentiation. Strong expression is also noted in astrocytic and ependymal tumors in addition to central neurocytoma. A mixture of antibodies to Chromogranin and Synaptophysin will increase the sensitivity.

Other synaptic vesicle proteins, such as Synaptic vesicle protein-2, Synaptogranin, and vesicle-associated membrane protein, are rarely used in routine immunohistochemistry.

### 14.1.3 Insulinoma-Associated Protein 1 (INSM-1)

| Insulinoma-associated protein 1 (INSM-1) | | |
|---|---|---|
| **Expression pattern:** nuclear | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Neuroendocrine tumors (NET and NEC) of different origin<br>– Extraskeletal myxoid chondrosarcoma | Pheochromocytoma, paraganglioma and neuroblastoma, pituitary adenoma, medullary thyroid carcinoma, Merkel cell carcinoma | Pituitary gland, thyroid C cells, pancreatic islet cells, adrenal medulla, GIT enterochromaffin cells, pineal gland |
| **Positive control:** pancreatic tissue | | |

**Diagnostic Approach** Insulinoma-associated protein 1 (INSM-1) is a transcriptional factor involved in the regulation of proliferation (repressor) and differentiation of neuroendocrine cells. INSM-1 is expressed in all neuroendocrine cells and tumors derived from these cells, including low-grade and intermediate neuroendocrine tumors (NET G1/2), small cell and large cell neuroendocrine carcinomas, paraganglioma, medullary thyroid carcinoma, and pituitary tumors (Fig. 14.1) [4–7]. Compared to other neuroendocrine markers, including chromogranin, synaptophysin, CD56, and NSE, INSM-1 showed a higher sensitivity and speci-

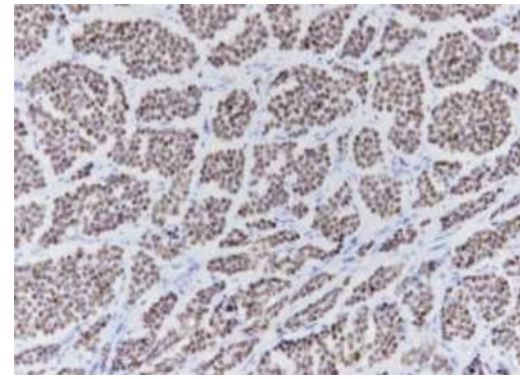


**Fig. 14.1** Neuroendocrine tumor (NET G2) with strong nuclear INSM-1 expression

ficity in many studies. INSM-1 is also found to be a specific marker for extraskeletal myxoid chondrosarcoma. INSM-1 is usually negative in non-neuroendocrine epithelial tumors and in melanocytic tumors.

### 14.1.4 Islet-1

The human insulin gene enhancer binding protein (Islet-1/ISL-1) is a transcription factor involved in the differentiation of sympathetic neurons and neuroblasts and neuroendocrine pancreatic cells. Islet-1 is a marker for different neuroendocrine tumors in addition to thyroid medullary carcinoma, pheochromocytoma, and paraganglioma. This transcription factor is listed in detail with the markers of pancreatic tumors (Chap. 8).

### 14.1.5 CD56

CD56 (N-CAM) is a transmembrane neural adhesion molecule involved in the development of neural cells and differentiation of neural tissue. Normally, CD56 is expressed on the membrane of neuroectodermal cells, NK cells, activated T cells, myoblasts, and skeletal muscle. CD56 is a sensitive but less specific marker for neuroendocrine cells and neuroendocrine tumors, especially small cell carcinoma. CD56 is listed in detail.

### 14.1.6 Neuron-Specific Enolase

| Neuron-specific enolase (NSE) γ-subunit | | |
|---|---|---|
| **Expression pattern:** cytoplasmic | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Neuroendocrine and neuroectodermal tumors | Melanoma, Merkel cell carcinoma, meningioma, renal cell carcinoma | Neurons, neuroendocrine cells, megakaryocytes, T lymphocytes, smooth and striated muscle |
| **Positive control:** appendix | | |

**Diagnostic Approach** Neuron-specific enolase (NSE) is a glycolytic enzyme catalyzing the reaction pathway between 2-phospho-glycerate and phosphoenolpyruvate, playing a role in intracellular energy metabolism. Enolases are homo- or heterodimers composed of three subunits—alpha (α) subunit, beta (β) subunit, and gamma (γ) subunit—whereas antibodies to the γ-subunit are the most commonly used. The γ-subunits are primarily expressed in neurons and normal and neoplastic neuroendocrine cells. Different expression levels are also found in megakaryocytes and T lymphocytes, in addition to striated and smooth muscle cells.

**Diagnostic Pitfall** NSE has a low specificity to neuroendocrine tumors ("nonspecific enolase") and is usually used as a screening marker; therefore, the diagnosis must be supported by other more specific markers.

### 14.1.7 Somatostatin Receptor Type 2

| Somatostatin receptor type 2 (SSTR2) | | |
|---|---|---|
| **Expression pattern:** membranous | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Neuroendocrine tumors (NET G1–3) and neuroendocrine carcinoma (NEC)<br>– Olfactory neuroblastoma<br>– Meningioma<br>– Follicular dendritic cell sarcoma | Pituitary adenoma, paraganglioma, pheochromocytoma, GIST, synovial sarcomas | CNS/cerebellum, neuroendocrine cells, kidney, follicular dendritic cells |
| **Positive control:** pancreas | | |

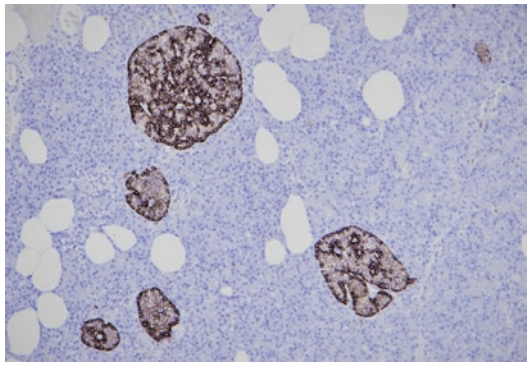


**Fig. 14.2** Pancreatic tissue with exocrine and endocrine components. SSTR2 strongly stains the α- and β-endocrine cells in pancreatic islets

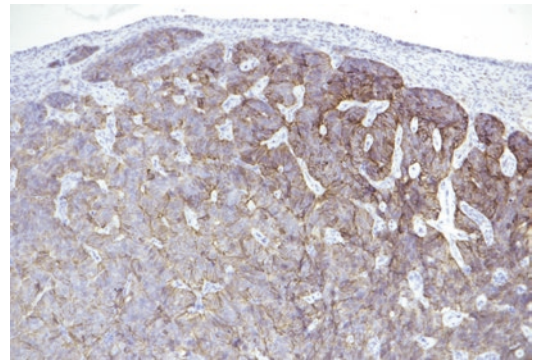


**Fig. 14.4** NET G2 (atypical carcinoid) of the lung with strong membranous SSTR2 expression

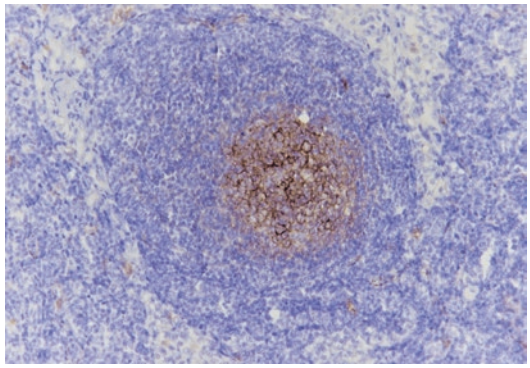


**Fig. 14.3** SSTR2 highlighting the follicular dendritic cells in lymphoid germ centers

**Diagnostic Approach** Somatostatin receptor type 2 (SSTR2) is a transmembrane G protein-coupled receptor for somatostatin-14 and somatostatin-28. SSTR2 inhibits the secretion of several hormones and other secretory proteins in several organs with high expression levels in the cerebrum, and neuroendocrine tissue, including α- and β-cells in pancreatic islets and to a lesser degree in all neuroendocrine cells of the gastrointestinal tract (Fig.14.2) and pituitary gland in addition to kidney and follicular dendritic cells (Fig. 14.3). In routine histopathology, SSTR2 is a diagnostic marker for neuroendocrine and endocrine tumors, whereas the expression intensity decreases with the dedifferentiation of the neuroendocrine neoplasia as the highest expression levels are noted in NET G1–2 (Fig. 14.4) and frequently disappear in high-grade neuroendocrine carcinomas. Consequently, SSTR2 is a prognostic factor for related tumors and a therapeutic target of somatostatin analog-based treatment.

SSRT2 is also a diagnostic marker for other endocrine tumors, meningioma, and follicular dendritic cell sarcoma. SSTR2 is negative in schwannoma and neurofibroma.

**Diagnostic Pitfalls** The expression of SSTR2 is found in other tumors with similar morphology, such as synovial sarcomas and gastrointestinal stromal tumors, and must be used in a panel with more specific markers.

### 14.1.8 Serotonin

Serotonin (5-hydroxytryptamin, Enteramin) is a neurotransmitter molecule synthesized in the peripheral and central nervous system and enterochromaffin cells in the gastrointestinal tract. Serotonin is a neuroendocrine marker mainly used for pancreatic and gastrointestinal neuroendocrine tumors.

### 14.1.9 S100

| S100 | | |
|---|---|---|
| **Expression pattern:** cytoplasmic/nuclear | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Melanomas, schwannoma<br>– Histiocytic (Langerhans cell) neoplasms<br>– Neuroendocrine tumors | Liposarcoma, malignant peripheral nerve sheath tumors, neurofibroma, neurilemoma, chondrosarcoma and chondroblastoma, clear cell sarcomas, myoepithelial tumors, granulosa cell tumor | Cells of neural crest (glial cells, Schwann cells, melanocytes, and nevus cells), chondrocytes, adipocytes, myoepithelial cells, macrophages, adrenal medulla and paraganglia, Langerhans cells, dendritic cells |
| **Positive control:** appendix | | |

**Diagnostic Approach** The S100 proteins are a family comprising about 25 homologous low molecular weight intracellular calcium-binding proteins encoded by different genes located at different chromosomes, mainly chromosome 1. S100 is normally present in cells derived from the neural crest, including glial cells, Schwann cells, melanocytes, chondrocytes, osteocytes, adipocytes, myoepithelial cells, dendritic cells, Langerhans cells, macrophages, and some types of epithelial cells. S100 is a widely used broad-spectrum marker, and different polyclonal or monoclonal antibodies directed to various members of the S100 family are available for routine immunohistochemistry.

**Diagnostic Pitfalls** S100 is a screening marker that lacks specificity, and the final diagnosis must be confirmed by additional more specific markers.

Further markers for endocrine and neuroendocrine tumors are listed in detail in related chapters.

## 14.2 Pituitary Gland Tumors

### 14.2.1 Diagnostic Antibody Panel for Tumors of the Anterior Pituitary Gland (Adenohypophysis)

Neuroendocrine markers (see previous chapter), cytokeratin profile, pituitary hormones (GH, PRL, TSH, ACTH, FSH, LH, α-SU), transcription factors (PIT-1, Tpit, GATA-2/GATA-3, and SF-1) [8].

The adenohypophysis is composed of six secretory cell types (α, β, δ, γ, ε cell), and all but one of them are able to produce only one of the anterior lobe hormones. The new classification of pituitary gland adenomas is based on the hormonal activity of the adenoma cells, which can be detected using specific antibodies to the pituitary gland hormones and hormone precursor molecules.

### 14.2.2 Pituitary Hormones

- Growth hormone (GH): GH is a 191 amino acid single chain polypeptide able to stimulate the release of insulin-like growth factor-1, which promotes the growth of long bones.
- Prolactin (PRL): PRL is a 198 amino acid polypeptide. Antibodies to PRL stain prolactin-producing normal and neoplastic cells of the pituitary gland. Prolactin-producing cells may also be found in prostatic glands.
- Thyroid-stimulating hormone (TSH): TSH is a glycoprotein consisting of the β- and α-chain regulating the T4 production in the thyroid gland.
- Adrenocorticotropic hormone (ACTH): ACTH is a 39 amino acid polypeptide that acts on the cells of the adrenal cortex. Besides cells of the adenohypophysis, ACTH can be synthesized by macrophages and lymphocytes in

response to stress. Pulmonary small cell carcinoma can also be positive for ACTH.
- Follicle-stimulating hormone (FSH): FSH is a glycoprotein consisting of the β- and α-chain regulating folliculogenesis, spermatogenesis, and proliferation of Sertoli cells.
- Luteinizing hormone (LH): LH is a glycoprotein consisting of the β- and α-chain regulating folliculogenesis and testosterone production in Leydig cells.
- α-Hormone subunit (α-SU): All glycoprotein hormones are composed of a 92 amino acid α-chain and a variable β-chain. The expression of the α-SU is found in most of the TSH-, FSH-, and LH-producing adenomas, whereas some pituitary gland adenomas exclusively express the α-SU.

## 14.2.3 Pituitary Transcription Factors

### 14.2.3.1 Pituitary Transcription Factor-1 (PIT-1, PUO1 F1)

PIT-1 is a member of the POU family of transcription factors regulating the differentiation of somatotroph, lactotroph, and thyrotroph cells of the pituitary gland and stimulating the synthesis of related hormones in these cells. Pit-1 is a marker for PIT1-lineage adenomas, including somatotroph, thyrotroph, lactotroph, and plurihormonal adenomas.

### 14.2.3.2 T-box Pituitary Transcription Factor (T-pit, T box 19)

T-pit is a transcription factor regulating the synthesis of pro-opiomelanocortin in corticotroph and melanotroph cells. T-pit is a marker for corticotroph adenoma and null cell adenoma (TPIT lineage adenomas).

### 14.2.3.3 Steroidogenic Factor 1 (SF-1)

This marker is listed in detail in a later chapter as a marker for adrenocortical tumors (see Chap. 14.6). SF-1 is strongly expressed in gonadotroph cells and is one of the markers for gonadotroph adenoma (SF-1 lineage adenoma).

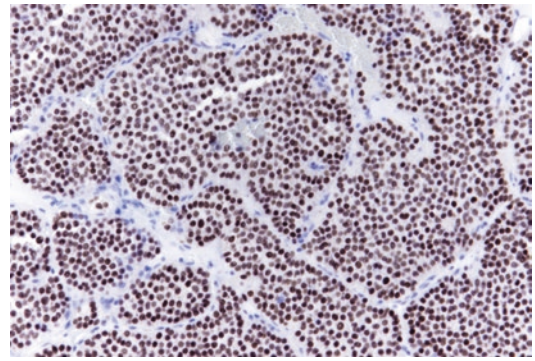


**Fig. 14.5** Pituitary adenoma with strong nuclear GATA-3 expression

### 14.2.3.4 GATA-2

GATA-2 is a transcriptional activator involved in the regulation of endothelin-1 gene expression in endothelial cells besides the differentiation and proliferation of hematopoietic and endocrine cells. GATA-2 is homologous to GATA-3 and, in immunohistochemistry, can be detected using the same antibody.

SF-1, in association with GATA-2/GATA-3 and estrogen receptor-α, regulate the differentiation of gonadotroph cells and are characteristic markers for gonadotroph adenoma (Fig. 14.5). TSH-producing and plurihormonal adenomas can also be positive for GATA-2/GATA-3. Somatotroph, lactotroph, and null cell adenomas lack the expression of both markers [9].

## 14.2.4 Diagnostic Antibody Panel for Tumors of the Posterior Pituitary Gland (Neurohypophysis)

GFAP, S100, TTF-1 (see also tumors of the central nervous system).

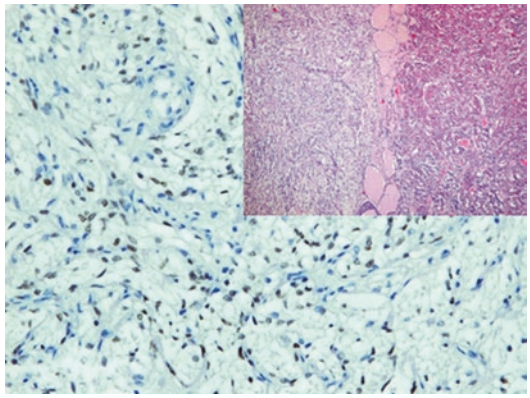


**Fig. 14.6** TTF-1 staining the cells of the neurohypophysis

#### 14.2.4.1 Thyroid Transcription Factor-1 (TTF-1)

TTF-1 was listed in detail as a marker for pulmonary and thyroid carcinomas (see Chap. 3). In addition to lung and thyroid cells, TTF-1 is also expressed in the cells of neurohypophysis (Fig. 14.6); consequently, TTF-1 is also a diagnostic marker for tumors derived from these cells, including pituicytoma and granular cell tumor of the sellar region [10, 11]. These tumors constantly lack the expression of Cytokeratins, which is important to consider in the differential diagnosis.

| Immunoprofile of pituitary gland tumors | | | | |
|---|---|---|---|---|
| Tumor type | + in >90% (+) | + in 50–90% (+/−) | + in 10–50% (−/+) | + in <10% (−) |
| A. Tumors of adenohypophysis | | | | |
| Pituitary adenoma (pituitary neuroendocrine tumor) General markers | Synaptophysin, chromogranin, INSM-1, NSE<br>Proliferation index (Ki-67):<br>In pituitary adenoma, <3%<br>In pituitary carcinoma, >12% | Pan-CK[a], EMA, GATA-3 | CD99 | Vimentin, CK5/6, CK7, CEA, Sox-10 |
| – Somatotroph adenoma | **GH**<br>**PIT-1** | Prolactin, TSH, FSH, LH, α-subunit, SSRT2, ER-α | | |
| – Lactotroph adenoma | **Prolactin**<br>**PIT-1** | α-Subunit, Galectin-3, ER-α | | |
| – Corticotroph adenoma | **ACTH**<br>**Tpit** | NeuroD1, α-subunit | | |
| – Gonadotroph adenoma | **LH**, **FSH**<br>**SF-1**, **GATA-2/GATA-3** | ER-α, α-subunit | | |
| – Thyrotroph adenoma | **TSH**<br>**PIT-1** | Prolactin, GATA-3, α-subunit | | |
| – Plurihormonal adenoma | | STH, TSH, LH, FSH, prolactin | | |
| – Null cell adenoma | Nonfunctional: no endocrine hormone secretion; no expression of pituitary transcription factors | | | |
| B. Tumors of neurohypophysis | | | | |
| Granular cell tumor of the sellar region (neurohypophysis) | **S100, TTF-1** | GFAP | TFE-3 | Neurofilaments, **Pan-CK**, HMB45, Olig-2, Synaptophysin, chromogranin, pituitary hormones |

| | | | | |
|---|---|---|---|---|
| Pituicytoma Grade 1 | MAP2, S100, **TTF-1**, vimentin Proliferation index (Ki-67): 1–2% | GFAP | EMA | Synaptophysin, chromogranin, Neurofilaments, Pan-CK, pituitary hormones, Olig-2, CD34 |
| Spindle cell oncocytoma | S100, **TTF-1**, bcl-2 | EMA | | Synaptophysin, chromogranin, Pan-CK, pituitary hormones |
| C. Tumors from the Rathke pouch epithelium | | | | |
| Craniopharyngioma | CK5/6, CK7, CK17, CK19, Claudin-1, β-catenin | p53 | CK18 | CK10, CK20, EMA, vimentin, GFAP |
| Pituitary blastoma | *In small cells:* Pan-CK, EMA *In secretory cells:* Synaptophysin, chromogranin, Pan-CK Proliferation index (Ki-67): 1–60% | | | Oct-4 |
| Rathke cleft cyst | Pan-CK, CK7, β-catenin | | | |

[a]Characteristic perinuclear expression pattern in somatotroph adenoma

## 14.3 Tumors of the Thyroid Gland

### 14.3.1 Diagnostic Antibody Panel for Tumors of Follicular Cell Origin

Thyroglobulin, thyroperoxidase, TTF-1, PAX-8, IGF2BP-1, cytokeratin profile [12].

### 14.3.2 Markers for the Evaluation of Malignancy

CD56, cytokeratin 19, Galectin-3, HBME-1.

### 14.3.3 Therapy-Related and Diagnostic Markers

BRAF-$_{V600E}$, NRAS-$_{Q61R}$, Trop-2, RET, ALK [13].

### 14.3.4 Diagnostic Antibody Panel for Tumors of C Cell Origin

Calcitonin, TTF-1, INSM-1, Islet-1, CEA, and other markers of neuroendocrine differentiation.

#### 14.3.4.1 Thyroglobulin

| Thyroglobulin | | |
|---|---|---|
| **Expression pattern:** cytoplasmic | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Follicular and papillary thyroid carcinomas | | Thyroid follicular cells |
| **Positive control:** thyroid tissue | | |

**Diagnostic Approach** Thyroglobulin is a glycoprotein synthesized by the thyroid follicular cells used as a substrate for the synthesis of thyroxin ($T_4$) and triiodothyronine ($T_3$). Thyroglobulin is a specific marker for thyroid follicular cells and follicular cell neoplasms. In diagnostic immunohistochemistry, it is recommended to use thyroglobulin in a panel with TTF-1 and PAX-8. Anaplastic thyroid carcinoma is usually negative for thyroglobulin. Thyroid parafollicular C cells and neoplasms originating from these cells constantly lack the expression of thyroglobulin.

Thyroperoxidase is a further specific marker for thyroid follicular cells. The expression of this

enzyme correlates with the differentiation grade of thyroid tumors and can be lost in poorly differentiated thyroid carcinomas.

#### 14.3.4.2 Thyroid Transcription Factor-1 (TTF-1)

TTF-1 is mentioned in detail with the markers of pulmonary tumors (Chap. 3). In addition to pulmonary adenocarcinoma, the expression of TTF-1 is characteristic for thyroid tissue and thyroid carcinomas. Follicular, papillary, and medullary thyroid carcinomas are typically strongly positive for TTF-1, whereas undifferentiated (anaplastic) thyroid carcinoma is usually negative (Fig. 14.7). In tumors with unknown primary, TTF-1 is to use in a panel with PAX-8 to discriminate between primary pulmonary adenocarcinoma and thyroid carcinomas.

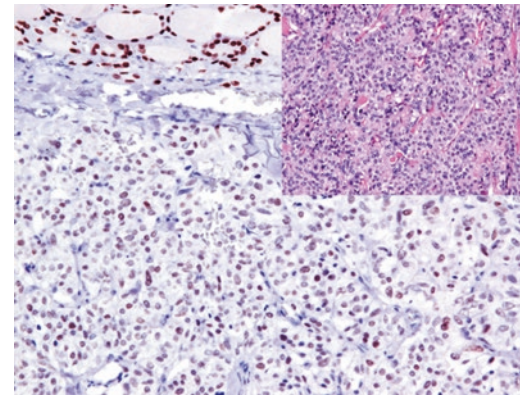


**Fig. 14.7** Medullary thyroid carcinoma. TTF-1 staining nuclei of the tumor cells. Note intensive nuclear staining in normal follicular cells

#### 14.3.4.3 Thyroid Transcription Factor 2 (TTF-2)

TTF-2 is a nuclear protein involved in the synthesis of thyroglobulin and thyroperoxidase, expressed in thyroid follicular cells and related thyroid tumors in addition to a small subset of parafollicular C cells, anterior pituitary gland, esophageal and tracheal mucosa, and seminiferous tubes [14]. Pulmonary parenchyma, gastrointestinal and hepatopancreatic epithelium, and corresponding tumors are constantly negative for TTF-2.

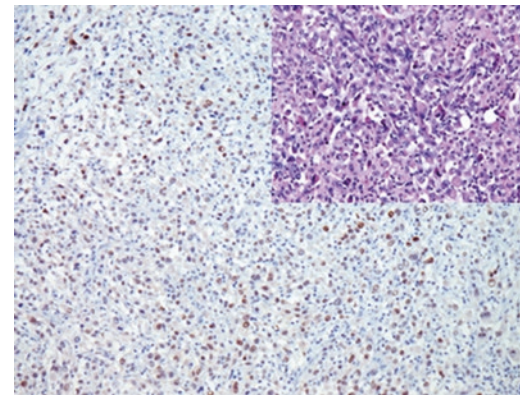


**Fig. 14.8** Nuclear PAX-8 staining of anaplastic thyroid carcinoma cells

#### 14.3.4.4 PAX-8

PAX-8 is a transcriptional factor involved in the fetal development of the brain, eye, thyroid tissue, and upper urinary system, as well as organs of Müllerian origin. PAX-8 labels normal thyroid follicular cells and more than 90% of papillary and follicular thyroid carcinomas, including Hürthle cell carcinoma in addition to thyroid squamous cell carcinoma and in the majority of poorly differentiated thyroid carcinoma and more than 50% of anaplastic thyroid carcinomas (Fig. 14.8). Medullary thyroid carcinoma is usually negative for PAX-8 or shows patchy weak expression. Pulmonary adenocarcinomas and breast carcinoma are constantly negative for PAX-8. It is important to consider that parathyroid tissue and parathyroid tumors, in addition to thymoma and other different neuroendocrine tumors in the head and neck region, may be positive for PAX-8. It is important to consider that PAX-2 shows in normal and neoplastic thyroid tissue a different expression behavior than PAX-8 and cannot be used as equivalent markers. PAX-8 is listed in detail with the markers of genitourinary tumors in a previous chapter.

#### 14.3.4.5 Insulin-Like Growth Factor 2 mRNA Binding Protein 1 (IGF2BP-1)

IGF2BP-1 is an oncofetal protein that regulates the transcription and splicing of different genes by binding to the mRNAs, including insulin-like growth factor 2 [15]. IGF2BP1 is found to be a marker for anaplastic thyroid carcinoma. Other carcinomas of thyroid follicular cells, including papil-

lary thyroid carcinoma, follicular thyroid carcinoma, and high-grade follicular cell-derived non-anaplastic thyroid carcinoma (poorly differentiated thyroid carcinoma), lack the expression of IGF2BP-1 [16].

#### 14.3.4.6 Galectin-3

Galectin-3 is 1 of the 14 members of the galactosidase binding protein family normally expressed in endothelial cells and peripheral nerves. The Galectin-3 expression is stimulated during the malignant transformation, which makes it a helpful marker for the diagnosis of different carcinoma types. Galectin-3 is positive in most papillary, follicular, and undifferentiated thyroid carcinomas, as well as in parathyroid carcinoma, head and neck squamous cell carcinoma, and colorectal and hepatocellular carcinoma.

#### 14.3.4.7 HBME-1

HBME-1 (Hector Battifora mesothelial cell 1) was initially recognized as a marker expressed on the microvilli of normal and neoplastic mesothelial cells. HBME-1 is also expressed on the membrane of different normal epithelial and carcinoma cells, including pulmonary, breast, pancreatic, and ovarian adenocarcinomas. Furthermore, HBME-1 is strongly expressed in papillary and follicular thyroid carcinomas but negative or weakly positive in hyperplastic thyroid tissue and benign thyroid lesions. Similar to CK19, CD56, and Galectin-3, HBME-1 may be helpful in differentiating between malignant and benign encapsulated thyroid lesions.

#### 14.3.4.8 Trophoblastic Cell Surface Antigen 2

| Trophoblastic cell surface antigen 2 (Trop-2) | | |
|---|---|---|
| **Expression pattern:** membranous/cytoplasmic | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Papillary thyroid carcinoma<br>– Gastrointestinal and pancreatic carcinomas | Carcinomas of the breast, lung, uterus, uterine cervix and ovaries, bladder, and prostate | Epithelium of salivary glands, pancreas, bile ducts, breast, uterus, prostate, squamous epithelium |
| **Positive control:** prostatic tissue | | |

**Diagnostic Approach** Trophoblast cell surface antigen 2 (Trop-2), also known as tumor-associated calcium signal transducer 2, is a type I transmembrane glycoprotein functioning as a calcium signal transducer. Low baseline Top-2 expression is found in different normal tissue types such as the breast, pancreas, ovaries, lung, and kidney. During malignant transformation, the expression of Trop-2 is upregulated, and overexpression of Trop-2 is noticed in different carcinoma types, including gastrointestinal, pulmonary, genitourinary, and breast carcinomas [17]. In the majority of tumors, the overexpression of Trop-2 correlates with aggressive behavior and poor prognosis.

In routine histopathology, Trop-2 is a helpful marker for the diagnosis of different histological types of papillary thyroid carcinomas. More than 90% of papillary thyroid carcinomas express Trop-2, while benign thyroid nodules, follicular adenomas, and follicular carcinomas usually lack the expression of this protein [18]. Trop-2 can be used in combination with CD56 and CK19.

As a cell surface protein, Trop-2 is an interesting target for specific humanized therapeutic antibodies and specific inhibitors in different carcinoma types exhibiting Trop-2 overexpression, such as triple-negative breast carcinoma (Fig. 14.9).

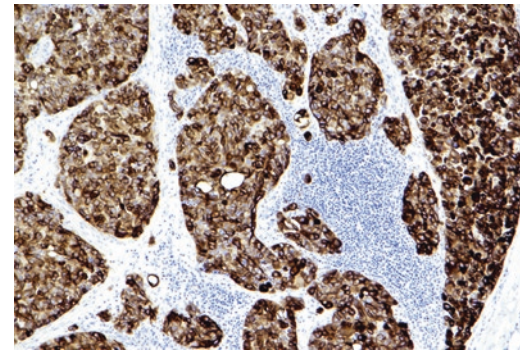


**Fig. 14.9** Metastatic triple-negative breast carcinoma exhibiting Trop-2 overexpression

#### 14.3.4.9 CD44v6

CD44v6 is an isoform of CD44 (heparan sulfate proteoglycan), a surface glycoprotein functioning as a cell-to-cell and cell-to-matrix mediator. CD44 is expressed in different carcinoma types, including breast, colonic, hepatocellular, and renal cell carcinomas, in addition to papillary and follicular thyroid carcinoma. In combination with other markers, CD44v6 can be a helpful marker to differentiate between papillary carcinoma and other benign thyroid lesions mimicking this carcinoma type. CD44 is also helpful in differentiating between reactive urothelium and carcinoma in situ (see respective section).

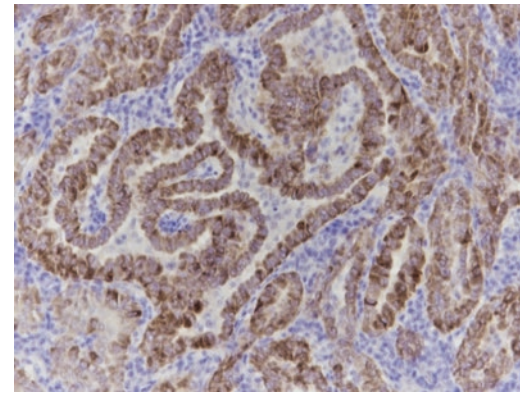


**Fig. 14.10** Papillary thyroid carcinoma exhibiting strong cytoplasmic staining with the antibody to BRAF-V600E

#### 14.3.4.10 BRAF

The RAF (**r**apidly **a**ccelerated **f**ibrosarcoma) kinase family includes three isoforms, ARAF, BRAF, and CRAF, which are cytoplasmic serine–threonine kinases that play an important role in the RAS–RAF–ERK kinase signaling pathway as the mitogen-activated protein kinase (MAPK) cascade. Among the three RAF kinases, mutations occur mainly in the BRAF gene located on chromosome 3, causing the activation of the MAPK signaling pathway and uncontrolled kinase activity affecting cell proliferation and differentiation [19]. BRAF mutations are among the most common mutations in human malignancies, found in ~50% of malignant melanoma, ~70% of papillary thyroid carcinoma, up to 10% of colorectal adenocarcinomas, >95% of hairy cell leukemia, ~65% of Langerhans cell histiocytosis, and >90% of papillary craniopharyngioma. About 60 variant mutations are described within the BRAF gene, whereas the most common mutation occurs at position 1799 in the nucleotide sequence, causing the substitution of thymine to adenine resulting in a valine to glutamic acid replacement in the encoded amino acid sequence at residue 600 (V600E). The BRAF-V600E variant makes ~99% of all BRAF mutations associated with papillary thyroid carcinoma and is found in about 50% of adult papillary thyroid carcinoma and ~95% of tall cell papillary thyroid carcinoma. BRAF-V600E negative papillary thyroid carcinoma is frequently associated with other mutations within the genes encoding further molecules of the MAPK signaling pathway, including RET, NTRK, and ALK. Different BRAF mutation variants are also found in poorly differentiated and anaplastic thyroid carcinomas but are rarely associated with radiation-induced thyroid carcinomas and are not characteristic for follicular thyroid carcinoma. The BRAF-V600E mutation is absent in follicular and Hürthle cell carcinoma as well as in medullary thyroid carcinoma.

In routine immunohistochemistry, the mutated V600E amino acid sequence can be detected using different specific antibodies and considered as a diagnostic marker and therapeutic target (Fig. 14.10).

**Diagnostic Pitfalls** The available antibodies can only detect a specific mutated amino acid sequence, mainly the BRAF-V600E variant. To detect other possible variants, the molecular sequencing of the complete BRAF gene is required.

#### 14.3.4.11 RAS

The Ras proteins (KRAS, HRAS, and NRAS) are a group of closely related proteins with high sequence homology expressed in all mammalian cells and encoded by different genes discussed in Chap. 35. In thyroid tumors, RAS mutations (mostly NRAS) are described as the second most common genetic mutations and play an important role in thyroid oncogenesis. The NRAS mutations are mainly found in association with thy-

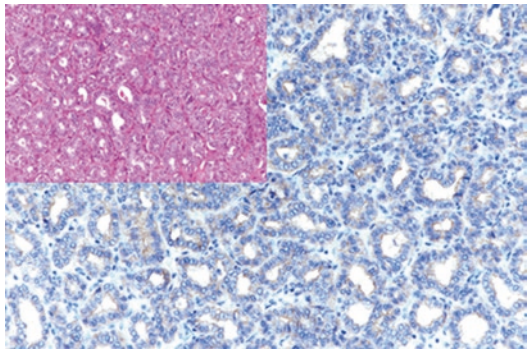


**Fig. 14.11** Papillary thyroid carcinoma exhibiting moderate cytoplasmic staining with the antibody to NRAS-Q61R

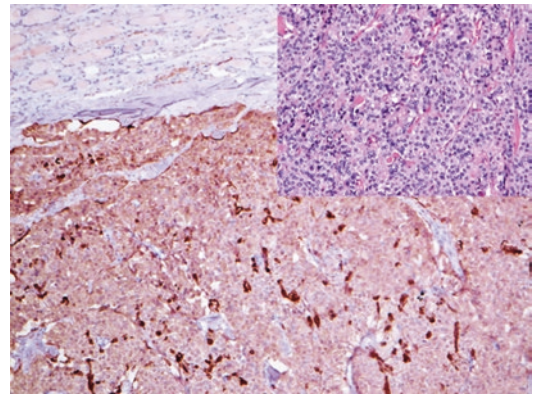


**Fig. 14.12** Medullary thyroid carcinoma exhibiting cytoplasmic expression of calcitonin in the tumor cells

roid tumors with follicular morphology, including follicular adenoma, 40–50% of follicular carcinoma, and 10–20% of follicular variant of papillary thyroid carcinoma. The NRAS-Q61R mutation is the most common variant found in up to 65% of NRAS mutated cases. The mutated NRAS-Q61R protein can be effectively detected by immunohistochemistry using specific antibodies (Fig. 14.11) [20].

### 14.3.4.12 Calcitonin

| Calcitonin | | |
|---|---|---|
| **Expression pattern:** cytoplasmic | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Medullary thyroid carcinoma | Neuroendocrine carcinoma | Thyroid parafollicular C cells |
| **Positive control:** thyroid tissue/medullary thyroid carcinoma | | |

**Diagnostic Approach** Calcitonin is a polypeptide hormone synthesized by the parafollicular C thyroid cells involved in the regulation of calcium and phosphorus metabolism, principally contracting the effect of parathyroid hormone. Calcitonin is a specific marker for the parafollicular cells and tumors originating from these cells, namely, medullary thyroid carcinoma (Fig. 14.12). Tumors originating from the thyroid follicular cells are constantly negative for calcitonin but also positive for TTF-1 and PAX-8. Best stain results are obtained using monoclonal antibodies.

**Diagnostic Pitfalls** Rare cases of neuroendocrine tumors such as neuroendocrine carcinoma of the larynx and pheochromocytoma are reported to be positive for calcitonin, whereas the latter is usually negative for TTF-1.

| Immunoprofile of thyroid tumors | | | | |
|---|---|---|---|---|
| Tumor type | + in >90% (+) | + in 50–90% (+/−) | + in 10–50% (−/+) | + in <10% (−) |
| Follicular thyroid adenoma | **Thyroglobulin**, thyroid peroxidase, **TTF-1**, **PAX-8**, Pan-CK | CK7 | CK19 | CK5/6, CK20, calcitonin, CD44V6, Trop-2, Galectin-3, $BRAF\text{-}_{V600E}$ |
| Follicular thyroid carcinoma/oncocytic carcinoma (Hürthle cell carcinoma) | **Thyroglobulin**, thyroid peroxidase, **TTF-1**, **PAX-8**, CK7, CK8, CK18, CD44V6, S100 | Galectin-3, HBME1, E-cadherin, bcl-2, vimentin | CK19, $NRAS\text{-}_{Q61R}$ | Calcitonin, CK5/6, CK20, Islet-1, INSM-1, Trop-2, CEA, PAX-2, $BRAF\text{-}_{V600E}$ |

| | | | | |
|---|---|---|---|---|
| Papillary thyroid carcinoma | **Thyroglobulin**, thyroid peroxidase, **TTF-1**, **PAX-8**, **Trop-2**, CK1, CK7, CK8, CK18, **CK19**[a], p63[a], Galectin-3[a], CD44V6, HBME-1 | CK5/6/14, EMA, CD15, **BRAF-$_{V600E}$**, CD44[a], vimentin | CD15, CK17, CD34, ER, PgR | CK20, CEA, calcitonin, Islet-1, INSM-1, CD56[a], Synaptophysin, chromogranin, PAX-2 |
| Follicular-derived carcinoma, high grade (poorly differentiated thyroid carcinoma) | **Thyroglobulin**, thyroid peroxidase, **TTF-1**, **PAX-8**, Pan-CK, Galectin-3, CD44V6 | Vimentin, bcl-2 | p53 | CK5/6, CK19, CK20, calcitonin, INSM-1 |
| Anaplastic thyroid carcinoma | Pan-CK, CK8/18, **IGF2BP-1** | CK19, **PAX-8**, CEA, p53, cyclin D1, CD10, vimentin | TTF-1, EMA, Galectin-3, bcl-2 | Thyroglobulin, calcitonin, INSM-1 |
| Medullary thyroid carcinoma | **Calcitonin**, **INSM-1**, **Islet-1**, chromogranin, Synaptophysin, **TTF-1**, CD56, Leu7, S100, NSE, **CEA**, vimentin (in spindle cell components), CK7, CK8, CK18, HER-2, Synapsin I | Bcl-2 | CK19, Galectin-3 | **PAX-8**, CK5/6, thyroglobulin, CK20, BRAF-$_{V600E}$ |
| Cribriform morular thyroid carcinoma | *Cribriform component:* TTF-1, β-catenin<br>*Morulae:* CK5, CD5, CD10, CDX-2 | | PAX-8, ER, PgR | Thyroglobulin, CK20, calcitonin, BRAF TTF-1, PAX-8, p40, p63 |
| Carcinoma showing thymus-like differentiation (CASTLE)/ intrathyroid thymic carcinoma | Pan-CK CK5/14, p63, p40, CD5, CD117 | | | TTF-1, calcitonin |
| Spindle epithelial tumor with thymus-like differentiation (SETTLE) | CK7 | CD117 | | TTF-1, calcitonin, CD5, S100 |
| Primary squamous cell carcinoma of the thyroid | CK5/6/14, p63, p40, **PAX-8** | | TTF-1 | |
| Hyalinizing trabecular tumor | **Thyroglobulin**, **TTF-1**, Ki-67 (MIB-1 clone)[b] | CK 7, Galectin-3 | | |

[a]See table below
[b]Atypical membranous and cytoplasmic stain patterns may be noted when the MIB clone is used as a characteristic stain pattern for this tumor type

Immunohistochemical markers for differentiation between papillary thyroid carcinoma (PTC), benign pseudopapillary hyperplasia (BPH), and follicular neoplasms (FN)

- **CK19:** positive in PTC but negative or weakly positive in FN, except for chronic lymphocytic thyroiditis (Fig. 14.13)
- **Galectin-3:** positive in PTC and follicular carcinoma but negative in benign thyroid tissue
- **CD56:** negative in PTC but positive in benign thyroid tissue, BPH, and FN (Fig. 14.14) [21]
- **p63:** focal expression in PTC; constantly negative in non-PTC lesions
- **Trop-2:** positive in >90 PTC but negative in follicular adenoma/carcinoma
- **HBME-**1: positive in PTC and follicular carcinoma but negative or weakly positive in benign thyroid tissue
- **BRAF-V600E:** positive in ~50% PTC but negative in FN and BPH (Fig. 14.10)
- **CD44:** positive in PTC and follicular carcinoma but negative or weakly positive in BPH and benign thyroid tissue

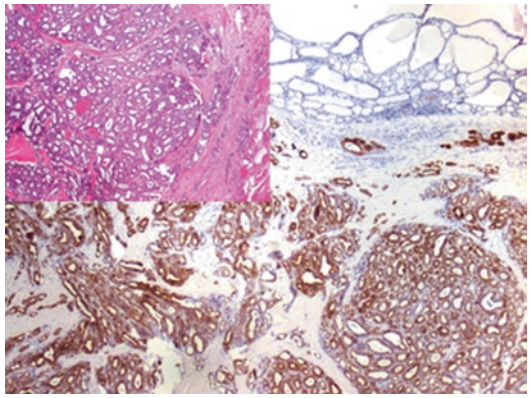


**Fig. 14.13** CK19 highlighting the cells of papillary thyroid carcinoma. Normal thyroid tissue lacks CK19 expression

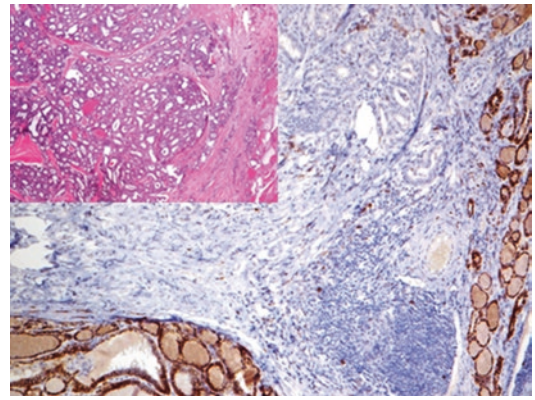


**Fig. 14.14** CD56 staining normal thyroid tissue, whereas areas infiltrated by papillary thyroid carcinoma lack CD56 expression

## 14.4 Tumors of the Parathyroid Gland

### 14.4.1 Diagnostic Antibody Panel for Parathyroid Neoplasms

Parathyroid hormone, PAX-8, GATA-3, CD4, Thyroglobulin, TTF-1 [22].

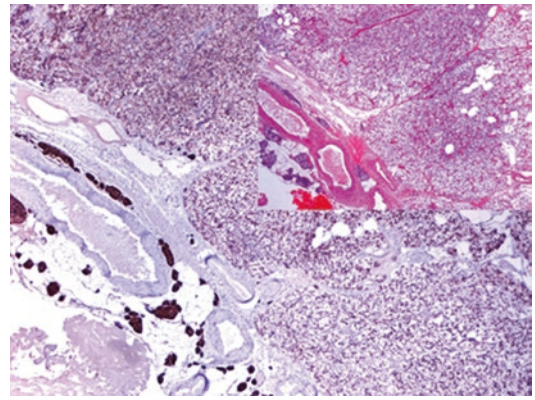


**Fig. 14.15** Parathyroid hormone labeling parathyroid tissue and cells of parathyroid adenoma

#### 14.4.1.1 Parathyroid Hormone

| Parathyroid hormone (PTH) | | |
|---|---|---|
| **Expression pattern:** cytoplasmic | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| Parathyroid tissue and neoplasms | Ovarian small cell carcinoma of hypercalcemic type, pheochromocytoma | Parathyroid chief cells, fetal tissue (CNS, lung, gastrointestinal tract) |
| **Positive control:** parathyroid | | |

**Diagnostic Approach** Parathyroid hormone (parathormone, PTH) is a polypeptide hormone secreted by the chief cells of the parathyroid glands. PTH and calcitonin are directly responsible for the regulation of calcium and phosphate levels in serum. Antibodies to PTH and related peptides are specific markers for the diagnosis of parathyroid neoplasms. PTH is helpful in recognizing ectopic parathyroid tissue and tumors, which may be situated in the mediastinum or intrathymic (Fig. 14.15).

**Diagnostic Pitfalls** Parathyroid chief cells usually rapidly discharge PHT after the synthesis, which may cause false negative immunohistochemical results. More challenging are nonsecretory clear cell parathyroid carcinomas, which may resemble metastatic renal cell carcinoma or any

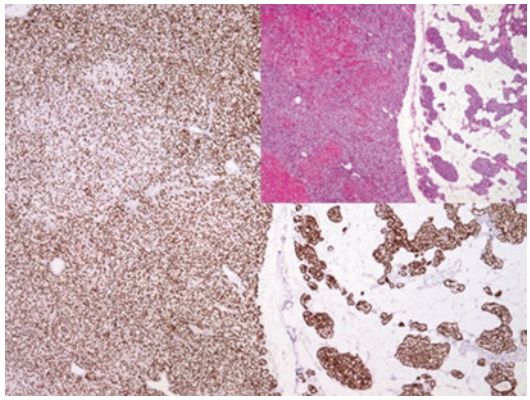


**Fig. 14.16** GATA-3 staining cells of the suppressed parathyroid gland (right) and neighboring parathyroid adenoma

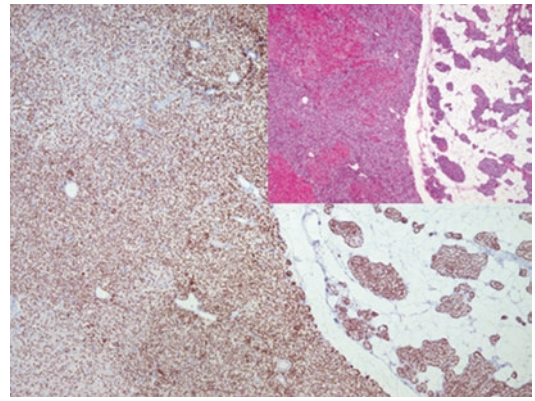


**Fig. 14.17** PAX-8 staining cells of the suppressed parathyroid gland (right) and neighboring parathyroid adenoma

other clear cell carcinoma. The diagnostic panel for thyroid/parathyroid tumors must include thyroid and parathyroid hormones in addition to other differentiation markers such as PAX-8 and GATA-3.

#### 14.4.1.2 Parathyroid Hormone-Related Peptide

This polypeptide (PtHrP) is a member of the parathyroid hormone family, also involved in calcium metabolism, and regulates the endochondral bone development. Antibodies to PtHrP stain parathyroid cells and parathyroid tumors in addition to several other malignant tumors such as breast carcinoma, cholangiocarcinoma, and transitional cell carcinoma, especially poorly differentiated types. PtHrP can also be used as a marker to discriminate between cholangiocarcinoma and metastatic colorectal adenocarcinoma [23, 24].

#### 14.4.1.3 PAX-8, GATA-3, and CD4

Both transcription factors were listed in detail in previous chapters as markers for breast, renal, and urinary tract tumors (Chaps. 10 and 12.1). PAX-8 and GATA-3 also label parathyroid tissue and parathyroid tumors, including adenoma and carcinoma, with the characteristic nuclear pattern, and can be used in a panel as parathyroid markers (Figs. 14.16 and 14.17) [25]. It is important to remember that PAX-8 labels also thyroid follicular cells and tumors.

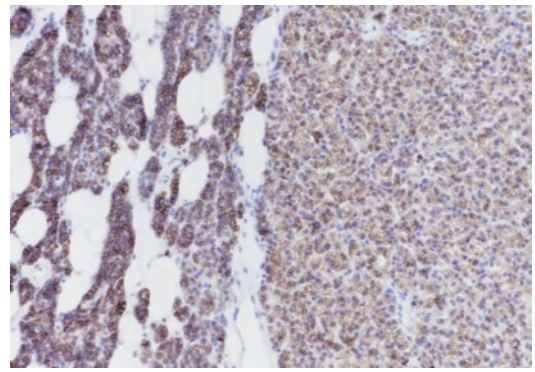


**Fig. 14.18** Parathyroid gland (left) and parathyroid adenoma (right) with strong membranous CD4 expression in both normal and neoplastic cells

CD4 is a marker for T lymphocytes listed. CD4 labels also the chief cells of the parathyroid gland. Parathyroid adenomas and carcinomas are also positive for CD4, and the expression intensity correlates with the differentiation grade of the neoplasia (Fig. 14.18).

| Immunoprofile of parathyroid tumors | | | | |
|---|---|---|---|---|
| Tumor type | + in >90% (+) | + in 50–90% (+/−) | + in 10–50% (−/+) | + in <10% (−) |
| Parathyroid adenoma/ parathyroid lipoadenoma | **PTH**, Synaptophysin, chromogranin, Neurofilaments, Pan-CK, CK8, CK18, CK14[a]. PAX-8, GATA-3, CD4 Proliferation index (Ki-67): <5% | CK19, RCC (gp200), vimentin | Cyclin D1, calcitonin, CK7, CK20 | **TTF-1**, thyroglobulin, INSM-1, CD56, CK5/6/14 |

| | | | | |
|---|---|---|---|---|
| Atypical parathyroid tumor and parathyroid carcinoma | Synaptophysin, chromogranin, Neurofilaments, PGP9.5, Pan-CK Proliferation index (Ki-67): >6% | **PTH**, CK19, PAX-8, GATA-3, cyclin D1[b], CD4, vimentin | Calcitonin, Galectin-3, p53, CK7 | Thyroglobulin, CK5/6/14, CK20, TTF-1, CD56[c], INSM-1 |

[a]Negative in parathyroid carcinoma
[b]See Fig. 14.19
[c]May be positive in oxyphil parathyroid adenoma

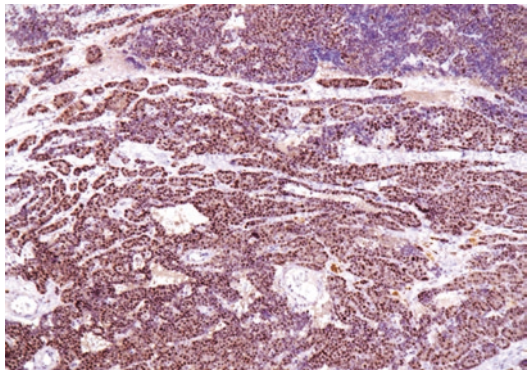


**Fig. 14.19** Parathyroid carcinoma with nuclear Cyclin D1 expression

## 14.5 Pancreatic Endocrine Tumors

### 14.5.1 Diagnostic Antibody Panel for Pancreatic Endocrine Tumors

Islet-1, PDX-1, insulin, gastrin, glucagon, somatostatin receptor, vasoactive intestinal polypeptide (VIP) and human pancreatic polypeptide (hPP), PAX-6, PAX-8, progesterone receptors, and general screening neuroendocrine markers.

The immunophenotype of pancreatic endocrine tumors and the description of related immunohistochemical markers are listed in the chapter on pancreatic tumors (see Chap. 8).

## 14.6 Tumors of the Adrenal Gland

### 14.6.1 Diagnostic Antibody Panel for Adrenocortical Tumors

Adrenal 4 binding protein (Ad4BP, SF-1), DAX-1, inhibin, Melan A, Calretinin, Synaptophysin, Podoplanin, and WT-1 [26].

#### 14.6.1.1 Adrenal 4 Binding Protein/Steroidogenic Factor-1

| Adrenal 4 binding protein/steroidogenic factor-1 (SF-1, Ad4BP) | | |
|---|---|---|
| **Expression pattern:** nuclear | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Adrenocortical tumors | Sex cord-stromal tumors (granulosa cell tumor, Sertoli cell tumor, fibroma and fibrothecoma), gonadotroph pituitary adenoma | Adrenal cortex, ovarian stromal cells, Sertoli cells, red pulp sinus cells of the spleen, gonadotrophic cells in the anterior pituitary gland |
| **Positive control:** adrenal gland | | |

**Diagnostic Approach** Adrenal 4 binding protein (Ad4BP), also known as steroidogenic factor 1 (SF-1), is a member of the orphan nuclear receptor family and is a transcriptional factor regulating steroidogenesis.

Initially, SF1 is expressed in the developing urogenital ridge; in adult tissue, it is constantly expressed in the pituitary gland, neurons of the ventromedial nucleus of the hypothalamus, adrenal cortex, testicular Sertoli and Leydig cells, granulosa cells, and different tumors derived from these tissue and cell types (Fig. 14.20). SF-1 is constantly negative in renal cell carcinoma, hepatocellular carcinoma, melanoma, and pheochromocytoma. Generally, the positivity to Synaptophysin, Melan A, Inhibin, D2–40, and

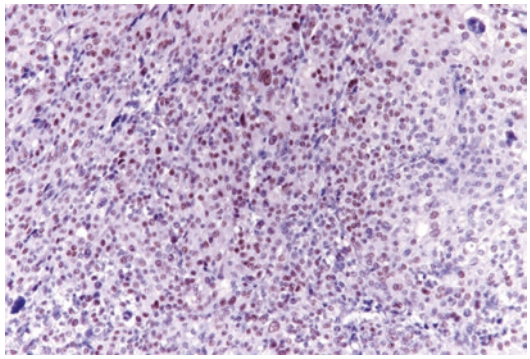


**Fig. 14.20** Nuclear SF-1 expression in the cells of adrenocortical adenoma

Calretinin and the co-expression of Vimentin support the adrenocortical origin of the tumor [27–29]. SF-1 is also helpful for the classification of pituitary adenomas as it is selectively expressed in gonadotroph adenomas.

**Diagnostic Pitfalls** Clinical and paraclinical data must be considered to diagnose metastatic adrenocortical carcinoma, as the morphology and immunoprofile of sex cord-stromal tumors may be very similar to those of adrenocortical tumors.

#### 14.6.1.2 DAX-1

DAX-1 is a nuclear receptor protein and a member of the orphan nuclear receptor family encoded by the NR0B1 gene (**n**uclear **r**eceptor subfamily **0** group **B** member **1**) located on chromosome Xp21 acting as a suppressor for the steroid hormone production in the adrenal cortex by inhibiting the effect of the steroidogenic factor 1 (SF-1) [30, 31]. Furthermore, DAX-1 plays an active role in the development of the hypothalamic–pituitary–adrenal–gonadal axis and the differentiation of osteoblasts. The expression of the DAX-1 transcription factor is restricted to steroid-producing cells, including those of the adrenal cortex, pituitary gland and hypothalamus, testis, and ovary. Similar to SF-1, DAX-1 is a marker of adrenocortical tumors and other types of ovarian, testicular, and breast tumors.

DAX-1 is also found to be a specific marker for Ewing's sarcoma due to the genetic alterations caused by the EWS/Fli-1 translocation prompting the expression of DAX-1 [32, 33].

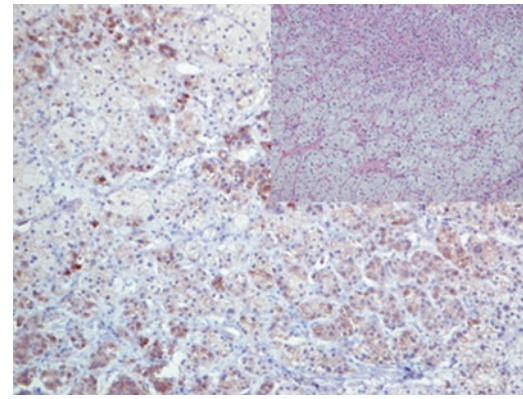


**Fig. 14.21** Adrenocortical adenoma exhibiting cytoplasmic expression of inhibin

#### 14.6.1.3 Inhibin

Inhibin is a glycoprotein hormone listed as a marker for sex cord tumors. Inhibin is normally expressed in the gonads and adrenal glands, whereas the strongest expression in the adrenal gland is found in the zona fasciculate and reticulares of the cortex. The adrenal medulla lacks the expression of Inhibin.

Besides testicular and ovarian sex cord tumors, Inhibin is an important marker for benign and malignant adrenocortical tumors (Fig. 14.21) [34].

#### 14.6.1.4 Melan A

Melan A is listed in the chapter on melanoma markers (Chap. 21). Melan A is also a marker for adrenal cortex cells and adrenocortical tumors. Characteristic for adrenal cortical cells and adrenocortical tumors is the strong granular cytoplasmic expression pattern of Melan A.

#### 14.6.1.5 CYP11B2

CYP11B2 is an enzyme that catalyzes the biosynthesis of aldosterone, normally expressed in the zona glomerulosa of the adrenal cortex. The immunohistochemical stain of the adrenal gland with the CYP11B2-specific antibodies labels the functional areas of aldosterone production in the adrenal cortex. CYP11B2 is an immunohistochemical marker for aldosterone-producing adrenal cortical adenoma.

CYP11B1 is a further enzyme of the adrenal cortex that catalyzes the biosynthesis of cortisol, physiologically expressed in the zona fasciculata/reticularis and related pathological lesions.

### 14.6.2 Markers and Immunoprofile of Tumors of the Adrenal Medulla and Extra-Adrenal Paraganglia

#### 14.6.2.1 Diagnostic Antibody Panel for Pheochromocytoma and Tumors of Extra-Adrenal Paraganglia

Chromogranin, Synaptophysin, INSM-1, Islet-1, CD56, GATA-3, NSE, S100.

These antibodies were listed in detail in other chapters (Figs. 14.22, 14.23 and 14.24).

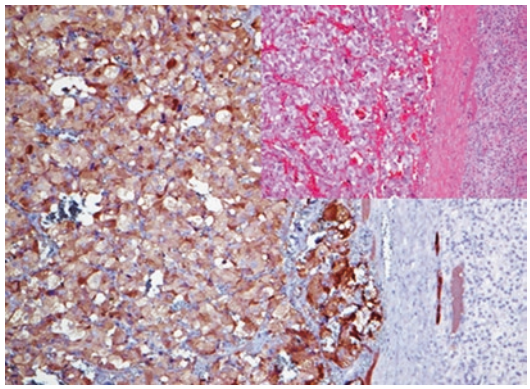


**Fig. 14.22** Pheochromocytoma with strong CD56 expression

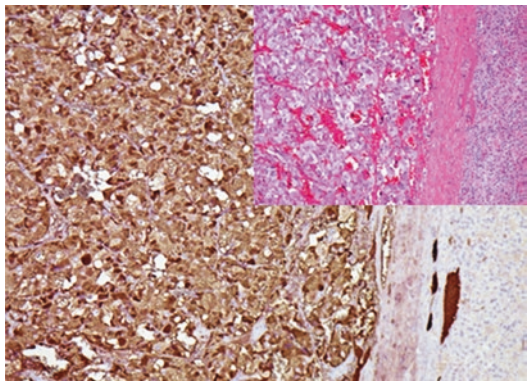


**Fig. 14.23** Pheochromocytoma exhibiting strong Synaptophysin expression

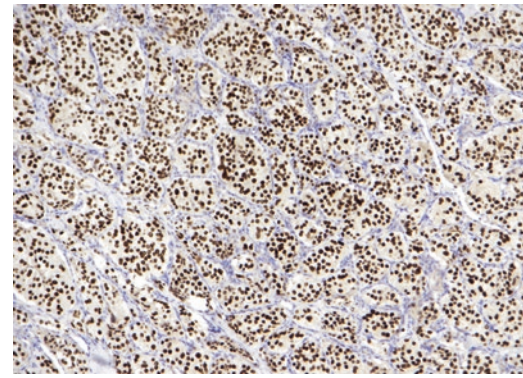


**Fig. 14.24** Extra-adrenal paraganglioma with strong nuclear INSM-1 expression in the tumor cells

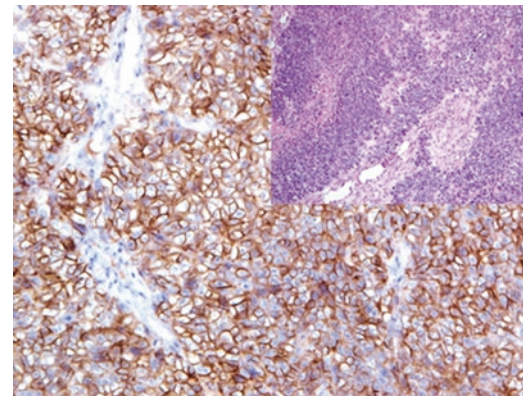


**Fig. 14.25** CD56 staining the membrane of olfactory neuroblastoma cells

#### 14.6.2.2 Diagnostic Antibody Panel for Neuroblastoma

Chromogranin, Synaptophysin, INSM-1, Islet-1, CD56, NSE, NB84, PGP9.5, PHOX2B, GATA-3, CD117, S100, and neurofilaments (Fig. 14.25) [35].

The tumors mentioned above are of neuroectodermal origin and usually lack the expression of cytokeratins, and the general neuroendocrine markers are characteristic for these tumors (Figs. 14.22, 14.23 and 14.24).

##### Neural Cell Adhesion Molecule (CD56)

CD56 is a member of the immunoglobulin superfamily clustered as CD56 functioning as a media-

tor of cell-to-cell adhesion and cell-to-matrix interaction, involved in the regulation of cell adhesion, synaptic plasticity, migration, proliferation, differentiation, and apoptosis. CD56 is an important molecule for developing and differentiating the nervous system. Normally, CD56 is expressed on neuroectodermal cells, glial cells, myoblasts, skeletal muscle, neuromuscular junctions, and tumors derived from these cell types (Fig. 14.24). CD56 is a helpful wide-spectrum marker for neural and neuroendocrine tumors. Furthermore, CD56 is also expressed on the NK cells and activated T cells playing an important role in the immune reaction. In routine immunohistochemistry, CD56 is used as a marker for NK neoplasms.

### NB84

| NB84 | | |
|---|---|---|
| **Expression pattern:** membranous | | |
| **Main diagnostic use** | **Expression in other tumors** | **Expression in normal cells** |
| – Neuroblastoma | Ewing's sarcoma/PNET, medulloblastoma, desmoplastic small round cell tumor | |
| **Positive control:** neuroblastoma | | |

**Diagnostic Approach** NB84 is a membranous antigen isolated from human neuroblastoma cells. It stains about 100% of differentiated and about 90% of undifferentiated neuroblastomas. NB84 is more sensitive but less specific than Synaptophysin [36]. For an appropriate diagnosis of adrenal or extra-adrenal tumors, a panel of three to four of the antibodies mentioned above is recommended.

**Diagnostic Pitfalls** NB84 may be positive in other tumors with similar morphology, including PNET and desmoplastic small round cell tumor. To exclude these tumors, an antibody panel that includes CD99 and different cytokeratins is required. It is essential to consider that about 5% of undifferentiated neuroblastomas lack the expression of NB84.

### Paired Mesoderm Homeobox Protein 2B (PHOX2B)

PHOX2B is a transcription factor encoded by the PHOX2B gene on chromosome 4p13, essential for the differentiation and maturation of sympathetic neurons and chromaffin cells. The expression of PHOX2B is limited to the cells of the autonomic nervous system, mainly to the cells originating from neural crest precursors. The expression of PHOX2B is demonstrated in all neuroblastoma, ganglioneuroblastoma, and ganglioneuroma cases as well as in about 40% of paragangliomas (Fig. 14.26).

Other small round blue cell tumors such as rhabdomyosarcoma, Ewing sarcoma, and different lymphoma types, in addition to epithelial neuroendocrine neoplasms, are negative for PHOX2B. Unlike GATA-3, epithelial tumors such as squamous cell carcinoma, different types

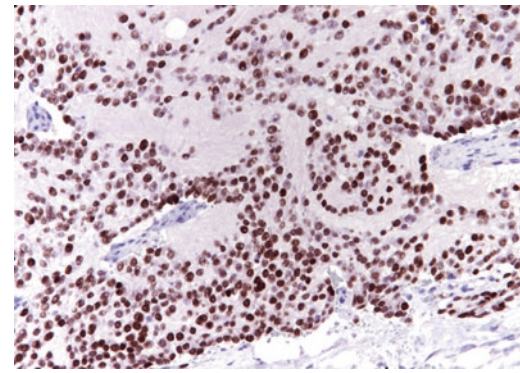


**Fig. 14.26** Nuclear PHOX2B expression in the cells of neuroblastoma

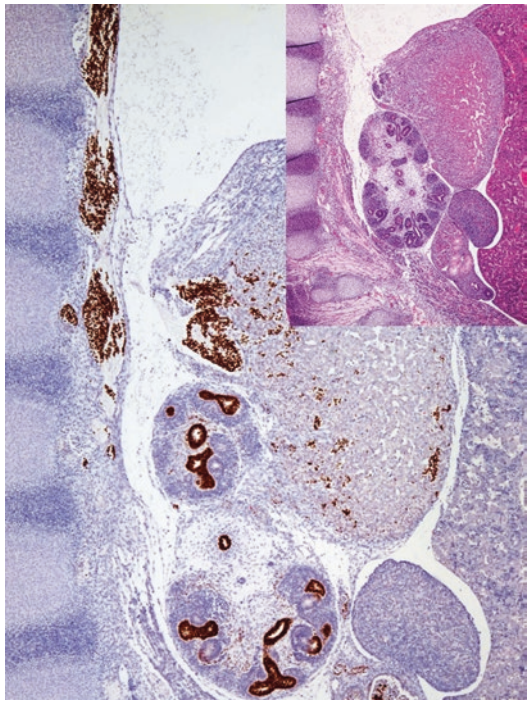


**Fig. 14.27** Section through a 12-week embryo showing paravertebral sympathicoblasts of neural crest labeled by GATA-3. These sympathicoblasts migrate into the dorsomedial part of the primordial adrenal gland to form the adrenal medulla. GATA-3 is also highlighting the urothelium of the collecting system of the kidney

of adenocarcinomas, and transitional cell carcinoma are negative for PHOX2B [37, 38].

### GATA-3

This transcription factor was listed in previous chapters as a marker for breast, salivary gland, parathyroid, and urothelial tumors (Chaps. 10). GATA-3 strongly labels the fetal sympathicoblasts and the chromaffin cells of the adrenal medulla and sympathetic paraganglia derived from sympathicoblasts (Fig. 14.27). Consequently, GATA-3 is a marker for tumors of the adrenal medulla and extra-adrenal paraganglia, including pheochromocytoma and neuroblastoma (Figs. 14.28, 14.29 and 14.30). Very low GATA-3 expression is also found in the adrenal cortex and adrenocortical tumors.

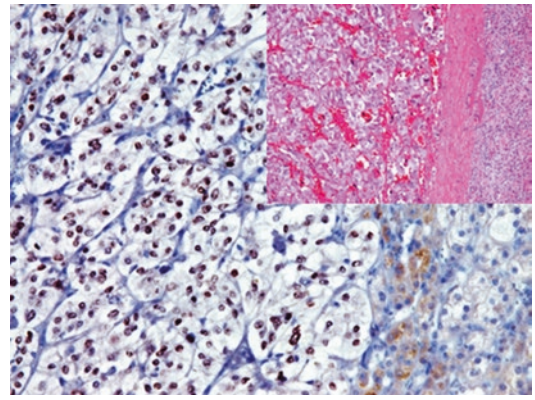


**Fig. 14.28** GATA-3 staining the nuclei of pheochromocytoma cells

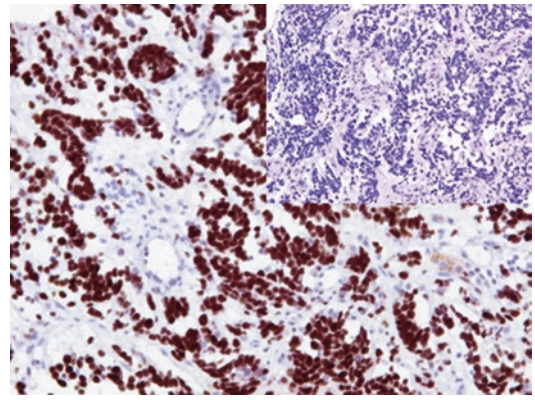


**Fig. 14.29** GATA-3 highlighting the nuclei of neuroblastoma cells in an adrenal gland biopsy

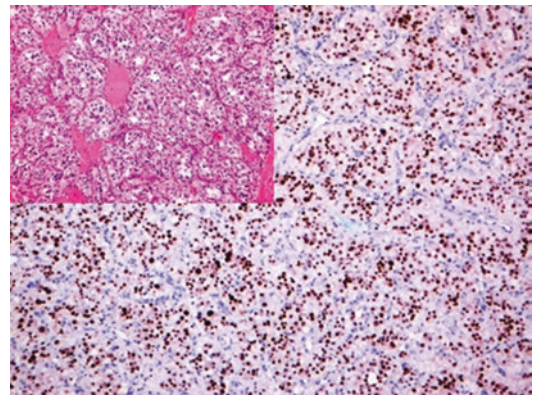


**Fig. 14.30** Paraganglioma. Tumor cells exhibiting nuclear GATA-3 expression

| Immunoprofile of adrenal gland tumors and tumors of extra-adrenal paraganglia | | | | |
|---|---|---|---|---|
| Tumor type | + in >90% (+) | + in 50–90% (+/−) | + in 10–50% (−/+) | + in <10% (−) |
| Adrenocortical adenoma/carcinoma | **Adrenal 4 binding protein (SF-1)**, **inhibin**, Melan-A<br>Proliferation index (Ki-67): in adrenocortical adenoma, <2.5%<br>In adrenocortical carcinoma, >4% | Synaptophysin, NSE, Calretinin, CD56, CYP11B2[a], vimentin | Pan-CK, CK5, bcl-2 | CK7, CK19, CK20, EMA, CEA, CD10, INSM-1, chromogranin, RCC, PAX-8 |
| Pheochromocytoma and tumors of extra-adrenal paraganglia (sympathetic and parasympathetic paraganglioma): | **INSM-1**, Islet-1, CD56, **chromogranin**, Synaptophysin, NSE<br>Proliferation index (Ki-67): in benign pheochromocytoma, <2%<br>In malignant pheochromocytoma[c], >3% | **S100**[b], SSTR2, GATA-3, bcl-2 | GFAP, vimentin, pan-CK, calcitonin | CK5/CK6, CK7, CK19, CK20, EMA, D11, PAX-8, CA IX, Melan-A |
| Neuroblastoma | **INSM-1**, **CD56**, NSE, Neurofilaments, PGP9.5, NB84, **GATA-3**, **PHOX2B**, vimentin | **S100**, ALK, Synaptophysin, chromogranin, CD117, Calretinin | Pan-CK, WT-1 | CK5/CK6, CK7, CK20, CD99 |
| Olfactory neuroblastoma (esthesioneuroblastoma) | **CD56**, CD57, NSE, Fli-1, PGP9.5, Neurofilaments, NB84, **S100** | SSTR2, Bombesin, Synaptophysin, chromogranin | Pan-CK | GATA-3, PHOX2B, EMA, WT-1, CD99, PAX-7 |

[a]Labels aldosterone-producing adrenal cortical tumors
[b]Strong nuclear and cytoplasmic S100 stain in sustentacular cells (Fig. 14.31)
[c]This criterion cannot be used exclusively to define malignancy

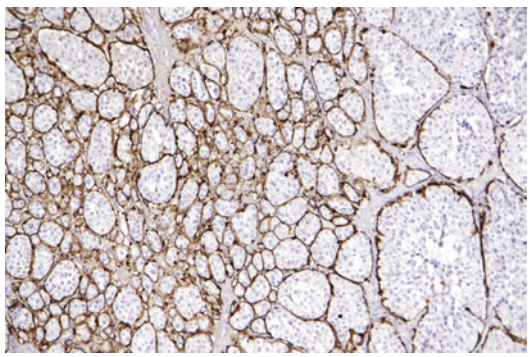


**Fig. 14.31** Extra-adrenal paraganglioma showing strong S100 expression in sustentacular cells

## 14.7 Diagnostic Antibody Panel for the Classification of Neuroendocrine Neoplasms: Neuroendocrine Tumors (NET G1, G2, G3) and Neuroendocrine Carcinomas (NEC) (Small and Large Cell Types)

The general neuroendocrine markers, including INSM-1, Chromogranin, Synaptophysin, NSE, S100, CD56, and Secretogranin and Somatostatin receptor (SSTR), are characteristic markers for

neuroendocrine neoplasms [27, 39, 40]. The mitotic proliferation and index estimated by PHH3 and Ki-67 are essential for tumor grading. The tissue-specific transcriptional factors such as CDX-2, SATB-2, PDX-1, PAX-6, Istet-1, TTF-1, OTP, and NKX3.1 in addition to the cytokeratin profile are helpful markers to ascertain the site of the primary tumor (see the chapter below).

## 14.8 Approach to the Diagnosis of Neuroendocrine Neoplasms (NET, NEC)

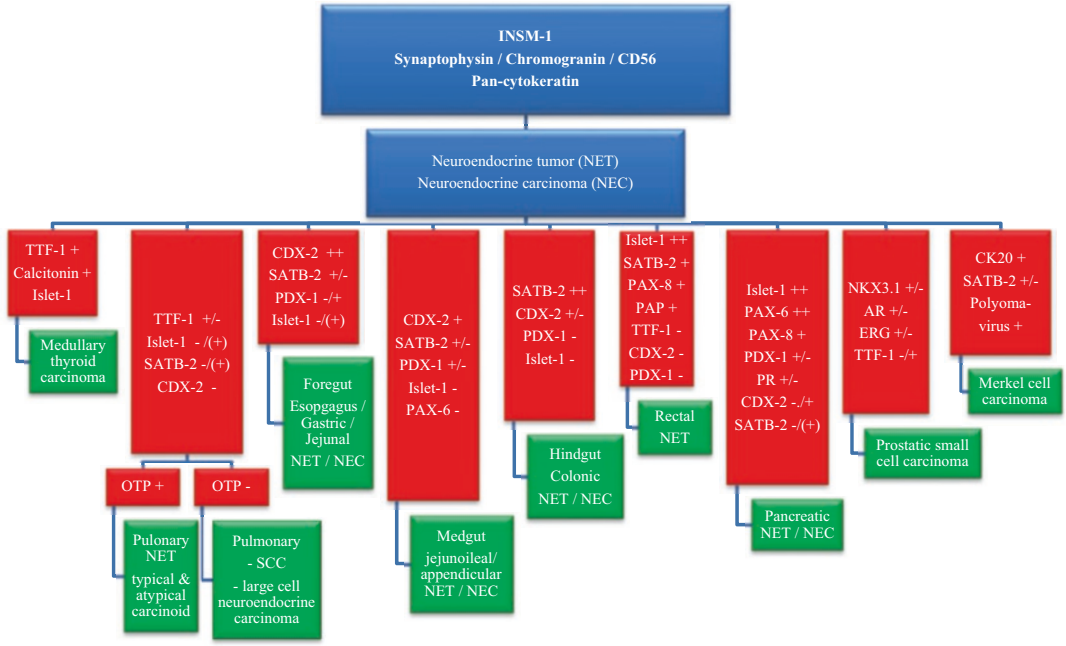
Neuroendocrine neoplasms are tumors that arise from the neuroendocrine cells that migrated from the neural crest and include the cells of the adenohypophysis, thyroid C cells, bronchial K cells, gastroenteropancreatic neuroendocrine cells, paraganglionic cells and cells of the adrenal medulla, urogenital neuroendocrine cells, and cutaneous Merkel cells. Neuroendocrine neoplasms are divided into two main groups with distinct morphological, genetic, biological, and clinical features: [41]

- Well-differentiated neuroendocrine tumors (NET) include three differentiation categories G1, G2, and G3.
- Neuroendocrine carcinomas (NEC) include both small and large cell types and always are G3.

**For the optimal diagnosis of this heterogeneous group and to determine the most probable site of origin, it is recommended to consider the following points**

- The morphological features of the tumor: neuroendocrine tumors (NET G1, G2, or G3) show the characteristic neuroendocrine growth pattern that includes insular, trabecular, acinar, or glandular growth patterns. Neuroendocrine carcinoma (NEC) shows an undifferentiated anaplastic morphology.
  - The epithelial nature of the tumor can be confirmed by the expression of one of the pan-cytokeratin markers or another cytokeratin like CK7/19/20, whereas the neuroendocrine tumors usually exhibit a characteristic dot-like or paranuclear cytoplasmic expression pattern. It is important to remember that neuroendocrine neoplasms of neuronal/neuroectodermal origin, such as paraganglioma, pheochromocytoma, and neuroblastoma, usually lack the expression of cytokeratins.
  - The neuroendocrine differentiation must be confirmed using one or more neuroendocrine markers (INSM-1, synaptophysin, chromogranin, CD56).
  - A panel of tissue-specific markers can be used to define the most probable site of origin (see Algorithm 14.1) [42–45].
  - Several markers may be useful to differentiate between NET G3 and NEC (see endocrine tumors of the pancreas; Chap. 8).

| Classification and immunophenotype of neuroendocrine neoplasms | | | | |
|---|---|---|---|---|
| Tumor type | + in >90% (+) | + in 50–90% (+/−) | + in 10–50% (−/+) | + in <10% (−) |
| Neuroendocrine tumors<br>NET G1 (carcinoid)<br>– NETG2 (atypical carcinoid)<br>– NET G3 | **INSM-1**, **Islet-1**, chromogranin, synaptophysin, somatostatin, CD56, Secretogranin, NSE, PGP9.5, Leu7 | Pan-CK, CK8, CK18, CK19, S100, SSTR2 | | CK5/CK6, CK7, CK20 |
| Neuroendocrine carcinoma<br>– NEC of small cell type<br>– NEC of large cell type | Proliferation/mitotic index (Ki-67 and PHH3)<br>NET G1 (carcinoid)—proliferation index (Ki-67): <3%; mitotic rate: <2/2 mm$^2$<br>NET G2 (atypical carcinoid)—proliferation index (Ki-67): 3–20%; mitotic rate: 2–20/mm$^2$<br>NET G3 and NEC—proliferation index (Ki-67): >20%; mitotic rate:>20/2 mm$^2$ | | | |




**Algorithm 14.1** Differential diagnosis of neuroendocrine neoplasms

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