Thyroid Neuroendocrine Neoplasms

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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

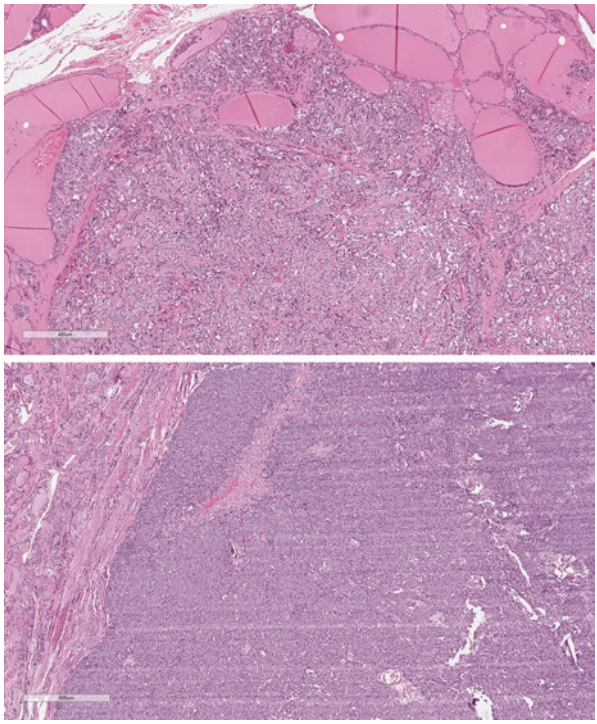

Fig. 7.1 Patterns of growth of medullary thyroid carcinoma. These tumors are usually infiltrative and grow around the follicles of the nontumorous thyroid (top), but occasionally they are well-delineated and expansile lesions that mimic thyroid follicular lesions (bottom)

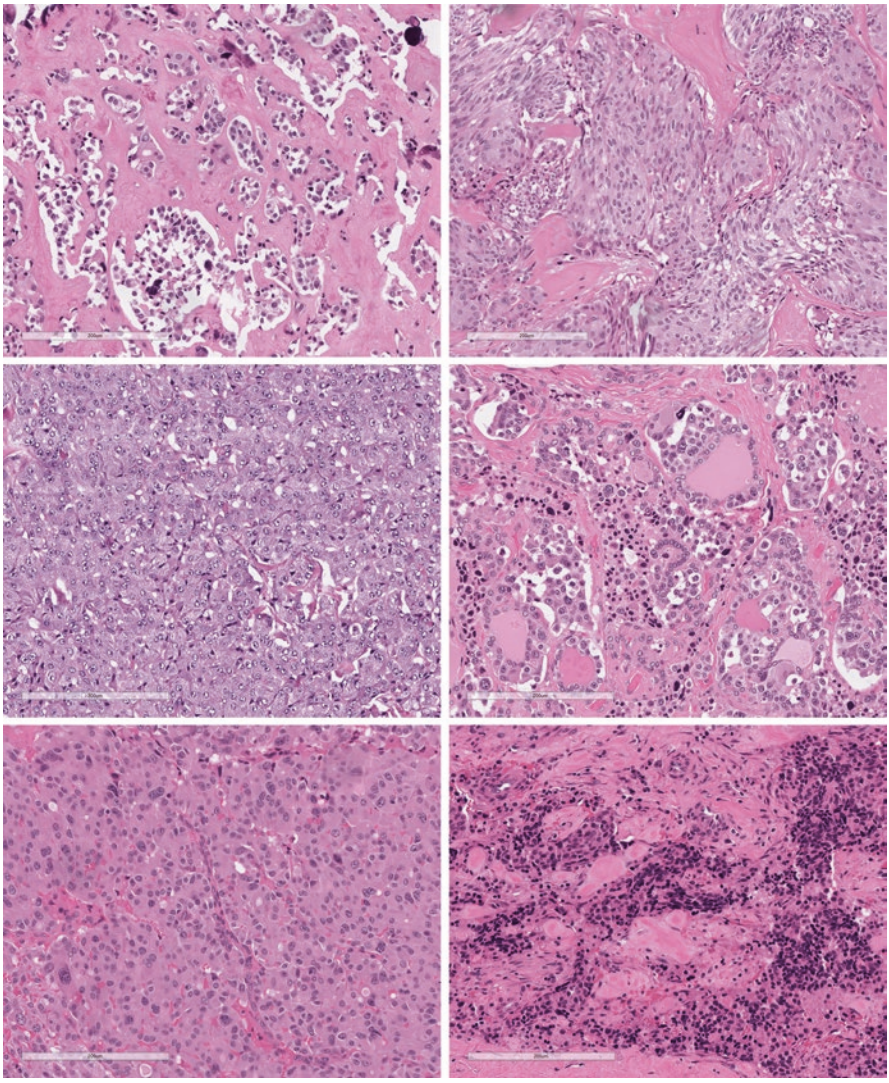

Fig. 7.2 Architecture and cytology of medullary thyroid carcinoma. The classical variant of this tumor is composed of small nests of discohesive cells in a stroma with amyloid and may have focal calcification (top left). Tumors with less amyloid usually have a more spindle cell morphology (top right). 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

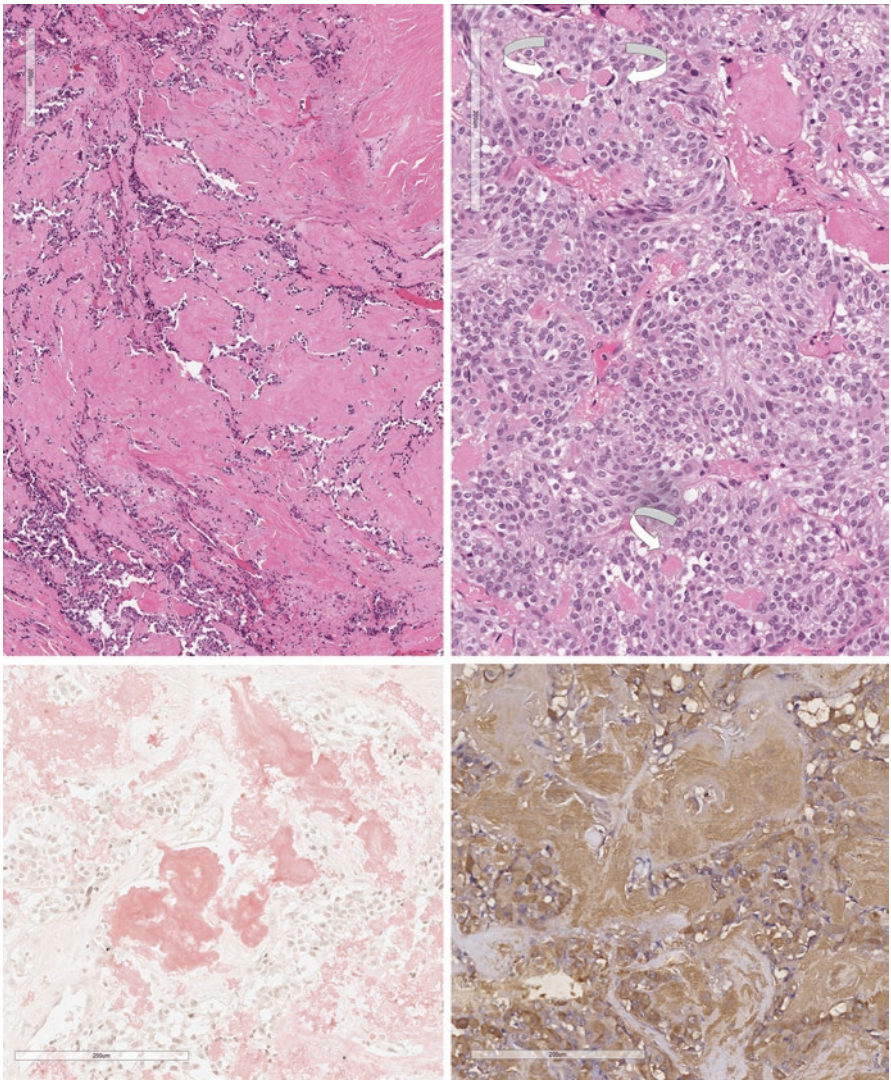

Fig. 7.3 Amyloid in medullary thyroid carcinoma. The presence of amyloid is identified in approximately half of these tumors. It is usually abundant (top left) but may be scattered and scant (top right); it may be found only within tumor cells that accumulate the material and rupture (top right, arrows). 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)

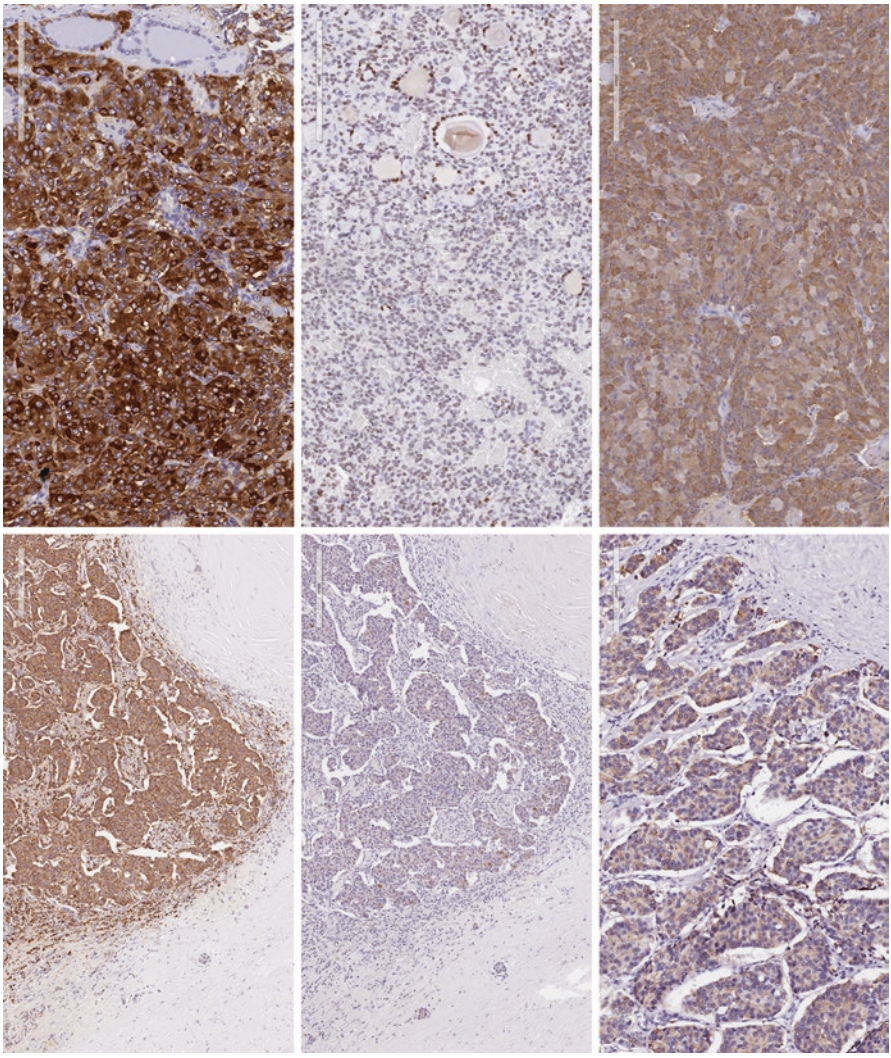

Fig. 7.4 Immunohistochemical profile of medullary thyroid carcinoma. These tumors stain strongly for chromogranin (top left) and may have variable nuclear reactivity for TTF1 (top middle). They usually have cytoplasmic positivity for calcitonin (top right), and they stain diffusely for carcinoembryonic 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

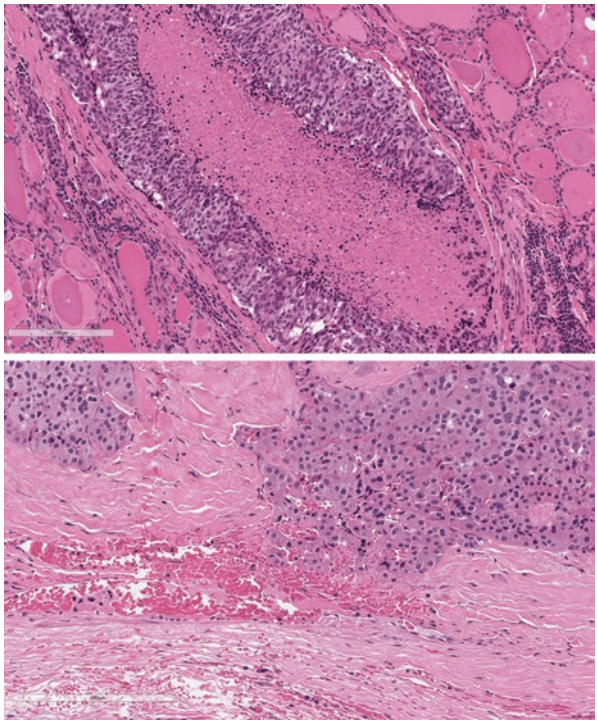

Fig. 7.5 Prognostic features in medullary thyroid carcinoma. The presence of extensive tumor necrosis (top) and angioinvasion, defined by tumor cells within vascular channels associated with thrombus (bottom), are adverse features seen within the primary tumor

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

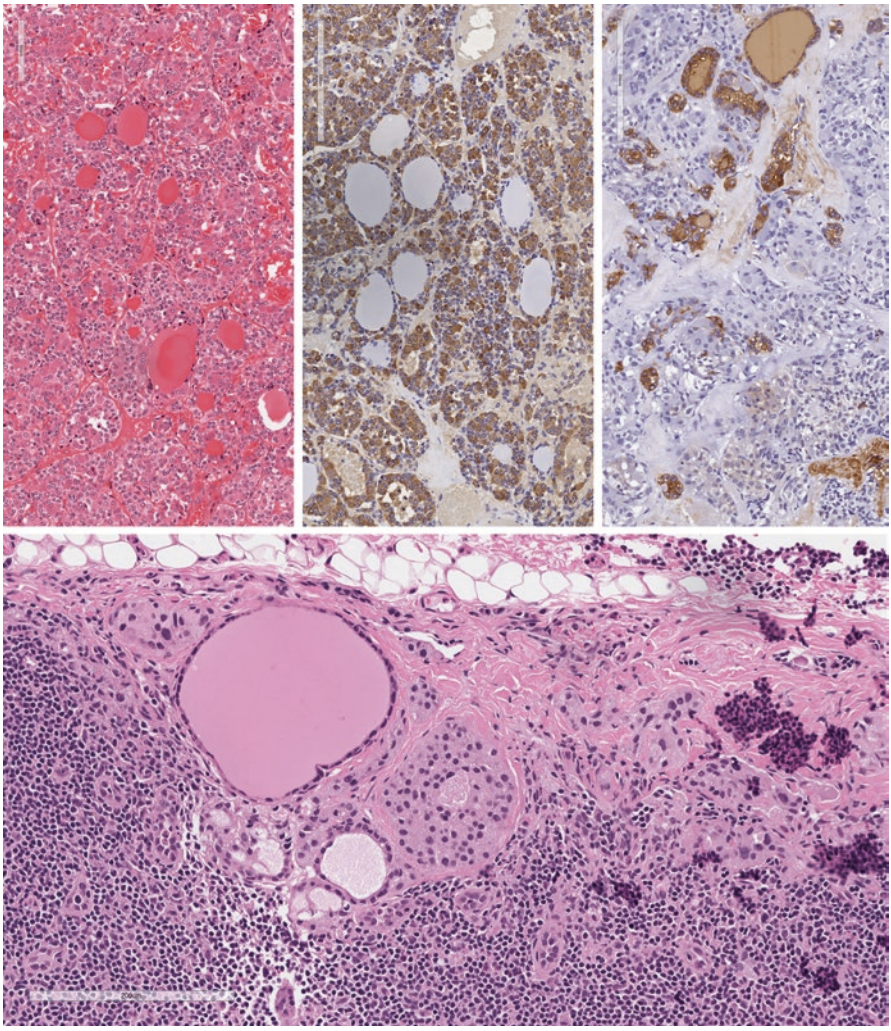

Fig. 7.6 Trapped thyroid or composite tumor? The presence of thyroid follicles within a medullary thyroid carcinoma (top left) does not indicate the presence of a composite tumor, since these lesions grow by surrounding the adjacent nontumorous gland. The follicular cells may even exhibit nuclear atypia 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

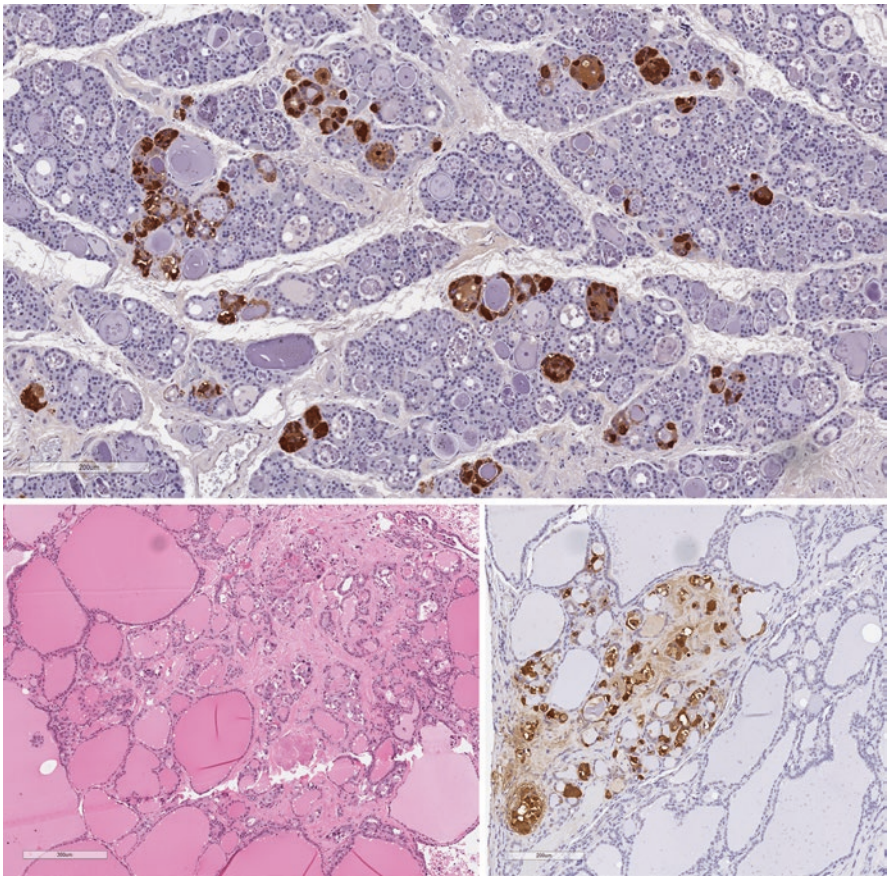

Fig. 7.7 C cell hyperplasia and micromedullary thyroid carcinoma. In patients with germline *RET* mutations, these precursor lesions develop multifocally throughout the thyroid. C cell hyperplasia is difficult to see on routine H&E staining, but immunohistochemistry for calcitonin (shown, top) or monoclonal 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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