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Friedhelm Raue
Editor

Medullary Thyroid Carcinoma

Biology—Management—Treatment

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Medullary Thyroid Carcinoma

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Foreword

Sixty-five years ago Hazard, Hawk, and Crile first described medullary thyroid carcinoma (MTC) as a specific histological entity. Initially, it was thought that the tumor only occurred sporadically; however, 48 years ago, Steiner and associates first reported that MTC also occurs as an integral part of a familial polyendocrine disorder, which they named multiple endocrine neoplasia (MEN) type 2, in contrast to the previously described MEN1. There are two subtypes of MEN2: MEN2A and MEN2B.

Although MTC accounts for less than 5 % of thyroid cancers, it has attracted an inordinate amount of interest for several reasons:

The most significant driver mutations causing MTC are known. Virtually all patients with hereditary MTC have germline mutations in the *RET* protooncogene, and almost all patients with sporadic MTC have either somatic *RET* mutations or somatic *RAS* mutations. Direct DNA analysis to detect *RET* mutations has proved invaluable in the early diagnosis of patients at direct risk for hereditary MEN2, and in the detection of hereditary MTC in patients with presumed sporadic MTC. Furthermore, there is a correlation between genotype and phenotype that is useful in predicting the clinical expression of disease in patients with the MEN2 syndromes.

MTC cells secrete the polypeptide calcitonin, which serves as a sensitive serum tumor marker for MTC. Youngsters who have inherited a mutated *RET* allele can be monitored by serum calcitonin measurements to determine the timing of a prophylactic thyroidectomy, which is curative when done before MTC develops or spreads beyond the thyroid gland. Serum calcitonin measurements are also useful for detecting persistent or recurrent MTC following thyroidectomy, and for monitoring response to therapies in patients with advanced disease.

Although total thyroidectomy is the treatment of choice for primary MTC, there had been no effective therapy for extensive regional or distant metastases until recently, when prospective randomized trials demonstrated prolongation of progression-free survival in patients treated with tyrosine kinase inhibitors, compared to placebo. At present, these novel agents are first-line treatment of patients with advanced MTC.

The International MEN Workshop was formed in 1984. The Workshop meets biannually and provides a valuable forum where clinicians and basic scientists meet

to discuss new developments in basic science and clinical research related to the MEN1 and MEN2 syndromes.

Dr. Friedhelm Raue, of the Department of Endocrinology at the University of Heidelberg, is an internationally respected investigator in endocrinology. He is the editor of this outstanding book on medullary thyroid carcinoma, and his co-authors are international leaders in the field of endocrinology and thyroid cancer.

The initial part of the book is devoted to MTC, beginning with a chapter on C-cell molecular biology and the molecular genetics of MTC, and continues with chapters on pathology, epidemiology and clinical presentation, imaging, and tumor markers. Subsequent chapters concern the MEN2 syndromes including the relationship between genotype and phenotype in patients with MEN2A and MEN2B, and the management of pheochromocytoma and hyperparathyroidism. The final chapters address the surgical management of MTC, the long-term evaluation of patients following thyroidectomy, and the use of tyrosine kinase inhibitors in the treatment of patients with advanced MTC.

This is an excellent book, primarily because of the expertise of the contributors and their thorough and timely coverage of all aspects of MTC and the MEN type 2 syndromes. This book will be a valuable resource for medical students, post-graduate students, basic scientists, clinical investigators, and practicing clinicians.

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Thyroid C-Cell Biology and Oncogenic Transformation

Gilbert J. Cote, Elizabeth G. Grubbs and Marie-Claude Hofmann

Abstract

The thyroid parafollicular cell, or commonly named "C-cell," functions in serum calcium homeostasis. Elevations in serum calcium trigger release of calcitonin from the C-cell, which in turn functions to inhibit absorption of calcium by the intestine, resorption of bone by the osteoclast, and reabsorption of calcium by renal tubular cells. Oncogenic transformation of the thyroid C-cell is thought to progress through a hyperplastic process prior to malignancy with increasing levels of serum calcitonin serving as a biomarker for tumor burden. The discovery that multiple endocrine neoplasia type 2 is caused by activating mutations of the *RET* gene serves to highlight the RET-RAS-MAPK signaling pathway in both initiation and progression of medullary thyroid carcinoma (MTC). Thyroid C-cells are known to express RET at high levels relative to most cell types; therefore, aberrant activation of this receptor is targeted primarily to the C-cell, providing one possible cause of tissue-specific oncogenesis. The role of RET signaling in normal C-cell function is unknown though calcitonin gene transcription appears to be sensitive to RET activation. Beyond RET, the modeling of oncogenesis in animals and screening of human tumors for candidate gene mutations have uncovered mutation of *RAS* family members and inactivation of Rb1 regulatory pathway as potential mediators of C-cell transformation. A growing understanding of how RET interacts with these

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pathways, both in normal C-cell function and during oncogenic transformation, will help in the development of novel molecular-targeted therapies.

Keywords
Thyroid C-cell · Parafollicular cell · RET · Medullary thyroid carcinoma · Mouse models

Contents

1 Introduction

The expression of calcitonin by medullary thyroid carcinoma (MTC) defines the C-cell (parafollicular cell) origin of this tumor and serves a valuable role in monitoring the progression of disease. C-cells comprise a minor population of the thyroid, representing approximately 2–4 % of the organ's cells, which also represents the relative frequency of MTC among all thyroid cancer types. The remaining majority of thyroid cells are follicular in origin. Despite a shared anatomical location, initiation and progression of MTC tumors from C-cells differ from follicular cell-derived tumors, of which papillary thyroid carcinoma is the most common. Immunofluorescent detection of calcitonin performed initially by Bussolati and Pearse localized expression to the thyroid C-cell and ultimately MTC cells (Bussolati et al. 1969; Bussolati and Pearse 1967). Our understanding of the initiating and driving events involved in the oncogenic transformation of the C-cell has been greatly facilitated through the discovery that activating germline mutations of the *RET* gene are responsible for hereditary forms of MTC (Donis-Keller et al. 1993; Hofstra et al. 1994; Mulligan et al. 1993). However, it remains unclear as to what drives thyroid C-cell sensitivity to RET activation, which in turn sets in motion the development of MTC. Predicting the occurrence of MTC in patients

with the germline RET mutation is important for clinicians attempting to treat the disease at its earliest onset. A RET mutation is also thought to play a role in 40 % of sporadic MTCs (Bamford et al. 2004). How an alteration in *RET* initiates C-cell transformation remains unclear in this setting as well. There exists a need to understand RET's mechanism of action further and to uncover other mediators of C-cell transformation and tumor progression. Expanding our understanding of the biology of the normal C-cell and of MTC will aid in the future treatment of this malignancy, by refining the timing of resection of the thyroid in hereditary cases and allowing the further development of novel, molecular-targeted therapies of advanced cases.

2 Defining the C-Cell

The thyroid gland consists of two endocrine cell types, the follicular cells that produce the thyroid hormones thyroxine (T4) and triiodothyronine (T3), and the parafollicular C-cells that synthesize calcitonin. It is well accepted that these two thyroid cell types have unique embryonic origins, although details of their differentiation and development during organogenesis remain somewhat controversial. However, tumors arising from the two cell types are clearly distinct entities with different treatment and prognosis.

The identification of the C-cell predates the discovery of calcitonin by nearly 90 years. As early as 1876, Baber had pointed out the presence in dog thyroid gland of a distinct group of "parenchymatous cells" which he described as markedly different from follicular cells in that they were large, rounded, frequently solitary cells with oval nuclei (Baber 1876). These cells have since been redescribed by numerous authors using various names such as "*ovoid* cells" (Bensley 1914), "interfollicular cells" (Takagi 1922) or "mitochondria-rich cells" (Seecof 1927). Nonidez (1932a, b) is perhaps most frequently credited with redefining these cells based on the intuition that they might be argyrophilic. Silver staining led him to visualize their sparse distribution throughout the thyroid gland and to describe these cells as parafollicular (Nonidez 1932a). Still, the role for these parafollicular cells remained unclear for decades, postulated to be either nonsecretory (Saito and Shibata 1957) or a stage in the life cycle of the follicular cells (Gabe 1959, 1961). However, with the application of electron microscopy, a more detailed examination of the parafollicular cell from several species uncovered a common abundance of granules believed to be secretory in nature (Ekholm and Ericson 1968; Young and Leblond 1963). Their ultrastructural and cytochemical characteristics led to the suggestion by Pearse (1966a, b) that parafollicular cells belong to a group of endocrine cells he termed "APUD" (amine precursor uptake and decarboxylation), all of endodermal origin (Copp et al. 1967; Moseley et al. 1968; Pearse and Carvalheira 1967; Stoeckel and Porte 1969; Tauber 1967) and each producing polypeptide hormones. Ultimately, immunohistological studies (Bussolati and Pearse 1967; Kracht et al. 1968a, b) provided the evidence that the hormone calcitonin was specifically secreted by the parafollicular cells and not by follicular

cells. This localization also fits well a previously proposed label of C-cells for calcitonin, which was introduced as a functional substitute for the existing misleading descriptive terms (Pearse 1966c). As such, parafollicular cells are now commonly referred to as thyroid C-cells given their primary functional role in calcitonin production. The hormone acts to reduce the serum calcium level, counteracting the effects of parathyroid hormone.

3 Tracing the Developmental Origin of the Thyroid C-Cell

A detailed discussion of the embryonic origins of the parafollicular C-cell and thyroid development is beyond the scope of this chapter. Readers are referred to several excellent reviews on thyroid development (Fagman and Nilsson 2010; Nilsson and Fagman 2013). The now classic chick-quail chimera studies by Le Douarin and Le Lievre continue to serve as the basis for defining the neural crest origin of C-cells (Le Douarin and Le Lievre 1970; Polak et al. 1974). Quail neural crest cells transplanted to chick embryos and identified by their characteristic chromatin structure were specifically demonstrated to invade the chick ultimobranchial gland and differentiate into C-cells based on calcitonin immunostaining. However, two major caveats of these studies need to be considered in the context of MTC. First is the fact that in lower vertebrates, including fish, reptiles, and birds, the C-cells comprise a separate ultimobranchial gland that exists separate from the thyroid (reviewed in Nilsson and Fagman 2013). Thus, a major difference in cellular migration exists beginning with mammals. While the relevance of this divergence to oncogenesis is not clear, it is interesting to note that nonthyroidal C-cell carcinomas have only been reported for zebra fish among the various lower vertebrates (Kent et al. 2012; Spitsbergen et al. 2012). Second, it is important to remember the limited availability of lineage-specific markers along with the capability to employ engineered genetic models to aid in cell lineage tracing at the time these studies were performed. However, the clear demonstration of calcitonin-producing cells within the avian ultimobranchial gland, coupled with the species-specific (chick-quail) cell-tracking studies, defined avian C-cells as of neural crest origin. These observations, in turn, provided the original basis to postulate a similar neuroectodermal origin for thyroid C-cells.

The evolutionary mechanism behind C-cell migration into the thyroid, as well as its potential functional roles beyond calcitonin production, remains unclear. In mammals, it is believed that a specific subpopulation of ectodermal-derived neural crest cells migrate to the most inferior pharyngeal arch to invade/merge with a pair of endodermal-derived ultimobranchial bodies (also referred to as the lateral thyroid anlage). The ultimobranchial bodies then serve to specifically carry the C-cell precursors into the developing thyroid gland. The ultimobranchial body origin of thyroid C-cells was originally suggested from light microscopic observations in dog thyroid (Godwin 1937) and first experimentally documented by the specific uptake of fluorescent amine (Pearse and Carvalheira 1967); subsequently, this role was

supported by demonstration of characteristics consistent with defining APUD cell types (Pearse and Polak 1971). Electron microscopy studies further identified cells with ultrastructural features of C-cells in the ultimobranchial glands prior to fusion with the thyroid gland (Jordan and Scothorne 1972). Despite these biochemical and structural features, the precursor cells within ultimobranchial bodies do not stain positive for calcitonin (Fagman et al. 2006; Westerlund et al. 2013). Therefore, unlike lower vertebrates, ultimobranchial bodies do not serve as a site of C-cell differentiation, but merely to ensure specific migration into the developing thyroid. This specific role is supported by several mouse knockout models demonstrating that failed or abnormal development of the ultimobranchial body causes aberrant localization or even loss of calcitonin-producing C-cells (Kusakabe et al. 2006; Liao et al. 2004; Manley and Capecchi 1998; Xu et al. 2002). Once within the thyroid gland, Mash1 and perhaps RET signaling pathways are associated with differentiation and induction of neuronal traits in the cells. In two independent studies, Mash1 null mice are severely impaired in their ability to generate mature C-cells (Kameda et al. 2007; Lanigan et al. 1998). The near or complete absence of C-cells in the Mash1 null mutants suggests that progenitors may degenerate before they become differentiated. Available evidence suggests that Mash1 enhances survival of C-cell progenitors by inhibiting apoptosis while promoting differentiation (Kameda et al. 2007). Surprisingly, the impact of loss of RET function is less severe. Unlike the complete loss of the neural crest-derived enteric nervous system associated with loss of Mash1, RET null mice reportedly demonstrate only a 37 % loss of C-cell number (Lindahl et al. 2000). This observation suggests that C-cell development may not be dependent on an intact RET neural crest cell lineage (see below).

The actual mechanisms involved in the direction of C-cell precursors to the ultimobranchial body and dissemination of these maturing C-cells within the thyroid remain unknown. Perhaps, an even greater area of controversy is the true origin of C-cell precursors. Circumstantial evidence based on genetic fate-mapping studies in mice suggests that mammalian thyroid C-cells might have an endoderm origin (Kameda et al. 2007). This challenges the prevailing concept of MTC being a neuroectodermal tumor. Lineage-tracing studies have invalidated a neural crest origin of gut endocrine cells, proving that endoderm stem cells can differentiate into both exocrine and endocrine phenotypes (reviewed in May and Kaestner 2010). Similar fate-mapping studies have also challenged C-cell origin in mice. In mice, using either Connexin43 or Wnt1 as neural crest cell markers failed to localize staining to the pharyngeal pouches or developing ultimobranchial bodies (Kameda et al. 2007). Unexpectedly, these areas were found to stain intensely for E-cadherin and to colocalize with calcitonin-positive cells following birth, suggesting an endodermal epithelial origin. Support for an endodermal origin of C-cell progenitors is also provided by a recent reexamination of the sonic hedgehog Shh−/− mouse (Westerlund et al. 2013). Shh is required for both migration and survival of neural crest cells (Ahlgren and Bronner-Fraser 1999; Brito et al. 2006; Testaz et al. 2001). In the absence of Shh, mature C-cells instead appear in ectopic locations. Calcitonin-positive C-cells are found to populate the regions deriving pharyngeal pouches that remain in the absence of ultimobranchial body formation. These

findings indicate that Shh determines the endoderm territory for C-cell differentiation and guides the migration of C-cell precursors into the thyroid, presumably by regulating the separation of glandular domains in the pharyngeal pouch endoderm.

In summary, the embryonic origin of thyroid C-cells, whether it is neural crest cell, endoderm, or perhaps both, remains a controversy. What is clear is that to date there are no reports of C-cells in the thyroid of mice lacking ultimobranchial bodies, indicating that thyroid progenitors from the midline anlage are not capable of generating C-cell lineage, and there are no examples of a normally formed thyroid completely devoid of C-cells, suggesting a yet-to-be-defined functional interaction between C-cells and follicular cells. However, while under normal circumstances C-cell progenitors differentiate within the thyroid, differentiation is not uniquely dependent on thyroid localization. Whether these developmental findings in mice can be translated to humans is unclear. A strict dependence on the ultimobranchial body for C-cell development may not exist. First, thyroid C-cells are not ablated in patients with DiGeorge syndrome (Pueblitz et al. 1993) in which not only do the thymus and parathyroid fail to develop, but also the ultimobranchial body is assumed to be missing due to defective development of all posterior pharyngeal arches and pouches (Liao et al. 2004). More recently, ectopic lingual thyroids located far away from the origin of the ultimobranchial body were also found to contain C-cells (Abu-Khudir et al. 2010; Vandernoot et al. 2012).

4 C-Cell Function

It is universally accepted that a primary function of the thyroid C-cell is to secrete calcitonin and to a lesser degree smaller quantities of several neuroendocrine peptides such as somatostatin, calcitonin gene-related peptide (CGRP), and serotonin (Fernández-Santos et al. 2012). In animals that have been examined to date, the secretion of calcitonin by C-cells is evolutionarily conserved beginning from teleost fish through mammalians. Furthermore, despite only 50 % conservation at the amino acid level, salmon calcitonin is functionally active in humans (Copp 1970). In all vertebrate species, calcitonin, along with parathyroid hormone and vitamin D, plays key roles in serum calcium homeostasis (for details, see Brown 2013; Davey and Findlay 2013). It is interesting to note evolutionarily that parathyroid gland development from gills occurs with tetrapods (Okabe and Graham 2004) and that integration of ultimobranchial C-cells in the thyroid coincides with that of mammals (Nilsson and Fagman 2013).

Calcitonin release from the C-cell is triggered primarily in response to elevated serum calcium, though other hormones including gastrin have been reported to stimulate release. Indeed, the sensitivity of C-cells to gastrin led to the pentagastrin stimulation test, which measures calcitonin elevations in MTC. This test has been replaced by calcium testing where pentagastrin is not available (Cooper et al. 1971; Hennessy et al. 1973). While the precise mechanism controlling calcitonin release has not clearly been elucidated, both the calcium-sensing receptor (CASR) and

cholecystokinin-B receptor (gastrin receptor) are expressed in MTC cells (Reubi and Waser 1996; Desai et al. 2014; Garrett et al. 1995). In MTC cells, the functionality of CASR has recently been demonstrated to be modulated though interactions with RAMP1, which act to regulate trafficking of the receptor to the cell surface (Desai et al. 2014). Unfortunately, these studies are complicated by the reliance on MTC tumor cell line models rather than normal C-cells. Much of the focus on C-cell function is a direct result from the development of calcitonin as a marker of tumor progression and the study of MTC tumor cell biology rather than normal cells.

In mice, calcitonin production begins around embryonic day 15 as the progenitor C-cells enter the thyroid follicular organization becomes apparent (Fagman et al. 2006; Westerlund et al. 2013). In humans, calcitonin positivity is noted by the end of the first trimester (Fisher 1986). In humans, C-cells are differentially distributed with the thyroid tending to be concentrated at a junction approximating the upper two-thirds of the thyroid (Wolfe et al. 1974). Overall, C-cells represent minority cell population typically situated basally in the epithelium, without direct contact with the follicular lumen. The mean C-cell densities in mouse ($\sim$220/mm^2) and rat ($\sim$450/mm^2) are considerably greater than the numbers observed in human thyroid glands (0–20/mm^2) (Wolfe et al. 1974; Bjerre Knudsen et al. 2010; O'Toole et al. 1985). Importantly, calcitonin and C-cells are not essential for normal development. Genetic knockout of calcitonin in zebra fish (Lafont et al. 2011) or mice (Thomas et al. 2001) does not affect animal viability or present an overt phenotype. However, challenges to calcium homeostasis do induce notable differences in bone formation. In humans, early prophylactic thyroidectomy in MEN2 patients is not associated with calcium metabolism or bone problems, however, other sources of calcitonin may compensate for this C-cell loss. Population studies have also demonstrated no effect on fracture rate in men having undergone thyroidectomy (Nguyen et al. 1997). Also, patients with calcitonin deficiency caused by thyroid dysgenesis do not present with alterations of bone development or bone mass (Daripa et al. 2004). Thus, the precise normal physiological role of both calcitonin and the thyroid C-cell remains to be completely elucidated.

5 Defining the C-Cell as the Origin of MTC

The earliest descriptions of MTC predate the characterization of calcitonin. They include descriptions of argyrophilic tumors in dog (Nonidez 1932b), "microadenomas" or "light cell" tumors in rat (Axelrad and Leblond 1955; Doniach 1953), and ultimobranchial carcinomas in bulls (Jubb and Mc 1959). The first detailed description in humans along with their specific classification as "medullary" thyroid carcinomas is generally credited to Hazard et al. (1959). However, the recognition that MTC is derived from thyroidal C-cells was not made until several years later. Through comparison of thyroid tumors derived from rats, dogs, and humans, it became apparent that MTC was a histological class of tumors that was distinct from

papillary, follicular, and anaplastic tumors (Williams 1966). The linking of tumor formation to C-cell hyperplasia (CCH) in rats led to the postulation that all tumors of this classification are C-cell-derived. It was the identification that thyroidal calcitonin was derived from the C-cell (Bussolati and Pearse 1967), that ultimately led to this cell being identified as the definitive origin of MTC through immunohistochemical localization (Bussolati et al. 1969), and that elevated plasma calcitonin levels served as a sensitive tumor marker (Melvin and Tashjian 1968; Woodhouse et al. 1969). These findings represented a paradigm shift in the treatment of familial MTC, the presence of elevated calcitonin allowing early diagnosis of CCH and MTC in affected family members (Melvin et al. 1971) often prior to clinical or radiographic methods of detection.

The availability of calcitonin antibodies provided a tool to confirm the C-cell origin of tumors in several animal species. Interestingly, despite serving as an early model for the purification of calcitonin, ultimobranchial tumors have not been reported in salmon, though they are known to occur in zebra fish (Kent et al. 2012; Spitsbergen et al. 2012). A recent examination found ultimobranchial tumors to occur at a frequency of $\sim$1 % in a population of 702 fish or $\sim$5 % of all tumors observed (Spitsbergen et al. 2012). Among all species evaluated for MTC, rats are noted to have the highest incidence of spontaneously occurring tumors. It is remarkable to consider that 10–20 % of Wistar rats (Lindsay and Nichols 1969; Martin-Lacave et al. 1999), approximately 20 % of Long–Evans rats (DeLellis et al. 1979), 50–70 % of Wag/Rij rats (Boorman et al. 1972; Lausson et al. 1995), and 78 % of Sprague Dawley rats (Fritz et al. 2002) develop MTC as adults. This compares to a reported frequency of less than 1 in 2500 for mice (Ward et al. 1979) and 1 in 6700 in humans. Sporadic MTC accounts for approximately 5 % of the reported 298 cases per 100,000 thyroid cancer cases observed in 2012 (Ferlay et al. 2015). These differences in MTC incidence provide strong evidence for the role of genetic drivers in C-cell tumorigenesis.

6 Molecular Pathway Dysregulations in Human C-Cell Oncogenic Transformation

Dysregulation of pleiotropic growth factors and their receptors is a common feature of carcinogenesis and contributes to proliferation, antiapoptosis, and invasive behavior of the tumor cells. Relevant mutations observed in C-cell neoplasia mainly involve the RET receptor and the RAS downstream effector, but mutations in the components of other pathways are also encountered, albeit less frequently. Indeed, these molecules are instrumental for progenitor cell survival, normal C-cell development, and migration from the neural crest. The pathways they trigger are normally tightly regulated. Dysregulation of these factors and their pathway components is therefore connected with essential MTC properties such as tumor cell proliferation, cell cycle checkpoints, and invasive behavior.

6.1 RET Signaling

The RET receptor is encoded by the *RET* proto-oncogene localized on human chromosome 10q11.2. This gene encodes a single-pass transmembrane protein that belongs to the receptor tyrosine kinase (RTK) family (Pasini et al. 1995). The extracellular region of the protein contains cadherin-like domains, while the intracellular region contains the active tyrosine kinase domain. RET is activated through extracellular binding of the glial cell line-derived neurotrophic factor (GDNF) family of ligands, which include GDNF, neurturin (NRTN), persephin (PSPN), and artemin (ARTN) (Fig. 1). In order to stimulate RET tyrosine kinase activity, these growth factors need first to form a complex with glycosylphosphatidylinositol (GPI)-anchored coreceptors belonging to the GDNF receptor-α family (GFRA1-4). The growth factor–GFRA complex then recruits RET, which dimerizes and is activated by transautophosphorylation. The RET receptor contains multiple tyrosine sites within its intracellular domain that upon phosphorylation mediate differential downstream signaling (Liu et al. 1996; Kawamoto et al. 2004) (Fig. 2). Located within the activation loop of the RET kinase adjacent to the ATP-binding pocket, the Y905 site serves a key role in RET receptor kinase activation (Knowles et al. 2006). Its phosphorylation serves as the initiating event for additional phosphorylation. Tyrosine 1062 (Y1062) is a multidocking site for proteins containing a phosphotyrosine-binding (PTB) domain (Asai et al. 1996). Once recruited, these docking proteins will activate the RAS-MAPK and

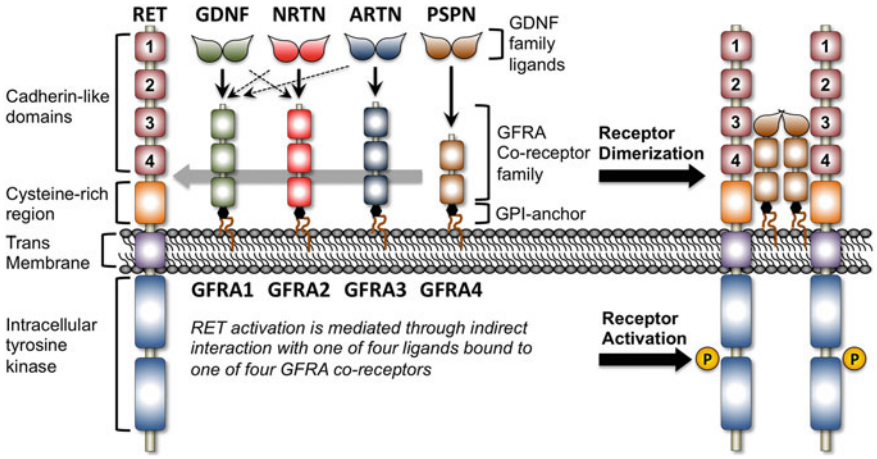

Fig. 1 RET receptor activation. Shown is a schematic figure outlining the basic structural organization of the transmembrane receptor tyrosine kinase RET and the pathway to its activation. One of the four GDNF family ligands is recruited to the receptor upon binding to its cognate coreceptor. *Solid arrows* indicate the primary ligand/coreceptor interactions. *Dotted arrows* show secondary binding interactions. Generation of the heterotrimeric complex allows for receptor dimerization causing kinase activation and autophosphorylation (P). The predominant receptor complex observed in MTC cells is comprised of PSPN and GFRA4

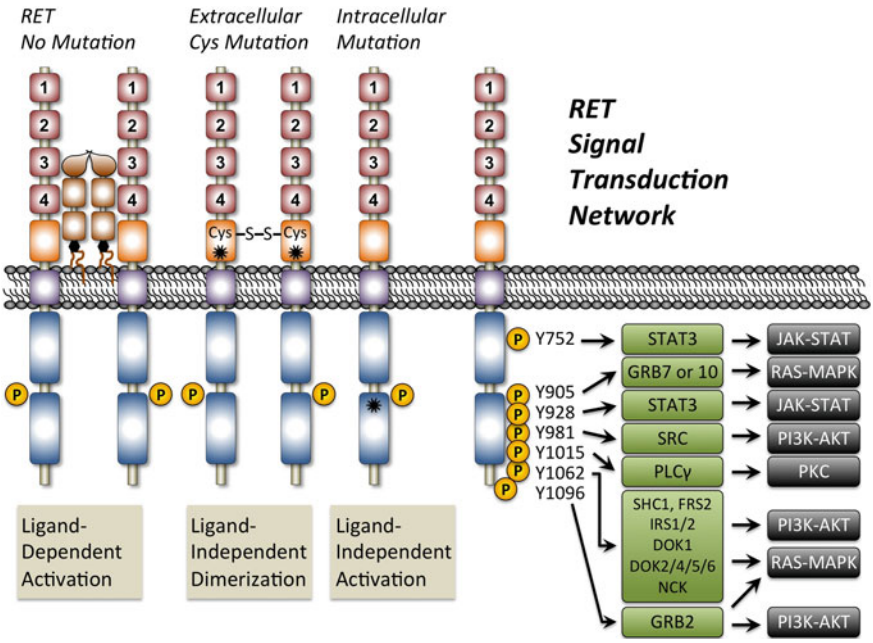

Fig. 2 Mechanism of oncogenic RET activation and the signal transduction network. Shown is a schematic figure outlining the normal ligand-dependent activation of RET compared with ligand-independent mechanisms resulting from receptor mutation. The *asterisks* show the general location of two common RET activating mutations. In all cases, RET activation is associated with phosphorylation (P) of tyrosines (Y) at seven possible positions located within the intracellular domain. Note that Y1096 only exists on the long isoform of RET. Tyrosine phosphorylation results in the recruitment of adaptor proteins (*green boxes*) that lead to the activation of multiple signaling pathways including JAK/STAT, PKC, PI3 K/AKT, and RAS/MAPK

PI3K-AKT pathways. The Y1096 site, which exists only on the long isoform of RET, also couples to the RAS-MAPK and the PI3K-AKT pathways. Finally, Y981 and Y1015 are coupled to other important signaling molecules such as SRC and PLC-γ, respectively (Borrello et al. 1996; Encinas et al. 2004). For a more extensive discussion of RET signaling and its role in disease, the reader is referred to the following excellent recent reviews (Mulligan 2014; Plaza-Menacho et al. 2014).

6.1.1 Attenuated RET Signaling

RET signaling is crucial for mammalian embryonic development as mouse models lacking Ret function die before birth. The mutant embryos exhibit abnormal kidney development, and aberrant ureter, ovary, muscle, and intestine morphology. Ret signaling seems also essential for neurogenesis and establishment of neuronal populations of the central nervous system. In addition, Ret activation is necessary for the development of neural crest-derived lineages, in particular the enteric nervous system, adrenal medulla chromaffin cells and thyroid C-cells (Plaza-Menacho

et al. 2014). Notably, loss of Ret, but not Gfra4 or Pspn, is associated with C-cell reductions in adult mice (Lindahl et al. 2000; Lindfors et al. 2006). Within the nervous system, GDNF/RET signaling is crucial to the maintenance of the nigrostriatal pathway by preventing cell death and onset of Parkinson's disease (Kramer et al. 2007). It is also essential for the survival of motor neurons; alterations in the pattern of phosphorylation lead to amyotrophic lateral sclerosis (Luesma et al. 2014). RET signaling also maintains the enteric nervous system, and loss of RET function in these neurons leads to Hirschsprung's disease, a condition characterized by the absence of enteric ganglia (Luesma et al. 2014). Importantly, this role of RET is reproduced in both Ret knockout (Schuchardt et al. 1994) and Ret knock-in mice with Ret C620R mutations (mixed Hirschsprung's/MTC phenotype) (Carniti et al. 2006; Yin et al. 2007). Finally, it is important to remember that Gdnf/Ret signaling has a clearly established role in driving spermatogonial stem cell self-renewal in the testis and is therefore essential to male fertility (Meng et al. 2000; Hofmann 2008; Hofmann et al. 2005; Braydich-Stolle et al. 2007). Thus, in hereditary RETopathies or in therapies targeting RET activity, cell types beyond the thyroid C-cell must be considered.

6.1.2 Oncogenic RET Signaling

Several known mutations can convert the RET receptor into a dominant transforming oncogene, resulting in neoplasms affecting multiple cell types and organs. While RET mutation has been primarily associated with thyroid cancer, somatic structural RET alterations or changes of its expression have been described in lung, breast, and pancreatic cancers (Esseghir et al. 2007; Gainor and Shaw 2013; Ito et al. 2005; Kohno et al. 2012; Lipson et al. 2012; Morandi et al. 2013; Plaza-Menacho et al. 2010; Sawai et al. 2005). Germline activating mutations of RET are the cause of multiple endocrine neoplasia type 2 (MEN2), which is discussed in detail within other chapters of this book. Activating mutations of the RET oncogene fall into two major classes, extracellular and intracellular (Fig. 2). The extracellular cysteine-rich domain of RET is most frequently mutated at cysteine 634 (85 % of the cases), but mutations also occur distributed among all cysteines (C609, C611, C618, C620, C630) (Figlioli et al. 2013). Intracellular RET mutations are observed in the RET kinase domain and have a variable impact on RET activity. The most frequent mutation observed is M918T, which is found in patients with MEN2B and somatically in sporadic MTC. The molecular mechanism by which *RET* mutations trigger transformation has been elucidated (Santoro et al. 1995; Arighi et al. 2005; Santoro and Carlomagno 2013). As discussed, the normal route to activation involves binding of GFRA1-4/ligand complex to RET, which promotes dimerization and in turn autophosphorylation of the receptor. Mutations present in the extracellular domain are known to target highly conserved cysteine residues. Because these residues are normally involved in intramolecular disulfide bond formation, mutation generates a free sulfhydryl group. Mutant RET receptors in close proximity will then form intermolecular disulfide bonds, which function to stabilize dimerization even in the absence of the ligand (Fig. 2). This will therefore

constitutively activate autophosphorylation and the intracellular pathways downstream of the receptor (Santoro et al. 1995; Asai et al. 1995; Borrello et al. 1995). In contrast, mutations in the intracellular tyrosine kinase domain of RET are known to target regions associated with the ATP-binding pocket, activation loop, and substrate binding pocket (Santoro et al. 1995; Salvatore et al. 2001; Songyang et al. 1995). Depending on their specific location, they induce structural changes and alter RET substrate specificity. This results in phosphorylation of alternative intracellular proteins. Therefore, the mutated receptor no longer needs dimerization to become active. RET mutations are often an initial step in the oncogenic process.

6.2 RAS Signaling

The RAS family of proteins consists of small GTPases that act as binary molecular switches downstream of ligand–receptor interactions (Malumbres and Pellicer 1998; Wennerberg et al. 2005). They are mainly activated through growth factors binding to RTKs, such as RET. RAS proteins are anchored to the plasma membrane through prenylation, which is indispensable to their function (Buss et al. 1989; Carboni et al. 1995; Cox et al. 1992). RTK autophosphorylation enables interaction with the adaptor protein GRB2 (Fig. 3). GRB2 is bound to SOS, a guanosine nucleotide exchange factor (GEF) that activates RAS by stimulating the release of guanosine diphosphate (GDP) to allow binding of guanosine triphosphate (GTP) (Cherfils and Zeghouf 2013). Therefore, upon stimulation by an activated receptor, RAS switches from the inactive "off" form (GDP bound) to the active "on" form (GTP-bound). Binding of GTP induces a conformational change leading to an increase in RAS affinity and binding to downstream mediators such as RAF or PI3K family members depending on the cell status. RAF is a serine–threonine kinase. RAS acts to recruit it to the plasma membrane and promotes dimerization and activation (Avruch et al. 2001). Activated RAF phosphorylates and activates the kinase MEK, which in turn phosphorylates and activates ERK kinase. Activated ERK phosphorylates a number of substrates, including other kinases and transcription factors, which will induce cell cycle progression (McCubrey et al. 2007). In the other main pathway, activation of PI3K will induce phosphorylation of AKT, leading to inhibition of several tumor suppressors (e.g., p21, BAD) or activation of the mTORC1 complex (Fig. 3) (Ma and Blenis 2009; Rodriguez-Viciana et al. 1994; Sarbassov et al. 2005; Staal 1987; Vanhaesebroeck et al. 2010) (Fig. 3). Note that activation of the PI3K/AKT pathway also occurs independently of RAS through direct and indirect binding of RTKs (Castellano and Downward 2011). The ultimate outcome is regulated induction of cell survival, growth, and migration. Components of these main signaling cascades are often mutated in cancer, with approximately one-third of human tumors expressing a constitutively activated mutant form of the RAS protein (Stephen et al. 2014). For a more extensive discussion of RAS signaling and targeting in cancer, the reader is

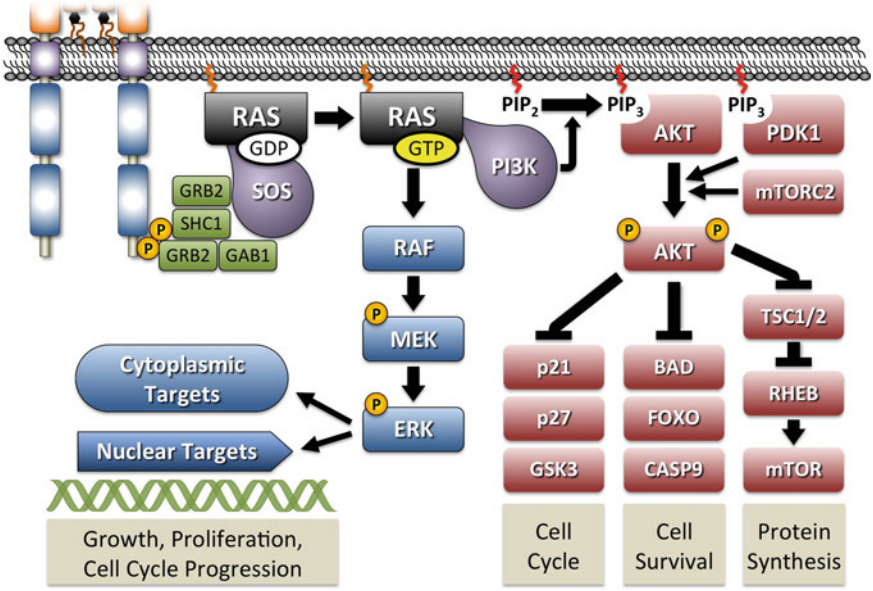

Fig. 3 RAS signaling pathway. The figure shows the activation of RAS initiated through interaction with phosphorylated RET. As shown in Fig. 2, signal transduction is primarily mediated by tyrosines 1062 and 1096 through adaptor proteins GRB2, SHC1, and GAB1. The guanine nucleotide exchange factor SOS is responsible for initiating the formation of active GTP-bound RAS. Active RAS is capable of activation of MAPK signaling through RAF or PI3K activation leading to AKT signaling. Major components of both pathways are shown with their specific outcome of regulating cellular functions indicated

referred to the following excellent recent reviews (Stephen et al. 2014; Prior et al. 2012; Cox et al. 2014; Pylayeva-Gupta et al. 2011; Malumbres and Barbacid 2003).

6.2.1 Attenuated RAS Signaling

There are approximately 30 members of the RAS family, but the most clinically relevant are H-RAS, N-RAS, and K-RAS (Wennerberg et al. 2005). In mice, *Kras* knockout is embryonic lethal, whereas *Hras*, *Nras*, and *Hras–Nras* double knockouts survive normally (Esteban et al. 2001; Johnson et al. 1997; Koera et al. 1997; Umanoff et al. 1995). A thyroid C-cell phenotype has not been reported in any of these mouse models. Surprisingly, knockout of Nras, but not Hras or Kras, in combination with Rb1 haploinsufficiency is associated with a pronounced MTC phenotype in mice (see below). In human cell lines, somatic cell knockout of oncogenic *KRAS* has been accomplished in human colon cancer cells (Shirasawa et al. 1993). Upon *KRAS* gene disruption, these cells lost anchorage independence and grew slower than the parental cells in vitro and after injection into the flank of nude mice. They showed reduced expression of *C-MYC*, a transcription factor normally stabilized by phosphorylation in response to activation of the RAS/ERK

pathway (Sears et al. 2000). To date, there have been no reports examining the impact of RAS loss in human MTC cell lines.

6.2.2 Activated and Oncogenic RAS Signaling

The RAS signal transduction pathway has been extensively studied in the context of tumorigenesis since the recognition that somatic gain-of-function mutations were responsible for cellular transformation. It is interesting to note that germline RAS gain-of-function mutations are observed in two separate genetic syndromes, both without a predominant cancer phenotype. Noonan syndrome, which is associated with heterozygous mutation of *KRAS* or *NRAS*, is characterized by learning disabilities, skin lesions, heart and facial abnormalities, cryptorchidism, and of potential relevance short stature with craniofacial anomalies (Cirstea et al. 2010; Schubbert et al. 2006). A role for the C-cell in any of these phenotypic features has not been reported. Costello syndrome is caused by heterozygous germline mutations at amino acid positions 12 and 13 in *HRAS* (Aoki et al. 2005). The result is a net increase in the active GTP-bound form of RAS. Interestingly, these positions are also the most frequently mutated positions in oncogenic RAS, and patients with Costello syndrome are at increased risk of neural crest tumors, although there have been no reports to date of MTC (Morandi et al. 2013; Gripp 2005). Those interested in RASopathies are referred to exhaustive reviews (Rauen 2013; Bezniakow et al. 2014).

Somatic gain-of-function mutations in *RAS* genes that promote cancer were identified more than three decades ago (for a review, refer to Malumbres and Barbacid 2003). Mutations are found in each of the three major isoforms and nearly all occur in the highly conserved codons 12, 13, and 61 (Prior et al. 2012; Cox et al. 2014). Mutation in these codons alters both nucleotide-binding properties and GTPase properties of the resulting RAS proteins. The primary outcome is a constitutive activation because RAS remains in the active, GTP-bound form (Milburn et al. 1990). Driver mutations involving RAS codons 12, 13, and 61 have been reported in a broad spectrum of cancer types, including MTC. The widespread involvement of RAS in tumorigenesis prompted the first MTC screen for somatic driver mutations in 1989 (Okazaki et al. 1989). The discovery of a single *HRAS* codon 61 mutation found in one of 18 tumors suggested that RAS mutation was a rare or low-frequency event. Three subsequent studies examining all three RAS family members in 30 MTC failed to uncover a single somatic mutation (Bockhorn et al. 2000; Horie et al. 1995; Moley et al. 1991). This lack of RAS involvement in C-cell oncogenesis fit well with in vitro studies performed during the same period, demonstrating that oncogenic HRAS and CRAF decreased proliferation through induction of neuroendocrine differentiation rather than enhancing tumorigenicity (Carson-Walter et al. 1998; Nakagawa et al. 1987). However, these results were in conflict with transgenic mouse model data, demonstrating that C-cells were sensitive to activated MAPK-induced tumorigenesis (Johnston et al. 1998; Schulz et al. 1992). More recently, the widespread availability of tools for somatic mutation analysis has led to a reexamination of RAS involvement in C-cell tumorigenesis.

By focusing on identifying driver mutations in MTC tumors lacking somatic RET mutation, Moura et al. (2011) uncovered an unexpectedly high fraction of RAS mutation. Subsequent to these findings, several other studies have reported the presence of somatic RAS mutation (Agrawal et al. 2013; Boichard et al. 2012; Ciampi et al. 2013; Schulten et al. 2011). As seen in other tumor types, mutations are nearly exclusive to RAS codons 12, 13, and 61. The currently reported distribution of mutations seen in MTC differs from what is seen in other thyroid cancers with targeting of the *HRAS* gene, and specifically codon 61 mutations, occurring most frequently. In nearly all cases reported to date, RET and RAS mutations are mutually exclusive, supporting the belief that RET-mediated oncogenic transformation occurs separately from RAS.

6.3 Other Pathways

RET and RAS pathways have emerged as predominant oncogenic pathways for the C-cell; however, mutation of these genes currently only explains only about 60 % of MTC. The search for other major drivers of transformation has been largely unsuccessful. This failure may be explained in part by the lack of large unbiased search for new drivers. To date, only a single study reporting exomic sequencing was performed, which included only 25 MTC tumors (Agrawal et al. 2013). An alternative explanation is that a significant proportion of C-cell oncogenesis is driven by gene copy number changes rather than gene mutation. A role for copy number changes is supported by high-density comparative genomic hybridization (CGH) array studies (Flicker et al. 2012; Ye et al. 2008) and the unexpected MTC phenotype observed in numerous mouse models with allelic loss (see Table 1). From these studies, the retinoblastoma tumor suppressor (RB1) pathway has emerged as a route to C-cell oncogenesis in mice and should be considered a potentially important pathway in humans. Indeed, a recent association study specifically identified cell cycle genes in MTC development (Barbieri et al. 2014). The pathway primarily is made up of cyclin-dependent protein kinases (CDKs), cyclin-dependent kinase inhibitors (CDKNs), Cyclins (A, D, and E), RB proteins, and the E2F—family of transcription factors (Knudsen and Wang 2010) (Fig. 4). The pathway plays a critical role in cell cycle checkpoint regulation, which safeguards appropriate cell division; alterations in one or more of various components may lead to aberrant cell proliferation and development of cancer. Cell cycle progression is positively regulated by Cyclins activated by CDKs and negatively regulated by CDKNs. The activity of both is in turn regulated by environmental and cellular signals. For example, MAPK signaling leads to increased Cyclin D expression, while p53 activation enhances p21 function. The CDKN family members thus play a central role in preventing inappropriate cell division through negative CDK regulation and G1 cell cycle arrest. The CDKNs are made up of two major families—the INK4/CDKN2 family, which includes p15, p16, p18, and p19, and the Cip/Kip/CDKN1 family that includes p21, p27, and p57. The absence of

Table 1 Mouse models of MTC

Genetic target	C-cell phenotype	Reference
RET		
Human RET C634R Tg-RET C634R short isoform Rat CT/CGRP promoter	MTC in 3 of 4 founder lines CCH or MTC in 93 % of mice MTC in 8, 13, and 14 months in each founder	Michiels et al. (1997)
Human RET C634R Tg-RET C634R long isoform MoMuLV LTR promoter	Two founder lines only 1 with MTC Line 121 (22 copies) MTC in 100 % of mice by 9 months	Kawai et al. (2000)
Human RET M918T Tg-RET M918T short isoform Human CT/CGRP promoter	MTC in 3 founder lines, highest in line 42 CCH in 77 % of line 42 mice by 8 months MTC in 13 % of line 42 mice by 11 months	Acton et al. (2000)
MEN2B M919T knock-in $ret^{MEN2B/+}$ $ret^{MEN2B/MEN2B}$	Nodular CCH in 14 % at 8–12 months Nodular CCH in 60 % at 6–10 months	Smith-Hicks et al. (2000)
MEN2A C620R knock-in $ret^{C620R/+}$	No thyroid phenotype out to 24 months Homozygous is a neonatal lethal	Carniti et al. (2006)
MEN2A C620R knock-in $ret^{C620R/+}$	CCH in 48.4 % compared to 22.8 % in control mice at 20–30 months	Yin et al. (2007)
Human RET C634R Tg-RET C634R long isoform Human CT/CGRP promoter CT-2A-3 (1 copy)	MTC in 0 FVB/n mice at 43 weeks MTC in 14 % of BALB/c mice at 43 weeks MTC in 64 % of C57BL/6J 9 mice at 43 weeks MTC in 98 % of CBA/ca mice at 43 weeks	Reynolds et al. (2001), Cranston and Ponder (2003)
Tg-RET M918T; p18+/+ No Tg-RET; p18+/− Tg-RET M918T; p18+/− No Tg-RET; p18−/− Tg-RET M918T; p18−/− No Tg-RET; p18+/−; p27+/− Tg-RET M918T; p18+/−; p27+/− No Tg-RET; p18−/−; p27+/− Tg-RET M918T; p18+/−; p27−/−	MTC in 0 % of mice by 12 months MTC in 0 % of mice by 12 months MTC in 26 % of mice by 12 months MTC in 11 % of mice by 12 months MTC in 43 % of mice by 12 months MTC in 0 % of mice by 12 months MTC in 27 % of mice by 12 months MTC in 88 % of mice by 9 months MTC in 100 % of mice by 9 months	van Veelen et al. (2008)
RAS		
Tg-v-Ha-Ras Rat CT/CGRP promoter v-Ha-ras-p21	MTC in 5 founder lines Expanded lines with 1 and 12 copies MTC in 85 % of mice at 6–12 months	Johnston et al. (1998)

(continued)

Table 1 (continued)

Genetic target	C-cell phenotype	Reference
Other genes		
Tg-SV40 T-antigen Rat CT/CGRP promoter SV40 T-antigen	MTC in 5 founder lines MTC in 100 % of mice with death by 3 months	Baetscher et al. (1991)
Tg-mos Moloney virus LTR Mouse mos	MTC in 3 of 4 founder lines MTC in 4–63 % of mice by 8 months	Schulz et al. (1992)
Rb1+/−1t19neo	CCH in 43 % of mice	Harrison et al. (1995)
Rb1+/−	MTC in 96 % of mice by 77 weeks	Zhou et al. (2005)
Rb1 D326V/+ (M1326) Rb1 intron 10 3′ss/ + (M1033) Rb1 intron 21 5′ss/ + (M1032)	No thyroid tumors MTC in 90 % of mice by 70 weeks MTC in 90 % of mice by 70 weeks	Toki et al. (2014)
Rb1+/− Rb1+/−; p53+/− Rb1+/−; p53−/−	MTC in 70 % of mice over 6 months MTC in 83 % of mice over 6 months MTC in 60 % of mice by 6 months	Williams et al. (1994)
Rb1+/− Rb1+/−; p53+/−	MTC in 0 % of mice by 18 months MTC in 37 % of mice by 16 months	Harvey et al. (1995)
Rb1+/−; p53+/−	CCH or MTC in 40 % of mice	Coxon et al. (1998)
Rb1+/− p27−/− Rb1+/−; p27+/− Rb1+/−; p27−/−	MTC in 60 % of mice by 12 months MTC in 30 % of mice by 15 months MTC in 80 % of mice by 15 months MTC in 77 % of mice by 7 months	Park et al. (1999)
Rb1+/−; Men1+/−	No effect on RB1 phenotype	Matoso et al. (2008)
Rb1+/−; Msh2−/−	No effect on RB1 phenotype	Nikitin et al. (2002)
p18−/− p27−/− p18−/−; p27+/− p18−/−; p27−/−	CCH 12 %, nodular CCH 2 % in mice over 12 months CCH 4 % of mice by 12 months CCH in 46 %, nodular CCH 8 % in mice by 9 months CCH in 81 %, nodular CCH 6 % in mice by 3.5 months	Franklin et al. (2000)
p18−/−; men1+/− p27−/−; men1+/−	Nodular CCH in 80 % of mice by 22 months Nodular CCH in 6 % of mice by 12 months	Bai et al. (2007)
p18−/− p18−/−; p53−/−	Nodular CCH in 2.4 % of mice at 14 months Nodular CCH in 5 % of mice at 8 months	Damo et al. (2005)
Tg-TetOp-p25-GFP; Tg-NSE5021	MTC in 100 % of mice by 16 weeks	Pozo et al. (2013)
Rb1+/− Rb1+/−; E2f1+/− Rb+/−; E2f1−/−	MTC in 53 % of mice by 500 days MTC in 6 % of mice at 575 days MTC in 0 % of mice at 700 days	Yamasaki et al. (1998)
Rb1+/−	MTC in 40 % of mice at 210–270 days	Ziebold et al. (2003)

(continued)

Table 1 (continued)

Genetic target	C-cell phenotype	Reference
Rb1+/−; E2f3+/− Rb1+/−; E2f3−/−	MTC in 88 % of mice at 210–270 days MTC in 100 % of mice at 150–270 days	
Rb1+/− Rb1+/−; Nras+/− Rb1+/−; Nras−/−	Nodular CCH or MTC in 32 % of mice MTC in 50 % of mice, significantly larger tumors MTC in 100 % of mice, significantly larger tumors	Takahashi et al. (2006)
PRLR−/−	MTC in 29 % of mice at 12 months	Kedzia et al. (2005)

CDKN suppression leads to increased phosphorylation of RB, which disrupts an inhibitory RB-E2F interaction. Once E2F is free of RB suppression, it functions to transcribe genes that are required for progression through S phase. The aberrant loss of CDKNs and RB has been associated with the development and progression of numerous cancer types and thus these proteins serve the role of tumor suppressors (Knudsen and Wang 2010; Zhu et al. 2014; Chinnam and Goodrich 2011; Di Fiore et al. 2013). Given the fundamental role that the *RB1* gene played in the establishment of "two-hit" hypothesis of retinoblastoma (Knudson 1971), it was surprising that knockout of *Rb1* in mice was initially associated with the development of pituitary tumors and subsequently CCH (Jacks et al. 1992; Williams et al. 1994; Harrison et al. 1995). Indeed, it is clear from several studies that Rb1 plays a central role in mouse C-cell tumorigenesis (see next section). Despite this observation, a direct role for RB1 loss in human C-cells has not been found. Studies that including *RB1* sequence analysis failed to find mutation in 46 MTC tumors examined (Agrawal et al. 2013; Kanagal-Shamanna et al. 2014; Simbolo et al. 2014). A separate study found positive RB1 staining in 46 MTC tumors (Holm and Nesland 1994). When considered together, all these studies suggest that RB1 inactivation specifically induces C-cell oncogenesis but that mutation of the *RB1* gene is a low-frequency event.

In a similar manner, tumor modeling in mice has implicated roles for the RB1 regulatory CDKNs: p18 (CDKN2C), p21 (CDKN1A), and p27 (CDKN1B) in C-cell oncogenesis (see below). A role for CDKNs in MTC is supported by the observation that genetic loss of chromosome 1p (p18) is a frequent event in MTC (Mathew et al. 1987) and that reproducible losses targeting other CDKN genes are observed in recent CGH array studies (Flicker et al. 2012; Ye et al. 2008). Indeed, loss of the p18 region has been seen in 20 % of MTC tumors examined by CGH. A direct role for specific p18 loss in human MTC is also supported by a single study that found p18 mutations in 4 of 30 tumors examined (van Veelen et al. 2009). A caveat of these studies in that mutations were observed in patients with RET driver mutations, two of which were germline C634W mutations arguing against a driver role in human tumorigenesis. Additionally, an earlier study that included 50 MTC tumors found no p18 mutations (Holm and Nesland 1994), while a recent

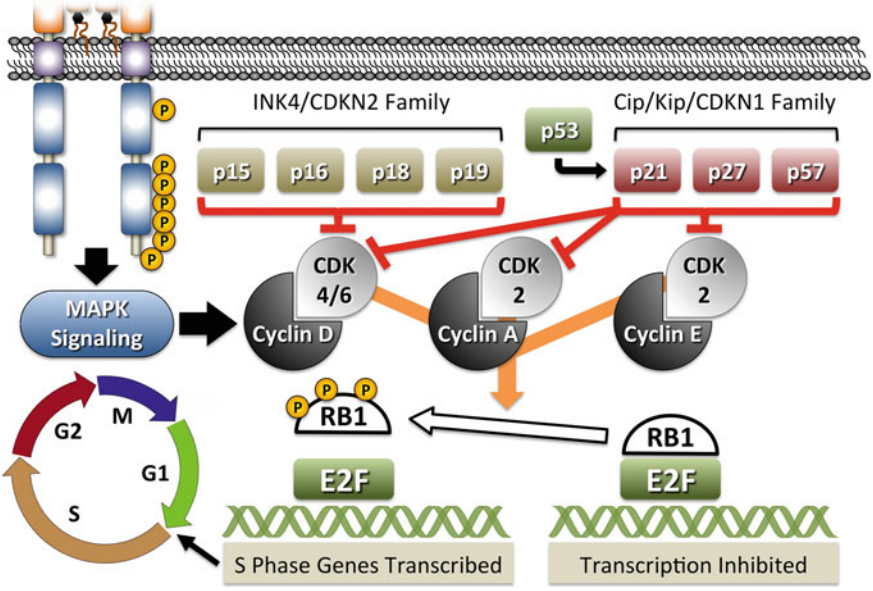

Fig. 4 RB1-mediated pathway of cell cycle regulation. The figure shows a schematic representation of major proteins involved in RB1-mediated cell cycle regulation. RB1 is responsible for inactivation of E2F transcription factors involved in the expression of proteins required for G1 to S transition. Phosphorylation (P) of RB1 results in release from E2F and activation of transcription. RB1 phosphorylation is positively regulated through induction of Cyclins and CKDs, such as RET-mediated increase of Cyclin D, and negatively regulated through activation of CDK inhibitory proteins, the CDKN2 and CDKN1 families

exomic study that included 17 tumors also found no mutations in any of the CDKN genes (Agrawal et al. 2013). The incongruity that exists between CGH and mutation studies suggests that as observed in mice, CDKN haploinsufficiency is sufficient to promote C-cell oncogenesis, or the inactivation of the remaining allele by mutation is a rare event. In support of a haploinsufficiency model, reduced p27 expression is significantly correlated with larger MTC tumor size and elevated serum calcitonin (Ito et al. 2005). Further investigation is needed to elucidate the role of the Rb pathway in MTC oncogenesis, a worthwhile endeavor given that targeted therapies, which function through direct CDK inhibition, are being developed to exploit these paths across many human cancers.

7 Modeling MTC in Animals

The first widespread animal models of MTC were the Long–Evan, Wag/Rij, and Sprague Dawley rat strains. The high incidence of spontaneous MTC tumors in these mice provided the tools for development of transplantable tumors and establishment of MTC cell lines (Muszynski et al. 1983; Zeytinoglu et al. 1980,

1983). However, despite the high frequency of MTC in these rat strains, the underlying genetic cause has only been identified in Sprague Dawley rats as mutation of the p27 gene (Cdkn1b) (Pellegata et al. 2006). For the Long–Evans and Wag/Rij rat strains, we can only say that germline mutations of the RET gene do not play a role (Fritz et al. 2002; De Miguel et al. 2003). With the development of tools to manipulate the mouse genome, rats were largely replaced as disease models for many disorders including MTC. This section focuses on engineered mouse models of MTC. A summary of mice with a reported MTC phenotype is provided in Table 1.

7.1 RET

Activating mutations within the *RET* gene were identified as a genetic cause of MTC in human in 1993 (Donis-Keller et al. 1993; Mulligan et al. 1993). With this discovery began the work to generate genetic mouse models to better understand the pathogenesis of the disease. Transgenic mice employing the CT/CGRP promoter for cell specificity predominate early models, which focused on the common RET mutations of C634R and M918T (Acton et al. 2000; Michiels et al. 1997; Reynolds et al. 2001). A single transgenic line has been created using nonspecific Moloney murine leukemia virus LTR (Kawai et al. 2000). A direct comparison of these models is difficult, given that in addition to the variable number of copies integrated, different transcription promoters were used along with expression of either long or short RET isoforms. Despite the variations, all mice were found to develop CCH as a precursor to MTC (Table 1). Curiously, unlike humans, the progression of MTC in mice expressing RET M918T is less aggressive than that observed in C634R models; this finding may relate to the use of the short RET isoform. Genetic knock-in models, which are clearly a more accurate representation of human disease, actually fail to develop MTC (Carniti et al. 2006; Yin et al. 2007; Smith-Hicks et al. 2000). However, similar to human disease, the M919T (in mice RET M919T is equivalent to the human MEN2B M918T) model develops earlier onset of CCH and pheochromocytoma compared to MEN2A models. Furthermore, MEN2A models display a pathology consistent with Hirschsprung's disease, which is associated with the specific RET C620R mutation.

The differential onset of MTC in RET transgenic mice and failure to develop tumors in knock-in models argue for a role beyond aberrant RET activation in C-cell oncogenesis. Indeed, in comparing the models, the site of transgene integration may be more telling than expression levels. Two studies also support that RET-mediated oncogenesis requires a cooperative genetic background. First, Cranston et al. demonstrated that the development of MTC was highly strain specific. Using mice with a single RET C634R insertion driven from the CT/CGRP promoter, tumor production varied from zero in FVB/n mice to nearly 100 % in CBA/ca mice (Table 1) (Cranston and Ponder 2003). Of note, the RET MEN2B knock-in mouse model exists in an FVB/n mixed background. The second study is

an extensive comparison of RET M918T-mediated tumorigenesis in mice with copy number variations of the cyclin-dependent kinase inhibitors p18 (cdkn2c) and p27 (cdkn1b) (van Veelen et al. 2008). This work demonstrates clear synergism between RET activation of MAPK pathways and deregulation of cell cycle progression (Table 1). These findings further correlate with the high frequency of allelic loss observed in chromosome 1p, where p18 maps, in human MTC tumors (Flicker et al. 2012; Ye et al. 2008) and the more aggressive phenotype observed in patients with coincident somatic M918T mutation and p18 loss (unpublished observations).

7.2 RAS

The first description of HRAS activation as an oncogenic driver of C-cells actually predates the discovery that RET germline mutations cause MEN2 (Okazaki et al. 1989). The development of a transgenic mouse with C-cell-specific expression of activated v-ha-ras occurred much later (Johnston et al. 1998). The v-ha-ras transgenic mouse most closely resembles the RET line generated by Michiels et al. (1997) in that both employ the rat CT/CGRP promoter and SV40 polyadenylation sites. Tumor formation in v-ha-ras mice is similar to that observed in RET transgenic mice (Table 1). Tumors were preceded by CCH, were calcitonin positive, and found to be invasive in older animals. Importantly, these findings are consistent with RET tumorigenesis being mediated through a ras-dependent pathway, but are in opposition to the neuroendocrine differentiation observed with ectopic expression in TT cells (Nakagawa et al. 1987). However, despite the high frequency of MTC formation observed in this model, a comparable genetic knock-in, *hras* G12V, has no overt tumor phenotype in animals up to 18 months of age (Chen et al. 2009; Schuhmacher et al. 2008). Furthermore, Costello and Noonan syndromes, which are associated with germline *HRAS* and *KRAS*/*NRAS* mutation respectively, have no reported incidence of MTC (Rauen 2013; Bezniakow et al. 2014). Therefore, a clear understanding of the role of RAS activation in C-cell oncogenesis remains to be elucidated.

7.3 Other Mouse Models with a MTC Phenotype

Three engineered mouse models with MTC actually predate the first reported RET transgenic mouse (Michiels et al. 1997). These include two transgenic models, one overexpressing *c-Mos* (Schulz et al. 1992) and the other SV40 T-antigen (Baetscher et al. 1991), and the earliest genetic knockouts of the *Rb1* gene (Williams et al. 1994; Harrison et al. 1995; Harvey et al. 1995). Although the mechanism of *Mos*-mediated tumorigenesis was unclear, the authors speculated that *Mos* "may function in the same pathway that gives rise to MEN2." The subsequent finding that MOS, like RET, activates the MAPK pathway serves to underscore the importance of

MAPK activation in C-cell oncogenesis (Okazaki and Sagata 1995). For the remaining models, given that one of the actions of SV40 T-antigen is to inhibit Rb1 function (DeCaprio et al. 1988), these early findings served to identify regulation of cell cycle progression as a second key pathway of C-cell oncogenesis.

Since these initial studies, more than 20 engineered mouse models have been reported to have MTC as part of their phenotypic presentation (Table 1). These models nearly universally share aberrant upregulation of the MAPK pathway (Spry1) or targeted inhibition of RB1-mediated cell cycle control. A single exception is the observation that loss of prolactin receptor is associated with MTC formation (Kedzia et al. 2005). How loss of prolactin receptor causes C-cell transformation remains unclear though it is interesting that, like *Rb1* loss, animals develop pituitary hyperplasia (Schuff et al. 2002). In looking at the remaining mouse models, some generalized observations seem to emerge. First, the targeting of *p18* (Cdkn2c) or *p27* (Cdkn1b), which indirectly inactivates Rb1 through enhanced phosphorylation, reproduces the *Rb1*+/− MTC phenotype albeit at a reduced level. Furthermore, as predicted, the simultaneous targeting of these Cdkn's with Rb1 enhances C-cell oncogenicity; combination deletions with *p53* or *Men1*, which act further upstream of Rb1, have only minor impact on tumorigenesis; and inactivation of *E2f1*, which is a downstream target of Rb1, inhibits tumorigenesis (Fig. 4, Table 1). Finally, targeted inactivation of Rb1 protein, mediated through its phosphorylation by hyperactive Cdk5, results in early onset of aggressive MTC with 100 % penetrance (Pozo et al. 2013). Despite the above observations, two additional Rb1 mouse models raise questions regarding its role in C-cell oncogenesis. Similar to the actions of Cdk5, *Rb1* haploinsufficiency combined with either *E2f3* or *Nras* loss results in the formation of highly penetrance aggressive MTC (Ziebold et al. 2003; Takahashi et al. 2006). The counterintuitive nature of these two observations serves to underscore the need for further investigation of pathways leading to C-cell oncogenesis.

8 Considering Mechanisms of C-Cell Tumor Initiation and Progression

Unlike some cancers, a clear stepwise route to C-cell oncogenesis has not emerged. Hereditary MTC demonstrates that activating mutations of RET serve as a primary initiating event. This role of RET is further supported by the observed high prevalence (~40 %) of RET mutations in sporadic MTC (Hu and Cote 2012). Several models have been proposed for how RET might function in initiation of C-cell oncogenesis. First, inactivation of RET in MTC cell culture models causes a marked reduction in tumor cell growth supporting a mechanism of oncogene addiction (Drosten and Putzer 2006; Vitagliano et al. 2011). This model is in part supported by patient responses to targeted therapies (Elisei et al. 2013; Wells et al. 2012). A second proposal is that RET increases genetic instability. In patient tumors, RET mutation is associated with increases in copy number variants and

somatic mutations (Agrawal et al. 2013; Flicker et al. 2012; Ye et al. 2008). In one report examining sporadic MTC tumors using exomic sequencing, the mutation number was more than doubled in tumors with RET mutation (21.9 in RET+ vs. 8.4 in RET−) (Agrawal et al. 2013). In hereditary tumors, somatic mutation identified by exomic sequencing appears to be less frequent (Cai et al. 2015). Finally, a role for RET in altering the stem cell population needs to be considered in both hereditary and sporadic diseases. RET is known to play a central role in regulation of human spermatogonial stem cell self-renewal (Wu et al. 2009) and neural crest stem cell lineage derivation (Iwashita et al. 2003). In fact, somatic *RET* M918T mutation in male germ cells is associated with enhanced stem cell proliferation and provides a plausible mechanism for the paternal origin of MEN2B (Choi et al. 2012). While it remains to be formally examined, germline mutation of *RET* is also predicted to increase the neural crest progenitor cell population, thereby providing a larger pool of tumor initiating or the so-called cancer stem cells. A thyroid cancer stem cell model has been proposed for both medullary and nonmedullary thyroid cancers (Thomas et al. 2008; Zhang et al. 2006; Hardin et al. 2013). Support for a role in MTC derives from the observations that C-cells or their progenitors play a role in adult thyroid regeneration (Ozaki et al. 2012) and that *RET* mutation is associated with EGF/FGF-independent (Zhu et al. 2010), as well as 5-fluorouracil-resistant MTC tumorsphere growth (Kucerova et al. 2014).

Because MTC occurs in the absence of *RET* mutation, other pathways to C-cell oncogenesis must exist. Activation of RAS gene family members has emerged as a second oncogenic pathway with mutations observed in approximately 15 % of sporadic MTCs examined (Moura et al. 2011; Agrawal et al. 2013; Boichard et al. 2012; Ciampi et al. 2013; Schulten et al. 2011). A specific role for RAS as an initiator derives from the mouse models previously discussed, as patients with germline *HRAS* mutations (Costello syndrome) or *KRAS*/*NRAS* mutations (Noonan syndrome) have not been reported to develop MTC (Rauen 2013). Finally, no single gene has emerged as a common driver responsible for MTC without *RET* or *RAS* mutation. However, a focused examination remains to be performed. First, a recent comparison of Sanger-based sequencing with Ion Torrent AmpliSeq found a 30 % increase in the number of RET mutations detected (Simbolo et al. 2014). Furthermore, we have recently identified a case of RET gene fusion as a driver of tumorigenesis (Grubbs et al. 2015). RET fusions would go undetected by most DNA sequencing approaches. At the time of writing, only 5 such MTC tumors had been examined by exomic sequencing (Agrawal et al. 2013). The number of reported mutations in these 5 tumors ranged from 7 to 10, with only the *TDG* gene found mutated in more than one tumor. Unfortunately, Sanger sequencing could not confirm these specific *TDG* mutations, and *TDG* mutation was not in a separate larger validation set. While the small sample size may have prevented the discovery of other MTC genetic drivers, it is also possible that noncoding RNAs or gene copy number changes went undetected because of the limitations associated with the sequencing approach (Berindan-Neagoe et al. 2014; Yang and Lu 1839; Davoli

et al. 2013; Manikandan et al. 2012). Indeed, in mice, heterozygous loss of *Rb1* or *p27* in the absence of a second mutation of the remaining allele is sufficient to induce MTC (Williams et al. 1994; Harvey et al. 1995; Park et al. 1999; Zhou et al. 2005). Although clear links establishing a role for cell cycle regulators in human C-cell oncogenesis do not exist, there is supportive evidence. Copy number changes that would not be detected by exomic sequencing have been observed using CGH arrays (Flicker et al. 2012; Ye et al. 2008). There have also been association studies linking increased MTC risk to genes associated with cell cycle regulation (Barbieri et al. 2014; Ito et al. 2005; Pasquali et al. 2011; Sekiya et al. 2014), although they are not universally positive (Sekiya et al. 2014). These observations fit well with a newer model, suggesting that heterozygous gene loss is sufficient to drive cancer (Davoli et al. 2013; Manikandan et al. 2012). The observation that MTC tumorigenesis in mice is significantly enhanced when targeting multiple Rb1 pathway members, particularly in combination with the RET/RAS pathway, suggests that progression and aggressive cancer might be caused by simultaneous hits to both pathways.

9 Summary

The production and secretion of calcitonin in response to increased serum calcium remains as the only well-defined role of the thyroid C-cell. The specific linking of the C-cell as the origin of MTC through calcitonin production has served as the primary motivator for understanding the biology of this cell. Ironically, this role has proven to be physiologically redundant, as genetic ablation of calcitonin is relatively inconsequential (Hoff et al. 2002). Unfortunately, little is known about other possible physiological roles of the C-cell and the mechanisms behind its unique sensitivity to RET-mediated oncogenesis. Genetic modeling in mice is beginning to gain insight into both processes. Studies of thyroidal development have suggested a possible interactive relationship between C-cells and follicular cells within the thyroid microenvironment (Andersson et al. 2011). Tumor modeling in mice has demonstrated that site-directed mutation of MEN2B-equivalent M919T alone is insufficient to cause MTC, but does induce pheochromocytoma. Models with unexpected MTC phenotypes have also linked oncogenesis to the Rb1-mediated pathway of cell cycle regulation. Together these pathways appear to synergize in a manner that appears to preferentially target the C-cell. Whether it is these cellular pathways that provide the C-cell with specific sensitivity to oncogenesis remains an unanswered question. Indeed, a role for RET signaling in normal C-cell function remains to be elucidated. In the nervous system, RET plays an essential role during development and in neuronal survival. A parallel role may exist for the C-cell, or perhaps like for the spermatogonial stem cell, RET functions as a C-cell signal for stem cell maintenance (Mulligan 2014). Roles for C-cells and calcitonin in thyroid regeneration and goiter formation have been proposed (Ozaki et al. 2012; Lupulescu 1972). Such a role would certainly fit with dysregulation of these pathways

being associated with oncogenesis. Thus, while we have an ever-increasing knowledge base linked to the cellular drivers of C-cell oncogenesis, the primary role of these genes in normal C-cell physiology remains unclear. A greater understanding of the normal function of these genes is likely to contribute to defining the differences observed in the biology of MTC between mouse models and human, as well as offering greater insight into pathways of tumorigenesis beyond aberrant RET activation.

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Histopathology of C Cells and Medullary Thyroid Carcinoma

Kurt Werner Schmid

Abstract

The human thyroid gland contains less than 0.01–0.1 % calcitonin producing and secreting C cells, which in men are almost exclusively situated in an intrafollicular location; the vast majority of C cells are embryologically derived of remnants of the ultimobranchial body and ultimately of the neural crest, a small subset, however, is presumed to originate from endodermal stem cells. Thyroid tumours with C cell differentiation have been named medullary thyroid carcinoma (MTC); calcitonin is also produced and secreted by MTC which makes this peptide hormone a very useful serum marker both for early detection and clinical follow-up of patients with MTC. About 70–80 % of MTC are sporadic tumours, whereas 20–30 % are familial MTC which are autosomal-dominant inherited and caused by germline mutations of the RET proto-oncogene located on chromosome 10. This article summarizes the histological, immunhistochemical and molecular genetic features of C cells, C-cell hyperplasia (CCH) and MTC, emphasizing the role of diagnostic pathology.

Keywords

C cells · C-cell hyperplasia · Medullary thyroid carcinoma · Sporadic · Familial · MEN 2

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Contents

1 Introduction

The putative first description of C cells can be attributed to E. Cresswell Baber in the thyroid gland of the dog in 1876; he suspected that sheets of cells situated between the "vesicles" (i.e. follicles) represent the regeneration pool of the "vesicles" (Baber 1876). In 1894, Karl Hürthle described in his only publication dealing with the thyroid cell complexes in dog thyroid which he called "interfollicular epithelium" (Hürthle 1894). Later, these cells were confused with follicular-derived elements such as oncocytic cells, leading particularly in the Anglo-American usage to the still common but erroneous designation "Hürthle cells" as a synonym for oncocytes.

In 1932, José F. Nonidez identified in the thyroid of the dog the exclusively between the follicles of the thyroid situated C cells as a second component of the thyroid epithelium and coined them "parafollicular cells" (Nonidez 1932). However, in humans, normal C cells are predominantly located within the thyroid follicles (Fig. 1), with less than 1 % of C cells in a parafollicular location. Thus, the term "parafollicular cells" as a synonym for human C cells should be avoided.

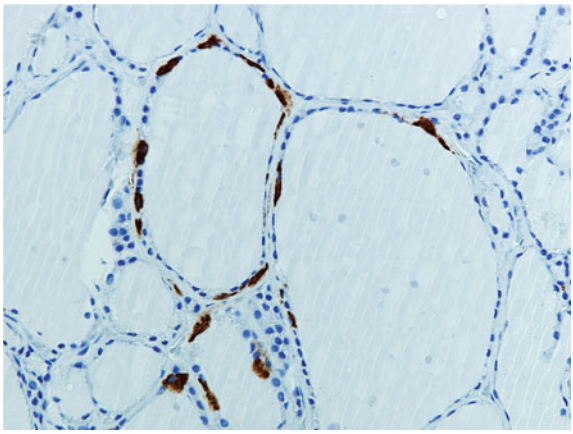

Fig. 1 Normal thyroid gland with immunohistochemically demonstrated C cells. The predominantly disperse distributed C cells are located intrafollicular (calcitonin immunohistochemistry, ×200)

Some decades later, electron microscopic studies supported the observation (DeLellis et al. 1978) that human C cells are indeed intrafollicular and separated from the thyroid interstitium by the follicular basement membrane. C cells contain moderately electron-dense larger (type I; 280 nm) and more electron-dense smaller (type II; 130 nm) secretory granules; ultrastructural immunohistochemistry demonstrated the peptide hormone calcitonin in both type I and type II granules. Besides, these granules C cells contain abundant endoplasmic reticulum and mitochondria. All these structures are also detectable in hyperplastic and neoplastic C cells.

2 Embryology of C Cells

The majority of C cells in the human thyroid gland are originating from primordial C cells of the neural crest, which migrate ventrally to the ultimobranchial body (a derivative of the ventral recess of the fourth pharyngeal pouch). These C cells are thus of neuroectodermal origin. During the descent of the thyroid, the ultimobranchial body is incorporated into both thyroid lobes, ideally leading to a disperse distribution of the C cells in preferred regions of the thyroid lobes (see below). Apparently in most humans, the ultimobranchial body is completely incorporated into the thyroid lobes. However, occasionally C cells remain along their migration path in extrathyroidal location and may give rise to pathological processes of C cells (hyperplasia and neoplasia) outside the thyroid (Hirsch et al. 2004; Smets et al. 1990). Disturbances in the intrathyroidal distribution result in focal accumulation of C cells (Gibson et al. 1980). Studies in thyroid glands of patients with DiGeorge syndrome confirmed on the one hand the origin of C cells from the ultimobranchial body (Pueblitz et al. 1993), and on the other hand, they suggest that a (small) subset of C cells most likely arise from endodermal stem cells (Harach 1997).

3 Normal Human C Cells

In the thyroid, three types of epithelial cells can be distinguished: follicular cells accounting for more than 99.9 % of all thyroid epithelia, C cells (0.01–0.1 %) and the only occasionally demonstrable so-called solid cell nests (SCN); the latter most likely represent remnants of the ultimobranchial body, which is supported by the calcitonin production of SCN. The number of C cells is higher in newborns than in adults and increases again after 60 years of age (O'Toole et al. 1985).

C cells are mainly confined to groups of thyroid lobules located centrally in the rear upper portions of both thyroid lobes. At the poles of the thyroid lobes, the isthmus and pyramidal lobe C cells are detectable in significantly reduced numbers or completely missing. Thus, medullary thyroid carcinoma (MTC) may occur in all parts of the thyroid; however, it can be found more frequently in areas of high C-cell concentration than in the peripheral thyroid.

Histologically, C cells are difficult to identify on routine H&E sections; usually, calcitonin immunohistochemistry is performed to demonstrate C cells on the light microscopical level (LiVolsi 1990). The majority of C cells (size up to 40 μm) are round or polygonal, found singly or in groups of 3–5 cells. A second type of C cells is spindled shaped with tapered ends (Ljungberg 1972; Schmid et al. 1992). Virtually, all C cells are situated within the follicular basement membrane.

4 Function of C Cells

The term "C cell" was introduced by Copp and Cameron in 1961, who postulated in isolated thyroid and parathyroid glands of dogs a type of cell producing and secreting a hormone "calcitonin" with calcium-lowering effect (Copp and Cameron 1961); however, initially, the parathyroid glands were assumed as the origin of the hormone (Copp et al. 1962). In 1964, it was recognized that calcitonin originates from the thyroid gland (Foster et al. 1964); in 1967 (Tauber 1967), the previously postulated "C cells" were morphologically demonstrated in the thyroid gland and their neuroectodermal origin was revealed. Already in 1966, E. Dillwyn Williams had predicted the until then postulated C cells as the source of MTC (Williams 1966). In 1973, Hubert J. Wolfe recognized that C-cell hyperplasia (CCH) is an obligatory precursor of hereditary MTC (Wolfe et al. 1973).

Almost all human C cells synthesize and secrete calcitonin; only in a small fraction of the C cells exclusively calcitonin gene-related peptide (CGRP) is detectable. Calcitonin is a 32-amino acid linear polypeptide hormone. Structurally, calcitonin has an intramolecular disulphide bridge (Cys-1 to Cys-7) and an amidated C-terminus; both are essential for the biological activity of the hormone. Disulphide bridge-free calcitonin is able to bind specifically to the calcitonin receptor; however, it acts at the receptor as a competitive antagonist. The neuropeptide CGRP consists of 37 amino acids and is encoded by the same gene as calcitonin; it occurs predominantly in the peripheral and central nervous system and to a lesser extent in the thyroid by selective mRNA splicing.

An increase of serum calcium in the blood, gastrointestinal hormones and pentagastrin (a synthetic oligopeptide with gastrin effect) stimulate calcitonin secretion. To lower the serum calcium level, calcitonin induces a decreased osteoclastic activity in the bone, an increased calcium excretion from the kidney and a reduced resorption of calcium in the intestine. However, the efficacy of calcitonin in the regulation of calcium in comparison with the other calcium-regulating hormones calcitriol and parathyroid hormone is relatively limited.

5 C-Cell Hyperplasia (CCH)

An increase in size and number of C cells is called CCH. The term CCH refers, however, to two different C-cell conditions with completely different pathological potential (Albores-Saavedra and Krueger 2001; LiVolsi 1997; Perry et al. 1996). Inherited "neoplastic C-cell hyperplasia", by definition always associated with germline mutation of the RET proto-oncogene, is the precursor lesion of familial MTC and can easily be identified on H&E sections (DeLellis et al. 2004). The enlarged, pale C cells are mainly located in areas of high C-cell concentration (Ting et al. 2015). Neoplastic CCH is found in prophylactic thyroidectomy specimens of asymptomatic carriers of the RET-mutation or patients with MTC usually in close vicinity of (multifocal) invasive familial MTC. Morphologically, neoplastic CCH is characterized by groups of atypical cells situated within the thyroid follicular basement membrane (focal CCH); these cells may encircle the entire follicular lumen (diffuse CCH; Fig. 2) and ultimately obliterate the follicular lumen partially or completely (nodular CCH; Fig. 3). Neoplastic CCH may be difficult to distinguish from SCN, palpation thyroiditis and occasionally papillary microcarcinoma (Elisei et al. 2008; Schmid and Sheu 2015).

In contrast, "non-MEN2-associated CCH" (Ting et al. 2015), previously named "sporadic" or "physiological" CCH (Perry et al. 1996), cannot be identified on H&E sections (DeLellis et al. 2004); its identification requires immunohistochemical calcitonin demonstration (Fig. 4). Non-MEN2-associated CCH is defined as an increase of normally appearing C cells; at 100-fold magnification, at least 50 C cells (calcitonin immunohistochemistry) have to be identified in areas of high C-cell concentration (Rosai et al. 1992). Occasionally, non-MEN2-associated CCH can also show focal obliteration of follicles. Non-MEN2-associated CCH has been described in association with a great variety of pathological conditions, including non-medullary thyroid tumours (Albores-Saavedra et al. 1988; Scheuba et al. 2000), autoimmune thyroiditis Hashimoto and thyroid non-Hodgkin-lymphoma (Baschieri

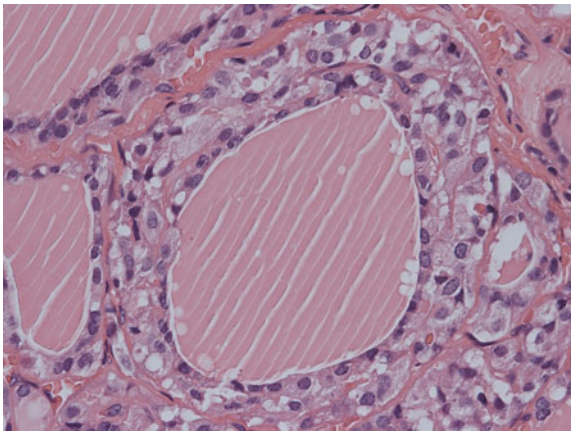

Fig. 2 "Diffuse" neoplastic C-cell hyperplasia (MEN 2A) with atypical cells encircling the entire follicular lumen (H&E ×200)

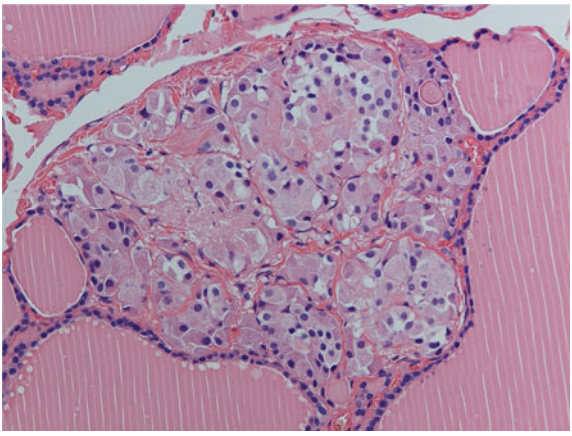

Fig. 3 "Nodular" neoplastic C-cell hyperplasia (MEN 2A) with complete obliteration of the follicle by atypical C cells. The follicular basement membrane is still intact (H&E ×200)

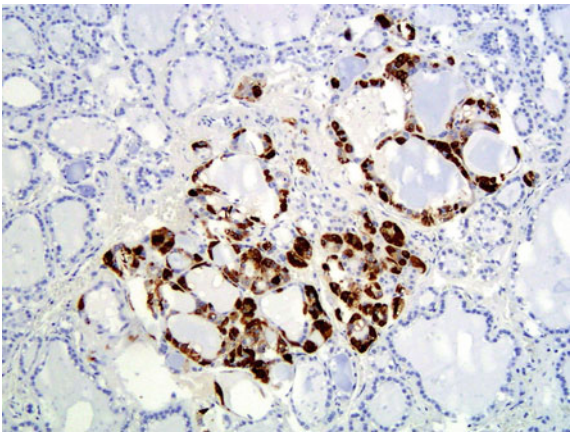

Fig. 4 "Non-MEN2-associated" C-cell hyperplasia, which cannot be demonstrated on H&E sections; the increased number (>50 at 100-fold magnification in areas of high C-cell concentration) of normally appearing C cells is demonstrated by immunohistochemistry (calcitonin antibodies; ×100)

et al. 1989), thyrotoxicosis (Scheuba et al. 2000), in the vicinity of SCN (Chan and Tse 1989), renal failure and calcium metabolism disorders. Up to 50 % of patients with nodular goitre (age- and sex-independent) show a morphologically detectable non-MEN2-associated CCH without increased serum calcitonin (Kaserer et al. 1998). To the best of knowledge, this form of CCH is not related to the development of sporadic MTC; so far, no somatic mutations of the RET proto-oncogene could be demonstrated in the C cells of "non-MEN2-associated" CCH (Saggiorato et al. 2007).

6 Transition of Neoplastic CCH to Familial MTC

The differential diagnosis of neoplastic CCH from microinvasive familial MTC may cause severe difficulties (DeLellis et al. 2004). Microinvasive MTC is characterized by defects of the follicular basement membrane (Rosai et al. 1992) which are virtually impossible to demonstrate on routine H&E sections. However, this is regularly accompanied by the development of stromal desmoplasia around the tumour cell nests which is a useful and reliable surrogate marker of tumour cell invasion (Sheu and Schmid 2010; Fig. 5). In some cases, the neoplastic C-cell aggregates are measuring several millimetres apparently without developing stromal desmoplasia (Fig. 6a, b); due to their size, these lesions are classified as MTC although no generally accepted consensus on this issue exists.

7 Medullary Thyroid Carcinoma (MTC)

Medullary carcinoma (MTC; ICD-O 8345/3) is defined as a malignant tumour of the thyroid gland showing C-cell differentiation (DeLellis et al. 2004). Up to 25 % of MTC is caused by a gain of function mutation of the RET proto-oncogene (familial MTC); familial MTC is preceded by neoplastic CCH hyperplasia. Sporadic (non-inherited) MTC has no defined precursor lesion; 40–60 % of sporadic MTC shows somatic mutations of the RET proto-oncogene (Elisei et al. 2008; Hofstra et al. 1994). The vast majority of MTC synthesizes and secretes the peptide hormone calcitonin. Thus, calcitonin is a very useful immunohistochemical markers for the histological diagnosis of MTC (Harach et al. 1992; Schmid and Böcker 1993). Rarely, however, MTC may be lacking immunohistochemically detectable calcitonin ("atypical MTC" (Schmid and Ensinger 1998)), and/or patients suffering from histologically proven MTC may lack pathologically elevated serum calcitonin ("non-secretory MTC" (Frank-Raue et al. 2013)).

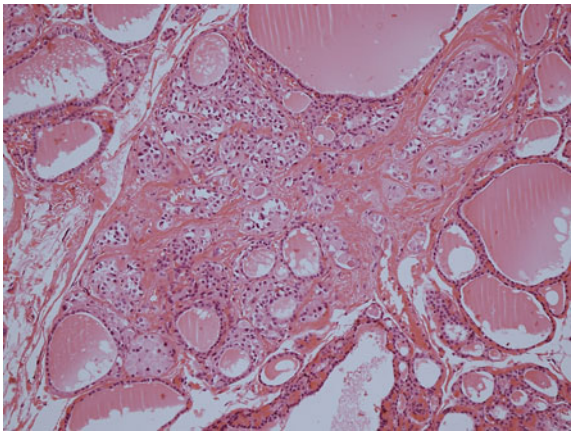

Fig. 5 Invasive familial MTC (MEN 2A) with stromal desmoplasia and tumour cell invasion. The MTC is measuring approx. 3 mm (H&E ×100)

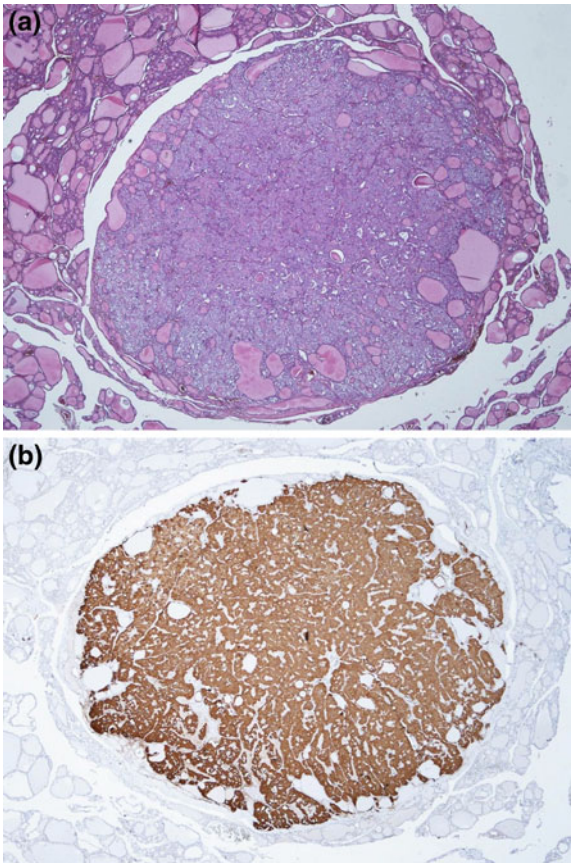

Fig. 6 **a** Familial MTC (MEN 2A) without stromal demoplasia or obvious invasive growth in the tumour periphery; the diagnosis of MTC is based on its size (5 mm). (H&E ×50). **b** Immunohistochemical calcitonin demonstration in the MTC depicted in Fig. 6a (×50)

In 1951, Robert C. Horn described (Horn 1951) a series of seven cases of a variant of thyroid carcinoma with solid growth pattern and amyloid, which he classified subsequently as poorly differentiated and highly malignant (Horn and Dull 1951). The term "MTC" was coined in 1959 by Hazard et al. based on a series of 21 thyroid carcinomas, again morphologically appearing as solid tumours with amyloid deposits; biologically, the tumours were considered to have an intermediate position between differentiated (papillary and follicular thyroid carcinoma) and anaplastic thyroid carcinomas (Hazard et al. 1959). In 1966, E. Dillwyn Williams recognized striking similarities between human medullary carcinoma and thyroid tumours in dog and rat originating from "parafollicular cells" (Williams 1966). He proposed that human medullary carcinoma might be derived from the to that time in men still postulated C cells, and he predicted that if the C cells were the source of calcitonin, medullary carcinoma might also produce this hormone. In 1968, Meyer and Abdel-Bari demonstrated electronmicroscopically secretory granules in the tumour cells as well as biochemically a 100-fold increased calcitonin concentration in the tumour compared to normal thyroid tissue (Meyer and Abdel-Bari 1968). Using

immunofluorescence, Bussolati et al. demonstrated in 1969 calcitonin in the cells of medullary(Bussolati et al. 1969).

In 1886, Felix Fränkel reported of an already 1884 performed autopsy of a 18-year-old female patient with bilateral adrenal tumours (then interpreted as an angiosarcoma) and goitre (Fränkel 1886); this is considered to be the first description of a case of multiple endocrine neoplasias type 2A. Over 120 years later, a germline mutation in the RET proto-oncogene at codon 634 TCG > TGG (C634W) was demonstrated on the still available tissue specimens of the patient; subsequently, descendants of brothers of the patient suffering from pheochromocytomas and MTC were identified (Neumann et al. 2007). John H. Sipple described in 1961 the combination of pheochromocytoma with adenocarcinoma of the thyroid (Sipple 1961); subsequently, the combination was recognized as the (autosomal-dominant inherited) syndrome of multiple neuroendocrine neoplasia (MEN) 2A (Steiner et al. 1968), in which the in all cases occurring MTC determines largely the patients' survival (Machens and Dralle 2006). The first description of a case with MEN 2B was made in 1922 by Wagenmann (1922) and Froebius (1922).

Up to date, no benign C-cell tumour has been defined. In familial MTC, malignancy is presumed when hyperplastic C cells show invasive growth through the follicular membrane which is accompanied by the development of a fibrous desmoplastic stroma (Rosai et al. 1992; Sheu and Schmid 2010; Fig. 5). This stromal desmoplasia is also observed in approximately 80 % of sporadic MTC. However, in the remaining 20 % of sporadic MTC as well as in a number of familial MTC, stromal desmoplasia is completely lacking (Koperek et al. 2008); in these cases, which are usually well circumscribed but not necessarily enveloped by a tumour capsule, the diagnosis of MTC (by definition a malignant tumour! (DeLellis et al. 2004)) is apparently rather based on the size of the C-cell tumour than the demonstration of unequivocal invasive growth (Synoracki et al. 2015; Fig. 6a, b).

7.1 Morphology of MTC

MTC is mainly located in the lateral upper two-thirds of the thyroid lobes, the area of highest C-cell concentration; the tumours range in size from barely visible to several centimetres in diameter (LiVolsi 1990). In >90 %, familial MTC is associated with (small) multifocal and bilateral tumours, whereas in sporadic MTC, multifocality is the exception (Rosai et al. 1992; Schmid et al. 2003). In the isthmic region or the thyroid periphery, MTC is located only occasionally. Macroscopically, MTC presents with a greyish-white to reddish-brown cutting surface; the tumours are often circumscribed and in rare cases even completely encapsulated. On the other hand, occasionally even very small tumours (<7 mm) may macroscopically already show infiltrative borders (Chan 2007). With increasing size, the tumours may regularly develop haemorrhage, cystic regressions and/or in large tumours central necrosis (DeLellis et al. 2004).

The histopathological appearance of MTC is exceptionally variable (DeLellis et al. 2004; LiVolsi 1990; Rosai et al. 1992; Synoracki et al. 2015). MTC may mimic a broad variety of thyroid and non-thyroid tumours; thus, every unusual thyroid tumour should be investigated using calcitonin immunohistochemistry in order to prove or exclude MTC (Schmid et al. 2003). The typical MTC shows a solid and compact growth pattern of nests of polygonal- and spindle-shaped tumour cells which regularly freely infiltrate into the surrounding non-neoplastic thyroid tissue. The cytoplasm of the tumour may appear granulated, and the predominantly uniform round-to-oval nuclei show a coarsely granulated chromatin ("salt-and-pepper pattern"). The number of mitosis found is quite variable. The tumour stroma may show delicate collagen bands without stromal desmoplasia; MTC without stromal desmoplasia is statistically significantly associated with a very low potential for metastasis (Koperek et al. 2008; Scheuba et al. 2006). So far, no specific immunohistochemical and/or molecular markers have been found further characterize MTC without stromal desmoplasia (Koperek et al. 2007, 2009, 2011). In the majority of MTC (approx. 80 %), stromal desmoplasia, indicating invasive growth of tumour cells, can be demonstrated; however, the extent of stromal desmoplasia may vary considerable.

Amyloid deposits are present in 60–85 % of MTC cases. The demonstration of amyloid may be strongly suggestive for the diagnosis of MTC; however, since amyloid can be found in a variety of non-C-cell differentiated thyroid lesions, the diagnosis of MTC cannot be based exclusively on the demonstration of amyloid.

7.2 Histological Variants

The papillary or pseudopapillary variant of MTC is characterized by the presence of true papillae or artificial pseudopapillary structures; the tumour cell nuclei are lacking the nuclear features of papillary carcinoma (Fig. 7). MTC with glandular

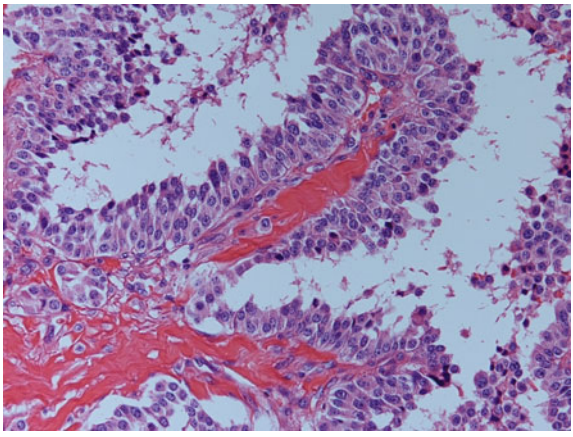

Fig. 7 Papillary structures in a sporadic MTC; the tumour cell nuclei show a coarsely granulated chromatin ("salt-and-pepper pattern") without the morphological nuclear features of papillary thyroid carcinoma (H&E ×200)

features (follicular or trabecular) may cause differential diagnostic problems with adenomas and follicular carcinomas (Fig. 8). The insular variant of MTC may resemble poorly differentiated thyroid carcinoma or classical carcinoid. Giant cell and spindle cell (Fig. 9) variants of MTC may be confused with anaplastic thyroid carcinoma; although these variants of MTC may show a less favourable outcome, the prognosis is still much better than that of anaplastic carcinoma. Small-cell variant of MTC, which is more commonly associated with tumour necrosis than other variants of MTC, is also associated with a poorer prognosis. The oncocytic variant of MTC may be misdiagnosed as oncocytic (Hürthle cell) variants of other thyroid tumour entities; since oncocytic thyroid tumours with follicular cell differentiation may show a false-positive calcitonin immunoreactivity (due to the antibody clone used), the diagnosis of the oncocytic variant of MTC should be confirmed by additional immunohistochmical markers [e.g. chromogranins,

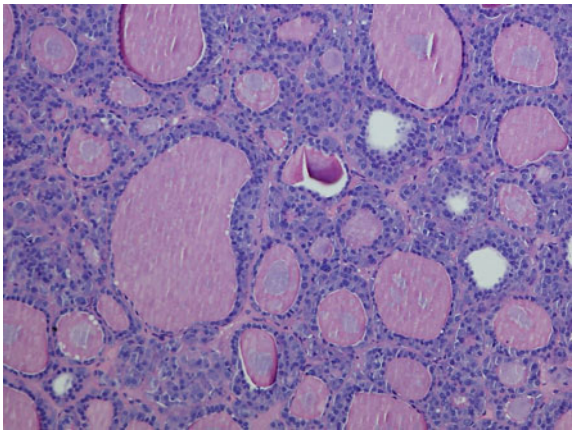

Fig. 8 Glandular (follicular) features in a sporadic MTC; this growth pattern may cause differential diagnostic problems with follicular neoplasms (H&E ×100)

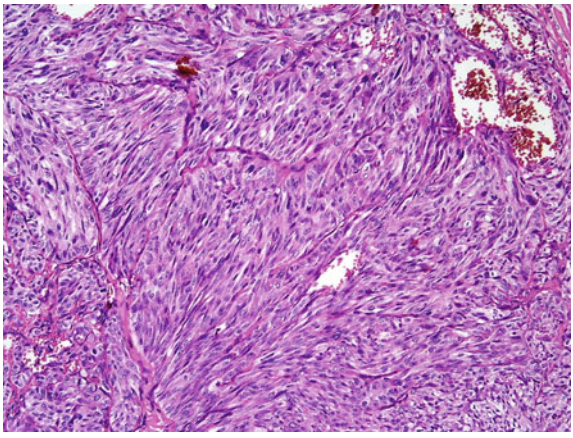

Fig. 9 Spindle cell variant of MTC may be confused with anaplastic thyroid carcinomas (H&E ×200)

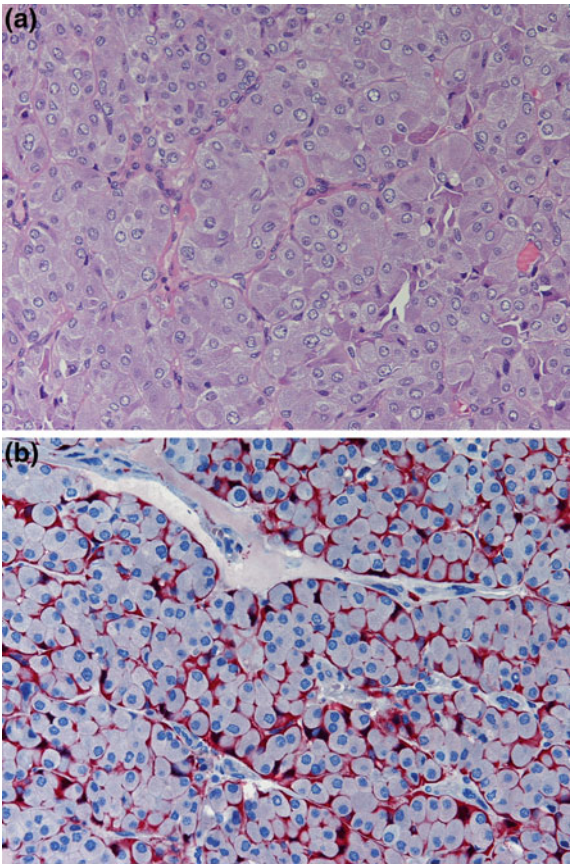

Fig. 10 **a** Paraganglioma-like variant of MTC (H&E ×200). **b** Numerous S-100-protein positive sustentacular cells in a paraganglioma-like variant of MTC (S-100-protein immunohistochemistry, ×200)

synaptophysin, carcinoembryonic antigen (CEA)]. The paraganglioma-like variant of MTC (Fig. 10) contains numerous S-100-positive sustentacular cells; in contrast to this MTC variant, the rare primary intrathyroidal paraganglioma is negative for cytokeratins. The angiosarcomatoid variant of MTC (Fig. 11) is characterized by a variable number of vascular spaces containing erythrocytes. Rarer variants of MTC include the clear cell, squamous cell, melanin-producing and amphicrine variants. However, it has to be emphasized that the morphological variants of MTC rather highlight the danger of a misdiagnosis then represent "real" entities with a defined biological behaviour.

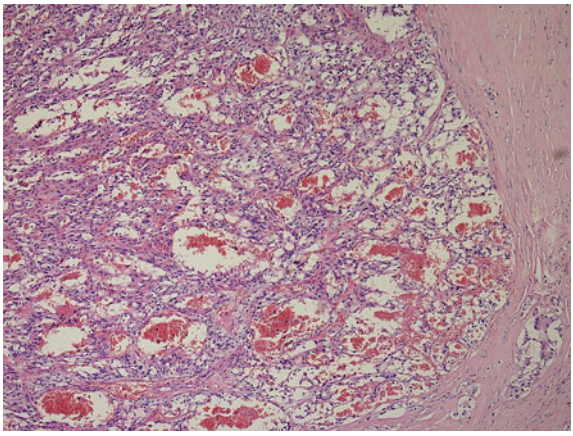

Fig. 11 Angiosarcomatoid variant of MTC with prominent vascular spaces containing erythrocytes (H&E ×100)

7.3 Immunohistochemistry

Calcitonin is expressed, although in considerable different quantity, in the vast majority of MTC (DeLellis et al. 2004; Harach et al. 1992; Krisch et al. 1985; Lloyd et al. 1983; Schmid and Ensinger 1998); by combined use of calcitonin and chromogranin A antibodies virtually, all MTC can be immunohistochemically detected (Harach et al. 1992). MTC expresses general neuroendocrine markers such as chromogranins (Schmid et al. 1987), synaptophysin and others (Chan 2007). Additionally, a broad variety of peptides (somatostatin, ACTH, serotonin, gastrin, bombesin, calcitonin gene-related peptide (CGRP) and others) can be demonstrated (Chan 2007; Schmid and Böcker 1993). CEA is expressed in most cases; in less differentiated MTC, CEA is usually stronger expressed than calcitonin (Lloyd et al. 1983; Schmid et al. 2003; Schröder and Klöppel 1987). Nuclear TTF-1 and Islet-1 expression can be found in most MTC (Agaimy et al. 2013). S-100 positive sustentacular cells can be regularly found in MTC (more often in familial than in sporadic MTC (Matias-Guiu et al. 1998)). Numerous S-100 positive sustentacular cells are the immunohistochemical hallmark of the paraganglioma-like variant of (sporadic) MTC (Bockhorn et al. 2005). Rarely, MTC may (almost completely) lack calcitonin and CEA expression (Bockhorn et al. 2004; Krisch et al. 1985); these tumours are referred to as "atypical MTC" (Schmid and Ensinger 1998). Even rarer patients with histologically proven MTC may lack pathological elevation of serum calcitonin ("non-secretory MTC" (Frank-Raue et al. 2013)); in these cases, MTC may immunohistochemically either show very few individual and weekly calcitonin-positive cells (as in "atypical MTC"), and in other cases, however, an unequivocal stronger calcitonin immunoreactivity can be demonstrated in the majority of tumour cells (Frank-Raue et al. 2013). Completely, calcitonin-negative MTC may strongly express CGRP (Nakazawa et al. 2014). Calcitonin-negative

tumours of the thyroid with neuroendocrine features (immunohistochemistry) may represent metastases from other neuroendocrine carcinoma (predominatly from lung or gastrointestinal tract), an intrathyreoidal located parathyroid tumour or rare primary paraganglioma of the thyroid.

8 Extrathyroidal MTC

Due to the possibility of an incomplete incorporation of the ultimobranchial body during thyroid, decent rarely C cells may remain in an extrathyroidal location (in the region of the larynx and pharynx); these C cells can give rise to carcinomas with the morphological, immunohistochemical, biological and genetic features of a MTC (Hirsch et al. 2004; Insabato et al. 1993; Smets et al. 1990; Sweeney et al. 1981).

9 Clinical and Prognostic Features of MTC

Non-specific clinical symptoms are thyroid enlargement, dysphagia, hoarseness and enlarged lymph nodes; the development of severe watery diarrhoea in approximately 40 % of MTC is however specific (Liu et al. 2011). The survival of patients with MTC significantly depends on tumour expansion at the time of diagnosis. MTC confined to the thyroid shows a long-term survival of >95 % (Girelli et al. 1998), whereas MTC associated with metastasis in cervical and/or upper mediastinal lymph nodes at the time of diagnosis has significantly worse prognosis (Erovic et al. 2012); distant metastases occurring in the course of the disease are predominantly found in the lungs, liver, bone and adrenal glands. In general, MTC is a very slow-growing tumour, and patients can still survive even with distant metastases for several decades; compared with the normal population, however, disease-related mortality is significantly increased even 10 years after the diagnosis of MTC (Bergholm et al. 1997).

Currently, the only curative therapeutic approach of MTC is the complete surgical removal of the primary tumour (and its lymph node metastases). For early detection of MTC, systematic routine calcitonin screening in patients with thyroid nodules has been introduced (Karges 2010; Vierhapper et al. 1997). The clinical management of affected members from known MEN 2 families crucially depends on the location of the RET proto-oncogene mutation (ATA risk levels A–D (Cooper et al. 2009); Table 1); ideally, prophylactic thyroidectomy is performed prior to the development of invasive MTC from neoplastic CCH (Table 2; Cooper et al. 2009; Sheu and Schmid 2010; Ting et al. 2015; Wohllk et al. 2010).

Table 1 Risk assessment (risk levels A–D) of MTC in MEN 2

ATA risk level	Exon	Mutation	MTC earliest age onset (years)	MEN2 phenotype
A	8	G321R C515S 532 duplication G533C	61 35 19 21	FMTC FMTC FMTC 2A/FMTC
	10	R600Q K603Q Y606C	46 35 58	FMTC FMTC FMTC
	11	635/insertion ELCR; T636P K666E	9 35	FMTC 2A/FMTC
	13	E768D N777S L790F	22 60 12	2A/FMTC FMTC 2A/FMTC
	14	V804L V804M	12 6	2A/FMTC 2A/FMTC
	15	S891A	13	2A/FMTC
	16	R912P	14	FMTC
B	10	C609R/G/S/Y C611R/G/F/S/W/Y C618R/G/F/S/Y C620R/G/F/S/W/Y C630R/F/S/Y	27/5/17/14 ?/28/41/47/79/7 8/9/34/9/26 6/44/40/24/37/18 1/?/39/22	2A/FMTC 2A/FMTC 2A/FMTC 2A/FMTC 2A/FMTC
	11	633/9 bp-duplication 634/12 bp-duplication	? 14	2A/FMTC 2A
	13/14	V804M/V778I	35	FMTC
C	11	C634R C634G/F/S/W/Y	1.25 (15 months) 25/7/23/3/5	2A 2A/FMTC
D	14	V804M/E805K V804M/Y806C	50 23	2B 2B
	14/15	V804M/S904C	34	2B
	15	A883F	10	2B
	16	M918T	0.75 (9 months)	2B

ATA risk level: *A* low risk, *B* intermediate risk, *C* high risk and *D* highest risk
FMTC familial MTC (MTC-only-syndrome); now regarded as a variant of MEN 2A
Modified according to Synoracki et al. (2015), Wohllk et al. (2010)

The insights gained into the constitutive activation of the RET kinase led to the introduction of targeted therapies for locally advances and/or metastatic MTC. Currently, two tyrosine kinase inhibitors are approved in Germany: vandetanib acts an inhibitor of RET kinase, but also of VEGFR and EGFR signalling pathways (Elisei et al. 2013). Cabozantinib is an inhibitor of the VEGFR 2 as well as VEGF and MET tyrosine kinases; additional effects were observed on RET, KIT and FLT3-AXL kinases (Wells et al. 2012). Whether molecular profiling of MTC has

Table 2 Recommendations for screening procedures and time of prophylactic thyroidectomy

Mutation locations	Exon 10	Exon 11	Exons 13–15	MEN2B
Age of prophylactic thyroidectomy	Consider surgery before 5 years or delay if stringent criteria are met[a]	Before 5 years	Beyond 5 years, if stringent criteria are met[a]	As soon as possible and within the 1st year of life
Screening for MTC with calcitonin and ultrasound	>3–5 years	>3–5 years	>3–5 years	6 months, if surgery has not been done yet
Screening for pheochromo-cytoma	Start at 20 years periodically	Start at 20 years periodically	Start at 20 years periodically	Start at 8 years periodically
Screening for pHTP	Start at 20 years periodically	Start at 20 years periodically	Start at 20 years periodically	–
Earliest progression to N1/M1 (age)	21/22 years	5/15 years	10/36 years	2.7/5 years

[a]Normal annual basal/stimulated serum calcitonin, normal annual neck ultrasound, less aggressive MTC family history and family preference (Wells et al. 2012)
Modified according to Cooper et al. (2009), Synoracki et al. (2015), Wohllk et al. (2010)

the potential to offer prediction of treatment response is yet unclear; however, the primarily with a less favourable clinical course associated somatic mutations of the RET proto-oncogene in MTC (Elisei et al. 2008) may probably mediate a better response to targeted therapies.

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Epidemiology and Clinical Presentation of Medullary Thyroid Carcinoma

Friedhelm Raue and Karin Frank-Raue

Abstract

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor originating from the thyroid C cells producing mainly calcitonin (CTN) used as tumor marker. MTC occurs either sporadic (75 %) or in a hereditary form (multiple endocrine neoplasia type 2, MEN2), due to germline mutations in the *RET* proto-oncogene. The discovery of an MTC in a patient has several diagnostic implications involving a specific strategy: preoperative evaluation of the tumor marker CTN and the extent of the disease, classification of MTC as sporadic or hereditary by DNA testing, and screening for associated endocrinopathies in hereditary MTC. Elevated CTN is a highly sensitive and specific tumor marker for diagnosis and follow-up of MTC. CTN is directly related to the tumor mass. In patients with nodular thyroid disease, diagnosis of MTC could be made by CTN determination as an indicator of tumor burden in conjunction with fine-needle aspiration. Patients with confirmed sporadic or hereditary MTC should have a total thyroidectomy and depending on the preoperative CTN value and the extent of disease additional dissection of the lymph nodes in the central and lateral neck compartment. In MEN 2 patients diagnosed by screening, the time of prophylactic thyroidectomy depends on RET mutation and CTN level.

Keywords

Calcitonin · RET proto-oncogene · Multiple endocrine neoplasia type 2 · Medullary thyroid carcinomac

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List of Abbreviations

MTC	Medullary thyroid carcinoma
MEN	Multiple endocrine neoplasia
FMTC	Familial medullary thyroid carcinoma
CTN	Calcitonin
RET gene	REarranged during Transfection gene
CEA	Carcinoembryonic antigen
CCH	C-cell hyperplasia
ICMA	Immunochemiluminometric two-site assays

Contents

1 Introduction

Medullary thyroid carcinoma (MTC) is a rare thyroid cancer that arises from the C cells or parafollicular cells of the thyroid and accounts for 3–8 % of all thyroid cancers (Davies and Welch 2006). MTC was first described as a distinct entity by Hazard et al. (1959). The serum calcium-lowering hormone calcitonin (CTN) is secreted by both benign and neoplastic C cells and is the main tumor marker for MTC (Giraudet et al. 2008; Machens et al. 2008). Most cases of MTC are sporadic

and approximately 25 % present as part of an autosomal dominant syndrome called multiple endocrine neoplasia type 2 (MEN2) (Raue and Frank-Raue 2010), which is caused by germline-activating mutations in the REarranged during Transfection (*RET*) proto-oncogene. Two distinct hereditary forms of MEN2 have been described based on their clinical phenotype: (i) multiple endocrine neoplasia type 2A (MEN2A) in association with MTC, and a variable degree of penetrance for pheochromocytoma and parathyroid disease; and (ii) multiple endocrine neoplasia type 2B (MEN2B) in association with MTC, pheochromocytoma, and mucosal neuromas. Genetic testing is available that detects nearly 100 % of mutation carriers, and such testing is considered the standard of care for all first-degree relatives of patients with newly diagnosed hereditary MTC (Task et al. 2009).

After the discovery of an MTC in a patient, the clinician completes the diagnosis by performing a preoperative evaluation of the extent of the disease, by classifying the MTC as sporadic or hereditary by DNA testing, and by screening for associated endocrinopathies if the MTC is hereditary. Sporadic MTCs are diagnosed as part of a workup for thyroid nodules. MTCs are usually discovered due to elevated CTN levels that are detected by screening patients who have thyroid nodules. Earlier identification of patients with MTC has led to the detection of preclinical disease, resulting in a higher cure rate of affected patients and a much better prognosis. However, persistent hypercalcitonemia is common after apparently complete surgical resection, and its management is controversial. The course of MTC is indolent, and survival rates depend on the tumor stage at diagnosis. Prognosis is good for MTC, which is usually a slow growing tumor and which has a 10-year disease-specific survival of about 75 %.

2 MTC Epidemiology and Classification

Thyroid cancer is the most common malignancy of the endocrine system and accounts for approximately 2 % of all new cancer diagnoses (Jemal et al. 2007). MTC is the third most common thyroid malignancy after papillary and follicular thyroid carcinoma. There has been a marked increase in thyroid cancer incidence in the last two decades which is almost entirely accounted for by papillary cancer. The increase in the incidence of thyroid cancer is mainly because of an increase in incidence of small papillary thyroid carcinomas, which have an excellent prognosis even when undetected (Colonna et al. 2007). There has not been a comparable increase in MTC (Netea-Maier et al. 2008); therefore, the relative incidence of MTC has now fallen from 9 % in the 1970s to approximately 3 % of all thyroid cancers, the absolute incidence remained stable (Netea-Maier et al. 2008; Ahmed and Ball 2011; Pacini et al. 2010).

MTC has both sporadic and hereditary/familial forms (Pacini et al. 2010) (Table 1). The majority of MTC patients have sporadic MTC (75 %), while 25 % suffer from hereditary MTC. The sex ratio in sporadic MTC is 1:1.3 (male:female), showing a slight female predominance, while familial cases have no sex predilection due to their autosomal dominant inheritance pattern (Raue 1998).

Table 1 Classification of medullary thyroid carcinoma (MTC)

Variety of MTC	Incidence	Age at clinical diagnosis	Associated endocrinopathies
Sporadic MTC	75	5th decade	None
Hereditary MTC	25		
– MEN2A	–23	3rd decade	Pheochromocytoma Parathyroid adenoma Cutaneous lichen amyloidosis
– MEN2B	–2	1st decade	Pheochromocytoma Mucosal neuroma

MEN2A multiple endocrine neoplasia type 2A

The highest incidence of sporadic disease occurs in the fifth decade of life, while hereditary disease can be diagnosed earlier if genetic and biochemical screening is performed.

The familial type of MTC is inherited as an autosomal dominant trait with nearly 100 % penetrance that is associated with MEN2 syndrome (Wells et al. 2013). Hereditary MTC is caused by germline-activating mutations of the *RET* proto-oncogene. There are two distinct hereditary types of MTC, and each variant of MEN2 results from different *RET* gene mutations, with good genotype/phenotype correlation. (1) MEN2A syndrome is characterized by MTC with a penetrance of nearly 100 % in combination with pheochromocytoma and tumors of the parathyroid. MEN2A is the most common form of all MEN2 syndromes, accounting for 95 % of all cases, and is further subdivided into 4 groups: (i) Classical MEN2A, which includes MTC with pheochromocytoma or hyperparathyroidism or both; (ii) MEN2A with Hirschsprung's disease; (iii) MEN2A with cutaneous lichen amyloidosis; and (iv) FMTC (familial MTC). FMTC, formerly considered a distinct variant of MEN2A, affects families as well as individuals who have *RET* germline mutations and who mainly show MTC (and rarely pheochromocytoma). Accordingly, FMTC should no longer be considered a distinct syndrome but rather should be considered to fall within the spectrum of MEN2A disease expression (Raue and Frank-Raue 2010; Wells et al. 2013, 2015). (2) MEN2B syndrome is rare (5 % of all MEN2 cases) and consists of MTC, pheochromocytoma, ganglioneuromatosis, and Marfanoid habitus. MEN2B is the most aggressive form of MEN2 syndrome.

These three varieties of MTC, i.e., the two hereditary forms and the one nonhereditary/sporadic form, are clinically distinct with respect to incidence, genetics, age of onset, association with other diseases, tumor histopathology, and prognosis (Table 1). Many patients with MEN2B show onset in the first year of life, presenting with MTC that is more aggressive and that has higher morbidity and mortality than MTC in patients with MEN2A. Often, these patients have no family history of the disease; accordingly, their tumors and characteristic appearance are due to de novo mutations that present as sporadic cases of potentially hereditary disease. In contrast, the clinical course of MTC in MEN2A is more benign than in MEN2B: It shows late onset or no clinically apparent disease, and the prognosis is

relative good. Therefore, a family history is often inadequate for establishing familial disease, and a more thorough evaluation by genetic and biochemical screening often reveals a family history of MTC in a patient originally thought to have the sporadic form of the disease.

The diagnosis of MTC in patients has changed in the last decade due to the use of CTN screening in patients with thyroid nodules and molecular screening for *RET* proto-oncogene mutations in patients with apparently sporadic MTC and in family members who are at risk of MTC. Earlier identification of patients with MTC has changed the presentation from clinical tumors to preclinical disease, resulting in a high cure rate for affected patients and a much better prognosis.

3 MTC Pathology and Staging

During development, embryonic C cells derived from the neural crest migrate into the last pharyngeal pouch and finally into the upper two-thirds of the thyroid. Accordingly, most tumors are located in this region. C cells account for about 1 % of thyroid cells and are much more numerous in males than in females (Guyetant et al. 1997). The histological appearance of MTC is enormously variable with regard to its cytoarchitecture (solid, trabecular, or insular) and cell shape (spindle, polyhedral, angular, or round). One characteristic is the presence of stromal amyloid in ~50–80 % of MTC patients. Amyloid deposits show positive staining with Congo red and bright green birefringence under polarized light. This feature was an auxiliary diagnostic criterion for MTC before the use of CTN immunohistochemistry (Khurana et al. 2004). Immunohistochemical staining with antibodies to CTN and thyreoglobulin may be extremely useful in difficult cases; as a general rule, MTC is thyroglobulin-negative and CTN-positive. MTCs will show positive immunostaining with antibodies to neuroendocrine factors such as chromogranin, synaptophysin, and neuron-specific enolase, as well as positive staining for somatostatin, bombesin, serotonin, and carcinoembryonic antigen (CEA) (Mendelsohn et al. 1984). MTC often has the histological features of other neuroendocrine tumors such as carcinoid and islet cell tumors.

Hereditary MTC characteristically presents as a bilateral, multifocal process with neoplastic C cell hyperplasia (CCH) in areas that are distinct from the primary tumor. Bilateral CCH is a precursor lesion to hereditary MTC with a penetrance approaching nearly 100 % in gene carriers (Etit et al. 2008). If patients with presumed sporadic MTC are found to have CCH or multifocal hyperplasia upon morphological examination of the entire gland, this should prompt analysis of germline DNA to detect mutations in the *RET* proto-oncogene. The CCH that occurs secondarily in association with hyperparathyroidism, chronic lymphocytic thyroiditis, renal insufficiency, and aging is not a premalignant condition (Biddinger et al. 1991; LiVolsi et al. 1973; O'Toole et al. 1985; Tomita and Millard 1992). CCH is defined as >40 C cells/cm^2 or at least three ×100 magnification fields containing >50 C cells. The time frame of the progression from CCH to microscopic carcinoma remains unclear but may take years (Machens et al. 2003).

MTC is characterized by early spread to locoregional lymph nodes, which often occurs before the primary tumor is diagnosed. Metastasis is found first in the central and lateral cervical and mediastinal lymph nodes of the neck in 10 % of patients with a micro-MTC operated on after discovery during familial screening, and in up to 90 % of patients operated on for clinical MTC. Metastases outside the neck and mediastinum can occur during the course of the disease in the lung, liver, and bone, and, less frequently, in the brain and skin. Distant metastases are observed at presentation in 7–23 % of MTC patients (Task et al. 2009). However, disease-specific mortality in MTC is relatively low.

Primary tumors are staged as T1 if they are <2 cm in size, T2 if 2–4 cm, and T3 if >4 cm or with minimal extrathyroidal extension. T4a and T4b tumors extend to structures outside the thyroid gland (Table 2). The staging system for MTC was updated by the AJCC/UICC in 2009 (Edge et al. 2010). Postoperative staging is helpful for distinguishing low-risk versus high-risk patients with MTC. However, the TNM classification lacks important prognostic factors, such as gradations of age and postoperative serum CTN levels. Lymph node metastases are not categorized by number and compartment but only according to the location of nodes inside (N1a) or outside (N1b) the central neck. Survival in MTC is intermediate between that for well-differentiated and poorly differentiated or anaplastic thyroid cancer. The overall survival of patients of all stages in larger series is 81–97 % at 5 years and 43–91 % at 10 years (Rendl et al. 2008). Survival at 10 years is 98–100 % for patients with stage I disease and 21–46 % for patients with stage IV disease (Raue 1998), illustrating the often long disease course, even in patients with known metastatic disease. The initial clinical stage remains highly predictive of future mortality and is the strongest predictor of survival.

4 Biochemical Markers of MTC

The primary secretory product of MTC is CTN, a 32-amino acid monomeric peptide that results from the cleavage and posttranslational processing of procalcitonin, a precursor peptide derived from preprocalcitonin. Measurement of CTN with immunochemiluminometric two-site assays (ICMAs) remains the most sensitive and specific way to test for intact, monomeric CTN (Kratzsch et al. 2011). With ICMAs, cross-reactivity with procalcitonin or other CTN-related peptides is largely eliminated. This is important because sepsis or other general inflammatory conditions may cause profound elevation of procalcitonin in tissues that do not normally transcribe the CTN gene (Becker et al. 2004; Whang et al. 1998). The test is widely available, accurate, reproducible, and cost-effective. Normal CTN levels are below 10 pg/ml. Current reference ranges for serum CTN vary according to sex and are higher in men than in women, almost certainly due to a larger C cell mass in men compared to women (8.4 pg/ml in men and 5.0 pg/ml in women, depending on the specific assay) (Guyetant et al. 1997; Kratzsch et al. 2011; Basuyau et al. 2004). Basal serum CTN levels are markedly elevated in children under 3 years of age and

Table 2 American Joint Committee on cancer TNM classification (thyroid cancer) (Edge et al. 2010)

Primary tumor (*T*)	
Note All categories may be subdivided: *s* solitary tumor and *m* multifocal tumor (the largest determines the classification)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor 2 cm or less in greatest dimension, limited to the thyroid
T1a	Tumor 1 cm or less, limited to the thyroid
T1b	Tumor more than 1 cm, but not more than 2 cm, in greatest dimension, limited to the thyroid
T2	Tumor more than 2 cm, but not more than 4 cm, in greatest dimension, limited to the thyroid
T3	Tumor more than 4 cm in greatest dimension, limited to the thyroid, or any tumor with minimal extrathyroid extension (e.g., extension to sternothyroid muscle or perithyroid soft tissues)
T4a	Moderately advanced disease
	Tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve
T4b	Very advanced disease
	Tumor invades prevertebral fascia or encases carotid artery or mediastinal vessels
Regional lymph nodes (*N*)	
Regional lymph nodes are the central compartment, lateral cervical, and upper mediastinal lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
N1a	Metastasis to Level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes)
N1b	Metastasis to unilateral, bilateral, or contralateral cervical (Levels I, II, III, IV, or V) or retropharyngeal or superior mediastinal lymph nodes (Level VII)
Distant metastases (*M*)	
M0	No distant metastasis
M1	Distant metastasis
Anatomic stage/Prognostic group	
Stage I	T1, N0, M0
Stage II	T2, N0, M0
	T3, N0, M0
Stage III	T1, N1a, M0
	T2, N1a, M0
	T3, N1a, M0
Stage IVA	T4a, N0, M0
	T4a, N1a, M0
	T1, N1b, M0

(continued)

Table 2 (continued)

Primary tumor (*T*)	
	T2, N1b, M0
	T3, N1b, M0
	T4a, N1b, M0
Stage IVB	T4b, Any N, M0
Stage IVC	Any T, Any N, M1

especially in those under 6 months of age (less than 40 pg/ml in children under 6 months of age and less than 15 pg/ml in children between 6 months and 3 years of age) (Basuyau et al. 2004) (Table 3).

Basal CTN concentrations usually correlate with tumor mass but also reflect tumor differentiation. Notably, they are almost always high in patients with palpable tumors (Cohen et al. 2000; Machens and Dralle 2010). Similarly, elevated plasma CTN levels following surgery to remove the tumor are indicative of persistent or recurrent disease. About 34–44 % of patients are biochemically cured by surgery, as indicated by undetectable serum CTN levels (Cohen et al. 2004). Slightly elevated postoperative CTN levels signal occult MTC, which often shows long-term survival due to an indolent course of disease. Other patients develop rapidly progressing disease, leading to death from distant metastases. It remains difficult to predict what will happen in most individual cases. However, it is well established that persistent and increasing CTN levels adversely affect life expectancy. A twofold rise in CTN, termed the CTN doubling time, in <6 months is a bad prognostic factor, with a 10-year survival of 8 %. In contrast, a longer CTN doubling time of >2 years is associated with longer survival (10-year survival of 100 %) (Giraudet et al. 2008; Barbet et al. 2005). Accordingly, the CTN doubling time seems to be the most powerful prognostic indicator in MTC. In studies that evaluate the serum levels of both CTN and procalcitonin in patients with MTC, CTN shows equal or superior diagnostic accuracy (Walter et al. 2010; Algeciras-Schimnich et al. 2010; Machens et al. 2014).

Provocative stimulation of CTN release using pentagastrin or calcium can be performed during follow-up to confirm the absence or presence of residual tumor and to identify patients with MTC during the evaluation of thyroid nodules as part of the screening procedure. The test is administered by administering 0.5 μg pentagastrin/kg body weight as an intravenous bolus over 5–10 s or by giving calcium gluconate 2.5 mg/kg body weight as an intravenous infusion over 30 s. CTN levels are measured 2 and 5 min after initiation of the infusion. For patients with MTC recurrence or persistence, the peak observed after pentagastrin stimulation is usually 5–10 times higher than basal levels. However, patients with normal or undetectable basal and stimulated postoperative CTN levels are probably disease-free (Lorenz et al. 2013; Colombo et al. 2012; Doyle et al. 2009). The stimulation tests have some limitations because pentagastrin is no longer available

Table 3 Clinical application of basal calcitonin (CTN) measurement in sporadic medullary thyroid carcinoma (MTC)

Indication	Clinical interest	Considerations	Cutoff values, pg/ml/month	References
Healthy controls	Reference range	Assay-dependent – Females – Males – Children <6 months – 6 months–3 years	<5.0 <8.4 <40 <15	Kratzsch et al. (2011) Basuyau et al. (2004)
Screening in thyroid nodule	Early detection of MTC	Females Males	>20 >26 >100 >68	Machens et al. (2009) Mian et al. (2014) Machens et al. (2009) Mian et al. (2014)
Extent of tumor before OP	Only thyroid Cervical LN – ipsilateral central,lateral – Contralateral, mediast. Distance metastases	Before first operation	<20 <200 <500 >500	Machens and Dralle (2010)
Follow-up	Definition of cure Resid./recurr. disease Imaging possible	After first operation	Not detect. Elevated >150	Elisei and Pinchera (2012), Engelbach et al. (2000) Giraudet et al. (2007)
Reoperation	Possibility of cure Palliative OP, distant metastisis		<500 >1000	Machens and Dralle (2013)
Prognosis	High risk Low risk	CTN doubling time	<6 months >24 months	Giraudet et al. (2008), Barbet et al. (2005), Meijer et al. (2010)

OP operation

in many countries, the stimuli can have an adverse effect, and there is no clear cutoff value to discriminate normal and CCH cases from MTC patients. In addition, the sensitivity and specificity of the ICMA assay has increased in recent years, providing more information about basal CTN values (Colombo et al. 2012) and making provocative testing no longer necessary in most cases (Mian et al. 2014).

4.1 CTN Screening

Measurement of plasma CTN is part of the routine evaluation of patients with thyroid nodules. Up to 3 % of patients with thyroid nodules have pathological CTN concentrations, and about 0.6 % have an MTC (Costante et al. 2007). The prevalence of MTC was nearly 100 % when basal CTN levels were >100 pg/ml and pentagastrin-stimulated CTN levels were >1000 pg/ml as measured with specific and sensitive ICMAs (Scheuba et al. 2009).

When using basal CTN as a screening tool, one must take into account that CTN can also be slightly elevated in patients with various clinical conditions who present with no clinical evidence of MTC. These conditions include normal elevation during early childhood and pregnancy and pathological elevation in patients with chronic renal failure or autoimmune thyroiditis, in those who take proton pump inhibitors or who smoke, and in patients with small-cell and large-cell lung cancers, prostate cancer, mastocytosis, and various enteric and pulmonary neuroendocrine tumors (Whang et al. 1998; Schuetz et al. 2006; Machens et al. 2000; Toledo et al. 2009). Notably, the serum CTN levels in patients with various non-MTC malignancies do not increase in response to calcium or pentagastrin stimulation, and compared to MTC, the tumors usually produce less CTN per gram of tissue. The presence of heterophilic antibodies can also interfere with ICMA testing, causing falsely elevated CTN values (Karanikas et al. 2004). With the improved sensitivity of new specific two-site assays, basal and stimulated CTN tests have similar accuracy, the number of false-positive elevated CTN results has decreased, and the relevance of stimulated CTN is reduced (Mian et al. 2014). Notably, many increases in CTN levels are unrelated to MTC. In particular in the 10–30 pg/ml range for basal CTN, such increases are commonly caused by C-cell hyperplasia and are not related to MTC.

The determination of the levels of CTN mRNA or CTN gene-related peptide mRNA as extracted from peripheral blood represents a diagnostic tool that is an alternative to basal and stimulated CTN measurement. These determinations have a higher positive predictive value than do basal or stimulated CTN levels (Camacho et al. 2013). Careful evaluation of CTN in nodular thyroid disease allows early diagnosis of MTC, with a reduction in primary tumor diameter at first diagnosis (Machens and Dralle 2010), early curative surgery, and a reduction in the significant mortality associated with this malignant tumor (Costante et al. 2007; Niccoli et al. 1997; Ozgen et al. 1999; Hahm et al. 2001; Elisei et al. 2004). Accordingly, measurement of plasma CTN in patients with thyroid nodules has been advocated as a routine procedure by some European consensus groups (Pacini et al. 2006). Compared with fine-needle aspiration, the sensitivity of the CTN measurement for preoperative diagnosis of MTC is higher (approximately 100 % sensitivity and 95 % specificity) (Bugalho et al. 2005; Hasselgren et al. 2010; Papi et al. 2006). There are a number of other proteins, including carcinoembryonic antigen (CEA), PDN-21 (katacalcin) (Blind et al. 1992), chromogranin A (Blind et al. 1992),

neuron-specific enolase (Grauer et al. 1987), somatostatin (Grauer et al. 1995), and ACTH, that are sometimes produced and secreted by MTC and that could serve in some cases as tumor markers for diagnosis and follow-up.

4.2 Carcinoembryonic Antigen

Neoplastic C cells also produce CEA, which can be used as a prognostic marker during the follow-up of individuals with MTC (Machens et al. 2007; Busnardo et al. 1984; Wells et al. 1978). CEA is not a specific biomarker for MTC and is not useful in the early diagnosis of MTC. However, serum CEA levels might be used for the risk stratification of individuals with known MTC. CEA levels >30 ng/ml are suggestive of lymph node metastases in the ipsilateral central and lateral neck compartments, while levels >100 ng/ml correlate with contralateral lymph node metastases and distant metastases. CEA values >30 ng/ml correlate with low cure rates (Machens et al. 2007). Some patients with progressive disease demonstrate increasing serum CEA levels that are associated with stable or declining serum CTN levels. This is considered an indication of a poorly differentiated MTC and is supported by CEA and CTN immunohistochemistry findings (Mendelsohn et al. 1984).

4.3 Nonsecretory MTC

MTC without CTN and CEA secretion has been reported, but it is rare. The prevalence of nonsecretory MTC in a series of 839 patients was low (0.83 %) (Frank-Raue et al. 2013). Nonsecretory MTC cannot be detected by serum CTN screening as an integral part of the diagnostic evaluation of thyroid nodules. However, it is detected more often postoperatively at advanced tumor stages because of its characteristic histology and can be confirmed by positive immunohistochemistry for CTN, CEA, and chromogranin A. In some cases, CTN or CEA levels may rise during follow-up but to an extent that is entirely disproportionate relative to the tumor mass (Bockhorn et al. 2004; Dora et al. 2008). Nonsecretory MTC is a rare disease with a poor prognosis, and sometimes it shows advanced dedifferentiation. The aggressive biological behavior of the tumor is characterized by poorly differentiated histology, a high Ki-67 proliferation index, and a high proportion of *RET M918T* mutated cells. Nonsecretory MTC is markedly heterogeneous in its histological and immunohistological appearance and in its clinical course and prognosis (Frank-Raue et al. 2013).

5 Molecular Pathogenesis and Genetic Abnormalities in MTC

5.1 Germline RET Mutations

The gene responsible for MEN2 was discovered in 1985 (Takahashi et al. 1985) and localized to centromeric chromosome 10 (10q11.2) by genetic linkage analysis in 1987 (Mathew et al. 1987). Activating germline point mutations in the *RET* proto-oncogene were identified in (Donis-Keller et al. 1993). Analysis of the RET gene in families with MEN2 revealed that only affected family members had germline missense mutations in 8 closely located exons.

The RET gene has 21 exons and encodes a single-pass transmembrane receptor tyrosine kinase that appears to transduce growth and differentiation signals in several developing tissues, including those derived from the neural crest, the branchial arches, and the urogenital system (Pachnis et al. 1993). RET is expressed in cells such as C cells, which are the precursors of MTC, as well as in pheochromocytomas. The RET gene codes for a receptor with three domains: a large extracellular cysteine-rich domain, which is thought to be involved in ligand binding; a short transmembrane domain; and a cytoplasmic tyrosine kinase (TK1 and TK2) domain that is activated upon ligand-induced dimerization. Hereditary MTC is caused by autosomal dominant gain-of-function mutations in the *RET* proto-oncogene that affect exons 5, 8, 10, 11, and 13–16. Mutation of the extracellular cysteine at exon 11, codon 634, causes ligand-independent dimerization of receptor molecules, enhanced phosphorylation of intracellular substrates, and cell transformation. Mutation of the intracellular tyrosine kinase (codon 918) has no effect on receptor dimerization but causes constitutive activation of intracellular signaling pathways and also results in cellular transformation (de Groot et al. 2006). There is a significant age-related progression from C-cell hyperplasia to MTC that correlates with the transforming capacity of the different *RET* mutations.

Mutation analysis has identified over 100 different missense mutations, duplications, insertions, and deletions involving RET that are associated with the development of MEN2 (Raue et al. 2012; Margraf et al. 2009). Although there is some overlap between *RET* mutations and the resulting clinical subtype of MEN2, 85 % of patients with MEN2A have a mutation in codon 634 (exon 11). Mutations in codons 609, 611, 618, and 620 account for an additional 10–15 % of cases. Pheochromocytoma is associated with codon 634 and codon 918 mutations in approximately 50 % of patients, and in 20 % of patients, pheochromocytoma is associated with mutations in exon 10 (codons 609, 611, 618, 620) or exon 15 (codons 791, 804) (Quayle et al. 2007). Hyperparathyroidism in MEN2A is most commonly associated with codon 634 mutations and in particular with the C634R mutation. In FMTC, germline mutations are distributed throughout the *RET* gene, with many in exon 13 (codons 768, 790, 791), exon 14 (codons 804, 844), and, rarely, exon 10 (codons 618, 620). More than 95 % of MEN2B patients have mutations in codon 918 (exon 16), but mutations are rarely identified in codon 883

in exon 15. Approximately 75 % of patients with MEN2B have mutations that occur sporadically as de novo mutations, almost always from the paternal allele (Gimm et al. 1997; Eng et al. 1994; Schuffenecker et al. 1997). The association between disease phenotype and RET mutation genotype has important implications for the clinical management of MEN2 patients and their families. In particular, there is a correlation between specific germline *RET* mutations and the age of onset with the aggressiveness of MTC development and the presence of nodal metastases (Task et al. 2009).

5.2 Somatic RET Mutations

Approximately 23–60 % of sporadic MTCs have an acquired RET codon 918 somatic mutation in tumor tissue that is termed somatic mutation T918M. This mutation is identical to the germline mutation found in MEN2B. Patients with sporadic MTC with T918M have more aggressive tumor growth and a poor prognosis (Schilling et al. 2001; Romei et al. 1996; Marsh et al. 1996; Moura et al. 2009; Elisei et al. 2008; Eng et al. 1996). The prevalence of somatic *M918T RET* mutations varies depending on tumor size: small tumors (<1 cm) rarely have the mutation (11.3 %), while T918M is found in 58.8 % of patients with tumors >3 cm (Romei et al. 2012). The discovery that mutation-positive and mutation-negative regions can coexist in the same sporadic MTC tumor suggests that such genetically heterogeneous MTCs may not be clonally derived from a single initiating tumor cell with a RET mutation. RET does not seem to be the early initiator of tumor growth in sporadic MTC; rather, RET is activated later in oncogenesis as a driver of tumor growth, and other genes must play a significant role in MTC onset.

Mutations in codons 618, 630, 634, 768, 804, and 883, as well as partial deletion of the RET gene, have been identified in a few tumors (Moura et al. 2011). It was recently discovered that 18–80 % of sporadic MTCs lacking somatic *RET* mutations have somatic mutations in *KRAS, HRAS,* or, rarely, in *NRAS* (Moura et al. 2011; Boichard et al. 2012; Ciampi et al. 2013). No other common genetic mutation has been detected in subsequent exome sequencing studies of MTCs (Agrawal et al. 2013).

6 MTC: Clinical Syndrome and Diagnostic Procedure Workup

6.1 Clinical Presentation

The most common clinical presentation of sporadic MTC is an indolent and usually solitary single nodule or thyroid mass with or without associated lymphadenopathy in the neck. Typically, the nodule or mass is found incidentally during routine examination or is an incidental finding during an imaging examination of the neck. Diagnosis of the sporadic form of MTC is usually established late in life

(approximately during the fifth or sixth decade), but age of onset shows a wide range. The presentation of sporadic MTC does not differ from that observed for papillary or follicular thyroid carcinoma. The patient may present with no symptoms or with local symptoms related solely to the neck mass, and presentation may include findings such as dysphagia, dysphasia, and dyspnea. Metastases to cervical and mediastinal lymph nodes are found in two-thirds of the patients at the time of initial clinical presentation (Moley et al. 1999; Scollo et al. 2003; Machens et al. 2002; Scheuba et al. 2007). In contrast, when MTC was diagnosed by CTN screening, the diameters of the tumors were <10 mm, and only 11 % had cervical nodal involvement (Scheuba et al. 2007). Distant metastases to the lung, liver, and bone occur late in the course of the disease. At presentation, about 5 % of patients have distant metastatic disease (Pacini et al. 2010). Patients sometimes present with painful bone metastases. Diarrhea is the most prominent of the hormone-mediated clinical features of MTC, while flushing can be present but is rare; notably, diarrhea is often seen in patients with advanced disease. In addition, occasionally tumors secrete ACTH ectopically, causing Cushing's syndrome (Barbosa et al. 2005). An MEN2 index patient might present with Hirschsprung's disease or with a cutaneous lichen in the back region. In a young patient, facial features that include a centrofacial ganglioneuroma of the lip or tongue may suggest MEN2B.

6.2 Family History

A patient with a palpable anterior neck mass and an associated endocrine neoplasm (pheochromocytoma, hyperparathyroidism) and/or a suggestive family history of thyroid tumors, early sudden death (suggestive of pheochromocytoma), or nephrolithiasis (primary hyperparathyroidism) in first-degree relatives might be an index patient with MEN2.

6.3 Ultrasonography

In general, a thyroid nodule that is identified by physical examination is subsequently evaluated by ultrasonography and radioisotopic scanning (Fig. 1). MTC shows hypoechogenic regions, sometimes with calcifications; however, there are no specific ultrasound features that are pathognomonic for thyroid cancer. Some nodules that have a higher risk of malignancy show ultrasound characteristics that include hypoechogenicity, microcalcifications, irregular margins, predominantly central vascularization, and the presence of enlarged neck lymph nodes. Nevertheless, there are no differences in the echogenicity or in the presence or type of calcifications between MTC and papillary thyroid cancer (Lee et al. 2010; Kim et al. 2009; Saller et al. 2002). One important limitation of ultrasound is that it is operator-dependent; therefore, the results vary according to the operator's experience.

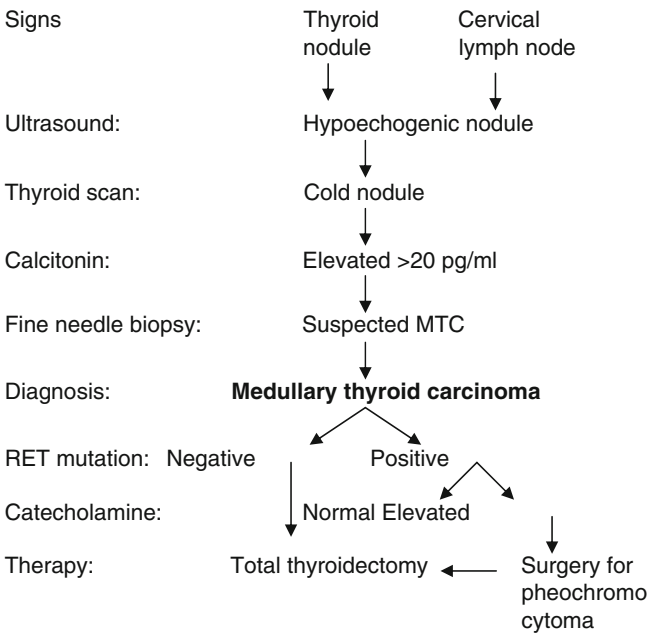

Fig. 1 Clinical evaluation of patients who are at risk for medullary thyroid carcinoma (MTC)

Rarely, a diagnosis of MTC is suggested by the presence of dense calcifications that are seen on X-rays or during imaging of the anterior neck. Plain X-ray film images of the neck sometime reveals a characteristic dense, coarse calcification pattern. Thyroid scans almost never show trapping of radioactive iodine or technetium (cold nodules). MTC has also been detected as an incidental finding on PET scan (Van den Bruel et al. 2002; Nam et al. 2007).

6.4 Fine-Needle Aspiration

Cytological examination of a hypofunctional, hypoechogenic nodule will lead to a strong suspicion or to a correct diagnosis in most cases of sporadic MTC. The sensitivity of fine-needle aspiration varies widely in clinical practice from 50 to 80 %, depending on the diagnostic accuracy of the technique, on the challenges of selecting appropriate nodules in a multinodular goiter for fine-needle aspiration, and on the experience of the cytopathologist (Tee et al. 2007; Trimboli et al. 2015). Higher sensitivity can be obtained by the addition of immunohistochemical staining for CTN (Bhanot et al. 2007). If clinical suspicion for MTC is high (e.g., the patient has diarrhea, flushing, and a palpable thyroid nodule), CTN can be measured in the washout of the fine-needle aspiration biopsy needle (Kudo et al. 2007), although this technique may not be readily available in many commercial laboratories.

Differential diagnosis between MTC and other malignant thyroid neoplasms, particularly follicular lesions, might be difficult because the cytological findings can be similar (Forrest et al. 1998). Other methods that might improve the diagnosis of MTC include assessing the level of CTN mRNA expression and determination of somatic RET mutation; however, these are not part of the standard diagnostic routine (Bugalho et al. 2000).

6.5 CTN Measurement

Plasma CTN measurement can clarify the diagnosis of MTC in patients with nodular thyroid disease. In the presence of a palpable MTC, the plasma CTN concentration will usually be greater than 100 pg/ml, and CTN levels correlate significantly with tumor size (Cohen et al. 2000). However, in the 10–100 pg/ml range, the percentage of MTC increases, but other differential diagnoses have to be taken into account. According to the basal CTN levels, the positive predictive values of this test are 8.3, 25, and 100 % for CTN levels of 20–50, 50–100, and >100 pg/ml, respectively (Costante et al. 2007). The CTN cutoff levels used to separate normal and CCH cases from MTC cases range from 20 to 100 pg/ml, depending on the study design, the assay, and the sex of those tested (Colombo et al. 2012; Mian et al. 2014; Rink et al. 2009). The indication for serum CTN measurements in thyroid nodule assessment remains controversial, especially for the older assays. This controversy is due to uncertainty about the best CTN threshold for distinguishing occult MTC from CCH (Costante et al. 2007; Machens et al. 2009; Chambon et al. 2011), the high false-positive rate (59 % or higher) in some studies due to C-cell hyperplasia (Machens and Dralle 2012). Recently, sex-specific cutoff values have been proposed to improve the accuracy of basal CTN levels for mandating total thyroidectomy. For women, basal CTN levels >20 or >250 pg/ml after pentagastrin stimulation are proposed; for men, basal CTN levels >80 or >500 pg/ml after pentagastrin stimulation are proposed (Machens et al. 2009). Using these values, the positive predictive value for MTC is 88 %. Other studies have reported similar results, with basal CTN values of >26 and >68 pg/ml in women and men, respectively, and stimulated CTN levels of >79 and >544 pg/ml in women and men, respectively (Machens et al. 2009) (Table 3). The positive predictive value of basal CTN and stimulated CTN values is similar, indicating that basal CTN values are at least as good predictors of MTC as stimulated CTN levels for the diagnosis of MTC. This suggests that serum CTN assays with improved functional sensitivity can be used instead of the stimulation tests. Using this threshold, the benefit of surgical intervention clearly outweighs the risk of harm. A careful evaluation of serum CTN measured by modern two-site automated chemiluminescent assay systems, together with fine-needle aspiration and imaging procedures, will provide enough information to make a sound final decision about surgery. In patients with CTN values below <30 pg/ml in women and <60 pg/ml in men, our practice is to follow patients conservatively with ultrasound

and basal CTN measurements, while considering surgery for progressively increasing CTN plus suspicious ultrasound or fine-needle biopsy findings during follow-up.

The CEA level is elevated in most cases with clinically evident tumors. If it is elevated, further investigation, including the use of imaging modalities, may be necessary for evaluating the extent of metastatic disease.

6.6 Imaging

In order to document local invasion or metastasis in the neck or distantly in patients with preoperative serum basal CTN levels >500 pg/ml, imaging modalities are needed in addition to ultrasound to assess the possibility of metastatic disease. However, in patients with a thyroid nodule and no evidence of systemic disease, screening for distant metastases is not indicated preoperatively unless the basal serum CTN level is markedly elevated (Machens and Dralle 2010). The sensitivity, specificity, and diagnostic accuracy of ultrasound for the preoperative detection of neck metastases are superior to those of computed tomography (CT) (Ahn et al. 2008). In addition, CT of the chest and liver or contrast-enhanced liver magnetic resonance imaging (MRI) are suggested. In patients with suspected skeletal metastases, MRI may be superior to other imaging modalities (Mirallie et al. 2005). We do not recommend 18-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) imaging or ^{18}F-dihydroxyphenylalanine F-DOPA-PET/CTS receptor imaging for routine use in initial screening for metastatic disease. The sensitivity of FDG-PET scanning for detecting metastatic disease is variable (Oudoux et al. 2007; Giraudet et al. 2007) but improves with higher CTN levels (sensitivity of 78 % for basal CTN values >1000 versus 20 % for values <1000 pg/ml, respectively) (Ong et al. 2007). Unfortunately, no single procedure provides optimal whole-body imaging. The use of somatostatin receptor scintigraphy is not currently recommended for routine initial screening for metastatic disease (Task et al. 2009; Frank-Raue et al. 1995). Scanning may be more useful in localizing residual or recurrent disease after primary therapy.

6.7 RET Proto-oncogene Mutations

Genetic testing for RET mutations in patients with elevated CTN levels may be helpful in apparently sporadic cases of MTC. Specifically, germline testing for mutations in the *RET* proto-oncogene can distinguish sporadic MTC from hereditary MTC. Germline *RET* mutations have been identified in about 6–7 % of clinically apparent sporadic MTCs (range, 1.5–24 %) (Eng et al. 1995; Zedenius et al. 1994; Wohllk et al. 1996; Elisei et al. 2007). In one report, 35 of 482 patients (7.3 %) with apparently sporadic MTC had mutations, and in 18 of the 35, relatives were identified as gene carriers (Elisei et al. 2007). A total of 75 % of the familial

MTC cases have no prior family history. Thus, if a mutation is found, it implies that the disease is hereditary and that the family should be screened as well. The RET mutations that are found are often less penetrant mutations, either inherited or de novo. Therefore, germline *RET* mutation testing should be performed in all patients with newly diagnosed C-cell hyperplasia or apparently sporadic MTC. Such testing should include sequencing of exons 5, 8, 10, 11, and 13–16 of the *RET* gene. The RET mutation analysis might guide the choice of therapeutic procedures and, consequently, change the natural course of the disease and help establish disease prognosis.

7 Sporadic Versus Hereditary MTC

The clinical presentation and manifestation of hereditary MTC in index cases often does not differ from that in patients with sporadic MTC. Both the personal and the familial medical history should be investigated carefully in all patients with MTC in order to identify familial disease. MTC is often the initial manifestation of MEN2 syndrome, as the other manifestations, pheochromocytoma and hyperparathyroidism, develop later in the course of the disease (Kouvaraki et al. 2005). Less common presentations of MTC include recognition during a search that is initiated after an associated disease, such as bilateral pheochromocytoma or multiglandular hyperparathyroidism. The diagnosis of familial MTC in index cases is often made postoperatively when histological examination shows multifocal bilateral MTC accompanied by diffuse C-cell hyperplasia. There are some rare variants of MEN2A, including MEN2A with cutaneous lichen amyloidosis and FMTC (or MEN2A) with Hirschsprung's disease. MEN2B has an atypical phenotype with visible physical manifestations such as raised bumps on the lips and tongue (due to cutaneous neuromas), ganglioneuromas throughout the gastrointestinal tract, and a Marfanoid habitus (long, thin extremities, an altered upper-lower body ratio, slipped femoral epiphysis, pectus excavatum) with skeletal deformations and joint laxity. These patients have disease onset in the first year of life with the most aggressive form of MTC.

DNA testing has become the optimal test for early detection of MEN2 especially in "at-risk" families. At present, genetic testing is performed before the age of 5 years in all first-degree relatives of an index case (directly after birth for relatives of MEN2B patients). Mutations in the RET proto-oncogene can be used to confirm the clinical diagnosis and to identify asymptomatic family members with the syndrome. Those who have a negative test can be reassured and require no further biochemical screening.

The age of onset of MTC and tumor aggressiveness in MEN2 depends on the codon that is mutated. This genotype–phenotype correlation is the basis for stratifying mutations into three risk levels for MTC development and growth. Decision making in the clinical management of MEN2 patients depends on this risk-level classification, particularly the decisions about the timing of prophylactic

thyroidectomy and the extent of surgical resection in presymptomatic *RET* mutation carriers (Wells et al. 2013). Additionally, the preoperative serum CTN levels and ultrasound examination must be taken into account when planning the extent of surgery.

8 Preoperative Assessment

When it is undiagnosed, preoperative MTC can have some adverse consequences, such as missing underlying pheochromocytoma or hyperparathyroidism and choosing a suboptimal extent of surgery (Ahmed and Ball 2011). Therefore, preoperative assessment of individuals with suspected or confirmed MTC should be performed to establish the extent of thyroid disease and to identify the eventual presence of associated comorbidities such as hyperparathyroidism and/or pheochromocytoma in MEN2 cases. Given the possibility that any patient with MTC may have MEN2, pheochromocytoma and hyperparathyroidism have to be excluded prior to thyroidectomy by measuring fractionated plasma metanephrines and serum calcium. In a patient with negative *RET* proto-oncogene testing and no family history of MEN2 syndrome, biochemical testing for coexisting tumors is not required. If pheochromocytoma is diagnosed, it has to be operated before thyroidectomy because of the high risk of anesthesia in cases with an undiagnosed adrenal tumor.

Patients with palpable thyroid nodules suspected to be MTC have a high rate (73–75 %) of lymph node metastases (Moley and DeBenedetti 1999). The basal serum preoperative CTN level is also useful in determining the extent of lymph node involvement. One study found no risk of lymph node metastases when the preoperative serum CTN level was <20 pg/ml (Machens and Dralle 2010). Basal serum CTN levels exceeding 20, 50, 200, and 500 pg/ml were associated with metastases to lymph nodes in the ipsilateral central and lateral neck, the contralateral central neck, the contralateral lateral neck, and the upper mediastinum, respectively. Similar information can be gained from preoperative serum CEA levels (Machens et al. 2007). In order to avoid repeat neck operations and associated complications, dissection of the lymph node compartments is best accomplished during the initial operation. The surgeon should base the extent of the initial surgery on the frequency and pattern of lymph node metastases relative to the location and size of the primary MTC, ultrasound findings, and serum levels of CTN and CEA.

9 Initial Treatment for Patients Who Undergo MTC Surgery

The definitive treatment for MTC is surgery, regardless of whether the MTC is sporadic or familial, primary or recurrent, restricted to the thyroid gland or extending beyond the thyroid. Several studies have shown that survival in patients with MTC is dependent upon the adequacy of initial surgical procedure. The appropriate surgery for MTC is total thyroidectomy and careful lymph node dissection of the central and, if necessary, the lateral compartment of the neck (Machens and Dralle 2010). Central lymph node dissection is necessary for tumor staging and prevention of later midline complications related to local metastatic disease. Less aggressive surgery is indicated in cases with advanced local disease and/or distant metastases to achieve local disease control while preserving the patient's voice, deglutition, and parathyroid function while focusing mainly on the patients' quality of life (Tuttle and Ganly 2013; Brauckhoff et al. 2010). If there is no evidence of local lymph node metastases during the primary surgical procedure, a surgical cure is likely and further neck dissection is probably unnecessary. The preoperative CTN level correlates with the extent of disease, and one study found that no patients with CTN levels <53 pg/ml had lymph node metastases (Yip et al. 2011). Another study found that no patients with CTN <20 pg/ml had lymph node metastases (Machens and Dralle 2010). Knowing the preoperative CTN levels allows the pretherapeutic risk of locoregional lymph node involvement to be assessed. Patients with CTN levels in the range of 20–53 pg/ml may safely forgo systematic lymph node dissection.

Total thyroidectomy is absolutely necessary in hereditary cases because of the bilateral and multifocal nature of MTC. If the initial surgical procedure was inadequate, reoperation with an appropriate surgical procedure is indicated. In contrast, unilateral lobectomy is sufficient in a patient with sporadic MTC showing a single, unifocal tumor that is limited to the thyroid gland with tumor-free surgical margins and undetectable plasma CT levels postoperatively.

Recommendations for the timing of prophylactic thyroidectomy in MEN2 patients are based on a model that utilizes genotype–phenotype correlations to stratify mutations into three risk levels, namely moderate, high, and highest risk (Task et al. 2009). For patients with moderate risk-level mutations, the decision regarding the age at which prophylactic thyroidectomy should be performed is no longer based upon genotype alone. Instead, this decision is currently driven by additional clinical data, the most important being basal or stimulated serum CTN levels (Machens et al. 2003; Frank-Raue et al. 2006; Skinner et al. 2005; Niccoli-Sire et al. 1999). Surgery may be postponed until the patient has an abnormal basal CTN level or C-cell stimulation test result.

Surgery for pheochromocytoma in MEN2 should precede surgery for MTC. Before adrenalectomy, all patients should receive the appropriate pharmacotherapy (alpha- and beta-adrenergic antagonists). Approximately one-third of patients who undergo a unilateral adrenalectomy will eventually require a second operation for

contralateral pheochromocytoma, but this may not occur for many years. During this time, the patient will not be steroid-dependent. Adrenal cortical-sparing laparoscopic or retroperitoneoscopic adrenalectomy is the procedure of choice for unilateral and bilateral adrenalectomy for preventing adrenal insufficiency (Walz et al. 2010; Brunt et al. 1996).

The parathyroid glands in MEN2 patients are frequently found to be enlarged at thyroidectomy for MTC and should therefore be carefully evaluated during surgery. The goal in MEN2 patients with primary hyperparathyroidism is to excise the enlarged glands and to leave at least one normal parathyroid gland intact. If they are all enlarged, subtotal parathyroidectomy or total parathyroidectomy with autotransplantation should be performed (Raue et al. 1995; Kraimps et al. 1996; Scholten et al. 2011).

10 Initial Evaluation and Treatment of Postoperative Patients

After total thyroidectomy, all patients should receive adequate L-thyroxine replacement therapy with the goal of maintaining serum TSH levels in the euthyroid range. Since MTC is not derived from follicular thyroid cells, suppressing TSH is not necessary. These patients need to be monitored carefully for postoperative hypoparathyroidism. Transient hypocalcemia is not uncommon; however, treatment with oral calcium and calcitriol is indicated in patients who become symptomatic and have persistently prolonged hypocalcemia.

All patients with MTC should undergo CTN and CEA determination at regular intervals after total thyroidectomy. CTN and CEA levels decline progressively and variably after surgery, with some patients reaching the nadir 3 months postoperatively (Elisei and Pinchera 2012; Brauckhoff et al. 2001). Normal basal and pentagastrin-stimulated CTN levels, which are seen in about one-third of operated patients, suggest a tumor-free state associated with a favorable outcome; such patients require no further treatment (Elisei and Pinchera 2012; Engelbach et al. 2000). They should be followed-up at half-yearly intervals with physical examination, CTN determination, and testing to ensure adequate thyroid hormone replacement therapy.

If the primary operation was inadequate and elevated CTN levels persist, if there is no evidence of distant metastases, and if local disease is found in the neck and/or mediastinum, reoperation is advocated using meticulous dissection and microsurgical techniques. A successful cure, even long after the primary operation, is possible in a small number of patients by systematic lymph node dissection of all compartments of the neck and mediastinum, with the complete removal of the lymphatic and fatty tissue between important anatomic structures. If >5 lymph node metastases were dissected in the previous surgery, the biochemical cure rate falls to 5 %. When preoperative serum CTN levels exceed 1000 pg/ml, biochemical cure is rare (Machens and Dralle 2013). If distant metastases are found, there is no

indication for surgical intervention unless the patient develops diarrhea or local complications. In such a case, tumor debulking or maintenance of local control in the neck may be beneficial.

Perhaps, the most difficult problem associated with the management of MTC is the question of what to do for a patient with persistently elevated plasma CTN levels after an adequate surgical procedure. A thorough evaluation should be performed to define the extent of local and distant metastatic disease if CTN is >150 pg/ml. In patients with only slightly elevated CTN (<150 pg/ml), localization is often unsuccessful (Giraudet et al. 2007). Localization of metastases or recurrence can be detected by different imaging methods. After these diagnostic procedures, a decision regarding further treatment must be made. In patients who remain CTN-positive with evidence of noncurable and nonoperable disease (diffuse distant metastases) or occult disease (no local recurrence is found and the operation was adequate), close observation of changes in serum CTN und CEA concentration is required. Many patients exhibit a remarkably stable course, and no further treatment is recommended; for these patients, a "watchful waiting" approach is advocated, as the experience with nonsurgical therapy in the management of slow growing metastatic MTC has been disappointing. In patients whose disease shows rapid and steady progress, e.g., doubling of tumor marker values in less than one year, intervention with tyrosine kinase inhibitors can be considered a palliative therapeutic modality (Giraudet et al. 2008; Barbet et al. 2005; Wells et al. 2012).

11 Conclusion

There are several steps that must be taken to perform a full diagnosis of MTC. First, its sporadic or hereditary nature is determined by RET mutation analysis. Second, the extension of the tumor must be determined in order to plan the best therapeutic procedure. Surgery represents the only curative therapeutic strategy, and cure of MTC is possible in MEN2 patients by the use of prophylactic thyroidectomy. In patients with sporadic MTC, the clinical diagnosis of MTC is often too late for a cure, as the tumor has already metastasized beyond the thyroid. Therefore, early diagnosis is critical. We recommend basal CTN testing, ultrasound of the neck, and fine-needle biopsy to exclude or confirm MTC in patients with thyroid nodules.

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Medullary Thyroid Carcinoma: Imaging

Stefan Delorme and Friedhelm Raue

Abstract

Imaging plays an important role in early detection and staging of medullary thyroid carcinoma (MTC) as well as in follow-up to localize early recurrence. MTC is a rare, calcitonin-secreting thyroid malignancy often diagnosed by ultrasound and calcitonin screening as part of the routine workup for any thyroid nodule. If calcitonin is elevated, imaging studies are needed for preoperative staging, which dictates surgical management. This can be done by ultrasound of the neck and abdomen. Computed tomography (CT) or magnetic resonance imaging (MRI) studies for more distant disease are done preoperatively if calcitonin levels are higher than 500 pg/ml. Neither FDG-PET/CT nor F-DOPA-PET/CT are used routinely for preoperative staging but may contribute in doubtful individual cases. Postoperative elevated calcitonin is related to persistence or recurrence of MTC. Imaging studies to localize tumor tissue during postoperative follow-up include ultrasound, CT, MRI as well as PET studies. They should be used wisely, however, since treatment consequences are often limited, and even patients with persistent disease may survive long enough to accumulate significant radiation doses. Imaging studies are also useful for diagnosis of associated components of the hereditary MTC such as pheochromocytoma and primary hyperparathyroidism (pHPT).

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List of Abbreviations

MTC	Medullary thyroid carcinoma
CTN	Calcitonin
US	Ultrasound
MRI	Magnetic resonance imaging
CT	Computed tomography
PET	Positron emission tomography

Contents

1 Preoperative Imaging at Initial Diagnosis of MTC

1.1 Imaging the Primary Tumor

The diagnosis of sporadic medullary thyroid carcinoma (MTC) usually results from a routine workup of a thyroid nodule, i.e., palpation, ultrasound, 99mTc-pertechnetate scintigraphy, fine needle aspiration biopsy, and serum calcitonin (CTN). MTC is highly suspected when CTN levels are elevated in a patient who has a thyroid nodule. Further, diagnostic procedures such as fine needle biopsy, imaging, and molecular-genetic analysis of the *ret* protooncogene are necessary. The clinical presentation and manifestation of hereditary MTC in index cases often does not differ from that in patients with sporadic MTC, while hereditary MTC with

proved *ret* mutation is diagnosed in a preclinical stage without any morphological changes in the thyroid. Imaging plays a critical role both in early detection and accurate staging of MTC, which dictates surgical management, as well as in follow-up to localize early recurrences, and in the assessment of tumor response to therapy (Wells et al. 2015).

1.2 Ultrasound of the Primary Tumor

The first imaging modality used in MTC is usually ultrasound, most commonly for the workup of a palpable thyroid nodule. Less frequently, screening of a MEN2 family, cervical lymphadenopathy, or symptomatic distant metastases give rise to the examination. MTC shares the sonographic features of other thyroid carcinomas. In a study by Choi et al., 47 % of MTCs had ill-defined or spiculated margins, 97 % were hypoechogenic compared to the thyroid tissue, 39 % had microcalcifications, and 28 % had a more or less pronounced hypoechoic halo (Choi et al. 2011) in accordance with similar previous studies (Kim et al. 2009) and a recent meta-analysis (Wolinski et al. 2014). In other terms, virtually all MTCs are hypoechoic, and microcalcifications as well as ill-defined or spiculated margins are highly suggestive of malignancy, without favoring MTC over other differentiated thyroid carcinomas (Figs. 1 and 2). Microcalcifications in particular are reported to be very specific for malignancy and to correspond to clusters of psammoma bodies (Triggiani et al. 2008). A hypoechoic halo, often believed to indicate a benign nodule, seems to be of little help in this context. A breech of the thyroid capsule and a frank lymphadenopathy indicate an advanced stage of the disease. A remarkable feature of MTC is its striking hypervascularity, which can easily be depicted with color Doppler sonography, and which it shares with other neuroendocrine tumors and their metastases. Nevertheless, this will again not favor MTC over other thyroid

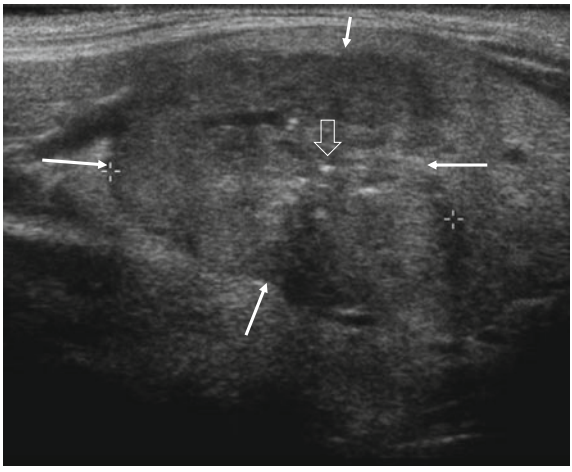

Fig. 1 Longitudinal US section of the left thyroid lobe, showing a sharply but irregularly delineated, hypoechogenic medullary thyroid carcinoma (*arrows*) with internal microcalcifications (*open arrow*)

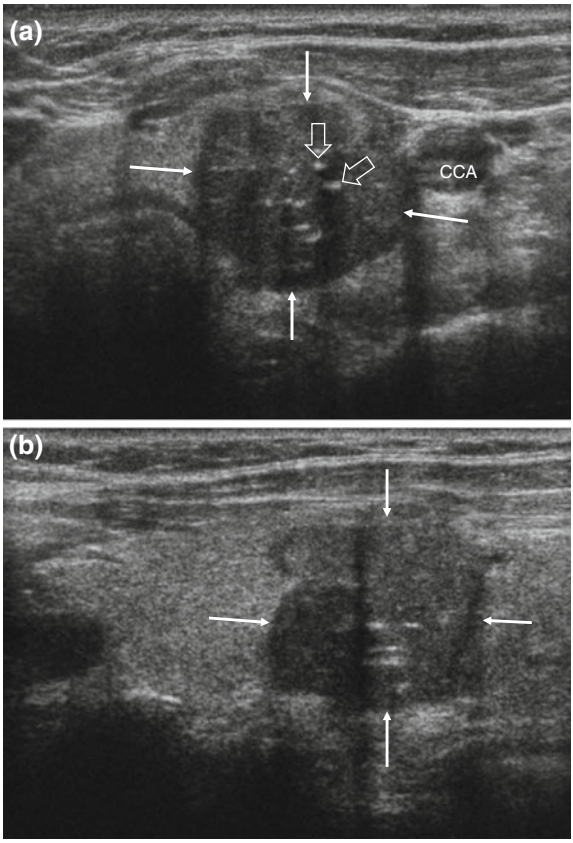

Fig. 2 Transverse (**a**) and longitudinal (**b**) US section of the left thyroid lobe in a different patient with medullary thyroid carcinoma, showing a well-defined tumor with irregular contours (*arrows*) and internal microcalcifications (*open arrows*), still confined to the thyroid. *CCA* common carotid artery

neoplasms, which are likewise hypervascular (Foschini et al. 2007). In this context, a ring arrangement of vessels is more typical of a benign nodule than of a carcinoma (Zhang et al. 2010).

Usually, the primary workup will result in a nodule that is possibly malignant, of whichever histology, and should be resected. It is nevertheless desirable to establish the likely diagnosis preoperatively so that the primary operation will meet oncological standards and include a systematic lymph node revision. Once the indication for resection is clear, serum CTN should therefore be obtained, if not already done.

1.3 Staging—Ultrasound of Cervical Lymph Node Metastases

Patients with preoperative serum basal CTN levels >500 pg/ml will undergo imaging studies in addition to thyroid and lymph node ultrasound for possible local invasion, metastatic cervical lymph nodes, or systemic metastatic spread. If the basal serum CTN level is not markedly elevated, patients with a thyroid nodule

need no preoperative staging for distant metastases unless there is other clinical suspicion of systemic disease (Wells et al. 2015; Machens and Dralle 2010).

Owing to its high spatial resolution in B-mode and color Doppler imaging, the sensitivity, specificity, and diagnostic accuracy of ultrasound for **cervical lymph node metastases** are superior to those of computed tomography (CT) (Ahn et al. 2008).

Lymph node metastases are most commonly found in the medial and infrahyoid cervical compartment, along the carotid artery and jugular vein, in the jugular fossa, and behind the medial third of the clavicles. In more advanced stages, they will lie in the lateral cervical triangle and the mediastinum. The suprahyoid levels 1 through 3 are less commonly involved.

For cervical lymph nodes, high-resolution ultrasound, using linear probes with 7 MHz and above, is the most sensitive and specific imaging modality and superior to CT or magnetic resonance imaging (MRI). High-end ultrasound units with 12–14-MHz transducers and sensitive color Doppler capabilities are best suited.

Discrimination of reactive, benign lymph nodes from metastases is a common problem. That a lymph node is there, or that it is enlarged, does not necessarily mean that it is metastatic. In fact, there is a broad overlap in size for benign and malignant lymph nodes. For discrimination, shape, internal structure, degree of hypervascularity, and internal vessel architecture must be assessed.

Size, shape, and border: In the literature, a transverse diameter of 10 mm is reported as a "diagnostic cutoff." In MTC, many metastases are in fact smaller, and we do not use formal size criteria. Benign cervical lymph nodes have typically an oval shape, with their longitudinal being more than twice the transverse diameter. A rather round shape is one feature of possible malignancy and so is the presence of internal microcalcifications or atypical inclusions (Figs. 3, 4, 5, and 6). Benign lymph nodes will have a clear capsule and be sharply delineated. A blurred border

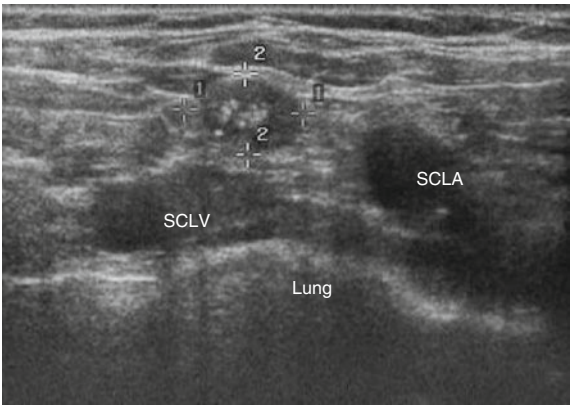

Fig. 3 Transverse US section of the right supraclavicular fossa in a patient with biochemical evidence of metastatic MTC, showing a hypoechogenic lymph node (*markers*) without echogenic hilum, but with round shape and microcalcifications, indicative of metastatic involvement. *SCLV* subclavian vein, *SCLA* subclavian artery

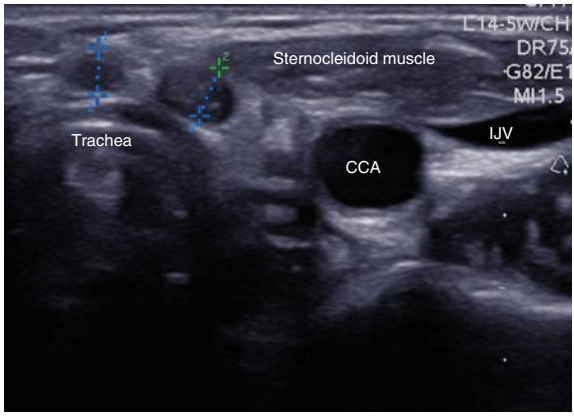

Fig. 4 Transverse US section of the former thyroid bed in a patient who had thyroidectomy for MTC and has biochemical evidence of progressive metastatic disease. Two round lesions (*markers*) at the anterior surface of the trachea without echogenic hilum, compatible with small subcentimeter metastases. Note that this location would be absolutely atypical for benign lymph nodes. *CCA* common carotid artery, *IJV* internal jugular vein

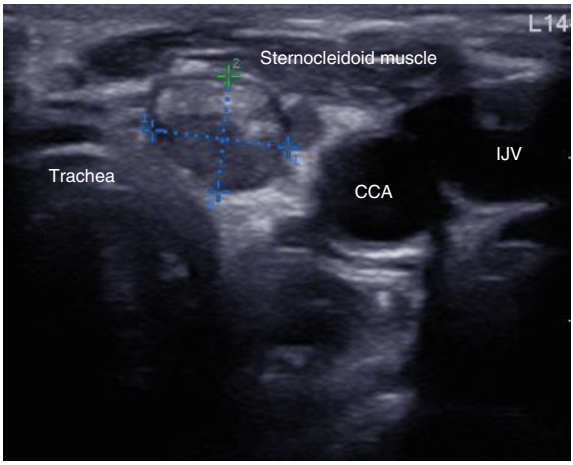

Fig. 5 Transverse US section of the former thyroid bed in a patient who had thyroidectomy for MTC and has biochemical evidence of progressive metastatic disease. Round lesion (*markers*) with a coarse hyperechogenic inclusion, highly suggestive of metastatic disease. *CCA* common carotid artery, *IJV* internal jugular vein

may indicate an extracapsular spread of a metastasis, but this is less common than, e.g., in squamous cell carcinomas.

Benign lymph nodes have a typical *internal echostructure*—a hilum and a peripheral follicular zone. The hilum is hyperechoic and often very thin. In very

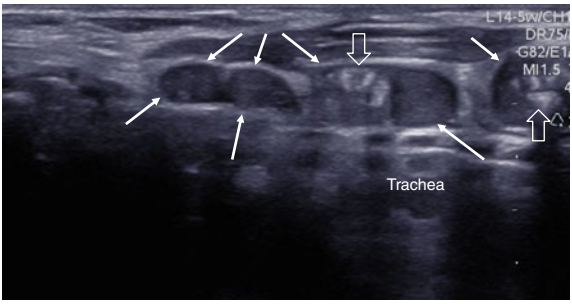

Fig. 6 Longitudinal US section along the anterior left surface of the trachea in a patient who had thyroidectomy for MTC and has biochemical evidence of progressive metastatic disease. There is a chain of suspicious lesions (*arrows*), *round* or *ovoid* in shape, some with coarse echogenic inclusions (*open arrows*), highly suggestive of metastatic disease

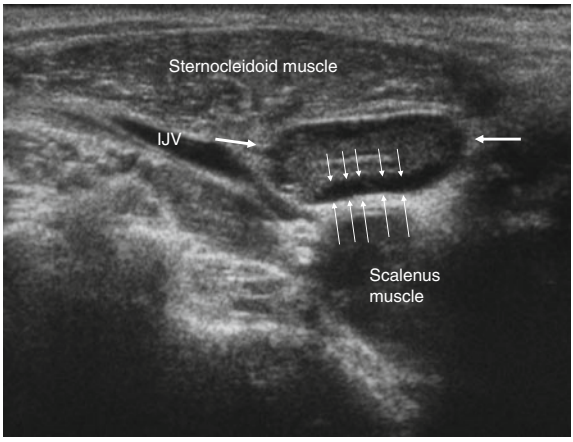

Fig. 7 Benign reactive lymph node (*arrows*) in a healthy individual without known malignant disorder. There is a marked hypoechogenic rim (*thin arrows*) all along the capsule, which is the strongest indicator of benign inflammatory changes and probably anatomically related to hyperplasic follicles. *IJV* internal jugular vein

slim lymph nodes, it cannot be seen. In case of inflammatory changes, there will be a thin, hypoechoic rim extending all along the capsule which corresponds to the follicular zone (Fig. 7). Both a hilum and a peripheral hypoechoic rim are very reliable indicators that an enlarged lymph node is benign. Often, however, the lymph node parenchyma is simply homogeneously hypoechoic. MTC metastases often have an intermediately bright "salt-and-pepper" echostructure without any normal anatomic components.

Vascularity: One unique feature of MTC is its markedly increased vascularity, which it shares with most other neuroendocrine tumors. As a result, blood flow will

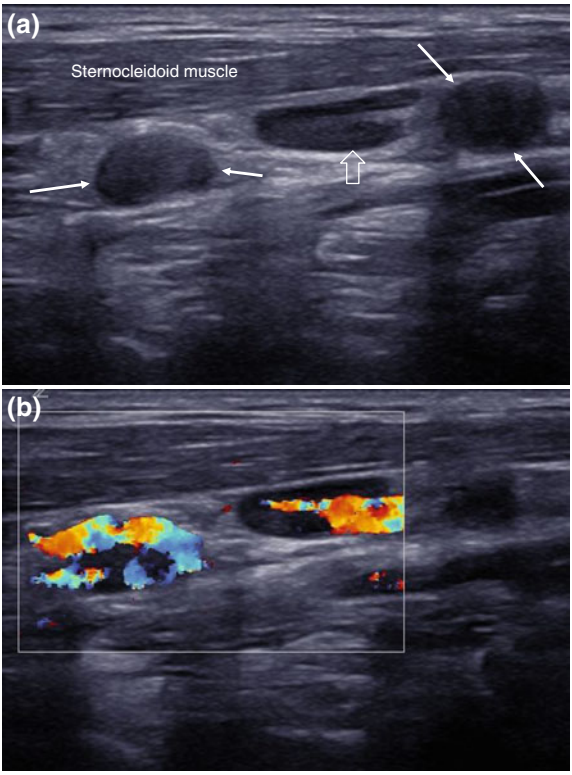

Fig. 8 Longitudinal B-mode (**a**) and color Doppler (**b**) image the left neck, lateral to the great vessels, showing two round, probably metastatic lymph nodes without hilum (*arrows*) as well as a longitudinally shaped, lymph node with preserved echogenic hilum and without criteria of suspicion (*open arrow*). Color Doppler imaging shows vessels in the hilum of the possibly benign lymph node in the middle and along the periphery of the suspicious ones

be detected with color Doppler ultrasound in nodules as small as few millimeters in diameter—which is uncommon in benign lymph nodes. Markedly inflammatory lymph nodes will also have increased blood flow. Nevertheless, their internal vessel architecture will be preserved: They have a single vascular pole at the hilum, and all internal vessels originate from there, showing a typical, treelike pattern. Metastatic lymph nodes show an atypical vascular pattern, with irregular vessels and multiple feeding vessels entering from the capsule (Figs. 8 and 9).

1.4 Computed Tomography/Magnetic Resonance Imaging

Additional CT studies of the chest and liver or contrast-enhanced MRI of the upper abdomen are suggested in patients with advanced locoregional disease.

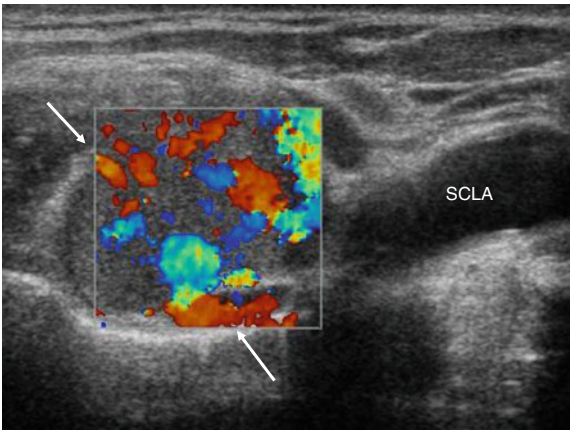

Fig. 9 Transverse US section of the right lateral supraclavicular fossa, showing a large metastasis due to MTC (*arrows*) with "salt-and-pepper" appearance and markedly increased perfusion with irregular vessel arrangement. *SCLS* subclavian artery

CT scans are obtained after i.v. injection of iodinated contrast medium with 3–5 mm slice thickness from the skull base to the sternal manubrium with the arms along the body, and with the arms above the head from the upper thoracic aperture to the lower renal pole. For the liver, an additional scan should be obtained during the portal phase (usually 60–90 S post-injection), which can be extended down to the pelvis if needed clinically. Modern multislice CT scanners will allow reconstruction of coronal and sagittal slices from an isotropic primary dataset, as well as thick slab maximum intensity projections (MIP) of the lung, which facilitate the detection of lung nodules. Single-slice or even non-helical CT scanners should no longer be used.

In CT, benign and metastatic lymph nodes are often similar in appearance—round and smoothly delineated. The ovoid shape of benign lymph nodes can only be appreciated if coronal reconstructions are available, since their axis is caudocranial. A blurred border of lymph nodes is a specific sign of malignancy but is only seen in very advanced disease or with locally very aggressive tumor variants. Remains only the transverse diameter as a criterion, 10 mm being most commonly used to discriminate benign and malignant lymph nodes. A criterion of malignancy that is not very frequently seen in MTC is a rim enhancement (Fig. 10), which is indeed uncommon for benign lymph nodes. Usually, the direction of metastatic spread is downstream, the central, infrahyoid, supraclavicular, and jugular compartments being most frequently involved (Fig. 11). However, there may also be an upstream spread, with metastases, e.g., at the skull base (Fig. 12).

Despite its higher soft tissue contrast, MRI has no clear advantages over CT, except that it is free of ionizing radiation (Fig. 13). The lower neck and upper thoracic aperture in particular pose problems in MRI since here the complex geometrical shape of the body causes magnetic field inhomogeneities. These can often

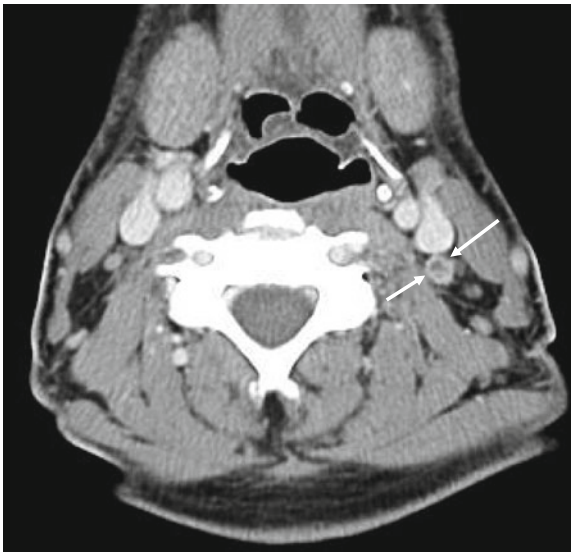

Fig. 10 Contrast-enhanced CT of the upper neck, showing a thin rim enhancement in a suspicious lymph node on the left side (*arrows*)

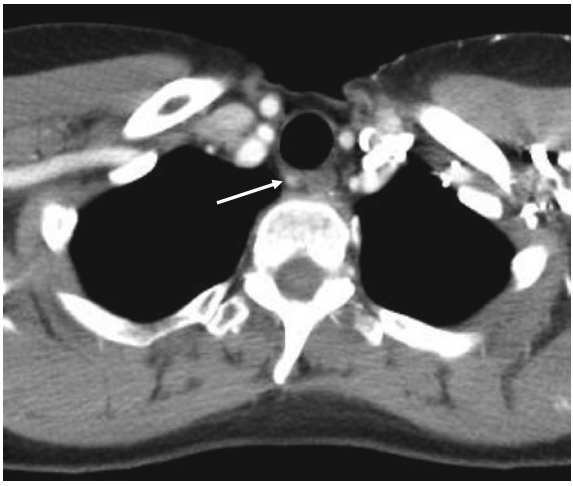

Fig. 11 Contrast-enhanced CT of the jugular fossa, showing a subcentimeter, retrotracheal lymph node metastasis due to MTC (*arrow*). This lesion was inaccessible to ultrasound, due to its location

not be entirely compensated with the scanner's shimming algorithms, and, as a result, the image quality is sometimes unsatisfactory. This concerns fat-suppressed images in particular, since fat suppression will only work if the local magnetic field is as expected. MRI studies of the mediastinum are frequently deteriorated by motion artifacts, caused by breathing and cardiac pulsations, although technology has improved significantly over the past years. Standard MRI series will consist of transverse and coronal T1- and T2-weighted as well as short tau inversion recovery (STIR) images, as well as contrast-enhanced, fat-suppressed T1-weighted images.

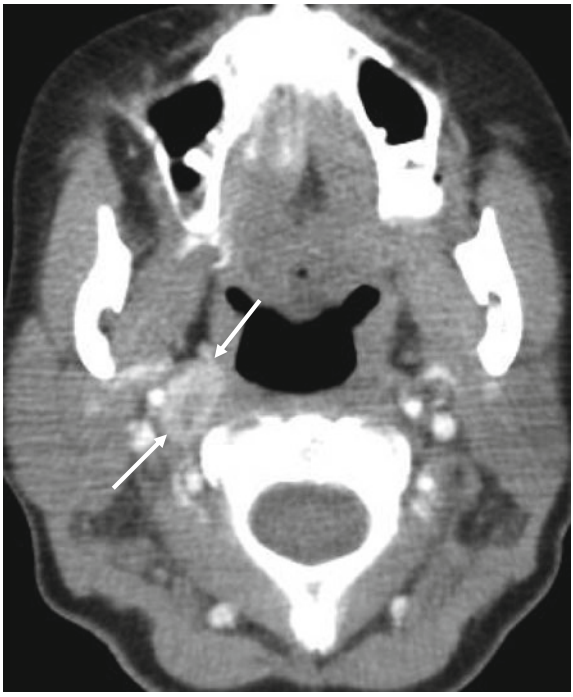

Fig. 12 Contrast-enhanced CT of the upper neck, showing a high parapharyngeal lymph node metastasis due to MTC (*arrows*)

Diffusion-weighted images may be added, because they may highlight possible metastases in unusual locations that might otherwise have escaped attention.

1.5 Imaging of Lung Metastases

The method of choice for detecting lung metastases is spiral CT, preferably using a multislice helical scanner and a collimation of 1 mm or less. From the thin-slice source images, "thick slab maximum intensity projections" (MIP) should be generated, which is possible on the scanner's console itself. On MIPs, it is easier than on the source images to discriminate small nodules from vessel sections (Fig. 14). MRI may depict nodules of more than 1 cm in diameter but is clearly not sensitive enough to be used alone for initial staging.

1.6 Imaging of Liver Metastases

With all imaging modalities, staging for liver metastases is complicated by the high prevalence of benign lesions, such as hemangiomas, focal nodal hyperplasias, and focal sparing in fatty liver. In abdominal ultrasound, the echogenicity of liver metastases cannot be predicted in general. Hypoechogenic nodules in an otherwise

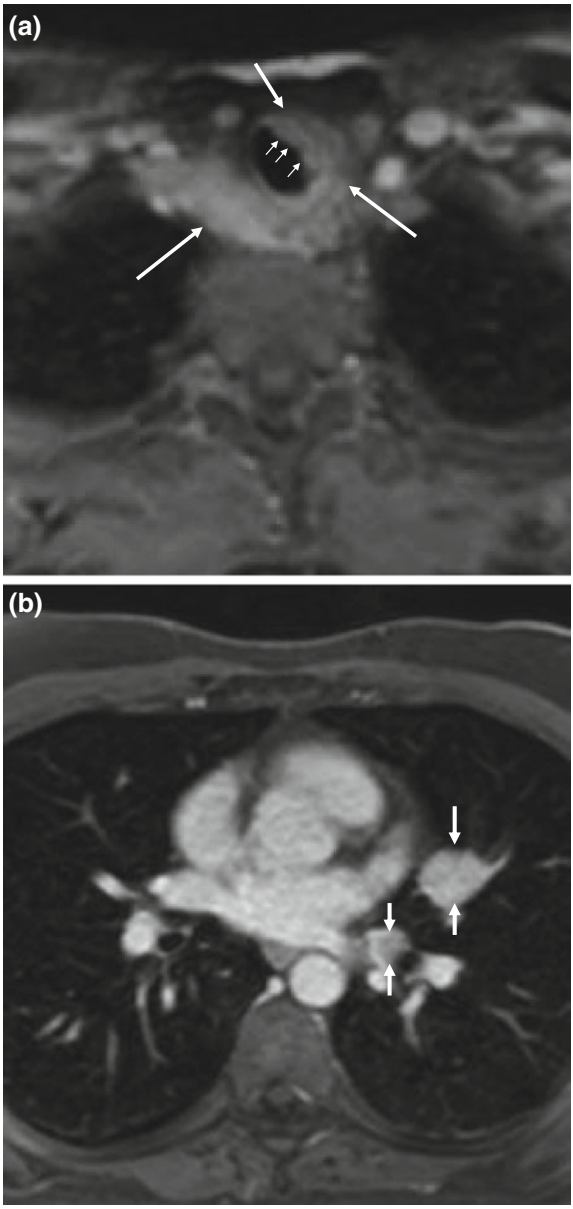

Fig. 13 Contrast-enhanced, transverse fat-saturated MR images at the level of the jugular fossa (**a**) and the left atrium (**b**) in a patient with persisting and progressive metastatic MTC. There is semicircular encasement of the trachea and esophagus in the upper thoracic aperture (*arrows*) and evidence of direct infiltration of the tracheal wall (*small arrows*), and as well metastatic disease in the mediastinum and left hilum (arrows)

normal (i.e., non-fatty) liver are likely to be metastases. Isoechogenic metastases may be hard to visualize if they are not demarcated by a hypoechogenic halo. Such a halo, however, is very specific for malignancy and usually rules out a benign nodule. Hyperechogenic metastases may be mistaken for hemangiomas. Nevertheless, there appear to be some peculiarities for MTC patients with liver

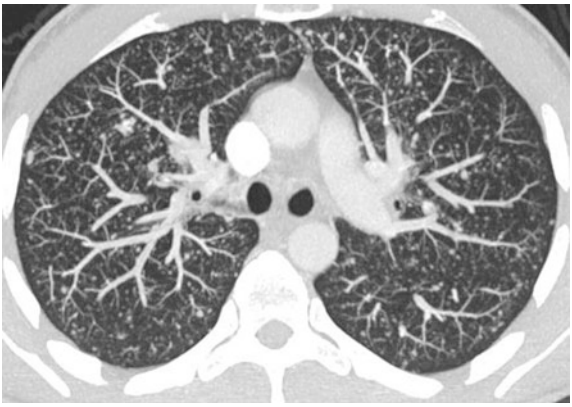

Fig. 14 CT of the chest in a patient with lung metastases due to MTC. Thick slab maximum intensity projection (*MIP*), generated from submillimeter transverse high-resolution slices, shows obvious miliary spread of metastases few millimeters in size. The MIP reconstruction allows a visually better discrimination of vessel sections (which due to the projection are longitudinally shaped) and nodules, which still have a round shape

metastases. Leclere et al. (1996) report that the vast majority of them had hyperechoic metastases (Fig. 15) and that 40 % of the metastases were calcified. Such calcifications may range from small echogenic spots inside a nodule to entirely calcified nodules with posterior acoustic shadowing. Hardly, any other benign condition tends to calcify in a similar fashion. In a fatty liver, the usual echogenicity-based criteria must be used with caution, since almost all focal lesions, benign and malignant ones, will appear darker than the parenchyma—except for calcifications (Fig. 16). Large metastases may become centrally necrotic and exhibit an echolucent zone (Fig. 17). The sensitivity of abdominal ultrasound for detecting metastasis depends on lesion size, their echogenicity (and thereby their conspicuity), the patient's constitution and scanning conditions, and of course the examiner's experience. As a rule of thumb, the sensitivity will not be higher than 60 %, and lesions smaller than 1 cm will only rarely be detected, particularly not with US (Fig. 18). Contrast-enhanced ultrasound (CEUS), although rather used for workup of unclear lesions than in staging, has a significantly higher sensitivity—in fact, it is comparable to CT or MRI and also helps to differentiate between benign and malignant lesions. However, it is not commonly used for staging, since contrast-enhanced CT is performed anyway.

In unenhanced CT, metastases will be less dense than the liver parenchyma, whose density is in part influenced by its iron content. In practice, however, no unenhanced series will be obtained. Instead, the chest CT part of the staging examination will be extended down to the mid-abdomen, followed by a portal phase scan. While 1/4–1/3 of the liver's blood supply is arterial and 2/3–3/4 is portal, the blood supply of malignant lesions is exclusively arterial. As a result, liver metastases will stand out hypodense during the portal phase of contrast enhancement.

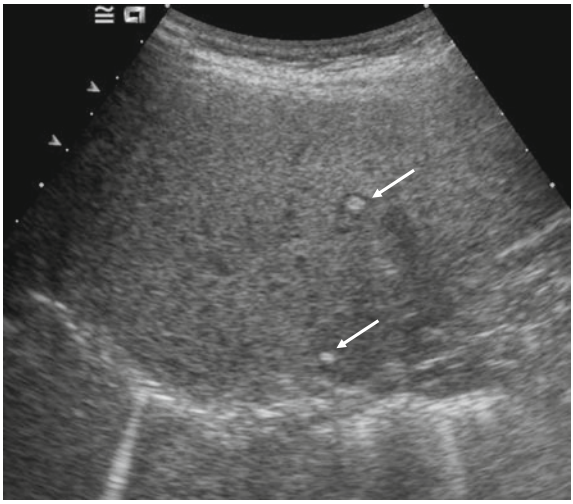

Fig. 15 Transverse US section of the subdiaphragmatic dome of the right liver lobe. Hyperechogenic metastases with thin hypoechogenic halo due to MTC (*arrows*)

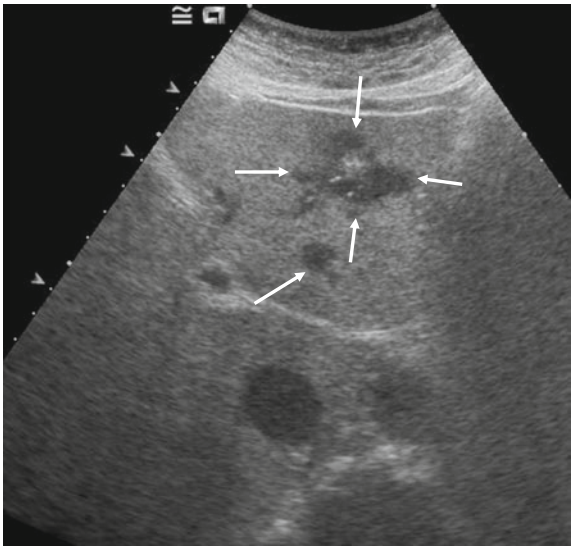

Fig. 16 Transverse US section of the liver segments 1, 2, and 3 in a patient with metastatic MTC and known fatty liver disease. There are two metastases (*arrows*) that stand out hypoechogenic in a bright liver, a small one with round shape and a large, irregular one with calcifications

Theoretically, hypervascular lesions, such as metastases from neuroendocrine tumors, may "flash" up during the arterial phase after contrast agent injection. This "flash", however, lasts only short, and the scan cannot be timed precisely enough to depict it reliably. In practice, both arterial and portal enhanced scans will be reviewed. The enhancement patterns are important criteria to differentiate metastases from benign liver lesions (hemangiomas, focal nodal hyperplasias (FNH), and cysts), which are common in healthy persons. Hepatic metastases from MTC often have a peculiar tendency to calcify, which at the beginning is more conspicuous at

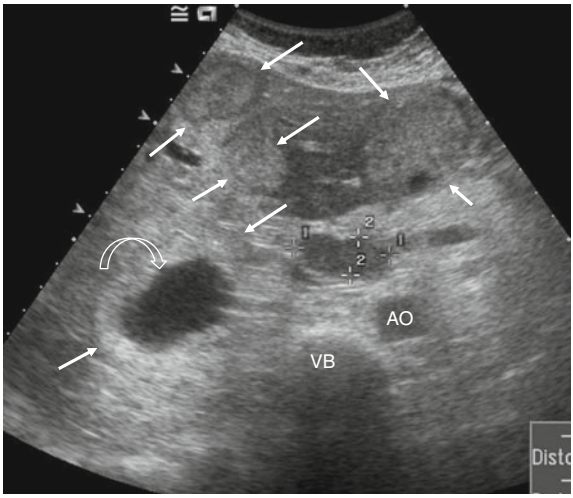

Fig. 17 Transverse US section in the epigastrium showing multiple, large, mostly hyperechogenic metastases (*arrows*) in both liver lobes, one with a central necrosis (*curved arrow*) along with a lymph node metastasis in the porta hepatis (*markers*). *AO* aorta, *VB* vertebral body

ultrasound than in CT. Not uncommonly, patients with MTC will be found with few or numerous strongly hyperechogenic lesions in ultrasound, which initially are often considered to be hemangiomas. However, they are more hyperechogenic than hemangiomas, and they become more over years. There are no reports on this peculiarity in the literature. Histologic proof of these lesions has not been obtained on large scale, but since they become more over time, they must be considered metastatic (Fig. 19). According to own experience, the progression of such entirely calcified lesions is often slow, and the serum markers (CTN and CEA) are only moderately elevated. We have been following these patients over years and even decades, without them becoming symptomatic. Liver metastases with predominant soft tissue components appear to be far more aggressive.

In MRI, liver metastases will be hypointense in T1-weighted and slightly hyperintense in T2-weighted images. After contrast agent administration, the enhancement pattern will be analogous to that in CT. We have little experience with MRI in entirely calcified metastases. Those few we have seen were hypointense in both weightings, as expected, and showed no measurable contrast agent uptake, which, as we believe, reflects their low aggressiveness.

There is so far only little experience with diffusion-weighted imaging (DWI) in MTC. We see no role for the workup of an unclear thyroid nodule, since the existing algorithms are clear and efficient. Since metastases from MTC, like those from most other neoplasms, will have a restricted diffusion, they will be hyperintense on DWI images with moderately high B-values (e.g., 800 at 1.5 T). This is not specific, particularly not in lymph nodes, but DWI images with background suppression will let candidate lesions stand out in good contrast and draw the reader's attention to a lesion that should be examined closer on other images, and possibly with ultrasound. Occasionally, we have seen metastases that were only seen with

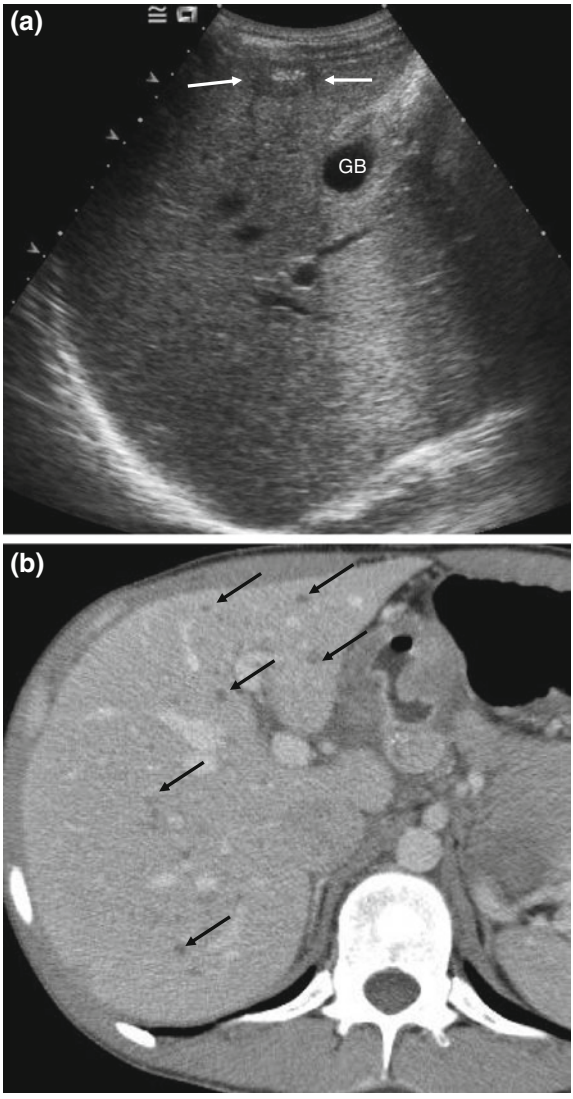

Fig. 18 Intercostal US section of the right liver lobe in a patient with metastatic MTC (**a**) and contrast-enhanced CT of the right liver lobe (**b**). US shows a single, partially calcified metastasis (*arrows*). CT reveals that there are innumerable small metastases (*black arrows*) that were invisible in US

difficulty in other series or in CT. DWI series should nowadays be a standard part of every MRI examination for cancer staging or follow-up.

There are no larger systematic reports on the accuracy of imaging modalities for liver metastases in MTC specifically—most reports are related to colorectal neoplasms. Here, the sensitivity of both helical CT and MRI is reported to be below 70 % (Bipat et al. 2005), but newer developments in CT and MRI have taken place after this publication. CEUS is highly sensitive and offers great capabilities in differentiating metastases from benign liver lesions, but may suffer from limited visibility of the entire liver, e.g., in obese patients (Cantisani et al. 2014).

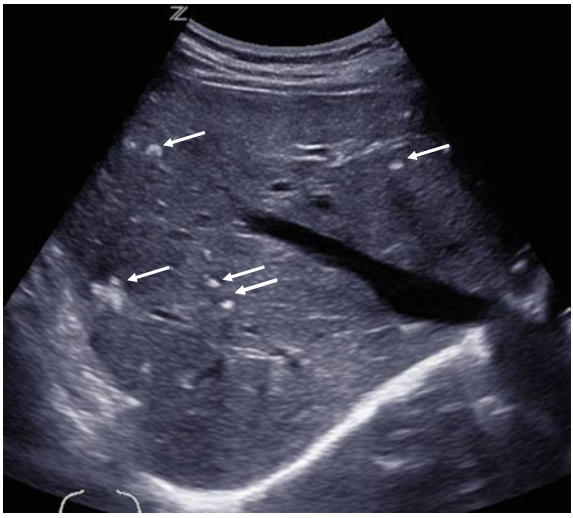

Fig. 19 Transverse US section of the right liver lobe in a patient with known metastatic MTC. There are multiple small calcifications (*arrows*) which have been followed for over 15 years and have shown only a slow progression

1.7 Imaging of Skeletal Metastases

Whenever skeletal metastases are considered, MRI may be superior to other imaging modalities (Mirallie et al. 2005), since it will reveal bone marrow deposits that have not yet altered the mineralized bone. Nevertheless, bone scintigraphy is a cheap and reasonably sensitive alternative if high CTN levels or an elevated alkaline phosphatase suggests bone metastases. We do not recommend 18-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) imaging or ^{18}F-dihydroxyphenylalanine F-DOPA-PET/CT receptor imaging for routine use in initial screening for metastatic disease. The sensitivity of FDG-PET scanning for detecting metastatic disease is variable (Oudoux et al. 2007; Giraudet et al. 2007) and improves with higher CTN levels (sensitivity of 78 % for basal CTN values >1000 vs. 20 % for values <1000 pg/ml, respectively) (Ong et al. 2007). Unfortunately, optimal whole-body imaging cannot be achieved with a single modality. Somatostatin receptor scintigraphy is currently not recommended for routine initial staging for metastatic disease (American Thyroid Association Guidelines Task et al. 2009; Frank-Raue et al. 1995) but may be more useful in localizing residual or recurrent disease after primary therapy.

2 Additional Imaging Studies in MEN2

Patients with MEN2 (according to their family history, genetic testing, or visble stigmata) should be evaluated for parathyroid adenomas and pheochromocytoma (Raue et al. 1995; Quayle et al. 2007; Machens et al. 2013). Therefore, serum calcium, phosphate, and parathyroid hormone levels as well as catecholamine metabolites in plasma as well as in urine are part of the routine laboratory workup.

The primary imaging study for parathyroid adenomas is ultrasound, which is highly sensitive for orthotopic adenomas, but requires a highly skilled examiner (Fig. 20). CT or MRI of the neck and mediastinum are clearly not as specific, lymph nodes being the main confounder. MIBI scintigraphy may be added if laboratory findings indicate primary hyperparathyroidism (pHPT), even if a probable adenoma is seen at ultrasound—simply, because multiple adenomas are more frequent than in sporadic pHPT (Guerin et al. 2015).

With ultrasound, the right adrenal gland, even if normal, is easily visualized in the majority of patients through an intercostal access, but the left one is difficult to see. The best access is from the epigastrium where it lies in the niche between the pancreatic tail, the aorta, and the upper left renal pole. There, a normal gland is still difficult to see, but a tumor may be depicted. Whenever the pancreatic tail is obscured by gas in the stomach or the bowel, ultrasound is unsuitable for assessing the left adrenal gland, and additional CT or MRI studies are warranted. With these, both adrenals can be assessed without difficulty.

The echographic appearance of pheochromocytomas is variable. Typical features are small echolucent inclusions (Fig. 21), which correspond to focal necrosis and lack contrast uptake at CT or MRI. All other plain solid adrenal tumors must be differentiated from common benign, non-functioning adenomas. Elevated catecholamines/nephrines in serum or urine are strong indicators of pheochromocytoma. A dedicated MRI technique is in-phase/opposed-phase imaging which serves to depict a diffuse lipid component in tissue. A clear lipid component definitely rules out pheochromocytoma and also metastases from whichever origin. If this is not seen, the diagnosis is open, pheochromocytoma or metastasis being possible diagnoses (Fig. 22). Still there are benign adenomas without a marked lipid component. Metaiodobenzylguanidine (MIBG) scintigraphy will be reserved for patients with normal appearing adrenal glands despite suspicious laboratory findings, for localizing catecholamine-producing tumors in ectopic locations (Ilias and Pacak 2004; Jacques et al. 2008; Ilias et al. 2007).

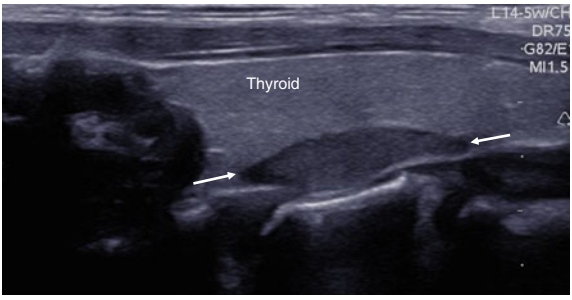

Fig. 20 Longitudinal US section over the right thyroid lobe. Typically shaped parathyroid adenoma (*arrows*) behind the otherwise normal thyroid lobe

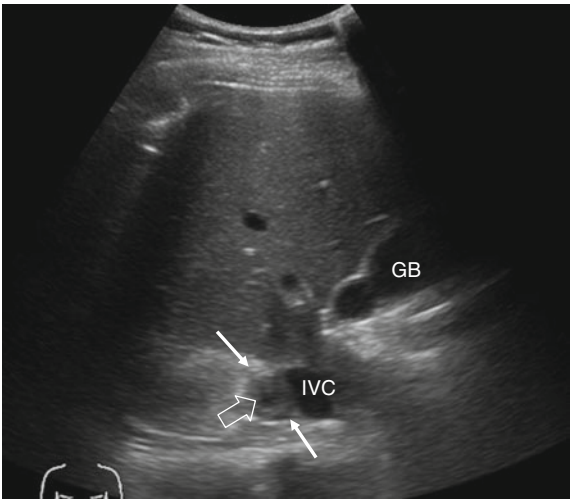

Fig. 21 Transverse intercostal US section in a patient with MEN2a and right-sided pheochromocytoma. In the adrenal bed, there is a round, hyperechogenic tumor (*arrows*) with a central echolucency (*open arrow*) which is pathognomonic for pheochromocytoma. *IVC* inferior vena cava, *GB* gall bladder

3 Postoperative Imaging and Follow-up of MTC

It is recommended to obtain a baseline ultrasound examination 3 months after surgery. Patients who according to their serum tumor markers are thought to have residual disease should also have a CT scan of the neck, chest, and upper abdomen, as outlined above.

After thyroid resection, the common carotid arteries lie directly adjacent to the trachea and the esophagus. All other anatomical structures should appear as usual, since classical radical neck dissections are no longer performed. Even with experienced surgeons, some residual thyroid tissue may be found, and this should not be mistaken for residual or recurrent local disease, at least not early after surgery and in the absence of extrathyroid extension of the primary tumor.

Whenever serum CTN and CEA levels are elevated, postoperatively persistent or recurrent MTC is suspected and ultrasound (US) of cervical lymph nodes is the method to start with (Kouvaraki et al. 2003). Suspicious findings may be confirmed by fine needle aspiration biopsy (Kudo et al. 2007). If serum CTN levels are higher than 150 pg/ml, additional imaging is indicated (Wells et al. 2015) for the commonest sites of distant metastasis, i.e., the liver (49 %), bones (45 %), and lungs (35 %) (Giraudet et al. 2007). In addition to ultrasound of the neck and abdomen, contrast-enhanced CT of the chest and abdomen, or MRI of the mediastinum and abdomen is being used, but also MRI of the pelvis and axial skeleton and bone scintigraphy, depending on local preferences or expertise.

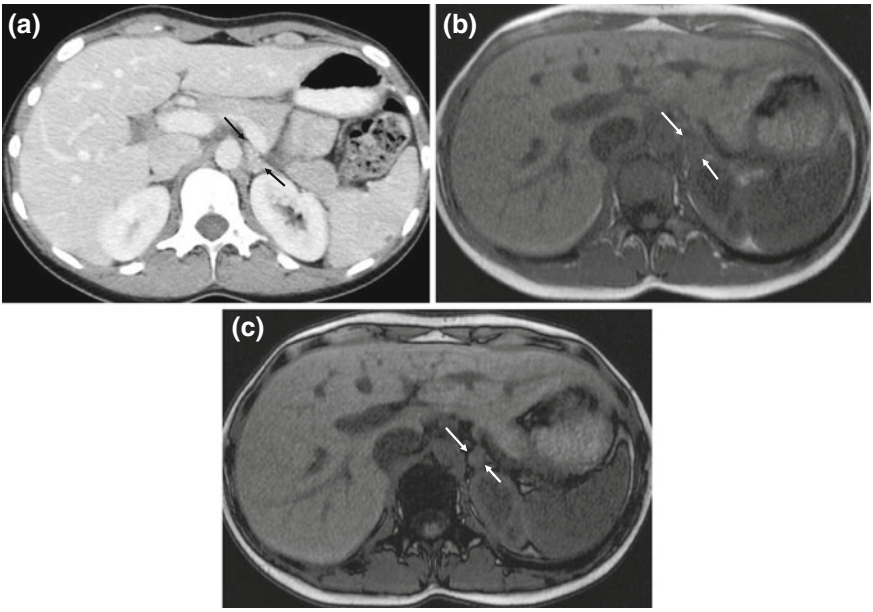

Fig. 22 Contrast-enhanced CT (**a**) in a patient with MEN2a and left-sided pathologically confirmed pheochromocytoma. CT shows an enlargement of the adrenal gland (*arrows*) with preserved shape, which by itself is an unspecific finding. Additional transverse, T1-weighted MRI images obtained in-phase (**b**) and opposed-phase show the tumor (*arrows*), and comparison of the two images reveals that there is no signal loss on opposed-phase images, thereby excluding a lipid containing adenoma

In one study from the literature, imaging was performed in 55 consecutive patients with MTC and elevated serum levels of CTN (median, 1250 pg/ml) and CEA (median, 37 ng/ml). Neck recurrences were demonstrated in 50 patients by US (56 %), CT (42 %), and FDG-PET/CT (32 %). Lung and mediastinal lymph node metastases were demonstrated in 55 patients by CT (35 and 31 %, respectively) and by FDG-PET/CT (15 and 20 %, respectively). Liver metastases were demonstrated in 41 patients by MRI (49 %), CT (41 %), US (41 %), and FDG-PET/CT (27 %). Bone metastases were found in 55 patients using FDG-PET/CT (35 %), bone scintigraphy (40 %), and MRI (40 %). Bone scintigraphy was complementary with MRI for axial skeletal lesions but superior for the detection of peripheral bone lesions. In 10 patients, no locoregional or distant recurrence was found despite elevated CTN levels (median 196 pg/ml). The authors concluded that the most efficient imaging procedures for detecting MTC at various sites were US for the neck, CT for the chest, MRI for the liver, and MRI and bone scintigraphy for the axial skeleton. FDG-PET/CT scan was less sensitive (Giraudet et al. 2007). The sensitivity of imaging for localizing metastatic disease ranges between 50 and 80 %, but is likely to be significantly lower in the setting of modestly elevated serum CTN values. Imaging with anti-CEA antibodies and scintigraphy with several tracers,

such as somatostatin analogues, metaiodobenzylguanidine (MIBG), dimercaptosuccinic acid and gastrin, are usually rather insensitive (Frank-Raue et al. 1995; Baudin et al. 1996; Behr et al. 1997; Clarke et al. 1988; Behr and Behe 2002).

The radiation doses are of concern if follow-up studies are regularly performed with CT, and doses will accumulate over years. Owing to the frequently slow progression of MTC, many patients will survive far longer than 10 years—long enough to experience secondary, radiation-induced neoplasms. Therefore, the need for CT or PET studies should be reflected for patients with low disease activity, or the protocol should at least be reasonably limited. The only organ for which CT is so far indispensible is the lung. With low-dose protocols, the effective equivalent radiation dose can be "tuned down" to less than 2 mSv, which is in the range of the natural exposition. Because of image noise, the mediastinum and upper abdomen cannot be assessed with this technique. For the neck, ultrasound is in our view superior to CT or MRI. For liver metastases, it is less sensitive than CT, but since the treatment options for distant MTC metastases are limited anyway, this may be acceptable. With appropriate technique, the mediastinum can be well examined with MRI. In practice, we could recommend regular ultrasound examinations of the neck and upper abdomen, low-dose CT of the lung, and MRI of the chest and upper abdomen, including DWI.

3.1 Role of PET/CT

Positron emission tomography (PET) appears promising in the diagnostic workup of MTC. As a biomarker of glycolysis, FDG ^{18}F-fluorodeoxyglucose uptake from MTC cells correlates with the degree of dedifferentiation and the proliferative activity, while the PET tracer ^{18}F L-dihydrophenylalanine (^{18}F-DOPA) has an intrinsically high affinity to neuroendocrine tumors. 18-FDG-PET/CT and ^{18}F-dihydroxyphenylalanine F-DOPA-PET/CT have been shown to be superior to conventional imaging procedures in detecting metastases in patients with MTC (Ong et al. 2007; Gourgiotis et al. 2003; Hoegerle et al. 2001; Rubello et al. 2008; Santhanam and Taieb 2014; Treglia et al. 2012), at least if the serum CTN levels are >500 pg/ml (de Groot et al. 2004). In another study, 100 % sensitivity was reached with F-DOPA-PET/CT in patients with CTN level >150 pg/ml (Luster et al. 2010). DOPA-PET is preferably used to assess the extent of the disease generally, while ^{18}F-FDG/PET seems to be better for identifying more progressive MTC (Oudoux et al. 2007; Verbeek et al. 2012; Koopmans et al. 2008). MTCs often exhibit high ^{18}F-FDG uptake in the later stages of disease and could be used as a surrogate for aggressiveness as well as short CTN doubling time. Sensitivity of ^{18}F-FDG/PET was 83 % for neck, 85 % for mediastinal, 75 % for lung, 60 % for liver, and 67 % for bone metastases; overall, sensitivity was 76 %. The standardized uptake value (SUVmax) correlated significantly with CTN doubling time (Oudoux et al. 2007). Therefore, FDG and F-DOPA-PET/CT appear complementary. Owing to the inter-

and intraindividual heterogeneity of this disease, a multimodality imaging approach is recommended for accurate anatomic localization of MTC lesion, especially if a reoperation is planned (Cheng et al. 2012).

The sensitivity of these tests in localizing metastatic disease ranges between 50 and 80 %, but is likely to be significantly lower in the setting of modestly elevated serum CTN values (Mirallie et al. 2005; Gourgiotis et al. 2003; Hoegerle et al. 2001; Koopmans et al. 2008). Patients with known but asymptomatic or small metastases or without frank progression, who do not receive any systematic treatment, should undergo repeated imaging every 6–12 months, but the intervals may deviate from this, depending on the doubling times of CTN or CEA. Only patients with significant tumor burden and those with symptomatic or progressive disease who are candidates for systemic therapy need follow-up at short intervals.

3.2 Decision to Treat and Determination of Response in Progressive MTC

Although MTC is generally regarded as a slowly progressive, indolent disease, patients with radiographic evidence of progressive MTC can have a substantial disease burden and high incidence of disease-related symptoms. Clinicians must decide which patients require therapy, balancing the often slow rate of tumor progression associated with a good quality of life, against the limited efficacy and potential toxicities of local and systemic therapies. Palliative therapy, including surgery, EBRT, or systemic therapy, should be considered in patients with metastases causing pain, mechanical compression, or signs and symptoms of hormonal excess. The optimal time to start treatment in patients with advanced MTC is controversial. The size and number of tumor foci, the rate of change of tumor volume during watchful waiting and rapid doubling times of serum Ctn and CEA levels (<6 month), are useful prognostic indicators.

The majority of clinical trials investigating cancer treatment utilize the Response Evaluation Criteria In Solid Tumors (RECIST) rules and guidelines, to standardize the measurement of solid tumors, to define the time to start treatment, and to determine whether the disease improves (complete or partial response), remains the same (stable disease), or worsens (progressive disease) (Eisenhauer et al. 2009). For RECIST measurements, up to 5 target lesions are defined (max. 2 per organ) that must be well enough defined to be unambiguously measured and be at least 10 mm in their longest diameter. Skeletal metastases are often excluded because reactive sclerosis may be hard to interpret and represent both progression and treatment-induced recalcification. An exception may be made for entirely lytic lesions with a clearly visible soft tissue component. The longest diameters of target lesions are added, and the sum of diameters will be the basis of response assessment. A drop in the sum by 30 % or more will be a partial response, and an increase by 20 % or more over the nadir will be a progression. Stable disease will be anything in between, and complete response is the disappearance of all lesions.

Apart from target lesions, nontarget lesions are defined, e.g., if there are more than 2 in one organ, if the maximum of 5 has already been reached, and for assessing ill-defined or irregularly shaped lesions which are difficult to measure, or for ascites, malignant pleural effusion, etc. Nontargets may but need not be measured, and they are reported to be present, unambiguously progressive (which may even override a target response), to have disappeared. Any new tumorous lesion will indicate a progressive disease. Special rules apply for lymph nodes. They must be measured in both axes, not only the longest one, and have a short-axis diameter of 15 mm to qualify for a target lesion and 10 mm to become a nontarget. For obtaining the sum of diameters, the short-axis diameter of lymph node targets will be taken, not the long one. A lymph node metastasis that becomes smaller than 10 mm short axis will be counted as "disappeared". Apart from targets and nontargets, "findings" may be reported, e.g., for clinically important but non-malignant changes, or to mark a lesion that not yet meets criteria for a target or nontarget, but which should be re-assessed at the next visit.

It is important to say, however, that RECIST or other similar systems are designed to document results within clinical trials and are too rigid to **govern** individual clinical decisions outside trials. They may, however support clinical work and also be a valuable help for radiologists in comparing studies in follow-up —particularly if dedicated software solutions are being used.

3.3 Therapy with Radiolabelled Substances

MIBG and somatostatin analogues can also serve as therapeutic agents if there is a clear uptake shown in the diagnostic scan. In metastatic disease, a therapeutic dose of 131I-labeled MIBG can be applied to MTC patients with metastatic disease as it is being done in pheochromocytoma or neuroblastoma patients. Some studies reported that MIBG therapy gives palliative response in approximately 60 % of patients, and measurable tumor shrinkage can be achieved in about 30 % of patients (Troncone et al. 1991; Castellani et al. 2008). Therapy with the somatostatin analogue 90Y-DOTATOC has induced a complete response in only 10 % of cases, with stable disease in 50 % and failed in 33 % of patients with metastatic MTC (Bodei et al. 2004).

4 Conclusion

Although US is a highly operator-dependent imaging modality, it is the method of first choice for the evaluation of thyroid nodules and detection of cervical lymph node metastases in the neck. CT and MRI can obtain detailed anatomic information of mediastinal and hilar lymph node metastases as well as parenchymal pulmonary metastases. The liver will be assessed by CT or MRI in combination with B-mode ultrasound, complemented by CEUS wherever needed. Nuclear imaging modalities

depend on functional parameters such as perfusion, receptor status, receptor–ligand interaction, and metabolism providing functional in vivo information of the whole body. Both imaging methods are necessary in the diagnostic workup and therapy planning of MTC patients.

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Calcitonin as Biomarker for the Medullary Thyroid Carcinoma

Yoon Ju Bae, Michael Schaab and Juergen Kratzsch

Abstract

Calcitonin (CTN) is a polypeptide hormone consisting of 32 amino acids with a disulfide bridge between position 1 and 7 that is mainly produced by the C-cells of thyroid gland. The measurement of CTN concentrations in blood reflects C-cell activity and is performed in general by immunoassay methods. However, there are analytical, physiological, pharmacological, and pathological factors that can influence results of serum CTN values. Due to the influence of these factors, there is a high variability in assay-dependent cutoffs used to discriminate between MTC, C-cell hyperplasia (CCH), and the absence of the pathological impairment of C-cells. There is a lot of evidence that the measurement of serum CTN concentrations in patients with thyroid nodules can lead to an earlier diagnosis of MTC or CCH than the exclusive use of imaging procedures and/or fine-needle aspiration cytology. Basal CTN concentrations higher than 60–100 pg/mL are highly indicative for the diagnosis MTC. In the range between cutoff and 60 pg/mL CTN, both MTC and HCC may be a relevant diagnosis. PCT and CTN appear to have a comparable diagnostic capability to diagnose MTCs. However, "positive" PCT values of more than 50 pg/mL may be reached also in subclinical infections and will lead, therefore, to an overdiagnosis of the tumor. Pentagastrin- or calcium-stimulated serum CTN concentrations higher than cutoff values might improve diagnostics of MTC, but the non-availability of the first and the lacking of relevant cutoff values for the second tool favors the use of only basal values currently.

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Keywords

Calcitonin · **Procalcitonin** · Immunoassay · Pentagastrin stimulation · Calcium stimulation · Cutoff

List of Abbreviations

AUC	Area under the curve
CTN	**Calcitonin**
CGRP	Calcitonin gene-related peptide
1,25–OH–Vitamin D3	Calcitriol
Ca	Calcium
CCH	C-cell hyperplasia
CKD	Chronic kidney disease
CV	Coefficients of variation
FNAC	Fine-needle aspiration cytology
H2RB	Histamine-2 receptor blockers
IRMA	Immunoradiometric assay
MTC	**Medullary thyroid carcinoma**
mRNA	Messenger ribonucleic acid
NID	Nichols Institute Diagnostics
PCTN	**Procalcitonin**
PG	Pentagastrin
PPV	Positive predictive value
PPI	Proton pump inhibitor
RIA	Radioimmunoassay
ROC	Receiver operating curve
RfB	Reference Institute for Bioanalytics
WHO	World Health Organization

Contents

1 Methods for CTN Measurement

1.1 Biochemical Background

The existence of calcitonin (CTN) as second hormone besides parathyroid hormone which is involved in regulation of calcium concentration was first demonstrated by perfusion experiments in dogs in the 1960s (Copp et al. 1962). Calcitonin is mainly produced by the C-cells also named parafollicular cells of thyroid gland. It is the product of posttranslational modifications of a 136 amino acid precursor protein called preprocalcitonin. The so-called mature form of calcitonin consists of 32 amino acids with a disulfide bridge between position 1 and 7. This intramolecular disulfide bridge as well as the proline amide at the carboxyterminal end is essential for its biological function (for review see Bringhurst et al. 2011).

The calcitonin gene is located on the short arm of chromosome 11 and consists of six exons (Fig. 1). Besides calcitonin, a second gene product called calcitonin gene-related peptide (CGRP) is produced by alternative splicing in a tissue-specific manner. In addition to CGRP, amylin, adrenomedullin and calcitonin receptor-stimulating peptide 1 belong to the calcitonin family. Calcitonin itself is generated through splicing and combining the first 4 exons of the transcribed mRNA. In the C-cells, calcitonin accounts for almost 95 % of all mature transcripts. The second gene product CGRP is produced by splicing together exons 1–3 and 5–6 (Fig. 1). The mature peptide consists of 37 amino acids and is expressed in numerous tissues, and it is the only transcript of the calcitonin gene found in neuronal tissue.

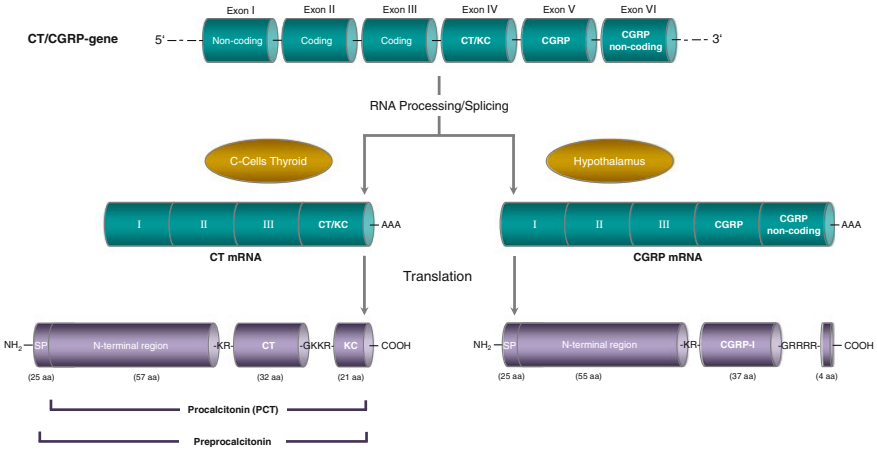

Fig. 1 Organization of the calcitonin/calcitonin gene-related peptide gene and its peptide hormone products. *CTN* calcitonin, *CGRP* calcitonin gene-related peptide, *KC* katacalcin, *SP* signal peptide, *K* lysine, *R* arginine, *G* glycine, and *aa* amino acid

Synthesis and secretion of calcitonin are tightly regulated: An almost linear correlation between calcium concentration and the amount of secreted calcitonin could be displayed in a porcine model (Garrett et al. 1995). In particular, the intracellular calcium concentration in the C-cells regulates the secretion rate of calcitonin possibly through the calcium-sensitive receptor, which is also expressed in the thyroidal C-cells. Glucocorticoids, CGRP, glucagon, gastrin, pentagastrin (PG), pancreozymin as well as β-adrenergic agents are also capable of inducing marked increases of calcitonin secretion (Care 1992). On the other hand, somatostatin, a hypothalamic hormone, which can be also secreted by the C-cells, inhibits calcitonin secretion. Calcitriol (1,25–OH–Vitamin D3), another player in the field of calcium homeostasis, may negatively influence the secretion of calcitonin by decreasing the amount of calcitonin mRNA.

It has been shown that calcitonin exerts its effects through the calcitonin receptor —a serpentine protein expressed at high levels in kidney and hypothalamus. In bone, CTN almost exclusively bounds to osteoclasts (Marx et al. 1972). In animal studies, numerous effects of calcitonin on calcium and bone metabolism could be demonstrated. For instance, calcitonin lowers tubular reabsorption of calcium when acutely administered (Friedmann and Gesek 1995). Furthermore, it impairs osteoclast-mediated bone resorption (Chambers et al. 1985). This anti-resorptive effect was especially pronounced in studies using salmon CTN which has a 50-fold higher potency than mammalian CTN (Chambers and Moore 1983; Zaidi et al. 2002). Because of these findings, CTN was and still is considered as functional counterpart of PTH. Although pharmacologic actions were studied extensively and seem to be clearly described, there is marked uncertainty about calcitonin's function in mammalian physiology (Davey and Findlay 2013; Hirsch et al. 2001). This is mainly based on the fact that patients with CTN deficiency following thyroidectomy do not display the expected osteoporosis and that bone mineral density was found decreased in individuals with medullary thyroid carcinoma (MTC) (Hurley et al. 1987; Zaidi et al. 2002).

Whereas there is an ongoing lively debate about its role as a regulator of calcium and bone metabolism, calcitonin's diagnostic importance as a tumor marker for MTC is undoubted. It is well known that patients with MTC display increased basal and pentagastrin or calcium stimulated CTN concentration compared to patients with C-cell hyperplasia (CCH) or healthy subjects (see Chaps. 2 and 3 of this article). Before the era of RET mutation identification, serum mature calcitonin measurements were the only mean to identify affected relatives in the case of familiar MTC (Bihan et al. 2003).

Besides the mature monomeric CTN (1–32 amino acids), several other isoforms, precursors, and metabolites are detectable in the circulation (Bucht et al. 1985; Deftos et al. 1975; Schifter 1993; Tobler et al. 1983). The half live of these isoforms distinctly differ between 15 min for mature CTN and several days for some CTN precursors (Becker et al. 2002). However, most of CTN immunoreactivity is referred to the intact molecule. Otherwise, patients with MTC display a big heterogeneity of calcitonin immunoreactivity (Austin and Heath 1981; MacIntyre et al. 1984; Snider et al. 1977; Tobler et al. 1983). Compared to healthy subjects, polymeric forms as

well as monomeric form of calcitonin can be found in patients with MTC. Polymeric forms of CTN in MTC patients may be a result of intermolecular disulfide bridges or disulfide bridges to unrelated proteins (Goltzman and Tischler 1978). Nevertheless, Austin and Heath (1981) could demonstrate that the polymeric isoforms are less bioactive than the monomeric one. In patients with chronic renal failure, monomeric CTN is relatively decreased, whereas increased concentrations of high molecular weight isoforms of CTN precursors and metabolites, probably generated through homo- and heteroaggregates, can be detected (Lee et al. 1977). Dialysis in patients with chronic renal failure contributes further to the heterogeneity of CTN, because high molecular weight forms are not dialyzable and accumulate due to the dialysis-associated calcium load. This should be considered when determining CTN concentration in patients with chronic renal failure. As mentioned before, measurements of CTN concentration are of considerable value in preoperative diagnosis of MTC (Bihan et al. 2003). Furthermore, preoperative mature CTN levels in MTC may also be indicative of tumoral volume and predictive of eventual cure when less than 50 pg/mL (Cohen et al. 2000). In addition, CTN is a marker for the postoperative prognosis of patients with MTC. In particular, monomeric CTN seems to be exclusively secreted by persisting tumor cells of the MTC (Engelbach et al. 1998). Hence, CTN concentrations of patients with total thyroidectomy measured with assays specific for the monomeric isoform suggest incomplete tumor extirpation (Engelbach et al. 1998). Thus, the heterogenic patchwork of CTN immunoreactivity should be clearly separated by the used assay system to obtain reliable values for diagnostic purpose (Bertagna et al. 1980; Heath and Sizemore 1979; Morimoto et al. 1981; Singer and Habener 1974; Sizemore et al. 1975; Voelkel and Tashjian 1971).

1.2 CTN Assay Methods

Measurement of CTN is described to be performed in general by immunoassay methods. The first radioimmunoassays (RIA) for CTN were established in the late sixties of the last century (Cooper et al. 1967; Deftos et al. 1968; Deftos 1971; Hirsch et al. 1964). These assays were competitive and exhibited frequently a low specificity. Later, two-sided immunoassays (immunoradiometric assays, IRMA) with a distinctly improved specificity became more and more accepted. These assays used two different antibodies directed against spatially separated, specific epitopes of the CTN molecule (Body and Heath 1984; Motté et al. 1988). Around the year 2000, assay manufacturers have moved from radiolabeled systems to fluorescent and chemiluminescent assays (Bieglmayer et al. 2002; d'Herbomez et al. 2001). Thus, the inconvenient radioactivity was substituted by labels with a somewhat higher signal efficiency to receive an improved analytical sensitivity. In the following years, the chemiluminescence test of the manufacturer Nichols Institute Diagnostics (NID) was considered as "gold standard" for CTN measurements as its functionality has been evaluated in a number of papers (Bieglmayer et al. 2007; Grauer et al. 1998; Engelbach et al. 2000). However, NID terminated

the production of their immunoassays recently. Moreover, due to the augmenting automation of hormone analytics in laboratory diagnostics of the last years, the number of fully mechanized systems increased noticeably and their analytical quality as well as clinical validity has been proven in recent papers (Biegelmayer et al. 2007; Kratzsch et al. 2011). Nevertheless, there are still some IRMAs on the market with a sensitivity and specificity that is approximately comparable to fully mechanized systems.

In the following important key points in assay, methodologies for CTN measurement are discussed:

Calibration: The unique calibrator 89/620 presented by the World Health Organization (WHO) is available as the ultimate basis preparation. Most of the manufacturers but also some in-house assays used calibrators that have been adjusted on this tool as a first precondition for the comparability of results from CTN assays (Camacho et al. 2014; Kratzsch et al. 2011).

Analytical sensitivity is a further important point of interest, especially for the detection of remaining tissue in the follow-up after thyroidectomy from MTC patients: The value of sensitivity was reported for most of the recently available assay methods in the range between 0.5 and 2.5 pg/mL (Camacho et al. 2014; d'Herbomez et al. 2001; Kratzsch et al. 2011; Roche cobas 2015).

Precision: Coefficients of variation (CV%) were described below 5.9 % for CTN concentrations of fully mechanized systems in the normal or minimal increased range of concentrations (Bieglmayer et al. 2007; Kratzsch et al. 2011). The respective data of manually handled assays were <12.9 % and thus in general, slightly higher (Bieglmayer et al. 2002; Camacho et al. 2014; Saller et al. 2002).

Cutoff and reference range: In the past, there was a consensus that patients with a calcitonin concentration higher than 10 pg/mL should undergo a PG test to exclude MTC (Motté et al. 1988). Recently, manufacturers of commercially available CTN assays revised this cutoff or decision point for the diagnosis of hypercalcitoninemia to values below 21 pg/mL, what is identical with the 95th or 97.5th percentile of reference or normal subjects. These data were gender-dependent with distinctly elevated values in male volunteers due to their higher thyroid volume. For males, commercially available CTN methods presented a cutoff in a range between 21 pg/mL (IRMA, Scantibody Laboratories) and 8.4 pg/mL CTN (IL2000, Siemens). In contrast, respective data for females were between 10 pg/mL (IRMA, Medipan) and 5.5 pg/mL (Liaison, Diasorin). In the literature, even higher cutoffs of up to 32.8 pg/mL for males and 14.6 pg/mL for females have been described (Rink et al. 2009).

Comparability of CTN concentrations from different assay methods: The above-mentioned differences in cutoff and reference range can be attributed to differences in the functionality of the assay constituents. Despite the widely use of the calibrators adjusted on the unique CTN WHO reference preparation 89/620, antibodies of commercially assays appear to present their interaction with a series of epitopes from different regions of the CTN molecule. Moreover, various labels for the signaling antibody may partially hamper antigen–antibody interactions. Nevertheless, numbers of preanalytical, analytical, and postanalytical influencing and interfering

factors may systematically modify assay results (see next chapter). The differences between samples measured by different assay methods can be estimated in external quality control (QC) surveys. The last German survey [Reference Institute for Bioanalytics (RfB), 4/2014] revealed CTN concentrations (mean ± standard deviation (SD)) for measurements of all participants (n = 170; 13 different methods) of 23.7 ± 3.38 pg/mL with a total CV of 14.3 % for sample A and of 35.3 ± 6.87 pg/mL with a total CV of 19.4 % for sample B. This data pretend an acceptable overall comparability of CTN data; however, a high intermethod variability between specific methods cannot be excluded. Thus, the mean of all measurements of the assay from the manufacturer with the highest CTN results of sample B was 42.5 pg/mL, whereas the assay with the lowest measuring values delivered a mean of only 24.3 pg/mL. A systematic bias was also shown for the comparison between assays with monoclonal and polyclonal antibodies (Tommasi and Raspandi 2007a, b). Moreover, results of CTN from different assay methods depend especially on the considered hormone concentration and on the source of sample used (Kratzsch et al. 2011).

1.3 Influencing Factors on CTN Measurement

The serum calcitonin concentration range of subjects without thyroid diseases is usually considered to be below 10 pg/mL (Costante et al. 2009). Calcitonin levels higher than cutoff may indicate preoperative MTC, postoperative recurrence of MTC, or untreated metastases (Hahm et al. 2001; Pacini et al. 1994). For some patients who have normal or slightly elevated basal calcitonin findings and clinically suspected MTC, calcitonin stimulation tests are performed, usually with PG or calcium (see Chaps. 2 and 3). Stimulated calcitonin values at PG test higher than 500 pg/mL are a clear indicator of MTC. However, intermediate calcitonin levels between 10 and 500 pg/mL cannot guarantee the presence of MTC due to various influencing factors.

Influencing factors on the determination of calcitonin concentration can result in false-positive diagnosis which might lead to unnecessary thyroidectomy or in false-negative diagnosis which can cause poor prognosis of MTC. Therefore, it is of significance to consider possible influencing factors in interpreting calcitonin results. In this chapter, the influencing factors are largely categorized into four sections: (1) analytical factors, (2) physiological factors, (3) pharmacological factors, and (4) pathological factors.

1.3.1 Analytical Factors

Sandwich immunoassays are applied when measuring calcitonin in the clinical laboratory. In immunoassays, falsely low results can be obtained due to unsuited storage conditions for the serum sample and the hook effect. Falsely elevated results can be obtained due to cross-reactivity with procalcitonin or interference by heterophilic antibodies.

CTN is one of the most susceptible biomarkers in laboratory routine analysis. At a room temperature of 20–25 °C, the CTN concentration remained constant only for a couple of hours. Accordingly, it is highly suggested to store any blood sample

containing the CTN molecule at a temperature between 4 and 8 °C for up to 6 h or in the frozen state for long-term storage (Kratzsch et al. 2011). Interestingly, 4 freeze/thaw cycles did not influence the CTN result.

Hook effect can be detected if the CTN result of a diluted sample is higher than that of the undiluted sample. The reason for such a particular effect is an overload in the binding capacity of the solid-phase adsorbed antibody due to an extraordinary high concentration of the analyte that inhibits binding activity of the labeled second antibody. Leboeuf et al. (2006) reported that a calcitonin IRMA exhibited such a hook effect and added that a falsely too low calcitonin result could dramatically change the prognosis of the patient. Tommasi and Raspanti (2007a, b) evaluated 3874 CTN measurements using a simultaneous polyclonal CTN IRMA that delivered 131 clinical serum samples above cutoff and, therefrom, 22 samples with a nonlinear relationship at several dilution steps. A nonlinear dilution can be indicative for a hook effect or for a dissociation of CTN aggregates. Although such effects occur infrequently in sandwich immunoassays, in suspicion of falsely decreased results, serial dilution of samples with increased CTN values should be performed until a stable quantitative response is achieved.

Procalcitonin is a peptide precursor of calcitonin molecule. One of the limitations in immunoassays is the cross-reactivity with the compounds which have structural similarity to the target molecule. Kratzsch et al. (2011) reported that the frequency of increased calcitonin concentrations was related to the degree of cross-reactivity exhibited by an specific CTN IRMA with PCTN. PCTN increased in patients with general infections but may also be elevated in states with non-infectious inflammation. Uhrova et al. (2011) demonstrated that there was visible interference of PCTN in the CTN determination in septic patients (PCTN > 0.5 ng/mL) with the IRMA method.

Interference by heterophilic antibodies in immunoassays is also a source of potential errors in calcitonin immunoassays. Heterophilic antibodies can link detection and signal antibody in sandwich immunoassays leading to enhanced immunoreactivity and consequently to increased measuring signals and analyte concentrations (Tate and Ward 2004). Papapetrou et al. (2006) suggested that the interference by heterophilic antibodies should be always considered when an unexpected increase in serum CTN concentrations is detected or when a patient has a high basal CTN and minimal CTN response to PG- or calcium stimulation tests. Interference by heterophilic antibodies can be also identified by nonlinearity in serial serum dilution results. In order to eliminate the interference by heterophilic antibodies, normal mouse gamma globulins or heterophilic blocking agents can be added.

1.3.2 Physiological Factors

Physiological factors which can influence the CTN concentrations can be viewed in light of age, sex, food intake, and lifestyle habit such as cigarette smoking or alcohol consumption.

CTN has been shown a progressive decrease with age: Concentrations were relatively high in neonates, declined from 6 months of age, and reached the adult

levels almost at the age of 3 (Basuyau et al. 2004). Reference intervals for children were rarely published for currently available assay methods: Only one paper suggested <40 pg/mL for children under 6 months of age, <15 pg/mL in children between 6 months and 3 years of age, and over 3 years of age the values should be indistinguishable from those observed in adults (Basuyau et al. 2004).

In adults, CTN was found generally higher in men than in women. Reference intervals are dependent on the used assay method and were suggested to be gender-dependent (see Sect. 1.2). A postmortem study has shown that physiologically men have twice as many C-cells as women (Engelbach et al. 2000). Sex difference in calcitonin is more dramatic during PG stimulation test (see Chap. 3). Heath and Sizemore (1977) showed that women had significantly lower CTN levels and a lesser response to both calcium- and PG stimulation tests than men with a statistical significance.

Zayed et al. (2013) reported that serum CTN concentration was significantly increased by food intake in healthy young subjects and revealed a circadian rhythm with increased values during the afternoon. This study suggests that timing of blood sampling relative to meals should be considered in CTN measurements. Polymeris et al. (2011) have shown that hyperinsulinemia during oral glucose tolerance test and high normal serum cortisol were also associated with increased secretion of CTN in normal subjects within the physiological range. Therefore, physicians should be aware that in states of acute hyperinsulinemia or high but even still within normal range measured serum cortisol, CTN results may be higher than expected without the presence of MTC or CCH.

d'Herbomez et al. (2007) reported that CTN levels were higher in smokers than in non-smokers. C-cells are distributed not only in the thyroid and the thymus but also in the liver, lungs, duodenum, and jejunum. It has been demonstrated that tobacco increased the number of neuroendocrine cells and the secretion of peptides such as CTN (Kapoor and Jones 2005). Alcohol was also shown to increase CTN (Kanis et al. 1979). Vantyghem et al. (2007) observed an increase in CTN concentrations in patients with chronic alcohol and smoking. Interestingly, the hormone concentration did not normalize after the withdrawal of alcohol. From this finding, it was suggested that smoking may play more predominant role than alcohol consumption in CTN elevation (Vantyghem et al. 2007).

1.3.3 Pharmacological Factors

Prolonged treatment with histamine-2 receptor blockers (H2RB) and/or proton-pump inhibitors (PPI), glucocorticoids, and several drugs have been associated with hypercalcitoninemia. CTN medication used for a therapeutic purpose should also be checked for the interpretation of CTN serum concentration.

H2RB/PPI drugs decrease gastric acid secretion in patients with hypergastrinemia, pernicious anemia, selective gastric vagotomies, and gastric operations. Inhibition of gastric acid elevates endogenous gastrin levels. Gastrin is secreted from G cells and known to stimulate CTN levels. PG used in the CTN stimulation test is a synthetic gastrin analog. Erdogan et al. (2006) found significantly higher

stimulated CTN levels in patients with H2RB and PPI therapy than controls. Chronic use of omeprazole has been reported to raise CTN levels after 2–4 months of therapy (Freston 1994; Klinkenberg-Knol et al. 1994).

Acute use of glucocorticoids has been shown to increase basal and calcium-stimulated CTN in patients with arteritis after 1 week of treatment with prednisolone (Mulder et al. 1990). However, CTN secretion capacity significantly decreased after 6 weeks of prednisolone therapy compared to the initial level. Exhaustion of CTN response system during the chronic use of glucocorticoids later contributes to the glucocorticoid-induced osteoporosis. For the treatment of glucocorticoid-induced osteoporosis, CTN can be used for a therapeutic purpose.

Other than these, ß-blocker, glucagon, enteroglucagon, and pancreozimine were reported to raise serum CTN levels (Toledo et al. 2009).

1.3.4 Pathological Factors

There are several physiological and pathological conditions in which CTN concentrations increase without the presence of MTC or CCH. Hypercalcitoninemia has been frequently reported in chronic kidney disease (CKD), neuroendocrine tumors, hypergastrinemia, hypercalcemia, and chronic autoimmune thyroiditis.

Among patients with CKD, CTN concentration was elevated in about 30 % of patients (Escalada et al. 1993; Garancini et al. 1983; Kotzmann et al. 1999; Kratzsch et al. 2011; Mulder et al. 1982; Niccoli et al. 1995). The amount of CTN in the circulation may increase due to the lowered clearance rate of CTN (Niccoli et al. 1995). Borchhardt et al. (2005) demonstrated that PG-stimulated CTN of around 100 pg/mL normalized after successful kidney transplantation. Martínez et al. (1983) reported that patients with CKD showed significantly lower calcium and CTN concentrations after receiving continuous ambulatory peritoneal dialysis.

Since C-cells are distributed throughout the neuroendocrine tissues, several types of neuroendocrine tumors of the lung or gastrointestinal tract have shown an elevated concentration of CTN (Becker et al. 2004). However, chronic hypercalcemic state can exhaust CTN reserves and diminish the CTN response to calcium stimulation test. Kim et al. (2014) reported such a case of prostatic and multiple bone metastases from medullary thyroid cancer, in which serum CTN levels were not significantly increased in response to an acute intravenous calcium injection.

Since gastrin is a strong stimulant of synthesis and stimulation of CTN, patients with hypergastrinemia may exhibit high concentrations of serum CTN (Toledo et al. 2009). In the same way, hypercalcemia caused by primary hyperparathyroidism, malignancy, vitamin-D metabolic disorders, or renal failure has been associated with hypercalcitoninemia (Toledo et al. 2009).

Chronic autoimmune thyroiditis has been reported leading to increased CTN. However, the findings were in part inconsistent. Uwaifo et al. (2001) reported that a patient with Hashimoto's thyroiditis showed an elevated calcitonin level with no evidence of MTC. Barbot et al. (1991) reported that 3 out of 24 patients with Hashimoto's thyroiditis showed high calcitonin levels. From pathological examination after thyroidectomy, high calcitonin levels were found to be a consequence of

extensive C-cell hyperplasia in Hashimoto's thyroiditis. Guesgen et al. (2013) reported there is no correlation between Hashimoto's thyroiditis and high calcitonin levels. Elevation of CTN in 1–3 % of patients with the same diagnosis has been observed by Kratzsch et al. (2011).

1.4 PCTN Assays

There is almost only one PCTN measurement principle commercially available that had been developed by the manufacturer "Brahms" in the year 2000 and which is sold by "Thermo Scientific" recently. Moreover, the two independent manufacturers, "USDN" and "Biomerioux", can deliver manually performed kits of this parameter (Chan and Gu 2011). An analytical sensitivity of 60 pg/mL as presented by the fully mechanized system, Kryptor (Thermo Scientific), is highly sufficient for the use of PCTN as biomarker for MTC diagnosis. To avoid purchasing a complete clinical analyzer only for PCTN measurement, the manually performed assays can be used alternatively with somewhat inferior quality control data (Steinbach et al. 2004). Reference cutoffs were set at 250 pg/mL or 500 pg/mL to indicate a potential bacterial infection or general inflammation, and levels higher than 500 pg/mL point a high probability of a bacterial infection. PCTN concentrations of non-MTC and of MTC patients correlated with CTN as calculated by "*r*" values between 0.36 and 1.0 in dependence on the concentration range of CTN values that were included into the calculation. The whole spectrum of data points delivered excellent correlation data (Kratzsch et al. 2011). Intra- and Interassay precision was found to be lower than 5 and 10 % for the mechanized as well as 7 and 17 % for the manually performed methods (Steinbach et al. 2004). The main advantage of using PCTN compared to CTN is the distinctly higher stability of the precursor molecule at room temperature and at 4 °C (Kratzsch et al. 2011; Steinbach et al. 2004). Thus, PCTN and CTN appear to have a comparable diagnostic capability to detect MTCs. However, "positive" PCTN values of more than 50 pg/mL may also be reached in subclinical infections and will lead, therefore, to an overdiagnosis of the tumor.

2 Screening for Medullary Thyroid Carcinoma in Patients with Struma Nodosa

2.1 CTN Screening

There is a lot of evidence that the measurement of serum CTN concentrations in patients with thyroid nodules can lead to an earlier diagnosis of MTC or CCH compared to the exclusive use of imaging procedures and fine-needle aspiration cytology (FNAC). In the following, some selected studies will be cited and issues about the diagnostic use of CTN as a screening tool will be discussed.

In one of the biggest studies, 10,864 patients with nodular disease were included during the years 1991–1998 in Italia (Elisei et al. 2004). Using CTN screening, the

prevalence of MTC was determined with 0.4 %. The observed positive CTN concentrations had higher diagnostic sensitivity than FNAC and allowed the diagnosis of MTC at an earlier stage compared to a group of MTC patients diagnosed only with FNAC and by postsurgical histological examination. Biochemical cure and tumor remission in the follow-up were clearly better in the group with CTN screening.

These data were supported by another large study with a cohort of 5817 consecutive patients demonstrating thyroid nodules (Costante et al. 2007). The authors estimated the positive predictive value (PPV) of CTN values in the preoperative diagnosis of MTC via gradual limits for this parameter: 100 % for CTN >100 pg/mL; 25 % for CTN between 50 and 100 pg/mL; 8.3 % for CTN between 20 and 50 pg/mL; and 23.1 % for CTN >20 pg/mL. The relatively low PPV values in the range between 20 and 100 pg/mL, due to the low incidence of MTC, caught up a confirmatory test like PG stimulation (PPV 40 % for CTN >100 pg/mL). The importance of this suggestion was supported by data of Hahm et al. (2001). From 1448 patients with nodular goiter screened for CTN, 56 revealed to have a basal elevation, but 46 had no evidence for a MTC. This deficit in the diagnostic value of CTN screening could be overcome by the confirmation test. The PG test, done in this study, demonstrated all of the 10 MTC patients with a CTN concentration higher than 100 pg/mL, whereas FNAC suggested MTC in only 2/9 patients. Contrasting results were delivered in a recent study that included 2773 patients before surgical treatment of nodular thyroid disorders and suspected MTC (Chambon et al. 2011). CTN screening was performed and PG stimulation was carried out if CTN was >10 pg/mL. Interestingly, the latter test delivered no further diagnostic information additional to the CTN screening value. MTC was observed in 12 patients, always present when basal CTN was >60 pg/mL ($n = 8$). In the range between 10 and 60 pg/mL, 4 patients had the diagnosis MTC. Two additional cases with micro-MTC were detected by histopathology only, and CTN was below 10 pg/mL in both cases. Comparable results were described for the investigation of 5920 thyroidectomies: A group of 14 patients with nodular thyroid disease and CTN concentrations between 20 and 100 pg/mL was identified (Boschin et al. 2014). MTC ($n = 9$) was diagnosed by FNAC and histopathology, whereas CCH revealed to be the finding in the remaining five. A PG test with CTN >100 pg/mL confirmed MTC in 5/9 cases but pointed to a questionable diagnosis MTC in 2/4 cases with HCC. Accordingly, a clear separation of these two diagnoses appeared to be impossible by PG test. Taken together all these and some other data [see reviews from Daniels (2011) and from Ahmed and Ball (2011)]: CTN screening in patients with nodular goiter delivers increased values for both MTC and HCC. Basal CTN concentrations higher than 60–100 pg/mL are highly indicative for the diagnosis MTC. In the range between cutoff and 60 pg/mL CTN, both MTC and HCC may be a relevant diagnosis. FNAC and stimulation test may but not must be preoperatively helpful tool for the further characterization of patient's disease. Accordingly, analysis of CTN is advisable in all patients with documented thyroid nodules according to the German evidence-based consensus recommendation (Karges et al. 2004) and the European Thyroid Cancer task force (Pacini et al. 2006), whereas the

European Thyroid Association weakened this recommendation to "nonstimulated CTN level may be considered before thyroid surgery for nodular goiter" (Gharib et al. 2010). As the low PPV of slightly elevated CTN values is still under debate, the American Thyroid Association Guidelines does neither recommend nor dis-advise this procedure (Cooper et al. 2009).

2.2 PCTN Screening

Procalcitonin was firstly described to be increased in 21 patients with MTC in 2003 (Bihan et al. 2003). In a larger study, PCTN concentrations were above a cutoff of 150 pg/mL in 83 out of 91 MTC patients with active disease (sensitivity 91 %), whereas only one patients with cured MTC (n = 42) revealed an increased PCTN concentration (Algeciras-Schimnich et al. 2009). Considering in parallel measured CTN values, 90 of 91 patients (sensitivity 99 %) had values higher than the gender-specific cutoff. Specificity was 96 % for PCTN and 99 % for CTN. In another study, patients with MTC (n = 60) were compared with controls for recurrence over 1, 5, 10, and 20 years (Walter et al. 2010). Thereby, PCTN (ROC analysis AUC = 0.89) demonstrated a slightly lower diagnostic accuracy for detecting MTC than CTN (ROC analysis AUC = 0.94). The optimal cutoff of 50 pg/mL PCTN delivered a diagnostic sensitivity and specificity of approximately 84 %. A recent study of Machens et al. (2014) included a distinctly higher number of untreated MTC patients (n = 112). Their ROC curves reflected a similar diagnostic accuracy of PCTN versus CTN for primary tumors at thresholds of 10 and 40 mm as well as at extrathyroidal extension, lymph node metastasis, and distant metastasis (AUC >0.84). Taken together, PCTN and CTN appear to have a similar diagnostic capability to detect MTCs. However, "positive" PCTN values of more than 50 pg/mL may be reached also in subclinical general infections and will lead to an overdiagnostic of the tumor (Kratzsch et al. 2011). Classical examples for this limitation are patients with CKD or hemodialysis. To further elucidate the role of PCTN as a biomarker for MTC diagnostics, larger studies with paralleled measurements of both parameters are necessary. Otherwise, as mentioned in Chap. 3, PCTN is by all means a useful alternative biomarker if CTN concentrations are increased, but patients have with questionable clinical circumstances (Kaczka et al. 2012; Kratzsch et al. 2011).

3 Stimulation Tests

3.1 Pentagastrin Stimulation Test

As mentioned in the chapters above, there is a consensus that CTN stimulation tests appear to be mandatory for the confirmation of the diagnosis MTC after screening for increased basal CTN concentrations. In the past intravenous PG stimulation was applied to increase a low PPV of below 10 % for basal CTN concentrations

between 20 and 50 pg/mL to diagnose MTC in the preoperative state (Costante et al. 2009). As any cutoff for the diagnostic decision between MTC and CCH or other control subjects depends on the used CTN assay method and the characteristics of investigated subjects, the suggested value of 100 pg/mL for CTN (Karges et al. 2004; Pacini et al. 2006) was a compromise from former studies to avoid permanently extensive assay and patient-specific evaluation procedures. Accordingly, there is a high variability in cutoffs used to discriminate between MTC and CCH mainly depending on the CTN secretion capacity of the tumor. Values of 205, 275, 560, and 1000 pg/mL were indicative for a PPV of around 100 %; whereas an overlapping range with CCH was found for 10, 175, 129, and 100 pg/mL as described in the same order by Gibelin et al. (2005), Milone et al. (2010), Scheuba et al. (1999), Constante et al. (2007). Alternatively, a twofold relative increase of CTN after PG was proposed as a criterion to distinguish between MTC and non-MTC in patients with increased basal CTN, as retrospectively investigated by histopathological examination after thyroidectomy. By this procedure, 81 out of 89 Patients with MTC were classified correctly, and 8 patients were misclassified (Machens et al. 2008).

Recently, the first ultrasensitive assays for CTN have been established demonstrating an analytical sensitivity of <1.5 pg/mL and a functional sensitivity <5 pg/mL. Such assays could be able to replace PG stimulation tests in the follow-up of patients with MTC after thyroidectomy. However, first data could not support this hypothesis, the sensitivity to detect C-cell disease remained lower than that of PG stimulation test (Pina et al. 2013).

3.2 Calcium Stimulation Tests

In addition to the US where PG for stimulation tests is generally unavailable, this stimulus became also inaccessible in Europe recently. Therefore, efforts for the reintroduction of calcium (Ca)-stimulated CTN measurement were undertaken in the last years. The first short-term Ca infusion tests were introduced in the seventies of the last century (Parthemore et al. 1974). Elemental Ca at a dose of 4.5 mg/kg was found to stimulate CTN in patients with MTC but not in normal subjects. Later, the dose was reduced to 2.3–2.5 mg/kg (Fugazzola 2013). The test was seldom used over the last 30 years, and no validated cutoff limits to distinguish preoperatively MTC from CCH or thyroid healthy subjects were established. In a recent most comprehensive cutoff study of Mian et al. (2014), thresholds for MTC of >79 pg/mL in women and >544 pg/mL in men were identified by ROC analysis in comparison to patients with immunohistochemical diagnosed CCH. Respective lower cutoffs (78.5 pg/mL for females and 102 pg/mL for males) and tremendously higher values (>184 pg/mL for females and >1620 pg/mL for males) were described in the literature, additionally (Colombo et al. 2012; Giovanella 2012). Such a high variation complicates the search for a consensus cutoff in analogy to the former 100 pg/mL CTN during PG stimulation. Interestingly, Mian et al. (2014) reported a PPV of basal CTN (>26 pg/mL for females and >68 pg/mL for males) that was at

least as good predictor of MTC as Ca stimulation. This finding suggests that basal serum CTN may replace CTN stimulation in defined conditions. Precondition therefore is a high functional sensitivity of the test method. Taken together these data, there is no clear alternative procedure for the PG stimulation available so far.

However, more recently, Thiem et al. (2014) reduced the dose for stimulation to 1 mg/kg Ca and received a comparable response of Ca and PG by this procedure for the first time. This low dose of Ca caused even less severe side effects than the standard dose of 0.5 μg/kg PG. As these findings were only the result of a pilot study in patients with chronic hemodialysis, large clinical studies with MTC and CCH patients have to confirm the promising approach.

3.3 Others

Besides PG and Ca stimulation tests, omeprazole tests were evaluated for their suitability in the diagnosis of MTC. However, omeprazole appears to be a less potent and sensitive secretagogue of CTN than PG (Vitale et al. 2002). Nevertheless, it may be necessary if PG or Ca tests are contraindicated or were refused by the patient. So far, clinical experience in test handling and interpretation of test results is insufficient. Only a couple of observations have been published until now (Erdogan et al. 1997; Vieira et al.2002).

4 Conclusions

- The heterogenic patchwork of CTN immunoreactivity consisting of monomers, dimers, and aggregates should be clearly unraveled by the used immunoassay system to obtain reliable and comparable values for diagnostic purposes;
- The effect of several influencing factors on the determination of calcitonin can result in false-positive diagnosis of MTC, which might lead to unnecessary thyroidectomy, or in false-negative diagnosis of MTC, which lead to a poor prognosis of this disease;
- Increased CTN concentrations in patients with suspected MTC had a higher diagnostic sensitivity than FNAC and allowed the diagnosis of MTC at an earlier stage;
- If PCTN will be used as an additional biomarker for the diagnostics of MTC, frequently increased values in subclinical general infections have to be taken into account; otherwise, MTC will be overdiagnosed
- At present, there is no acceptable alternative diagnostic procedure for PG stimulation of CTN available; accordingly, basal CTN serum values may replace the stimulation step if the assay method has a sufficiently high functional sensitivity.

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Hereditary Medullary Thyroid Cancer Genotype–Phenotype Correlation

Karin Frank-Raue and Friedhelm Raue

Abstract

During the last two decades, there has been a marked expansion of our knowledge of both the basic and clinical aspects of multiple endocrine neoplasia type 2 (MEN2). There are two clinically distinct types of MEN2 syndrome, termed MEN2A and MEN2B. Within MEN2A, there are four variants: (i) classical MEN2A, represented by the uniform presence of MTC and the less frequent occurrence of pheochromocytoma, or primary hyperparathyroidism, or both; (ii) MEN2A with cutaneous lichen amyloidosis; (iii) MEN2A with Hirschsprung's disease; and (iv) familial medullary thyroid carcinoma (FMTC), i.e., families or individuals with only MTC. MEN2B is associated with MTC, pheochromocytoma, and mucosal neuromas. Hereditary MTC is caused by autosomal dominant gain of function mutations in the *RET* proto-oncogene. Specific *RET* mutations may suggest a predilection toward a particular phenotype and clinical course with a strong genotype–phenotype correlation. Based upon these genotype–phenotype correlations, *RET* mutations are now stratified into three risk levels, i.e., highest, high, and moderate risk, based on the penetrance and aggressiveness of the MTC. Children in the highest risk category should undergo thyroidectomy in their first year of life, and perhaps even in their first months of life. Children in the high-risk category should have ultrasound of the neck and calcitonin (CTN) measurement performed prior to thyroidectomy. Thyroidectomy should typically be performed at the age of 5 or earlier, depending on the presence of elevated serum CTN levels. However, heterogeneity in disease expression and progression within these groups varies considerably. To personalize disease management, the decision regarding the

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age of prophylactic thyroidectomy is no longer based upon genotype alone but is currently driven by additional clinical data, the most important being serum CTN levels; specifically, the decision to perform thyroidectomy should err on the safe side if the CTN level is elevated but below 30 pg/ml, especially in the moderate risk group. Personalized management also includes decisions about the best age to begin biochemical screening for pheochromocytoma and primary hyperparathyroidism.

Keywords

Hereditary medullary thyroid carcinoma · Multiple endocrine neoplasia · Genotype–phenotype correlation · Calcitonin

List of Abbreviations

MTC	Medullary thyroid carcinoma
MEN	Multiple endocrine neoplasia
FMTC	Familial medullary thyroid carcinoma
CTN	Calcitonin
PHPT	Primary hyperparathyroidism
CCH	C-cell hyperplasia

Contents

1 Introduction

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant hereditary cancer syndrome. Accordingly, the offspring of a carrier have a 50 % risk of inheriting the disease. More than 20 years ago, the discovery that activating mutations in the RET gene cause MEN2 was one of the crucial moments in the cancer genetics (Mulligan et al. 1993; Donis-Keller et al. 1993) because it fundamentally changed our treatment of hereditary medullary thyroid carcinoma (MTC). Specifically, MEN2 is caused by missense mutations in the *RET* proto-oncogene that result in gain of function. Early prophylactic thyroidectomy based on genetic mutational analysis has significantly improved the overall survival. During the last two decades, there has been a marked expansion of our knowledge of the basic and clinical aspects of MEN2. Extensive studies of large families, often performed by national or international consortia, have led to the identification of new germline mutations, to changes in the spectrum of identified mutations, and to refinement of our knowledge about genotype–phenotype correlations.

There are two clinically distinct types of MEN2 syndrome, termed MEN2A and MEN2B. Within MEN2A, there are four variants: (i) classical MEN2A, represented by the uniform presence of MTC and the less frequent occurrence of pheochromocytoma, or primary hyperparathyroidism, or both; (ii) MEN2A with cutaneous lichen amyloidosis; (iii) MEN2A with Hirschsprung's disease; and (iv) familial medullary thyroid carcinoma (FMTC), i.e., families or individuals with only MTC. MEN2B is associated with MTC, pheochromocytoma, and mucosal neuromas (Wells et al. 2015). Specific *RET* mutations may suggest a predilection toward a particular phenotype and clinical course with a strong genotype–phenotype correlation. Based on a model that utilizes these genotype–phenotype correlations, RET mutations have been stratified into risk levels (Brandi et al. 2001; Kloos et al 2009). Recommendations regarding the timing of prophylactic thyroidectomy and the extent of surgery are based on classification of the RET mutations into these risk levels according to genotype–phenotype correlations. Genetic testing detects nearly 100 % of mutation carriers and is considered the standard of care for all patients with newly diagnosed MTC.

2 Genotype. The *RET* Proto-Oncogene: Structure, Function, and Genetic Abnormalities

The *RET* gene has 21 exons and encodes a receptor tyrosine kinase that appears to transduce growth and differentiation signals in several developing tissues, including those derived from the neural crest. The RET protein consists of an extracellular segment with a ligand-binding domain, a cadherin (Ca^{2+}-dependent cell adhesion)-like domain, and a cysteine-rich domain, which is close to the cell membrane. It has a single transmembrane domain and an intracellular segment with two tyrosine kinase subdomains, TK1 and TK2. The RET protein is activated upon

ligand-induced dimerization (Santoro et al. 1995) and binds ligands of the glial-derived neurotrophic factor (GDNF) family in conjunction with a co-receptor designated the GDNF-family receptor α (GFRα). RET is expressed in neuroendocrine cells, including the C-cells of the thyroid, which are the precursors of MTC, as well as in pheochromocytomas.

Hereditary MTC is caused by autosomal dominant gain of function mutations in the *RET* proto-oncogene. Mutations in the extracellular cysteine-rich domain in codons 609, 611, 618, 620, and 634 cause ligand-independent dimerization of the receptor molecules, enhanced phosphorylation of intracellular substrates, and cell transformation (Takahashi et al. 1998). A mutation in the intracellular tyrosine kinase subdomain (codon 918) has no effect on receptor dimerization but causes constitutive activation of intracellular signaling pathways and results in cellular transformation (Takahashi et al. 1998). There is a significant age-related progression from C-cell hyperplasia (CCH) to MTC that correlates with the transforming capacity of the respective *RET* mutations (Machens et al. 2003). MTC is generally the first neoplastic manifestation in patients with MEN2A because of its earlier age of onset and higher rate of penetrance compared with pheochromocytoma or parathyroid hyperplasia. This indicates that C-cells are more susceptible to oncogenic *RET* activation than adrenal medullary or parathyroid cells.

In 1987, genetic linkage analysis localized the MEN2 gene to centromeric chromosome 10 (10q11.2) (Mathew et al. 1987). Subsequently, point mutations in the *RET* proto-oncogene were identified in MEN2A, MEN2B, and FMTC in 8 exons located near this region (exons 5, 8, 10, 11, and 13–16) (Mulligan et al. 1993; Donis-Keller et al. 1993). Analysis of *RET* in families with MEN2A and FMTC revealed that nearly all of these families have germline mutations and that only those family members with the germline missense mutations have the disease. Somatic *RET* mutations, especially M918T, are present in 40–50 % of sporadic MTC tumor tissue. Tumors carrying a somatic codon 918 mutation were found to be more aggressive (Zedenius et al. 1994; Romei et al. 1996; Schilling et al. 2001) and more prevalent in larger tumors (Romei et al. 2012). This discovery prompted major advances in our understanding of the molecular genetic basis of MTC and significantly changed the clinical management of families with hereditary tumors. At present, mutation analysis has identified over 100 different missense mutations, duplications, insertions, and deletions involving *RET* in patients with hereditary MTC (Wells et al. 2015; Margraf et al. 2009).

2.1 New Mutations, Rare Mutations, and Polymorphisms

A sequence change in the RET gene is considered to be a causative MEN2 mutation if it segregates with the clinical expression of disease within a family, including at least two affected individuals with the MEN2A or MEN2B phenotype. In contrast, benign germline *RET* sequence changes that do not cause MEN2, such as p.G691S, p.L769L, p.S836S, p.S904S, and intron 14 c.2608-24G > A, are considered as

polymorphisms. If insufficient clinical information is available for a newly detected *RET* mutation (e.g., only one gene carrier has the disease or the mutation is detected incidentally as a *RET* sequence change without disease manifestation), a preliminary classification of "variant of unknown significance (VUS)" is suggested (Wells et al. 2015; Margraf et al. 2009). Prediction of disease association for novel mutations and uncertain gene variants may be performed using in silico and in vitro analysis. There is a positive correlation between the in silico risk score and in vitro focus formation units (Cosci et al. 2011). To improve the in silico algorithms, which use the physicochemical properties of selected amino acids, these algorithms were specifically trained using curated RET disease outcome databases (Crockett et al. 2011). The best way to confirm that a RET sequence change is causative for the disease is to demonstrate co-segregation with the disease in a large family.

3 Phenotype: Clinical Syndromes of MEN2

MEN2 (OMIM 171400) is an autosomal dominant tumor syndrome with an estimated prevalence of 1 per 30,000 in the general population. As noted above, MEN2 syndrome occurs in two clinically distinct varieties, MEN2A and MEN2B, with MTC as a common manifestation. The two subtypes of MEN2 differ with respect to incidence, genetics, age of onset, association with other diseases, aggressiveness of MTC, and prognosis (Wells et al. 2015; Brandi et al. 2001; Kloos et al 2009). Within MEN2A, there are four variants: classical MEN2A, MEN2A with Lichen amyloidosis, MEN2A with Hirschsprung's disease, and FMTC.

3.1 Classical MEN2A Syndrome

Classical MEN2A is the most common of the four MEN2A variants and is characterized by MTC in combination with pheochromocytoma and/or multiple tumors of the parathyroid glands in a single patient, or the presence of two or more tumor types in multiple members of a single family. In classic MEN2A, the frequency of MTC is nearly 100 %, while the frequency of pheochromocytoma and multiple parathyroid gland hyperplasia are 40–50 % and 10–20 %, respectively (Eng et al. 1996; Frank-Raue et al. 1996; Milos et al. 2008). However, the penetrance and aggressiveness of MTC and the frequency of pheochromocytoma and hyperparathyroidism vary according to the specific underlying mutation. In classic MEN2A, 95 % of the patients have RET germline mutations in codon 634 of exon 11 and in codons 609, 611, 618, or 620 of exon 10 (Raue and Frank-Raue 2012).

MTC is usually the first manifestation of MEN2A, and it develops between the ages of 5 and 25 (Machens et al. 2003). Affected individuals initially develop primary CCH, which progresses to invasive MTC. Familial primary CCH is a preneoplastic lesion, while the secondary CCH has no malignant potential. Secondary CCH has been described in combination with chronic lymphocytic

thyroiditis, with hypergastrinemia, as being near other follicular-derived thyroid tumors, and even with aging. C-cells secrete the 32-amino-acid glycoprotein calcitonin (CTN), which serves as an excellent tumor marker for MTC.

Before genetic screening became available, CTN determination in combination with pentagastrin stimulation was used to detect early abnormalities of the thyroid gland in MEN2 families. After 1993, the focus has been on genetic findings. However, during recent years, CTN has once again become an important marker in the management of hereditary MTC. The new CTN assays are more robust and precise for defining the upper limit of the reference range. While some clinical investigators feel that the sensitivity of the immunochemiluminometric two-site assay (ICMA) is such that provocative testing using pentagastrin is no longer necessary, others consider it useful in some clinical situations. Accumulating evidence shows that CTN levels can safely be used in determining the optimal timing for thyroidectomy in RET carriers, at least in the lower risk RET mutation group (Elisei et al. 2012, 2013). In addition, CTN/CEA doubling times are prognostic parameters (Barbet et al. 2005). Current reference ranges for serum CTN vary according to sex, being higher in men than women. Using these systems, the 95th percentile for serum CTN levels are about 5–6.5 pg/ml in women and about 9–10 pg/ml in men. The reference values for children are higher, with values up to 44 pg/ml in male neonates (Basuyau et al. 2004; Verga et al. 2006).

Pheochromocytoma is a tumor of the adrenal medulla. In MEN2A, pheochromocytomas are almost always benign and located in the adrenal glands, and in >50 % of patients they are bilateral. Pheochromocytoma in MEN2 most often manifests after a diagnosis of MTC, and pheochromocytoma tumor development is preceded by bilateral diffuse and/or nodular hyperplasia. Clinically, an underlying pheochromocytoma should be suspected when a patient exhibits symptoms of excessive beta-adrenergic activity from overproduction of epinephrine, such as tachycardia, intermittent headaches, or palpitations. Hypertension is more common when larger tumors are present. Among hereditary pheochromocytomas, the pattern of catecholamine production can depend on the underlying mutation (Eisenhofer et al. 2005), with tumors from patients with MEN2A producing epinephrine or a mix of epinephrine and norepinephrine in paroxysmal bursts (Pacak et al. 2009). Pheochromocytomas may secrete catecholamines episodically, but they metabolize catecholamines to metanephrines continuously; accordingly, the biochemical measurement of blood or urine catecholamine levels is poorly correlated with tumor size, whereas blood or urine metanephrines show strong correlation with tumor size (Eisenhofer et al. 2005). In MEN2 pheochromocytomas, chromogranin A and B and neuropeptide Y levels are much higher than in von Hippel-Lindau tumors (Pacak et al. 2009). In patients with biochemically proven pheochromocytoma, computed tomography and/or magnetic resonance imaging is used to localize the tumor. Computerized tomography has a 93–100 % sensitivity for localizing intra-adrenal tumors >0.5 cm (Ilias and Pacak 2004). No functional imaging is recommended if the tumor is less than 5 cm (Taieb et al. 2014). A pheochromocytoma should be resected before an MTC if both are present. The standard of care is preoperative preparation with α-adrenergic and, if necessary, β-blockade.

Unilateral adrenalectomy is performed in patients with a single pheochromocytoma, and subtotal adrenalectomy is a feasible procedure to preserve adrenocortical function (Scholten et al. 2011). Pheochromocytoma penetrance varies by *RET* mutation: penetrance is 50 % in 634 codon mutation carriers; about 20 % in exon 10 mutation carriers (Quayle et al. 2007; Frank-Raue et al. 2011); and usually <5 % in exon 13–15 mutation carriers.

Primary hyperparathyroidism (PHPT) is reported in 10–25 % of patients with MEN2A, but it is rarely the first manifestation of the disease (Raue et al. 1995; Kraimps et al. 1996). The hypercalcemia is usually mild, and most patients are asymptomatic. The mean age at diagnosis of PHPT in MEN2A patients is 38 years. Histologic findings are classified as chief cell hyperplasia, but clinical studies have described single or multiple "adenomas" in more than 50 % of patients. Compared to MEN1, PHPT in MEN2 is associated with lower persistence (3 %) and recurrence (12 %) rates (Raue et al. 1995). The results of clinical and biochemical studies indicate that MEN2A-related PHPT is generally not an aggressive disease. PHPT is seen in 20–30 % of patients carrying RET 634 mutation (Schuffenecker et al. 1998) and in 2–5 % of patients carrying exon 10 mutations (Frank-Raue et al. 2011).

3.2 MEN2A with Cutaneous Lichen Amyloidosis

Cutaneous lichen amyloidosis (CLA) in MEN2A is characterized by dermatological lesions that are particularly evident in the scapular region of the back, and histology shows the presence of amyloid in the dermis (Gagel et al. 1989). The initial symptom of CLA is intense pruritus that improves with sun exposure and worsens during periods of stress. Hyperpigmented lesions develop later, apparently secondary to scratching. The CLA may be present at a young age, prior to the onset of clinically evident MTC, thus serving as a precursor for the syndrome. MEN2A with cutaneous lichen amyloidosis is usually associated with mutations in codon 634 (Verga et al. 2003; Ceccherini et al. 1994), but it has been reported in one patient with a codon 804 mutation (Rothberg et al. 2009).

3.3 MEN2A with Hirschsprung's Disease

Hirschsprung's disease (HSCR), also termed aganglionic colon, represents the main genetic cause of functional intestinal obstruction, with an incidence of 1 per 5000 live births. The *RET* proto-oncogene is the major gene involved in the pathogenesis of both MEN2 and HSCR, but whereas activating mutations cause MEN2, inactivating mutations are involved in HSCR. These two diseases are only co-expressed in a very small number of families. HSCR occurs in approximately 7 % of patients with MEN2A who carry *RET* mutations in exon 10 (Frank-Raue et al. 2011). One explanation for the paradoxical effect, i.e., that the same mutation can be associated with a gain of function effect such as CCH and MTC as well as with a loss of

function effect like aganglionosis in HSCR, is that constitutive activation of RET may be sufficient to trigger neoplastic transformation of the C-cells and adrenal chromaffin cells yet be insufficient to generate a trophic response in precursor neurons due to a lack of expression of the RET protein at the cell surface (Asai et al. 2006). *RET* mutations in exon 10 are associated with HSCR in 5, 0, 2, and 13 % in codons 609, 611, 618, and 620, respectively (Frank-Raue et al. 2011). *RET* 791 mutations in HSCR have been described in rare cases (Vaclavikova et al. 2009).

3.4 FMTC Syndrome

FMTC is the mildest variant of MEN2. It has been diagnosed more frequently in recent years and is reported to account for 35–40 % of all MEN2 cases (Berndt et al. 1998; Elisei et al. 2007; Frank-Raue et al. 2007). With FMTC, there is a strong predisposition to develop MTC with a very low incidence of the other clinical manifestations associated with MEN2A. Defining and separating FMTC from MEN2A has been challenging, and the main concern is to mask the eventual identification of a pheochromocytoma. Longer follow-up and studies of other kindreds carrying the same mutation have made it clear that many kindreds who were first diagnosed as having FMTC instead have MEN2A. This is illustrated clearly by emerging facts over the years concerning RET mutation G533C in exon 8. In 2003, a large Brazilian six-generation family that included 76 gene carriers (29 with MTC) were described and diagnosed with FMTC (Da Silva et al. 2003); in 2006, two Greek families (20 carriers, 6 patients with MTC) were added (Kaldrymides et al. 2006), but no family members with pheochromocytoma (PHEO) or primary hyperparathyroidism (PHPT) were detected. Then, first in 2007 and then again in 2008 and 2012, Greek patients with PHEO and *RET* mutation G533C were reported (Bethanis et al. 2007; Peppa et al. 2008; Sarika et al. 2012). Further, in 2011, a PHEO was detected in the large Brazilian family (Oliveira et al. 2011). Accordingly, the *RET* mutation G533C was reclassified as MEN2A.

The criteria defining FMTC are various (Brandi et al. 2001; Eng et al. 1996; Wells et al. 2013). The original strict criteria, which failed when the 533 *RET* mutation was discovered, included the following: (i) more than 10 carriers, multiple >50 years, plus adequate medical history in order to exclude pheochromocytoma and HPT (Brandi et al. 2001); and (ii) a kindred with a minimum of 4 family members with MTC and no objective evidence of pheochromocytoma or HPT (Eng et al. 1996). More recently, investigators have used the term FMTC for (iii) kindreds who carry low-risk non-cysteine mutations in exons 13–15 who mainly manifest MTC and who show either a low or very low frequency of other endocrinopathies. If these extended criteria are used, the incidence of FMTC reaches the high numbers mentioned at the outset. Thus, clarification and standardization of the definition of FMTC is clearly needed.

Germline mutations in FMTC (as defined using the more extended criteria) are distributed throughout the RET gene, with several in exon 13 (codons 768, 790, and

791) and in exon 14 (codons 804, and 844). Some of these mutations have also been identified in families with MEN2A. Because FMTC shares a common genetic defect with MEN2A, it can be difficult to distinguish a family that initially appears to have FMTC from one with MEN2A, as the manifestation of pheochromocytoma and/or PHPT occurs later in the course of the disease.

3.5 MEN2B Syndrome

MEN2B syndrome is the most rare and aggressive form of MEN2 and accounts for about 5 % of MEN2 cases. It consists of MTC, pheochromocytoma, an absence of PHPT, visible physical stigmata such as raised bumps on the lips and tongue (due to cutaneous neuromas), ganglioneuromas of the intestine, and a Marfanoid habitus with skeletal deformations and joint laxity. Patients with MEN2B typically have disease onset in the first year of life plus a more aggressive form of MTC with higher morbidity and mortality rates compared to MEN2A patients. Patients with MEN2B often have no family history of the disease; in fact, in more than 90 % of cases, the syndrome is due to a de novo germline *RET* mutation. Early recognition of the pathognomonic signs and symptoms in these patients is crucial. Tearless crying (Brauckhoff et al. 2008) and constipation due to intestinal ganglioneuromatosis are expressed early on and may facilitate early diagnosis and surgical curability in MEN2B (Brauckhoff et al. 2014).

At least 95 % of the cases of MEN2B are attributable to a germline *RET* mutation in codon 918 that leads to this aggressive phenotype. Some families are reported to express the MEN2B phenotype with a more indolent variety of MTC and a *RET* mutation at codon 883 (Smith et al. 1997; Jasim et al. 2011). There are also patients with rare double *RET* mutations appearing in tandem on the same allele that involve V804M and either Q781R, E805K, Y806C, or S904C with atypical MEN2B (Miyauchi et al. 1999; Menko et al. 2002; Cranston et al. 2006).

4 Genotype–Phenotype Correlations in MEN2

There are clear associations between specific RET mutations (i.e., the MEN2 genotype) and the age of onset, the aggressiveness of MTC, and the presence or absence of other endocrine neoplasms, such as pheochromocytoma, PHPT, cutaneous lichen amyloidosis, and Hirschsprung's disease (i.e., the MEN2 phenotype). Extensive studies of large families, often performed by national or international consortia, have greatly enhanced our knowledge about genotype–phenotype correlations and MTC aggressiveness. Based on a model that utilizes these genotype–phenotype correlations, mutations have been stratified into three risk levels that reflect the penetrance and aggressiveness of MTC: highest risk, high-risk, and moderate risk (Wells et al. 2015) (Table 1).

Table 1 Risk groups of the *RET* mutations based on age at manifestation, the aggressiveness of medullary thyroid carcinoma and penetrance of pheochromocytoma or primary hyperparathyroidism (modified from Wells et al. 2015)

MTC risk group	Exon	Codon	Recommended age for genetic testing
Highest risk	16	918	As soon as possible (first year of life)
High-risk	11	634	3 years
	15	883	3 years
Moderate risk	8, 13–15	533, 609, 611, 618, 620, 790, 804, 891	5 years

Pheochromocytoma risk (%)	Exon	Codon	Recommended age for biochemical screening (years)
~50	16, 11	918, 833, 634	11
~20	10	609, 611, 618, 620	16
~5	13–15	804, 891	16

PHPT risk	Exon	Codon	Recommended age for biochemical screening (years)
~20 %	11	634	11
~5 %	10, 13–15	609, 611, 618, 620, 804, 891	16
No	16	918	–

4.1 Highest Risk Mutation (RET M918T)

Patients with codon M918T mutation and MEN2B have the highest risk of aggressive MTC occurring at a young age. Microscopic MTC was reported in a *RET* M918T gene carrier at 9 weeks of age (Shankar et al. 2012), and lymph node metastases have been diagnosed as early as 3 months of age (Zenaty et al. 2009). The vast majority (90–95 %) of patients carrying *RET* 918 mutations have de novo mutations (Brauckhoff et al. 2014) of paternal origin (Carlson et al. 1994). Because of this high proportion of de novo M918T mutations, early diagnosis via genetic screening is rarely possible. Similar to findings in the large series of Brauckhoff et al. (2014), in our own series of 21 patients with *RET* 918 mutations, the median age at MTC diagnosis, 13.9 years (0.9–30 years), was far too late. In both series, only about 20 % of patients were cured.

There are insufficient data concerning the clinical behavior of MTC in patients who have *RET* A883F mutations or in patients with double mutations involving *RET* codon V804M and either codon Y806C, S904C, E805 K, or Q781R.

4.2 High-Risk Group (RET 634; RET 883)

Mutations in *RET* codon 634 are characterized by an early age of MTC onset, and there is good evidence that there is significant age-related progression from CCH to MTC (Machens et al. 2003). Microscopic MTC has been detected in RET 634 mutation carriers as early as 10 months of age (Zenaty et al. 2009), and a larger series demonstrated that MTC associated with any mutation at codon 634 commonly appears before 10 years of age but is rarely associated with lymph node metastases in patients younger than 14 years (Machens et al. 2003).

4.3 Moderate Risk Group (Exon 10 RET 609, 611, 618, 620, and Exons 13–15)

The clinical course of MTC in patients with mutations in codons other than codons 634/918 varies widely. With lower risk *RET* mutations, the lifetime MTC risk is high, typically shows later onset, and is less aggressive compared with the high-risk and highest risk groups (Rich et al. 2014). The earliest ages at which MTC was reported in patients with *RET* 609, 611, 618, and 620 mutations (identified by family screening) were 5, 15, 8, and 5 years, respectively, while lymph node metastases were reported at the ages of 39, -, 11, and 10, respectively (Rich et al. 2014). For exon 10 mutations, the median age at manifestation of MTC is between 20 and 40 years, and lymph node metastasis rarely occurs before 30 years of age (Frank-Raue et al. 2011). The results of a multicenter study of 340 patients carrying exon 10 mutations show differences in the aggressiveness of MTC depending on the mutated codon (Frank-Raue et al. 2011). In order of severity from most to least severe, mutations in codons 620, 618, 611, and 609 result in earlier age at manifestation, more advanced tumor stage at manifestation, and decreased chance of cure. In carriers of *RET* mutations 533 and 804 that were identified by family screening, the earliest manifestations of MTC were reported at 21 and 16 years of age, respectively, and the median ages were 42 and 52 years, respectively. For carriers of *RET* mutations 533 and 804, the earliest ages at lymph node metastases were 22 and 31 years, with median ages of 59 and 53 years (Rich et al. 2014). The cumulative risk of MTC in children at the age of 20 was 10 % or lower for mutations in codons 533, 609, 611, 791, and 804 (Rich et al. 2014).

RET mutations Y791F and S649L are described as having variable phenotypes that are in most cases very mild (Berndt et al. 1998; Gimm et al. 2002; Frank-Raue et al. 2008). Accordingly, some questioned the pathogenicity of these RET mutations (Erlic et al. 2010; Toledo et al. 2015). In these mutations, thyroidectomy should not be based on mutation status alone but also on elevated CTN levels.

5 Clinical Implications

5.1 RET Testing

The benefits of *RET* testing are so well described that this genetic test is considered the standard of care in all patients with newly detected MTC and in all first-degree relatives of a patient with a known *RET* mutation. A family history is often inadequate for establishing the diagnosis of familial disease, and a more thorough evaluation by genetic and biochemical screening often reveals a family history of MTC in patients originally thought to have the sporadic form of the disease. About 1–7 % of apparently sporadic cases have identifiable *RET* mutations, including about 2–9 % that have de novo germline mutations (Kloos et al 2009). Presymptomatic identification of a *RET* mutation carrier is the predominant route to an MEN2 diagnosis in children. The most common practice for *RET* analysis includes DNA sequencing of exons 5, 8, 10, 11, and 13–16. It makes sense to perform targeted analysis of exons 15 and 16 in patients with the MEN2B phenotype, but analysis of the entire coding sequence of the *RET* gene should be reserved for patients with strong evidence suggesting a hereditary cause of the disease and negative findings when sequencing exons 5, 8, 10, 11, and 13–16. Prior to genetic testing, an experienced clinician or genetic counselor should communicate the risks, benefits, and limitations of this test. Thereafter, the patient or the patient's parents can provide informed consent. In families with hereditary MTC, genetic testing should be performed as soon as possible after birth for the highest risk group of *RET* mutations, at 3 years of age or before in children in the high-risk category, and at 5 years of age in children in the moderate risk category.

During recent years, there has been a change in the spectrum of *RET* mutations detected in patients with hereditary MTC. Specifically, there has been a shift from the "classical" mutation at codon 634 in exon 11 to more cases with mutations in exons 13–15 and less aggressive disease (Frank-Raue et al. 2007). Initially, the frequency of diagnosed *RET* mutations in patients with MEN2A was 85 % mutations in codon 634, with mutations in codons 609, 611, 618, and 620 accounting for the additional 10–15 % of cases (Eng et al. 1996). Our recent analysis of the *RET* proto-oncogene in patients with hereditary MTC provides evidence for this change in the spectrum of detected mutations. Exon 13–15 mutations, so-called rare mutations, were diagnosed in 39 % of families, exon 10 and 11 mutations in 54 %, and exon 16 mutations in 6 %. This change in the frequency of diagnosed mutations in MEN2A families from high-risk mutations to the so-called rare mutations in codons 13, 14, and 15 may impact the overall prognosis of hereditary MTC, i.e., improve the overall prognosis of hereditary MTC. The reasons underlying this change in mutation detection may include the routine *RET* diagnostics in all patients diagnosed with MTC, the discovery of hereditary cases in apparently sporadic (4–7 %) cases, and the extension of the analyses to included mutations other than the known "hot spots" (Berndt et al. 1998). In addition, there is a distinct distribution of *RET* mutations in different parts of the world that depend on the genetic

background of the local population, which may also affect the detection rates of specific *RET* mutations.

5.2 Prophylactic Thyroidectomy

Prophylactic or early thyroidectomy is the only curative therapy for MTC in *RET* gene carriers. The biological behavior of MTC, meaning the age at onset of MTC plus the aggressiveness of the disease, is mutation-dependent. These findings have led to stratification of RET mutations according to risk level (i.e., highest, high, and moderate), but heterogeneity in disease expression and progression within these groups varies considerably, especially in the moderate risk group. The optimal timing for thyroidectomy is a major consideration: Should the timing for a given *RET* mutation be based on the typical behavior of MTC in carriers of this *RET* mutation or on the earliest reported age at which MTC/metastases occur? Is the aim true prophylactic thyroidectomy (before manifestation of MTC) or is the aim long-term "biochemical" cure? Current discussions about performing prophylactic thyroidectomy in the first few years of life include: (i) concerns that the surgical and anesthetic risks outweigh the benefits of surgery at that age; (ii) concern for iatrogenic hypothyroidism (Frank-Raue et al. 2006); and (iii) the effectiveness of CTN for detecting clinically relevant MTC.

5.3 The Effectiveness of CTN for Detecting Clinically Relevant MTC

If the aim of prophylactic thyroidectomy is to remove the thyroid before MTC metastases occur and, concurrently, to decrease potential medical and surgical morbidity, the effectiveness of detecting clinically relevant MTC by CTN determination becomes crucial. Given our evolving understanding of MEN2A-related MTC, the decision regarding the age of prophylactic thyroidectomy is no longer based upon genotype alone. Rather, this decision is currently based on additional clinical data, the most important being basal or stimulated serum CTN levels. Data from the current literature show that basal CTN levels below 40 pg/ml and below 60 pg/ml are not associated with lymph node metastases; specifically, studies from France, Germany, and Italy show that all patients were disease-free after surgery when the basal pre-operative serum CTN level was below 40 pg/ml (Elisei et al. 2012; Machens et al. 2009; Rohmer et al. 2011).

Therefore, current guidelines state that the timing of prophylactic thyroidectomy should be based on the risk level of the particular RET mutation and on the CTN level; specifically, the decision to perform thyroidectomy should err on the safe side if the CTN level is elevated but below 30 pg/ml, especially in the moderate risk group. The recent version of the American Thyroid Association (ATA) guidelines included three risk groups for RET mutations, i.e., highest risk, high-risk, and

moderate risk categories. Children in the ATA highest risk category should undergo thyroidectomy in their first year of life, and perhaps even in their first months of life. Children in the ATA high-risk category should have ultrasound of the neck and CTN measurement performed prior to thyroidectomy. Thyroidectomy should typically be performed at 5 years of age or earlier, depending on the presence of elevated serum CTN levels. Children in the ATA-moderate category should have ultrasound of the neck and measurement of CTN prior to thyroidectomy. Thyroidectomy timing should most often be based on serum CTN levels; however, annual or biannual evaluations may extend the timing by several years or even decades (Wells et al. 2015).

5.4 Screening for Pheochromocytoma or PHPT

In addition to early treatment of MTC, the main concerns in a *RET* gene carrier are not to miss a pheochromocytoma diagnosis, because often there is a risk of lethal pheochromocytoma crisis during neck surgery or during childbirth, and to avoid overlooking primary HPT before neck surgery. Screening for pheochromocytoma should begin at the age of 11 for children carrying *RET* mutations in exons 16, 15, and 11 in codons 918, 833, and 634 and by 16 years of age in children carrying RET mutations in exons 5, 8, 10, and 13–15, e.g., in codons 609, 611, 618, 620, 804, and 891 (Table 1). Screening consists of measuring plasma levels of free metanephrines and normetanephrines, or 24-hour urine metanephrines and normetanephrines. Adrenal imaging with computerized tomography or MRI is indicated in patients with positive biochemical results. For practical reasons, patients should be screened for HPTH at the time of screening for pheochromocytoma; in MEN2B, no HPTH screening is necessary (Wells et al. 2015).

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Pheochromocytomas in Multiple Endocrine Neoplasia Type 2

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Abstract

Pheochromocytoma (PC) is a neuroendocrine tumor that originates from chromaffin cells of the adrenal medulla. The production of catecholamines, including epinephrine, norepinephrine and dopamine, may lead to haemodynamic instability. Over 30 % of PCs are associated with germline mutations, including re-arranged in transfection (*RET*) mutations seen in multiple endocrine neoplasia type 2 (MEN2) syndromes. Around 40 % of individuals with MEN2 develop PC, though it is rarely the presenting feature. Compared to sporadic PC, MEN2-associated PC is more likely to be epinephine secreting and demonstrate bilateral adrenal involvement, and is less likely to be malignant. The diagnosis of PC requires clinical suspicion and biochemical testing, followed by imaging studies. Novel nuclear medicine modalities, including FDG positron emission tomography (PET) and ^{68}Ga DOTATATE PET have added to the conventional techniques of ^{123} I-metaiodobenzylguanindine (MIBG) scintigraphy, computer tomography and magnetic resonance imaging. Treatment of PC is surgical and requires peri-operative alpha and, frequently, beta blockade. Novel surgical techniques, such as adrenal sparing surgery and a laparoscopic approach, have decreased peri-operative morbidity. Surveillance for PC is life long, due to the risk of metastatic disease.

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Keywords
Pheochromocytoma · Paraganglioma · MEN2 · Treatment

Abbreviations

ATA	American Thyroid Association
ATA HST	American Thyroid Association highest risk category
ATA MOD	American Thyroid Association moderate risk category
CT	Computer tomography
EPAS1	Endothelial PAS domain protein 1
^{18}F-FDG	^{18}F-fluorodeoxyglucose
^{18}F-DOPA	18-fluoro-dihydroxyphenylalanine
FH	Fumarate hydratase
^{68}Ga DOTATATE	Radioconjugate of radio-tracer Gallium 68 and somatatostatin analogue tyrosine-3-octreotate with chelating agent dodecanetetraacetic acid (DOTA)
^{68}Ga DOTANOC	Radioconjugate of radio-tracer Gallium 68 and somatatostatin analogue octreotide with chelating agent dodecanetetraacetic acid (DOTA)
^{68}Ga DOTATOC	Radioconjugate of radio-tracer Gallium 68 and somatatostatin analogue octreotide with chelating agent dodecanetetraacetic acid (DOTA)
H&E	Hematoxylin and eosin
HIF	Hypoxia inducible factor
hNET	Human norepinephrine transporter
IHC	Immunohistochemistry
^{123}I-MIBG	^{123}I-metaiodobenzylguanidine
KIF1Bβ	Kinesin family member 1B beta
MAX	MYC-associated factor X
MAPK	Mitogen activated protein kinase
MEN	Multiple endocrine neoplasia
MRI	Magnetic resonance imaging
MTC	Medullary thyroid cancer
NF1	*Neurofibromatosis type 1*
P13K	*Phosphatidylinositol-3-kinase*
PASS	Pheochromocytoma of the adrenal gland scaled score
PET	Positron emission tomography
PCs	Pheochormocytomas
PGL	Pargangliomas
PHD	Prolyl hydroxylase domain protein
PNMT	Phenylethanolamine N methyltransferase
RET	Re-arranged in transfection
SDHA	Succinate dehydrogenase subunit A
SDHAF2	Succinate dehydrogenase assembly factor 2

SDHB	Succinate dehydrogenase subunit B
SDHC	Succinate dehydrogenase subunit C
SDHD	Succinate dehydrogenase subunit D
SDHx	Succinate dehydrogenase subunits A, B, C, and D
TMEM127	Transmembrane protein 127
VHL	Von Hippel–Lindau

Contents

1 Introduction

Pheochromocytomas (PCs) are neuroendocrine tumors originating from the chromaffin cells of the adrenal medulla. The majority of PCs are functional, secreting the catecholamines epinephrine, norepinephrine, and dopamine and resulting in a constellation of symptoms with significant morbidity and mortality. The first PC was described by Felix Frankel in 1886 when he reported an 18-year-old girl with a one-year history of palpitations, headaches, vomiting, and pallor (Frankel 1886; Manger 2006). The first successful surgeries to remove an adrenal PC were performed by Roux in 1926 and Charles Mayo in 1927 (Manger 2006).

Symptoms that raise suspicion of catecholamine excess include flushing, palpitations, sweating, panic, headaches, and hypertension or postural hypotension. Increasingly, PCs are identified incidentally. The widespread use of routine radiological studies has led to unexpected adrenal lesions being reported in up to 4–10 % of all abdominal computer tomography (CT) and magnetic resonance imaging (MRI) studies (Grumbach et al. 2003; Kloos et al. 1995; Mansmann et al. 2004; Mantero et al. 2000; Terzolo et al. 2011), and 2.9–4.2 % of these adrenal incidentalomas have been reported to be PCs (Herrera et al. 1991; Mantero et al. 2000).

PCs and sympathetic paragangliomas (PGL) originate from chromaffin cells, which are generally uniform, polygonal, grow in nests, and have basophilic granules within the cytoplasm. There are also often intracytoplasmic hyaline globules. Nuclei are round or ovoid, with a large nucleolus, and inclusion-like structures. There are

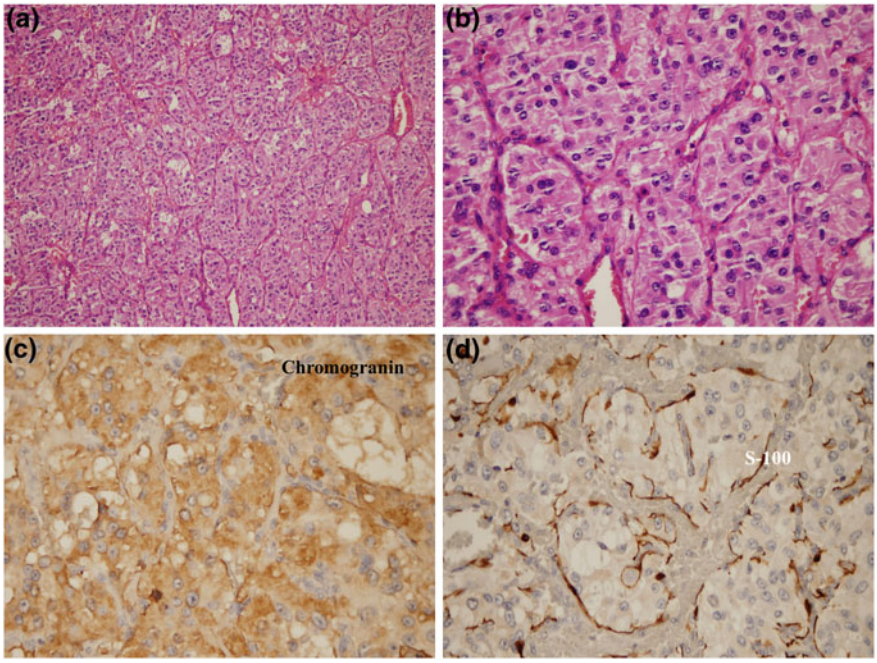

Fig. 1 Histology of pheochromocytoma. **a** Pheochromocytoma demonstrates a typical nested architecture (H&E, original magnification 100×). **b** At high power, the pheochromocytoma cells demonstrate a granular amphophilic cytoplasm and moderate nuclear pleomorphism (H&E, original magnification 400×). **c** Chromogranin immunohistochemistry demonstrates diffuse, strong granular cytoplasmic staining in neoplastic cells (Chromogranin IHC, original magnification 400×). **d** S100 labels the non-neoplastic sustentacular cells which outline the nests of cells (S100 IHC, original magnification 400×) (images courtesy of Associate Professor Anthony Gill, Anatomical Pathology Department, Royal North Shore Hospital, and University of Sydney)

very rarely mitotic figures. Immunohistochemical stains that are associated with PC and PGL include chromogranin, synaptophysin, S100, PAS+, catecholamine, neuron specific enolase, and neurofilament (DeLellis et al. 1984; Eisenhofer et al. 2004a; Salmenkivi et al. 2001; Wilson and Lloyd 1984) (Fig. 1a–d).

At present, there are no reliable histological biomarkers that predict malignant tumor behavior. After initial interest in the Ki-67 proliferative index (Bialas et al. 2013; Brown et al. 1999; Clarke et al. 1998; Elder et al. 2003; Jovanovic et al. 2012; Ohji et al. 2001; Semba et al. 2000), a more recent study has not been supportive (Ocal et al. 2014). The Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) was initially developed as a predictor of malignant potential. Calculated out of a maximum of 20 points, it utilizes the following histological features: vascular invasion (1 point), capsular invasion (1), periadrenal adipose tissue invasion (2), large nests or diffuse growth (2), necrosis (2), high cellularity (2), tumor cell spindling (2), cellular monotony (2), increased mitotic figures (2), atypical mitotic figures (2), profound nuclear pleomorphism (1), and hyperchromasia (1). A score of <4 was reported as

being likely benign, while ≥4 increased the likelihood of malignancy. Not all studies supported a correlation between PASS and clinical outcome, however (Agarwal et al. 2012; Jovanovic et al. 2012; Mlika et al. 2013; Ocal et al. 2014; Thompson 2002). New biomarkers that predict malignant tumor behavior are required.

While at present the majority of PCs are still considered sporadic tumors, studies of the genetics of tumorigenesis have led to recognition that over 30 % are associated with predisposing germline mutations, as part of various familial syndromes (Dahia 2014; Favier et al. 2014). Re-arranged in transfection (*RET*) mutations in MEN2 syndromes are associated with the development of PC. Individuals who are known to carry a *RET* mutation should undergo regular screening for PC from a young age, discussed below, with the goal of identifying presymptomatic disease that is readily surgically resectable, thus hopefully improving outcomes. Most MEN2-associated PCs are benign, but for those who do develop metastatic disease, treatment options are limited.

2 The Phenotype of MEN2-Associated Pheochromocytomas

Approximately 40 % of MEN2 patients will develop PC, although the likelihood varies with the particular *RET* mutation (Brandi et al. 2001; Raue et al. 1994). Medullary thyroid cancer (MTC) is typically the first identified feature of MEN2, while PC is rarely the presenting condition, reportedly diagnosed before MTC in only 12–25 % of cases (Modigliani et al. 1995b; Rodriguez et al. 2008; Welander et al. 2011).

MEN2-associated PCs generally arise in the adrenal glands, while paragangliomas (PGLs) are rare (Welander et al. 2011). As with other hereditary PCs, MEN2-associated PCs are more commonly multifocal and bilateral than sporadic PCs. Hereditary PCs also tend to be diagnosed earlier than sporadic PCs. Among the hereditary PC syndromes, those associated with causative genes in the kinase pathway, such as in MEN2 syndromes and neurofibromatosis, present later than those associated with causative genes in the oxygen sensing pathway, such as succinate dehydrogenase subunit B (SDHB)-associated hereditary PC/PGL syndromes and von Hippel–Lindau (VHL) disease (40 vs. 31 years $p < 0.001$) (Eisenhofer et al. 2011b).

MEN-2-associated PCs secrete epinephrine as the predominant catecholamine, along with its metabolite metanephrine, and lesser amounts of norepinephrine and normetanephrine. (Modigliani et al. 1995a) This biochemical secretion profile contrasts to sporadic, VHL-, SDHB- and SDHD-associated PCs, in which secretion of norepinephrine typically predominates (Benn et al. 2006; Neumann et al. 2002; Opocher et al. 2005). Catecholaminergic symptoms are characteristically more pronounced in MEN2-associated PCs, compared to VHL-associated PCs, due to the association of increased epinephrine with greater anxiety, palpitations, and episodic hypertension (Frank-Raue et al. 1996; Kaltsas et al. 2004; Pacak 2007). PC is

malignant in MEN2 in less than 5 % of cases (Gimm et al. 2004), in contrast to PCs in the SDHB-associated hereditary PC/PGL syndrome where malignancy is more common (Favier et al. 2014; Gimenez-Roqueplo et al. 2012).

A study of 85 patients with MEN2-associated PCs included 70 patients with MEN2A and 15 with MEN2B (Thosani et al. 2013). The median age at diagnosis of PC was 32 years. The initial manifestation of MEN2 was MTC in 60 % of patients, synchronous MTC and PC in 34 % and PC alone in 6 %. Seventy % of patients had bilateral PC and 82 % of these PCs were synchronous tumors. The median time between MTC and PC diagnosis was 5.4 years and the median time between diagnosis of first and second PC in those with metachronous PC presentation was 9.4 years (range 1.8–20.7 years). The commonest PC-associated *RET* mutation in MEN2, as discussed below, is *RET* codon 634, such that 50 % of these patients will develop PC. Other mutations associated with PC include codons 918, 883 (both associated with MEN2B), 630, and 666. There is a lower penetrance of PC in patients with exon 10 RET mutations (e.g., codons 609, 611, 618, and 620). Among the *RET* codon 634 mutation carriers in Thosani's study, the presence of PC was not associated with a later stage of MTC at the time of MTC diagnosis. Interestingly, the median survival of codon 634 MEN2 patients with PC was 499 months versus 444 months for those without PC ($p < 0.05$, albeit with a short follow- time for the latter group in this study). PC was unlikely to be a cause of death as only 2 patients in the study died of PC; these deaths were related to hypertensive crises either under anesthetic or with IV contrast administration. There were no cases of metastatic PC among the MEN2-associated PCs in this study.

3 The Molecular Genetics of Pheochromocytomas in Multiple Endocrine Neoplasia 2

PCs and PGLs represent the most heritable of all tumor types. The inherited predisposition to PC/PGLs was first recognized in 1993 and initially genetic mutations were thought to cause only 10 % of all PC/PGLs. Since then, a larger number of predisposition genes have been identified, such that more than 30 % of all patients presenting with PC and/or PGL will be found to carry a germline mutation (Dahia 2014; Favier et al. 2014).

To date, more than fourteen genes have been identified that show germline mutation in association with the development of PC/PGL. As well as the *RET* gene in MEN2 syndromes, mutations have been found in neurofibromatosis type 1 (*NF1*), Von Hippel–Lindau (*VHL*) disease, succinate dehydrogenase subunits A, B, C, and D (*SDHA*, *SDHB*, *SDHC*, and *SDHD*, collectively *SDHX*), succinate dehydrogenase assembly factor 2 (*SDHAF2*), prolyl hydroxylase domain protein enzyme 1 and 2 (*PHD1* and *PHD2*), transmembrane protein 127 (*TMEM127*), MYC-associated factor X (*MAX*), kinesin family member 1B beta (*KIF1Bβ*), endothelial PAS domain protein 1 (*EPAS1*, encoding *HIF2* α), and fumarate hydratase (*FH*) genes. Interestingly, these genes encode proteins with diverse

cellular function, but result in histologically identical tumors. A key paper by Dahia et al. (2005) divided tumors into two groups. Cluster 1 mutations were associated with upregulated hypoxia inducible factors (HIF) and other hypoxia responsive genes, whereas Cluster 2 mutations resulted in activation of protein kinase signaling pathways. Cluster 1 includes *VHL*, *SDHx*, and *PHD1* and *PHD2*, while cluster 2 comprises a more heterogenous group of genes including *RET*, *NF1*, *TMEM127*, and *MAX* (Burnichon et al. 2012; Dahia et al. 2005; Qin et al. 2010).

MEN2 results from a gain of function mutation in the *RET* proto-oncogene on chromosome 10q11.2, a 21 exon gene that encodes a tyrosine kinase receptor. This gene was initially discovered in 1985 (Takahashi et al. 1985). The receptor consists of an extracellular domain, a transmembrane domain, and an intracellular domain. Activation by ligand binding induces dimerization and downstream signaling via P13K-AKT and MAPK-ERK kinase pathways to modulate cell growth and differentiation (Treanor et al. 1996). *RET* mutations result in constitutive activation of the tyrosine kinase (Welander et al. 2011). These mutations are clustered in exons 8, 10, 11, 13, 14, 15, or 16.

4 Genotype and Phenotype Correlations in *MEN2*-Related Pheochromocytomas

Systematic analysis of kindreds affected by MEN2 has led to recognition of the strong genotype –phenotype relationship between *RET* mutations and specific clinical manifestations, including the age of onset and aggressiveness of MTC, and the likelihood of PC and primary hyperparathyroidism. This genotype–phenotype correlation has permitted the development of risk stratification guidelines that advise the timing of prophylactic thyroidectomy in asymptomatic *RET* mutation carriers, as discussed in Chap. 9, with the goal to remove the thyroid gland prior to the development of MTC. Different *RET* codon mutations are associated with variable penetrance of PC and primary hyperparathyroidism, and this can guide the age at which screening for PC and primary hyperparathyroidism should commence. The most recent American Thyroid Association (ATA) guidelines (Wells et al. 2015) have updated the 2009 guidelines (Kloos et al. 2009) which divided *RET* mutations into four levels of risk (A < B < C < D). ATA-D mutations (codons 918 and 883) are the most severe, being associated with MEN2B and the development of aggressive MTC in childhood, such that thyroidectomy is recommended within the first year of life for carriers. The 2015 guidelines refer to the highest risk category as "ATA-HST." MEN2B is associated with the development of PC in around 50 % of cases, while primary hyperparathyroidism is not seen. The high risk ATA-C group comprises *RET* codon 634 mutations. Codon 634 mutations are associated with an MEN2A phenotype, characterized by the early development of aggressive MTC, and a high risk of development of PC and hyperparathyroidism. The 2015 guidelines refer to this category as ATA-H. By contrast, the lower risk ATA-B and ATA-A groups have been grouped into a single category of

ATA-MOD, which consists of a number of *RET* codon mutations associated with a more variable phenotype. The ATA-MOD group is associated with the development of less aggressive medullary thyroid cancer, such that prophylactic thyroidectomy may be delayed beyond the age of 5 if certain strict clinical and biochemical criteria are met (Kloos et al. 2009). PC is seen less frequently in individuals with ATA-A and ATA-B (ATA-MOD) risk group *RET* mutations. Interestingly, PC can present at widely different ages in different members of "low PC risk" FMTC families with the same mutation (e.g., *RET* codon G533C). This has led the latest guidelines to recommend removal of the separate FMTC category which should be regarded now as a "low PC risk" variant of MEN2A.

Collaborative studies reporting the clinical data of MEN2-affected kindreds continue to provide an evidence base to guide age-dependent screening for PC. The highest risk *RET* mutations for the development of PC include 918, 634, and 630. Individuals with MEN2B carrying *RET* codon 918 mutations have a reported incidence of PC of around 50 %. Individuals carrying *RET* codon 634 mutations have been similarly reported to develop PC in around 50 % of cases, most frequently in the 3rd or 4th decade of life (Thosani et al. 2013). Guidelines published by the ATA proposed annual screening for PC commence at 11 years for the higher risk codon 918, 634, and 630 mutations (ATA-HST and ATA-H, and at 16 years for other mutations ATA-MOD) (Kloos et al. 2009; Machens et al. 2005). Biochemical screening for PC is discussed below.

5 Diagnosis of Pheochromocytoma

The diagnosis of PC is based on the clinical suspicion, biochemical testing, and then imaging studies (if biochemistry is positive), leading to surgery and histological evaluation (Lenders et al. 2014). Gene carriers in MEN2 families must not undergo general anesthetic for any surgery without prior PC screening. Episodic secretion of catecholamines by PC mean that the metabolites metanephrine and normetanephrine are more sensitive integrated measures of catecholamine production. *RET* mutation carriers should be screened at least annually for PC, with biochemical screening using either plasma metanephrines and normetanephrines (drawn from a supine patient after an overnight fast), or 24-h urinary fractionated metanephrines and normetanephrines (Eisenhofer et al. 2003). The recommended method of catecholamine measurement is via liquid chromatography with mass spectrometry (Eisenhofer and Peitzsch 2014; Weismann et al. 2014). Controversy persists regarding the relative merits of plasma versus urinary metanephrine measurements in screening for PC (Lenders et al. 2014), and in practice plasma and urine testing are complementary. Catecholamine levels have been shown to correlate poorly with tumor size, unlike plasma or urine catecholamine metabolite levels, which show a strong correlation (Eisenhofer et al. 2004b).

As discussed above, there are distinct patterns of plasma catecholamine o-methylated metabolites in patients with the hereditary PC syndromes. MEN2-associated PCs express phenylethanolamine N methyltransferase (PNMT), the enzyme that converts norepinephrine to epinephrine, hence the association with predominant epinephrine secretion and elevated metanephrines (Pacak et al. 2009). NF1-associated PCs show a similar profile, while the hypoxia gene (VHL and SDHB/D)-associated PCs produce elevated normetanephrines and methoxytyramine levels (Eisenhofer et al. 2011a).

In practical terms, MTC has generally developed and resulted in elevated plasma calcitonin levels by the time PCs present clinically in MEN2. Plasma-free metanephrine measurement has a sensitivity of 96–100 % and a specificity of 87–92 %, while 24-h urine fractionated free metanephrine measurement has a sensitivity of 92–99 % and specificity of 64–72 % in the diagnosis of PC (Sawka et al. 2003). Certain drugs are known to cause false elevations in catecholamines, including tricyclic antidepressants, beta blockers, phenoxybenzamine, and monoamine oxidase inhibitors. It is best to avoid these and retest subjects where possible. Medical conditions, including renal impairment and states of increased sympathetic activity such as heart failure and hypoglycemia, can also cause a physiological elevation in catecholamines. A number of provocation tests have been trialed in the past, including glucagon stimulation tests, clonidine suppression tests, and histamine stimulation tests (Lenders et al. 2010; Young 1997), but these have become largely redundant since advent of fractionated metanephrine testing. Serum chromogranin A is elevated in 48 % of patients with PC (Cotesta et al. 2005). Diagnostic utility of chromogranin A is, however, constrained by poor specificity due to its elevation in diverse conditions, including other neuroendocrine tumors, hyperparathyroidism, hyperthyroidism, gastrointestinal disorders including hepatitis, colon cancer and pancreatic disorders, cardiovascular disease, inflammatory diseases, renal failure, other cancers and the use of proton pump inhibitors (Modlin et al. 2010).

Once a biochemical diagnosis has been established, or in cases when there are symptoms of structural compression, anatomical and often functional imaging tests are required. Structural imaging for diagnosis of PC and PGL includes the use of CT and/or MRI. CT is usually the first choice for imaging of the chest, abdomen, and pelvis, whereas MRI is superior for imaging head and neck PGLs, more relevant to the hereditary PGL syndromes (Lenders et al. 2014). PCs enhance avidly after CT contrast administration and display a prolonged contrast washout phase with Hounsfield unit scores usually >30 (Ilias et al. 2007). CT has a sensitivity of 93–100 % for detecting tumors >0.5 cm diameter. An example of adrenal CT imaging in a man with known MEN2A syndrome who was found to have elevated urinary metanephrines on routine annual biochemical screen is shown in Fig. 2a. A second example of CT imaging in a patient presenting clinically with pheochromocytoma in MEN2A is shown in Fig. 2b. MRI is equally sensitive and may provide detail of the relationship between the tumor and its vasculature (Jacques et al. 2008). MRI is also recommended if there is an allergy to CT contrast dye, if

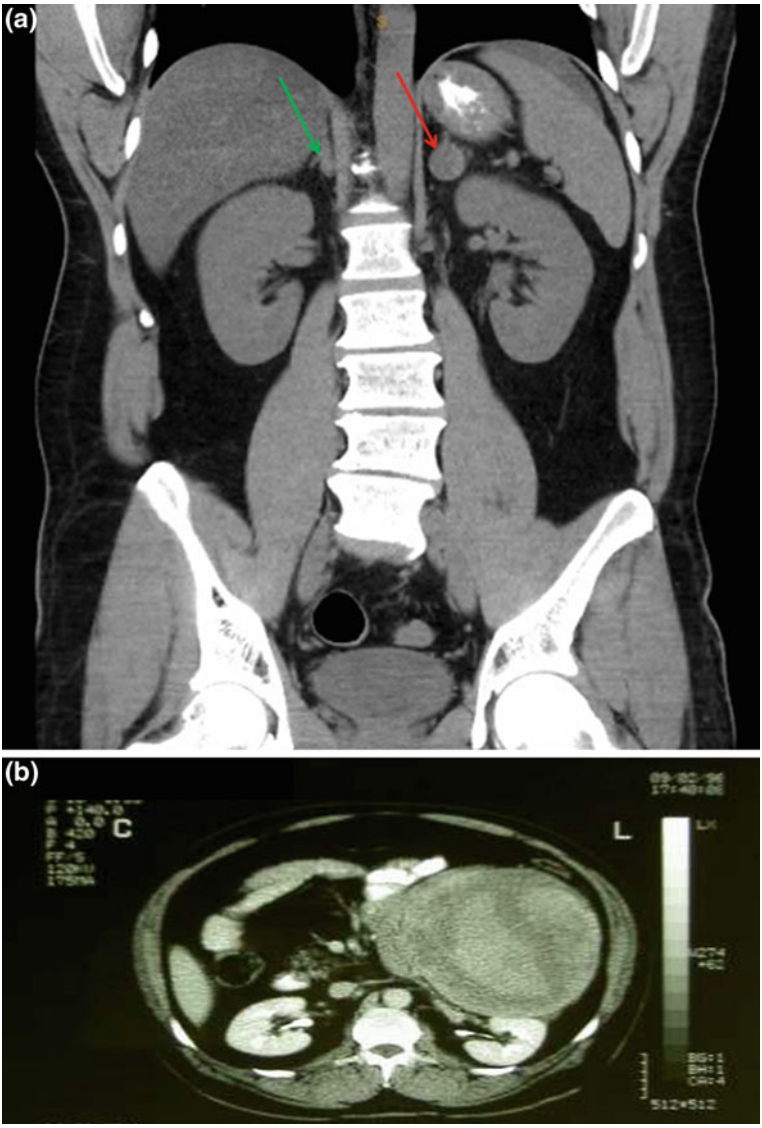

Fig. 2 Computer tomography (CT) of MEN2A-mutated pheochromocytoma. **a** Non-contrast CT coronal section through the abdomen of a patient with known MEN2A mutation, showing a 20-mm lesion in the left adrenal gland, Hounsfield units 38 (*red arrow*), and a 10-mm lesion in the right adrenal gland, Hounsfield units 35 (*green arrow*). **b** Contrast CT transverse section through the upper abdomen of a 60-year-old man who presented with flank pain, hypertension, and hemodynamic instability, showing a large left-sided retroperitoneal mass with extensive hemorrhage. Histopathology revealed a 2.1-kg pheochromocytoma. The patient was confirmed to have MEN2A syndrome, carrying a RET codon 608 mutation (case previously published in Learoyd et al. 2005; Evans et al. 1997)

there are surgical clips present, and to limit radiation exposure when imaging children. Most PCs will have heterogeneous, multiple high-intensity areas seen on T2 weighting, although this is not a universal finding. The specificity of CT and MRI varies in studies from 50 to 90 % (Mittendorf et al. 2007). False positivity can occur, especially when there has been previous surgery in the same location. Functional imaging is then needed to complement anatomical imaging.

Functional studies aid in the detection of extra-adrenal PGLs and of metastatic disease (Taieb et al. 2014). Functional imaging is therefore generally recommended for patients who are at risk of multiple tumors or metastases, who have a large primary tumor >10 cm, or where there is suggestion of recurrent disease (Lenders et al. 2014; Whalen et al. 1992). Historically, functional studies relied upon ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy. MIBG is a norepinephrine-like compound taken up by the human norepinephrine transporter (hNET) expressed in chromaffin tissue (Fig. 3a). Negative MIBG uptake in a structural lesion does not exclude the diagnosis of PC, particularly if the tumor is <3 cm (Kurisaki-Arakawa et al. 2014). MIBG scanning using either I^{123} or I^{131} has sensitivities of 60–95 %; I^{123} produces better image quality (albeit at higher cost) than I^{131}. MIBG scanning displays high specificity of 95–100 % but is less sensitive for metastatic or extra-adrenal disease. The specificity is complicated by the fact that up to 50 % of normal adrenal glands will demonstrate some physiological uptake of MIBG. Certain drugs will need to be withheld for 2 weeks prior to MIBG scanning including calcium channel blockers, tricyclic antidepressants, sympathomimetics, and combined alpha/beta blockers. MIBG imaging is particularly relevant when I^{131} MIBG is planned for therapy (Fig. 3b).

In recent years, positron emission tomography (PET) scanning has been increasingly studied, generally in combination with CT to enhance diagnostic accuracy. In imaging PC/PGL, these studies have evaluated the widely utilized, non-specific tracer ^{18}F-fluorodeoxyglucose (^{18}F-FDG), as well ligands specific to catecholamine metabolic pathways, such as ^{18}F-fluorodopamine and 18-fluoro-dihydroxyphenylalanine (^{18}F-DOPA). ^{18}F-FDG PET/CT is less specific, but is useful in detecting metabolically active lesions, particularly metastatic disease with high glucose metabolism. ^{18}F-FDG PET/CT is also more useful than MIBG in non-functional PGL (Ilias et al. 2003a; Lenders et al. 2014; Timmers et al. 2012). ^{18}F-FDG PET/CT may also be particularly useful for imaging *SDHB*-mutated tumors (Lepoutre-Lussey et al. 2015).

The PET isotope ^{18}F-fluorodopamine is taken up into catecholamine-producing cells by hNET, and initial studies showed promise with excellent sensitivity in PC/sympathetic PGL imaging; its utility is limited by availability. ^{18}F-fluorodopa enters neuroendocrine cells via an amino acid transporter and has also been reported to be a sensitive PET tracer for PC/PGL (Hoegerle et al. 2002; Ilias et al. 2003b).

PCs and PGLs also express somatostatin receptor subtypes 2, 3, and 5 (Mundschenk et al. 2003; Reubi et al. 1992), an observation utilized in functional imaging studies. Conventional ^{111}-Indium pentetreotide scintigraphy has shown less utility in PC/PGL, while newer PET-specific ^{68}Gallium-somatostatin analogues, such as ^{68}Ga-DOTATATE (Hofman et al. 2015), DOTANOC (Lopci et al.

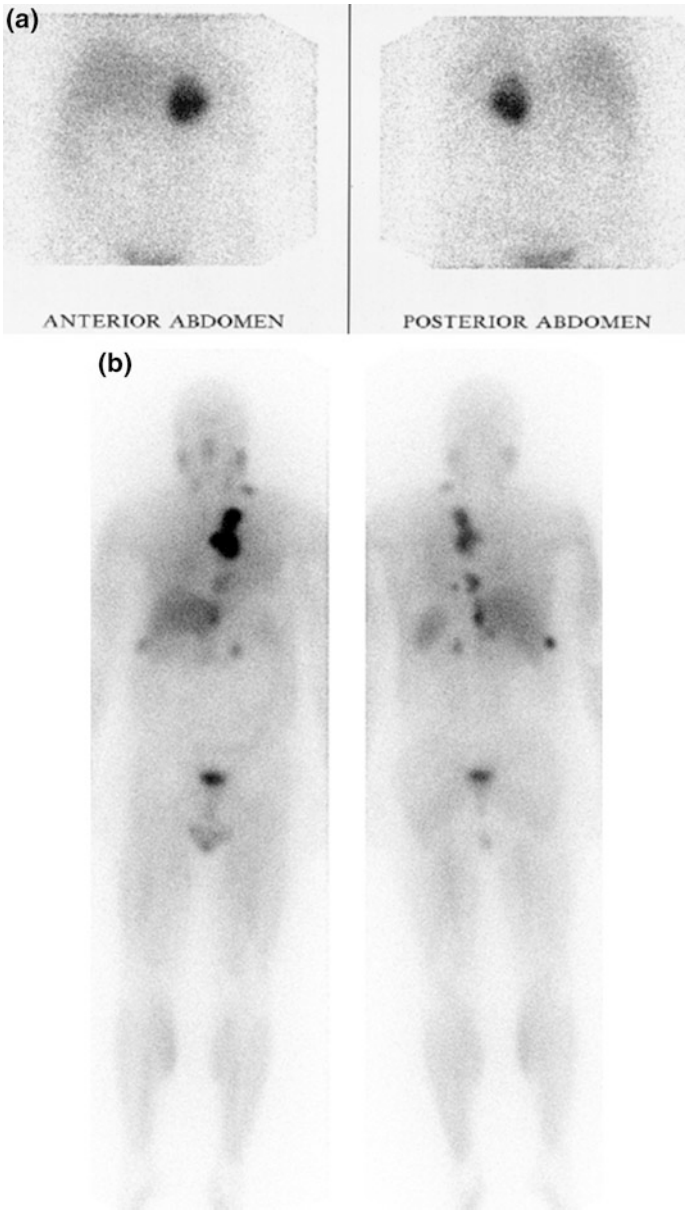

Fig. 3 ^{131}I-metaiodobenzylguanindine (I-^{131} MIBG) scintigraphy of RET-mutated pheochromocytomas. **a** Planar I-^{131} MIBG scintigraphy showing left-sided pheochromocytoma, with increased tracer accumulation. **b** Whole body I-^{131} MIBG scintigraphy following an 8 GBq therapeutic dose in a 52-year-old man with MEN2A, revealing metastatic deposits of MIBG-avid pheochromocytoma in the lungs, bones, and intra-abdominal viscera (images courtesy of Associate Professor Paul Roach, Nuclear Medicine Department, Royal North Shore Hospital, and University of Sydney)

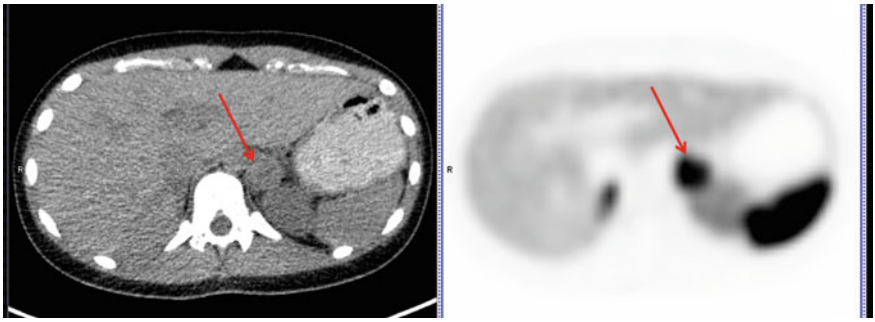

Fig. 4 ^{68}Gallium-somatostatin analogues (^{68}Ga-DOTATATE) positron emission tomography (PET) scan. *Ga*^{68}-DOTATATE PET scan in a patient with MEN2A showing a left-sided pheochromocytoma (*red arrow*), with physiological uptake in the right adrenal for comparison. Splenic uptake is also physiological (images courtesy of Associate Professor Paul Roach, Nuclear Medicine Department, Royal North Shore Hospital, and University of Sydney)

2013), and DOTATOC, are being explored and show promise in early studies (Rufini et al. 2013). An image of a ^{68}Ga-DOTATATE scan showing a left-sided pheochromocytoma is shown in Fig. 4.

The "flip-flop" effect that describes tumor heterogeneity, whereby rapidly progressive metabolically active tumor cells are better imaged with ^{18}F-FDG PET/CT, while better differentiated cells show greater avidity for 68Gallium- DOTA- or ^{18}F-fluorodopa, may not be as applicable to MEN-2 associated PCs as it is in the imaging of other neuroendocrine tumors.

6 Treatment

6.1 Perioperative Management of PC

Once a diagnosis of PC is made, surgical resection is the only curative option. Thus, early diagnosis of a solitary lesion is most likely to result in long-term remission. Lifelong follow-up is always required, as currently there are no reliable predictors of malignancy, and a second PC may present years later in MEN2. The latest ATA guidelines recommend that PC surgery should take place prior to thyroidectomy in MEN2 patients where MTC and PC are diagnosed simultaneously. It is also recommended that PC should be removed prior to embarking on pregnancy (Wells et al. 2015).

Preoperative medical treatment is required to prevent perioperative catecholaminergic crisis. The goals are to treat hypertension, expand the contracted plasma volume, and stabilize the heart rate in order to minimize hemodynamic disturbance both during induction of anesthesia and manipulation of the tumor and also following its resection. This should first be done using alpha-adrenergic receptor blockade (Pacak 2007; Weingarten et al. 2010; Young 1997). There are no

randomized controlled trials comparing the non-selective alpha-adrenergic blocker phenoxybenzamine to a selective alpha-1 blocker, such as prazosin, doxazosin, or terazosin. The non-competitive, irreversible alpha blockade provided by phenoxybenzamine does risk greater postoperative hypotension and, for this reason, some centers prefer use of a shorter-acting, competitive, selective alpha-1 blocker such as doxazosin. At least 7–14 days of alpha blockade treatment are typically required to achieve target blood pressure control of <130/80 mmHg lying and <100 mmHg systolic standing and to facilitate adequate blood volume expansion. Phenoxybenzamine is usually commenced at 10 mg twice daily, increasing every 2–3 days as tolerated up to a maximum of 1 mg/kg. Prazosin is administered as 2–5 mg two to three times a day, terazosin 2–5 mg/day, and doxazosin 2–8 mg/day (Pacak 2007). Beta blockade must never be started before adequate alpha blockade has been achieved, but may be required to control reflex tachycardia that develops, particularly with epinephrine-secreting tumors (Crago et al. 1967; Sibal et al. 2006; Wark and Larkins 1978). Cardioselective beta$_1$ adrenoreceptor blockers are preferred, such as atenolol 12.5–25 mg two to three times a day or metoprolol 25–50 mg three to four times a day. Combined alpha and beta blockers should be avoided, as the ratio of alpha:beta favors beta blockade and may trigger a catecholaminergic crisis. Labetalol, for example, provides an alpha:beta ratio of 1:5. If blood pressure and/or heart rate is unable to be controlled with these agents, or if patients have major side effects from alpha and beta blockade, other options for preoperative management include calcium channel blockers, such as amlodipine 10–20 mg, nifedipine 30–90 mg daily, or verapamil 180–450 mg daily (Lenders et al. 2014; Pacak 2007). Alternatively, calcium channel blockers may be used as additional agents in patients already alpha blockade. Use of calcium channel blockers as first-line therapy has been successful in patients with very mild hypertension, but this approach is discouraged unless the patient is very intolerant of alpha blockade.

To avoid postoperative hypotension, the goal is to achieve plasma volume expansion during the period of alpha blockade, both by encouraging increased oral sodium intake, and possibly with intravenous saline loading in the day prior to surgery. Practice varies between institutions, and randomized trials supporting this therapy are lacking. Blood pressure should be carefully monitored during surgery and in an intensive care unit postoperatively (Lenders et al. 2014; Pacak 2007).

6.2 Surgical Approach

It is crucial that patients are referred to a center experienced in adrenal surgery. Most such centers use minimally invasive (laparoscopic) adrenalectomy for PCs, although an open approach may be required for tumors larger than 6 cm in order to ensure complete resection and to avoid capsule rupture within the abdomen (Lenders et al. 2014). Laparoscopic approaches have only been compared in single center series and generally show better outcomes including less pain, less blood

loss, fewer days in hospital, without difference in mortality or recurrence (Agarwal et al. 2012; Shen et al. 2010). Perioperative mortality is approximately 1 % (Agarwal et al. 2012; Shen et al. 2010). A newer retroperitoneal approach has shown markedly less postoperative pain resulting in shorter hospital stays (Dickson et al. 2011; Epelboym et al. 2014; Walz et al. 2006; Yuan et al. 2014). Partial adrenalectomy has also been used in highly experienced centers, to spare the cortex and allow patients with bilateral disease or inherited syndromes to retain partial adrenocortical function (Iihara et al. 2003). This warrants further assessment in larger studies of MEN2-affected individuals, where malignant PC is unlikely but bilateral disease very common, and where adrenal insufficiency is inevitable after bilateral adrenalectomy. The cumulative recurrence rate for MEN2-associated PCs after adrenal-sparing surgery at 5 and 10 years is 38.5 %, including both ipsilateral and bilateral recurrence (Asari et al. 2006).

An individualized approach is recommended, balancing the risks of recurrence with those of chronic adrenal insufficiency (Asari et al. 2006). Expert panels have suggested it may even be appropriate to adopt a "watch and wait" strategy for asymptomatic patients with negative biochemistry and small tumors (<2 cm), such as those found in routine MEN2 screening (Lenders et al. 2014).

6.3 Postoperative Surveillance

The patient must be assessed for adrenal insufficiency in the immediate postoperative period, especially those who have had bilateral adrenal surgery of any nature. It is reported that between a third and a half of an adrenal gland is needed to preserve cortical function (Lenders review 2014), but isolated reports of smaller adrenal remnants providing adequate cortical function are described. In a recent series of 96 patients with hereditary bilateral PCs, predominantly MEN2 and VHL-associated, Grubbs et al. (2013) reported a steroid independence rate of 78 % and a recurrence rate of 7 % in the remnant adrenal at 3 years.

During postoperative follow-up, plasma or urinary fractionated metanephrines should be measured to verify complete resection and confirm remission. Recurrence may occur in up to 17 % cases, and lifelong surveillance is therefore required, as discussed above (Amar et al. 2005; Mannelli 2006). Similarly, although the malignant potential of *RET*-mutated tumors is low, the lack of a reliable biomarker of malignancy and the observation that metastases may only become apparent many years from initial diagnosis (Edstrom Elder et al. 2003; Goldstein et al. 1999; Plouin et al. 1997) also mean that long-term surveillance is crucial.

Structural and/or functional imaging is required if metanephrines remain elevated, or if metastatic disease is otherwise suspected.

7 Management of Metastatic Disease

RET-mutated PCs are only rarely malignant. As discussed above, metastatic disease has been reported in less than 5 % of cases (Lairmore et al. 1993; Modigliani et al. 1995a; Quayle et al. 2007; Rodriguez et al. 2008), in comparison with *SDHB*-associated PCs/PGLs, which are malignant in 30–70 % of cases, depending on the series (Favier et al. 2014). Malignancy is classified as the presence of tumor in tissue that does not normally contain chromaffin cells. Sites of malignant deposits include lymph nodes, bone, liver, lung, and rarely, brain, gastrointestinal tract, and other intra-abdominal viscera. Malignant disease is associated with a 5-year survival of 22 %, compared to 97 % for benign disease (Plouin et al. 1997).

Management of malignant PC can be extremely challenging. Palliative goals include management of blood pressure, heart rate, and catecholaminergic symptoms as well as control of local symptoms of metastases. Alpha and beta blockade is used, as discussed above. Local symptoms may be controlled with external beam radiotherapy, radiofrequency ablation, cryoablation, or transarterial chemoembolization, depending on the site of metastasis.

When metastatic deposits are more widespread, systemic therapies, such as iodine^{131}-MIBG therapy, cytotoxic therapy, and molecular therapy, may be required. Radionuclide therapy, in the form of radioactive iodine tagged to MIBG, is taken up by chromaffin cells and causes tissue destruction by beta radiation. Around 60–70 % of tumors respond by taking up the radionuclide (Pacak et al. 2005; Rutherford et al. 2014; van der Harst et al. 2001); lesions that have been previously irradiated by external beam radiation are less responsive (Fitzgerald et al. 2006). For rapidly progressive disease, combinations of cyclophosphamide, dacarbazine, vincristine, and doxorubicin have been most extensively studied for treatment of metastatic PC and PGL (Joseph 1967; Keiser et al. 1985). Rates of response are from 33 to 55 %, although others symptomatically improve with tumor size shrinkage (Ayala-Ramirez et al. 2012b; Huang et al. 2008; Tanabe et al. 2013).

Molecular-targeted therapy has been used in other neuroendocrine tumors with some success and has been trialed in PC and PGL. Sunitinib, a multikinase receptor antagonist that targets the actions of vascular endothelial growth factors 1 and 2, platelet-derived growth factor receptor and RET, showed partial responses in case reports, providing the basis for an international multicenter trial that is currently underway (First International Randomized Study in Malignant Progressive Pheochromocytoma and Paraganglioma, FIRSTMAPPP, clinical trial registration NCT01371201). This randomized, placebo-controlled, double-blind, phase II trial will evaluate progression-free survival using sunitinib (Ayala-Ramirez et al. 2012a; Jimenez et al. 2009; Joshua et al. 2009; Park et al. 2009). Other experimental agents undergoing clinical trial include everolimus, a mammalian target of rapamycin inhibitor.

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Primary Hyperparathyroidism in MEN2 Syndromes

Maria Alevizaki and Katerina Saltiki

Abstract

One of the components of t*ret*he classical form of MEN2 syndromes is primary hyperparathyroidism (PHP). It occurs in 20–30 % of the typical MEN2A syndrome. The prevalence is more rare in gene carriers as these frequently have familial MTC only. PHP is diagnosed more frequently in association with the exon 11, codon 634 mutation of the *ret* gene—so there is phenotype/genotype correlation. The clinical manifestations of PHP in MEN2 are usually mild and the peak age of diagnosis after the 3rd decade. The treatment is surgical excision of the enlarged gland(s). Although there can be multigland disease in the parathyroids, it is frequently the case that both hyperplasia and adenoma may coexist, or even a single adenoma may be found during the investigation and finally during the operation. Patients with MEN2 syndromes should be screened for PHP with serum calcium measurements. The intensity of the screening should be higher in those carrying the *ret* mutations most frequently associated with this manifestation.

Keywords

Hyperparathyroidism · Sestamibi scan · MEN1 syndrome · MEN2 syndrome · Parathyroid hormone · Hypercalcemia · Hypoparathryroidism · Minimally invasive surgery

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Contents

1 Introduction

Primary hyperparathyroidism (PHP) can occur in a familial form as a component of genetic syndromes with known or unknown etiology. It constitutes one of the clinical manifestations of the multiple endocrine neoplasia syndromes type 1 (MEN1) and type 2 (MEN2). While PHP occurs in the vast majority of cases who have MEN1, it is more rarely associated with MEN2 syndrome. The other components of MEN2 are medullary thyroid cancer (MTC) originating from thyroid C cells and affecting 95–100 % of patients with MEN2 and pheochromocytoma (PHEO) originating from adrenal medulla, affecting 40–50 % of patients with the typical MEN2 syndrome. The association of PHP with the concurrent development of MTC and PHEO which had already been described by Sipple in 1961 was recognized by Steiner et al. who also introduced the term "multiple endocrine neoplasia 2" (Steiner et al. 1968). The pathogenic genetic defect for MEN2 syndrome has been well characterized; it involves mutations in the *ret* proto-oncogene and is inherited in an autosomal dominant manner. In the case of MEN1, where PHP constitutes a major component, the pathogenic defect involves *menin*, which is a classical suppressive oncogene, and loss of heterozygosity is needed. So, the pathogenic mechanism of parathyroid involvement in the two MEN syndromes is different.

The prevalence of PHP has been reported between 20 and 30 % in MEN2A syndromes in various publications (Elisei et al. 2012). Recent large series reported a lower prevalence of only 4.2–8 % in *ret* gene carriers (Frank-Raue et al. 2011; Machens et al. 2013a; Uchino 2012). One possible explanation for this lower occurrence in the most recent series could be the application of genetic testing in patients with MTC which has recently become wider. This has led to the recognition of more familial cases that do not correspond to the classical full blown MEN2 syndrome. The majority of these new cases belong to the "familial MTC" subcategory of MEN2 syndromes which does not include PHP among its manifestations, and thus, the prevalence of PHP has become lower in the whole population of *ret* carriers (Romei et al. 2011; Machens et al. 2013b; Sarika et al. 2015).

In the inherited syndromes where hyperparathyroidism occurs, parathyroid cell hyperplasia frequently precedes the development of adenoma. However, both hyperplasia and adenoma may coexist, or even a single adenoma may be found during the investigation and finally during the operation. In the MEN syndromes,

there is an asynchronous and asymmetric hyperplasia of the parathyroid glands, and therefore, recurrences may occur. The recognition of this fact affects the decision to treat and the protocol to be followed during treatment (see below). In MEN1 syndromes, involvement of all 4 parathyroid glands is more frequent than in the PHP of MEN2, where single-gland adenoma with hyperplasia is more frequent.

2 Clinical Presentation

One of the clinical features characteristic of primary hyperparathyroidism in the context of inherited syndromes, and as such of the MEN2 syndromes, is the younger age at presentation compared to the sporadic form. One recent large study from Germany performed a detailed recording of the peak age at diagnosis of PHP in MEN2 patients, which differs slightly according to the ATA risk level of *ret* mutations. It appears that PHP is diagnosed between 21 and 45 years of age peaking at 36–40 years (Machens et al. 2013a). It has been reported that most of the patients are asymptomatic at PHP diagnosis (Schuffenecker et al. 1998). For this reason, the workup of a patient who is diagnosed with MEN2 syndrome should include investigations for diagnosing this "silent" PHP; these include measurements of serum calcium and albumin, and, when needed, measurement of parathyroid hormone. In the vast majority of MEN2 patients who have the full blown syndrome, MTC and PHEO precede the diagnosis of PHP by several years (Frank-Raue et al. 2011). However, there are few reports where PHP was the first clinical manifestation of MEN2 syndrome (Magalhaes et al. 2011; Mian et al. 2009).

3 Phenotype–Genotype Correlations in MEN2A Syndromes

Several studies have looked at associations between specific *ret* codons and the occurrence of PHP in familial MTC. The majority of studies have shown that there is a genotype/phenotype correlation concerning this manifestation of MEN2 syndrome. In fact, the majority of PHP cases are diagnosed in cases with mutations in exon 11 of the *ret* gene and specifically with the 634 codon mutation (Karga et al. 1998; Kraimps et al. 1996; Raue and Frank-Raue 2009; Valdes et al. 2015). One specific mutation in codon 634 (C634R) is associated with an even higher prevalence (Schuffenecker et al. 1998). Hyperparathyroidism has occasionally been reported in association with mutations in other *ret* gene codons such as codons 611, 618, 620, and 630. The mutations associated with PHP in MEN2 syndromes are presented in Table 1. It is interesting that some of the known *ret* mutations are associated only with MTC and PHEO but never with PHP. Specifically, the exon 16 codon 918 mutation, which is associated with the MEN2B syndrome, has not been associated with the occurrence of PHP. Apparently, the intracellular pathways activated with the various mutations vary, and, for unknown reasons, some of these do not favor the proliferation of parathyroid cells. The epidemiology of PHP within

Table 1 Specific *ret* codons where the coexistence of primary hyperparathyroidism (PHP) has been described in association with inherited medullary thyroid cancer (MTC)

ret codon A/A change in MEN2 syndromes	ATA risk level	Primary hyperparathyroidism
C609F/R/G/S/Y	B	
C611R/G/F/S/W/Y	B	
C618R/G/F/S/Y	B	
C620R/G/F/S/W/Y	B	
C630R/F/S/Y	B	Rare
D631Y	B	Rare
633/9 base pair duplication	B	
C634R	C	
C634G/F/S/W/Y	C	
C634Y/Y791F		Rare
634/12 base pair duplication	B	
S649L	A	Rare
E768D	A	Rare
L790F	A	Rare
Y791F	A	
V804L	A	
V804M	A	Rare
V804M+S904Cc,d	D	
S891A	A	
R912P	A	

The ATA risk level refers to aggressiveness of the MTC. Only a minority of these mutations' carriers develops PHP. Some mutations are only very rarely associated with PHP (*marked in table*). Data taken and modified from: National Cancer Institute database, Genetics of Endocrine and Neuroendocrine Neoplasias, genotype/phenotype correlations, and risk stratification www.cancer.gov

the MEN2 syndrome is difficult to elucidate, probably because its occurrence is rather rare and the diagnosis is dependent on various factors that are difficult to correct for (Machens et al. 2013b).

4 Diagnosis

The diagnosis of PHP in MEN2 does not differ from that in the sporadic form of the disease. Calcium levels corrected for albumin are abnormally elevated, and serum phosphate may be low. Parathyroid hormone measurement will confirm the diagnosis in case of hypercalcemia. Secondary hyperparathyroidism, usually due to vitamin D deficiency, should be excluded before the diagnosis of PHP can be made. This is also recommended in the European Thyroid Association Guidelines (Elisei et al. 2012). Localization studies include high-resolution ultrasound by an experienced observer and parathyroid scan using sestamibi. As in other cases of PHP, when the disease is asymptomatic, it is important to investigate whether any of the

complications accompanying PHP are present, such as hypercalciuria, nephrolithiasis, and osteoporosis. When the diagnosis of PHP is made before that of MTC, a combined thyroidectomy parathyroidectomy is performed (Elisei et al. 2012).

5 Treatment

MEN2 associated PHP is usually benign. The treatment of PHP is surgical. The decision on the surgical procedure to be followed is important because recurrence or persistence of disease may occur in cases where multiple gland involvement may go unnoticed. In the management of PHP in MEN syndromes in general, it is important to recognize single gland versus multiple gland involvement. Removal only of the enlarged glands is usually associated with good results (Kraimps et al. 1996; Dotzenrath et al. 2001; O'Riordain et al. 1993; Scholten et al. 2011). Over the years, several surgical procedures have been applied for the management of PHP, and these have recently been reviewed (Scholten et al. 2011). In some centers, the parathyroidectomy is performed together with the initial surgery for MTC (Scholten et al. 2011; Yoshida et al. 2009).

The surgical procedures that have been used are as follows: total parathyroidectomy with autotransplantation, subtotal parathyroidectomy, single-gland removal, or removal of only enlarged glands. The procedure to be followed depends on the number of glands apparently involved. As in other cases of PHP, the skills of the surgeon are of importance and high-volume surgeons should be undertaking the parathyroidectomy. Because of the possible involvement of multiple glands, all parathyroids should be identified during the operation. Results after surgery for PHP from various centers are shown in Table 2.

In the majority of young patients, the hyperparathyroidism is "asymptomatic" (Carling and Udelsman 2005). Some series report non-operations for PHP. This view is based on the fact that there is a substantial rate of both permanent hypoparathyroidism as well as recurrent/persistent hyperparathyroidism in most published series (Kraimps et al. 1996; Herfarth et al. 1996). This complication usually occurs after total parathyroidectomy. However, hypoparathyroidism has

Table 2 Recurrence/persistence of disease after surgery performed for primary hyperparathyroidism (PHP) in MEN2 syndrome according to the type of operation

			Type of parathyroidectomy		
Author (year)	*n*	Follow-up (years)	Selective	Subtotal	Total
O'Riordain et al. (1993)	18	5.8	0/9	0/7	0/2
Raue et al. (1995)	60	8	4/28 (14)	2/21 (9)	2/11 (18)
Herfarth et al. (1996)	34	11.4	6/21 (29)	2/8 (25)	0/5
Kraimps et al. (1996)	56	6.4	6/29 (21)	3/12 (25)	1/11 (9)
Dotzenrath et al. (2001)	7	3.5	2/5 (40)	0/1	0/1
Scholten et al. (2011)	16	9.6	1/6 (17) (MIP)	1/4 (25)	1/6 (17)

MIP minimally invasive parathyroidectomy

also been reported after subtotal parathyroidectomy, or even after excision of 2 glands (Herfarth et al. 1996). Re-operation can be performed when recurrent disease is diagnosed, but this is not always followed by cure. In these patients who have usually undergone thyroidectomy for the MTC, re-operation may occasionally be needed; satisfactory results have been reported in those with previous neck surgery even using minimally invasive methods (Dimas et al. 2012). In recent years, less invasive methods are frequently used (Scholten et al. 2011).

Successful use of calcimimetics has been reported in cases of disease persistence (Marcocci et al. 2009). The use of total parathyroidectomy with autotransplantation at the time of primary surgery is popular in some centers (Herfarth et al. 1996; Yoshida et al. 2009) but is not routine practice in Europe (Kraimps et al. 1996; Raue et al. 1995; Scholten et al. 2011).

6 Recommendations for Screening in *ret* Carriers

The American Thyroid Association (ATA) has suggested in the most recently published guidelines that all *ret* carriers should be screened for PHP. The various *ret* mutations have been classified according to the risk of aggressive MTC in risk groups A, B, C, and D, with the group D corresponding to the highest risk and the group A to the lowest risk. The ATA proposes to start the screening by age 8 in the cases carrying mutations in those codons (class C mutations) which are frequently associated with the occurrence of PHP (Kloos et al. 2009; Schuffenecker et al. 1998). It has been suggested that this may be too early especially because the overall median age at presentation appears to be 38 years (Alevizaki 2013; Carling and Udelsman 2005). Very few cases of PHP present before the third decade, and PHP appears to be generally mild in young subjects with MEN2 syndrome (Schuffenecker et al. 1998). The choice of the age for screening should probably take into account the earliest age and risk associated with the specific mutation. PHP may appear at 28 years in those carrying ATA class B mutations and at 38 years in class A mutations according to the ATA risk stratification (Frank-Raue et al. 2011; Kloos et al. 2009). The *frequency* with which patients with *ret* mutation should be screened for PHP has been discussed, and age and codon adjusted recommendations should probably be suggested for this issue (Alevizaki 2013; Machens et al. 2013b). Yearly checking is probably optimal for those carrying *ret* mutations in codons conferring high risk for PHP, as the calcium estimation is an inexpensive and simple blood test.

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Surgical Treatment of Medullary Thyroid Cancer

Andreas Machens and Henning Dralle

Abstract

Medullary thyroid cancer (MTC) can vary in tumor biology and progression. The most important indicator of distant metastases, determining clinical outcome, is lymph node metastasis to the neck and mediastinum. Surgical cure is within reach in node-negative tumors or node-positive tumors with fewer than 10 lymph node metastases. From a surgical point of view, compartment-oriented lymph node dissection, clearing gross, and occult metastases are important for locoregional tumor control. The discovery of missense germline mutations in the RET proto-oncogene and the close genotype-phenotype correlation in hereditary MTC promoted the worldwide breakthrough of prophylactic thyroidectomy. The best approach to hereditary MTC affords the DNA-based/biochemical concept, which is geared at limiting prophylactic surgery to total thyroidectomy at minimal surgical morbidity before the tumor can spread beyond the thyroid capsule. To improve outcome, routine calcitonin screening in nodular thyroid disease and DNA-based screening of the offspring in RET families are effective interventions.

Keywords

Medullary thyroid cancer · Lymph node metastases · Compartment-oriented lymph node dissection · RET proto-oncogene · Genotype-phenotype correlation · Prophylactic thyroidectomy

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Contents

1 Introduction

'Medullary' carcinoma of the thyroid was not completely unfamiliar to surgeons and pathologists in the nineteenth century (Gurlt 1861; Billroth 1869; Wölfer 1883; Stoffel 1910). Just 100 years ago, Burk (1901) gave a detailed account of a patient who had succumbed to this neuroendocrine malignancy. In his doctoral thesis submitted in 1901 to the Medical Faculty of the University of Tübingen, Germany, Burk reported a 12-year-old boy of '*slight build*' with 'an '*acute infrasternal angle'* and '*bumpy lips'* who had died of a '*metastatic amyloid tumor of the thyroid gland.'* The patient's age, phenotype, and the extent of metastatic disease support the notion that this was the first description of medullary thyroid cancer (MTC) in the context of multiple endocrine neoplasia type 2B (MEN 2B), a syndrome unrecognized at the time (Table 1).

It took another 60 years before MTC was classified as a tumor entity in its own right, setting it apart from follicular cell-derived thyroid cancer (Horn 1951; Hazard et al. 1959). The advent of immunohistochemical and radioimmunochemical techniques in conjunction with meticulous clinical observation resulted in the discovery of the hormone calcitonin in the 1960s (Copp 1962); the delineation of the parafollicular C(-alcitonin producing) cells within the thyroid gland (Foster et al. 1964; Pearse and Polak 1971); the development of sensitive radioimmunoassays for the determination of serum calcitonin (Tashjian and Melvin 1968; Tashjian et al. 1970); and the characterization of a heritable syndrome that is now commonly referred to as MEN 2B (Sipple 1961; Manning et al. 1963; Williams 1965).

Thirty years later, with increasing sophistication of molecular genetic technology, the RET proto-oncogene was identified in 1993 on chromosome 10q11.2 as the susceptibility gene of hereditary MTC (Donis-Keller et al. 1993; Mulligan et al.

Table 1 Milestones of medullary thyroid cancer

Period	Milestone
Mid-nineteenth century	Case reports on medullary thyroid cancer (MTC) as a rare tumor entity among young adults within the spectrum of thyroid cancers (Gurlt 1861; Billroth 1869; Wölfer 1883)
Turn of the century (~1900)	First case reports of MTC containing amyloid (Burk 1901; Jacquet 1906; Stoffel 1910), one of which is believed to represent the first description of MTC as a syndromic component of MEN 2B unrecognized at the time (Burk 1901)
1950s	Histopathological characterization of MTC (Horn 1951; Hazard et al. 1959)
1960s	Recognition of the parafollicular C-cells including its secretory product calcitonin (Copp 1962; Foster et al. 1964; Pearse and Polak 1971); first description of a radioimmunoassay for calcitonin (Tashjian et al. 1970); recognition of MTC as a syndromic component of MEN type 2 (Sipple 1961; Manning et al. 1963; Williams 1965)
1993	Identification of the susceptibility gene of hereditary MTC: heterozygous missense germline mutations in the RET proto-oncogene (Donis-Keller et al. 1993; Mulligan et al. 1993)
1994	Establishment of prophylactic thyroidectomy as definitive treatment of hereditary C-cell disease (Lips et al. 1994; Wells et al. 1994; Dralle et al. 1998)

1993). This seminal discovery paved the way for pre-emptive surgery, notably prophylactic thyroidectomy in asymptomatic gene carriers, eliminating the risk of MTC once and for all (Lips et al. 1994; Wells et al. 1994; Dralle et al. 1998; Machens et al. 2003; Skinner et al. 2005). In the following, the term 'prophylactic' is used to define removal of the thyroid gland before MTC develops or while it is clinically inapparent and confined to the thyroid gland.

MTCs differ in many ways from follicular cell-derived papillary, follicular, poorly differentiated, or undifferentiated thyroid cancers: not only in terms of embryological background, morphology, and causation but also functionally and regarding tumor biology. Because medullary cancer cells lack the sodium-iodine symporter necessary to concentrate iodine, radioiodine therapy remains ineffective in MTC so that surgery remains the mainstay of treatment.

All in all, MTC accounts for 5–10 % of all malignant thyroid tumors. Some 25–30 % of patients with MTC harbor missense germline mutations in the RET proto-oncogene. These mutations can be passed on to offspring, giving rise to MTC in a mutation- and age-dependent fashion. Hereditary, unlike sporadic, MTC can be connected to other neuroendocrine tumors, pheochromocytoma, and parathyroid hyperplasia or adenoma (primary hyperparathyroidism).

MTC has a proclivity for spreading early to lymph nodes and distant organs so that it is crucial to make the diagnosis as soon as possible. This is why timely diagnosis and surgical removal of the primary thyroid tumor along with the local lymph nodes take center stage in improving clinical outcome in MTC.

2 Clinical Work-Up

The availability of sensitive calcitonin assays, RET gene analysis, and high-resolution imaging (ultrasonography, computed tomography/magnetic resonance imaging, positron emission tomography) opened up three diagnostic avenues: (a) early recognition of sporadic MTC using calcitonin screening in patients with thyroid nodular disease; (b) RET gene analysis in kindred from known RET families; and (c) imaging for the staging of patients with clinically apparent MTC.

2.1 Early Recognition of Sporadic MTC Through Calcitonin Screening in Patients with Thyroid Nodular Disease

Calcitonin, procalcitonin, and to a lesser extent carcinoembryonic antigen (CEA) serum levels correlate fairly well with overall tumor mass. This is why serial measurements of these biomarkers afford early diagnosis of occult MTC in nodular thyroid disease and help determine the dynamics of tumor growth and progression in persistent disease. In patients with nodular thyroid disease, calcitonin screening has a greater sensitivity and specificity for MTC than fine-needle aspiration (Elisei et al. 2004). The widespread use of biochemical screening, coupled with early surgical intervention, was associated with a reduction in primary tumor diameter at first diagnosis (Machens and Dralle 2010b), and better clinical outcome of patients with sporadic MTC (Elisei et al. 2013). These results prompted several professional societies, including the German Association of Endocrine Surgeons and the European Thyroid Association, to recommend determination of calcitonin serum levels for routine use in patients with nodular thyroid disease (Karges et al. 2004; Elisei et al. 2012; Dralle et al. 2013b).

When basal calcitonin serum levels slightly exceed the upper normal limit of the assay, intravenous calcitonin stimulation tests with pentagastrin or calcium can facilitate distinction between C-cell hyperplasia and MTC (Elisei et al. 2004; Karges et al. 2004; Milone et al. 2010; Kratzsch et al. 2011), buttressing the case for surgery. In making that determination and planning, the extent of resection, the characteristics of calcitonin testing (Chambon et al. 2011; Doyle et al. 2009), gender and age dependencies (Machens et al. 2009a, b; Machens and Dralle 2009), and exceptionally calcitonin-secreting neuroendocrine primaries outside the thyroid gland (Machens et al. 2000b, c) need to be taken into account. Because the risk of MTC is increased beyond the 100 pg/mL mark after stimulation, thyroidectomy is generally advised for adults with stimulated calcitonin levels above that biochemical threshold (normal range of basal serum calcitonin <10 pg/mL) after due consideration to the gender-specific reference range of the calcitonin assay (Karges et al. 2004).

2.2 Screening for Gene Carriers from Established RET Families

Germline mutations in the RET proto-oncogene, inherited in an autosomal-dominant manner, may cause MTC in isolation or in concert with other neuroendocrine tumors. The time of malignant progression from C-cell hyperplasia to hereditary MTC and development of pheochromocytoma and parathyroid hyperplasia or adenoma is genetically encoded and largely framed by the underlying RET mutation (genotype–phenotype correlation) (Machens et al. 2003, 2005a, 2009b, 2013; Wells et al. 2015).

The risk of disease is extreme for MEN 2B [Online Mendelian Inheritance in Man (OMIM) #162300] patients harboring the M918T RET mutation that typically gives rise to MTC in early infancy and less frequently pheochromocytoma in adolescence or adulthood (Brauckhoff et al. 2004, 2008, 2014; Dralle et al. 2013a). Primary hyperparathyroidism is not an integral element of MEN 2B. Germline mutations in codon 634 most often underlie MEN 2A (OMIM #171400), encompassing MTC, pheochromocytoma, and primary hyperparathyroidism (Dralle et al. 2013a). Other RET mutations, specifically those in codons 533, 609, 611, 618, 620, 768, 790, 791, 804, and 891, carry moderate risks of disease and present with what clinically appears to be familial MTC (FMTC; OMIM #155240), an entity along the spectrum of disease expression in MEN 2A.

For MEN 2A, annual tumor growth in the thyroid gland was estimated at 0.4–0.5 mm in node-negative carriers with mutations in codon 634 (77 patients) and in codons 768, 790, 791, 804, or 891 (95 patients) (Machens et al. 2014a). In node-positive carriers, primary tumor growth was 2.6 mm per year for the former and 1.2 mm per year for the latter, more than 6-fold (2.6 vs. 0.4 mm) and more than 2-fold greater (1.2 vs. 0.5 mm) than in their node-negative peers. Node-positive carriers revealed an annual rate of lymph node metastasis of 0.6–0.7 nodes (Machens et al. 2014a).

Because lifetime risk of MTC for *RET* carriers can be >90 %, pre-emptive (‘*prophylactic*’) thyroidectomy is recommended worldwide for the condition (Lips et al. 1994; Wells et al. 1994; Dralle et al. 1998; Machens et al. 2003; Machens and Dralle 2009; Machens et al. 2009b; Wells et al. 2015) since the first RET germline mutations was detected in 1993 (Donis-Keller et al. 1993; Mulligan et al. 1993). Performance of preemptive thyroidectomy is contingent on prior evidence of one of the recognized RET mutations. Yet it is the patient’s basal calcitonin level, in conjunction with the position of his or her RET mutation and age that delimits the surgical window of opportunity. Based on the literature review, lymph node metastases may not be present as long as the basal calcitonin is <40 pg/mL (Wells et al. 2015). In this respect, it is worthy of note that children younger than 6 months of age may have a calcitonin reference range <40 pg/mL, which is reduced to <15 pg/mL in children between 6 months and 3 years of age (Basuyau et al. 2004). Children older than 3 years of age have the same calcitonin reference range than adults (<10 pg/mL).

Delaying thyroidectomy past that genetically encoded window of opportunity carries the risk that the MTC may spread beyond the thyroid gland.

2.3 Staging of Clinically Apparent MTC

Tumor staging is necessary to determine the extent of the operation as soon as the diagnosis of MTC has been established through fine-needle aspiration cytology or tissue biopsy from the primary tumor or neck nodes or based on excessive basal calcitonin levels. Depending on the age and inherited RET mutation, gene carriers also need a clinical work-up for associated pheochromocytoma and primary hyperparathyroidism (Machens et al. 2013). In patients with locally advanced MTC, invasion of the aerodigestive tract should be ruled out using computed tomography or magnetic resonance imaging. A clinical work-up for distant metastases should follow suit when imaging cannot clarify the technical resectability of tumor from the neck or mediastinum. Clinically apparent MTC has frequently spread to loco-regional lymph nodes. To reliably identify occult lymph node metastases, ultrasonography is not sensitive enough, certainly not for nodes lodging near or behind the thyroid gland or residing in the upper mediastinum. Owing to this methodological shortcoming, surgical treatment plans should be informed by the level of the basal serum calcitonin, as detailed below (central and lateral lymph node dissection only in the ipsilateral or also in the contralateral neck).

3 Sporadic MTC

3.1 Thyreoidectomy

Clinical evidence of MTC should prompt total thyroidectomy unless the thyroid primary is technically irresectable or the patient is inoperable. This line of reasoning is supported by the fact that (i) most patients' RET gene status is unknown before the initial operation (RET gene carriers always need total thyroidectomy because of the inherent risk of tumor multifocality), and (ii) multifocal tumors are present in some 10 % of patients with sporadic disease (Machens et al. 2007a). Under exceptional circumstances, lobectomy removing the thyroid primary can be adequate if and when (i) the diagnosis of MTC was made only after the operation, (ii) postoperative basal and stimulated calcitonin serum levels are within the normal range of the assay, and (iii) RET gene analysis comes back negative (Miyauchi et al. 1988, 2002).

3.2 Lymph Node Dissection

The absence of effective therapies other than surgery makes a strong case for the dissection of lymph nodes that are clinically manifest, visible on imaging, or suspect or involved on fine-needle aspiration cytology or tissue biopsy. For oncological reasons, this lymph node dissection must be commensurate with the extent of nodal spread and be carried out in a systematic fashion so that occult lymph node

metastases are not missed along the path of lymphatic dissemination. Because they frequently result in inferior tumor control (Dralle et al. 1994; Moley and DeBenedetti 1999; Scollo et al. 2003), selective forms of lymph node dissection have no role outside extraordinary settings like progressive distant disease.

MTC, having a propensity for occult lymph node metastases, always brings up the issue of lymph node dissection once the diagnosis has been confirmed. Although the case for systematic lymph node dissection is straightforward for gross nodal disease, it is less so for occult nodal disease. In the latter event, the risk of surgical morbidity must be carefully weighed against the risk of morbidity from the residual disease (Dralle and Machens 2013), which is negligible with slightly elevated postoperative calcitonin levels. Even though larger primary tumors and higher calcitonin serum levels often herald progressive disease (Machens et al. 2005b; Machens and Dralle 2010a), the pace of tumor progression cannot be predicted for a given patient. There is also evidence for a strong relationship between the number of lymph node metastases and the risk of distant metastasis, with 11–20 involved nodes implying intermediate risk and >20 involved nodes implying high risk (Machens and Dralle 2013a), and overall survival (Esfandiari et al. 2014). In a National Cancer Database study of 2968 patients with MTC, survival rates were 90, 76, 74, 61, 69, and 55 % for patients with lymph nodes resected and negative, cervical lymph nodes not resected, and 1–5, 6–10, 11–15, and ≥16 cervical lymph node metastases, respectively. More extensive neck dissection may improve survival in patients with slow-growing distant metastases, potentially preventing earlier death from locally invasive disease (Esfandiari et al. 2014).

Noteworthy are the strong correlations between the preoperative levels of basal calcitonin (Machens and Dralle 2010a) and procalcitonin (Machens et al. 2014b) serum levels on one hand, and primary tumor size, the number of lymph node metastases, distant metastases, and biochemical cure rates are on the other.

Basal calcitonin levels <20 pg/mL generally are unassociated with central neck nodes, rendering prophylactic central neck dissection unnecessary in the absence of clinically or ultrasonographically suspect nodes (Machens and Dralle 2010a). When basal calcitonin levels ranged between 20 and 200 pg/mL, the central and lateral compartments ipsilateral to the affected thyroid lobe were frequently involved, indicating a need for dissection of the central and lateral neck nodes on the side of the thyroid primary. Between 200 and 500 pg/mL, the lateral neck nodes on the opposite side were involved in 14 % of patients. Whether this rate warrants prophylactic dissection of these nodes or possibly a two-stage procedure for completion needs to be discussed with the patient, balancing the pros and cons of either approach (Dralle et al. 2013a; Machens and Dralle 2013b). Above the 500 pg/mL mark, upper mediastinal nodes were increasingly involved, as were distant organs.

When preoperative basal calcitonin serum levels exceed 200 pg/mL, a two-stage dissection of the opposite lateral neck (if this is the clinically inapparent left side of the neck) becomes an attractive option when the tumor arises from the right thyroid lobe. Lateral neck dissections in the right lateral neck entail a much lower risk of lymphatic leakage than in the left lateral neck where these complication rates may be as high as 3–8 % (Lorenz et al. 2010). In this specific scenario, it may be prudent

Table 2 Extent of lymph node dissection in sporadic MTC depending on preoperative basal calcitonin and primary tumor size

Basal calcitonin (<10 pg/mL)	<20	20–50	50–200	>200
Primary tumor diameter (mm)	<3	3–5	5–10	>10
Lymph node dissection (one-stage)	None	Ipsilateral central and ipsilateral lateral neck	Bilateral central and ipsilateral lateral neck	Bilateral central and bilateral lateral neck
Lymph node dissection (two-stage)				
Initially		Ipsilateral central neck	Bilateral central neck	Bilateral central and ipsilateral lateral neck
For completion (if needed)		Ipsilateral lateral neck	Ipsilateral lateral neck	Contralateral lateral neck (e.g., for right-sided primary tumors)

to refrain from prophylactic left-sided lateral lymph node dissection during the initial operation unless extensive nodal disease should be present in the central and right-sided lateral neck (Table 2).

With ***procalcitonin*** levels ≤1.0 ng/mL, lymph node metastases were present in the ipsilateral lateral neck, and with procalcitonin levels ≤0.25 ng/mL also in the ipsilateral central neck (Machens et al. 2014b). Above a procalcitonin threshold of 1.0 ng/mL, lymph node metastases emerged in the contralateral central and lateral neck, and above 5.0 ng/mL also in the upper mediastinum. When procalcitonin levels exceeded 1, 5, 10, and 50 ng/mL, biochemical cure rates declined to no more than 71, 36, 23, and 10 %, respectively (Machens et al. 2014b).

In the absence of infection, procalcitonin has diagnostic accuracy comparable to basal calcitonin across a wide spectrum of the disease. Because it does not need to be kept cool on ice or frozen during the entire process chain, procalcitonin is easier to manage at the community level (Machens et al. 2014b).

4 Hereditary MTC

4.1 Prophylactic Thyroidectomy in Asymptomatic Gene Carriers

The framework within which malignant transformation from C-cell hyperplasia to MTC develops is genetically encoded and largely dependent on the respective *RET* mutation, more specifically the affected codon (Machens et al. 2003; Machens et al. 2005a, 2009b, 2013). Among gene carriers from the same family, time to malignant

transformation and tumor progression within that genetically encoded frame (also dubbed 'window of opportunity') may vary greatly because of the play of chance or the effect of ill-defined 'modifying factors.' This is why age thresholds alone, prompting overtreatment as well as under treatment despite the close genotype-phenotype relationship, are unsuitable to delineate the optimal point in time for prophylactic thyroidectomy in individual gene carriers (Machens et al. 2009b). For this purpose, calcitonin serum levels, forming an integral element of the DNA-based/biochemical concept put forth in 2009 (Machens et al. 2009b), are more useful. In the presence of basal calcitonin serum levels within assay limits, pre-emptive thyroidectomy alone was never associated with increased postoperative calcitonin levels, regardless of the underlying RET mutation (Machens et al. 2009b; Elisei et al. 2012). In this setting, the thyroid primaries, having not been given enough time to grow larger and spread to lymph nodes, were still confined to the thyroid gland. RET carriers whose basal calcitonin serum levels are normal hence do not need lymph node dissection in addition, sparing them the incremental risk of hypoparathyroidism attendant to the procedure.

As a rare exception to the rule, carriers of a germline mutation in codon 918 need to undergo total thyroidectomy in early infancy. In MEN 2B, MTC develops so early that is surgically curable only within the first four years of life—barring unusual instances of cure in older children (Brauckhoff et al. 2004, 2008, 2014). Most M918T mutations arise de novo in >90 % of patients so that the family history is negative for MEN 2B most of the time. It is therefore of utmost importance to promptly recognize the premonitory symptoms characteristic of MEN 2B: '*crying without tears'* and severe constipation, conceivably caused by the increasing proliferation and thickening of corneal and gastrointestinal nerve sheaths (Dralle et al. 2013a).

On a technical note, preemptive thyroidectomy in children is demanding. Importantly, it should not be undertaken by surgeons who do not have the necessary surgical expertise at their disposal. Small children often reveal a very large thymus occupying a large portion of the neck, which occasionally is bigger than the thyroid gland itself. Great strides should be made to preserve the thymus, because it may house the lower parathyroid glands (Fig. 1). In a similar vein, pediatric parathyroids can differ tremendously from adult parathyroids in terms of location (intrathymic), size (smallness), and color (translucency), making it more difficult to distinguish them from the adjacent tissues (Brauckhoff et al. 2014). In an effort to minimize the risk of lifelong morbidity, it is critical to identify, with the aid of basal calcitonin serum levels, the best time for pre-emptive thyroidectomy (not too early, not too late), optimizing the chance of preserving the parathyroid glands.

4.2 Lymph Node Dissection in Symptomatic Gene Carriers and Index Patients with Hereditary MTC

As far as the pattern of lymphatic spread and clinical outcome are concerned, there is no difference between gene carriers with hereditary MTC and patients with sporadic MTC if equally large tumors are compared with each other (Fig. 2).

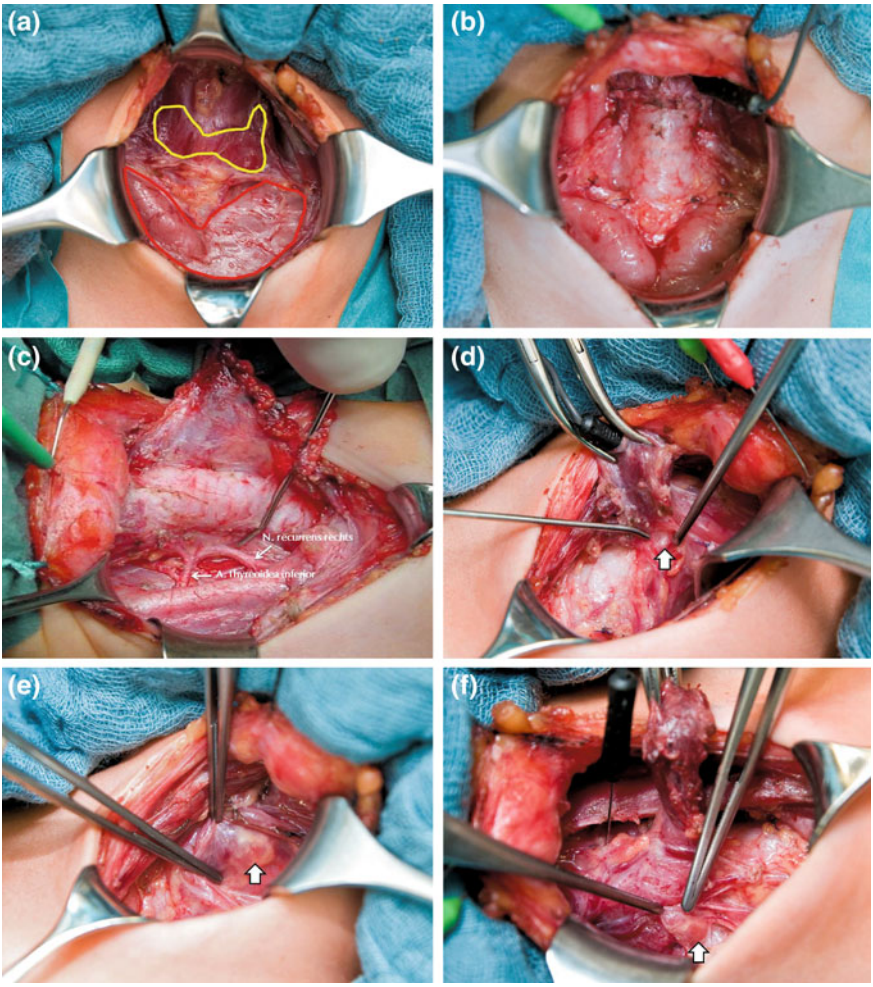

Fig. 1 Surgical anatomy of young children before and after prophylactic thyroidectomy: 4-year-old girl harboring a RET mutation in codon 634 (**a**, **b**, **d**, **e**, **f**), and 19-month-old boy with MEN 2B (**c**). Thymus (contoured in *red*) and thyroid gland (contoured in *yellow*) before (**a**) and after thyroidectomy (**b**); Recurrent laryngeal nerve in MEN 2B (**c**; thickened) and MEN 2A (**d**; normal-sized); Left upper (**e**) and right lower (**f**) parathyroid gland (*arrows*)

As detailed above in Sect. 3.2, it is recommended to perform total thyroidectomy with dissection of the central and lateral nodes on either side of the neck when the basal calcitonin serum levels are >200 pg/mL.

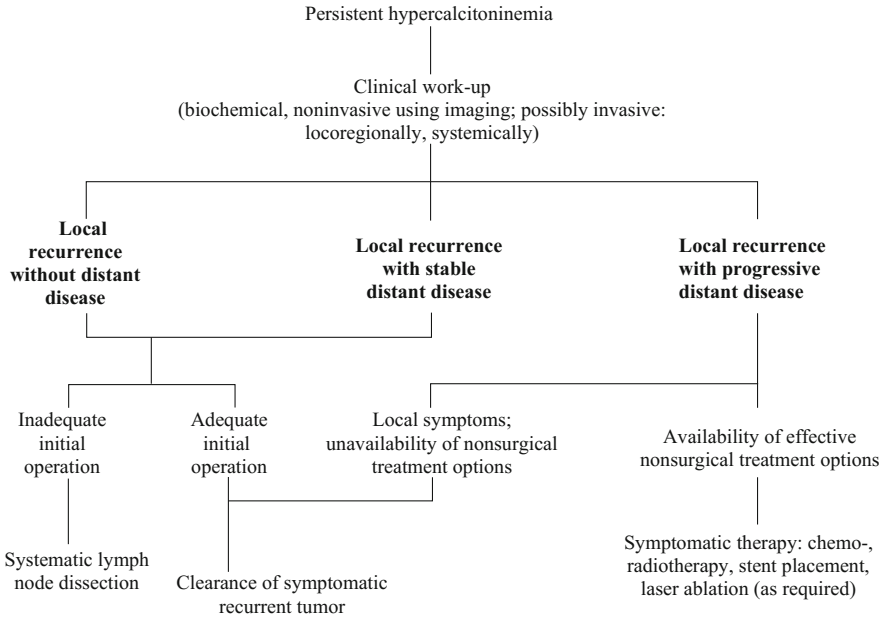

Fig. 2 Therapeutic algorithm for persistent hypercalcitoninemia

5 Reoperation for Recurrent or Persistent MTC

Because calcitonin is a highly sensitive tumor marker, postoperative evidence of elevated calcitonin level in a patient with histologically confirmed MTC heralds persistent disease. With the exception of poorly secreting MTC seen in 0.83 % of patients with MTC (Frank-Raue et al. 2013), the level of serum calcitonin accurately reflects overall tumor mass (also referred to as 'tumor burden').

Patients with residual lymph node metastases after initial thyroidectomy are likely to benefit from reoperation, as recently detailed (Machens and Dralle, Ann Surg 2013b). In 59 (44 %) of 133 patients who had no lymph node metastases removed at the initial operation, systematic central and lateral lymph node dissection attained biochemical cure. Conversely, biochemical cure was reached in only 12 (18 %) of 65 patients in whom 1–5 lymph node metastases had been previously cleared. If >5 lymph node metastases were dissected at prior surgery, the biochemical cure rate fell to 5 % (2 of 43 patients). When preoperative serum calcitonin levels exceeded 1000 pg/mL, biochemical cure was exceptional (1 of 76 patients). Based on these data, systematic lymph node dissection in patients who had inadequate neck surgery is worthwhile as long as the preoperative serum calcitonin level is <1000 pg/mL and no more than five lymph node metastases were removed. Beyond these thresholds, the focus of surgical treatment shifts to the maintenance of local control in the neck (Machens and Dralle 2013b).

Failure of elevated calcitonin serum levels to normalize after neck surgery signals persistent disease. This common phenomenon is encountered in >60 % of patients with MTC. Most occult calcitonin-secreting tumor cell deposits hide in lymph nodes and distant organs after lymphatic and hematogenous metastasis. MTC often grows into progressive metastatic disease before becoming clinically manifest or causing symptoms because of the space assumed or the onset of profuse diarrhea or hormonal symptoms. Less than 1 % of all patients with MTC, usually those with bulky disease, reveal hypercortisolism as a paraneoplastic syndrome with or without ectopic secretion of cortisol-releasing hormone (CRH) or adrenocorticotropic hormone (ACTH) (Barbosa et al. 2005). As long as metastatic MTC remains asymptomatic, 10-year survival rates are in excess of 80 %. This is why the need for reoperation must be deliberated in light of the circumstances of the case: more specifically, the patient's individual risk, type and extent of the preceding operations, and localizing studies of the recurrent tumor (Machens and Dralle 2013b).

Meticulous lymph node dissection will not result in normalization of postoperative serum calcitonin in as many as 40 % of patients with node-negative MTC and in as many as 90 % of patients with node-positive MTC (Machens et al. 2005b). Yet, many of these patients live on reaching 5-year and 10-year survival rates of 60–90 % (Pellegriti et al. 2003; Machens et al. 2007b). Because distant metastases visible on imaging are the single most determinant of cancer-specific mortality in MTC (Esik et al. 2002), radiological evidence of distant metastasis, in conjunction with progressive disease, can be a game changer, rendering not worthwhile an otherwise beneficial reoperation. Unlike reoperation in the neck that may be curative, chemoembolization (Lorenz et al. 2005), external beam radiation, radioligand therapy, chemotherapy, targeted therapies, and supportive medical treatment all are palliative forms of treatment.

5.1 Radiological Screening for Recurrent Disease

Conventional imaging methods, such as high-resolution ultrasonography in connection with fine-needle aspiration cytology, computed tomography, and magnetic resonance imaging, continue to be the mainstay of imaging for recurrent disease. Recently, advanced noninvasive radiological modalities, including ^{18}F-fluoro-deoxyglucose (FDG), ^{18}F-dihydroxyphenylalanine (F-DOPA), and somatostatin receptor positron emission tomography co-registered with computed tomography (PET/CT), have gained momentum because they diminish the need for invasive procedures such as selective venous catheterization, angiography, laparoscopy, and thoracoscopy (Ben et al. 1989; Frank-Raue et al. 1992; Tung et al. 1995; Mirallié et al. 2005; Szavcsur et al. 2005). These advanced technologies, pinpointing tumor deposits that are no larger than a few millimeters in size, have revolutionized the screening for recurrent disease.

These techniques not only enhanced the localization of small metastatic tumor deposits but also disclosed that up to 90 % of patients with elevated postoperative

calcitonin levels harbor previously undetectable systemic disease outside the neck (Mirallié et al. 2005; Szavcsur et al. 2005). Contrary to initial assumptions, a much higher percentage of those patients who end up with increased calcitonin serum levels in spite of adequate neck surgery set out with systemic disease.

5.2 Need for and Extent of Reoperation for Recurrent Disease

Because distant metastases in MTC occur almost never in isolation, there is rarely a need to extirpate tumor deposits from distant organs. Emblematic example includes resection of dominant liver and lung metastases or dissection of parahilar lymph nodes compromising the bronchial tree. Palliative surgery for metastatic MTC can make sense when (i) nonsurgical therapies cannot alleviate the condition; (ii) the patient is in reasonably good shape; and (iii) the surgical intervention offers at least temporary relief. To qualify, patients must meet all three preconditions, making surgical palliation of distant metastases a highly individual enterprise.

Although the patient's physical condition can enforce modification of the surgical treatment plan, three clinical scenarios of locoregional recurrent disease should be distinguished:

- Without radiological evidence of distant disease;
- With radiological evidence of stable distant disease; and
- With radiological evidence of progressive distant disease.

The surgical approach to recurrent thyroid disease is outlined in the treatment algorithm of Fig. 2. No reoperation is required for completion of an inadequate initial operation when stimulated calcitonin serum levels stay within the normal limits and the MTC is sporadic (Miyauchi et al. 2002). For hereditary MTC, it is essential to perform completion thyroidectomy to eliminate the malignant potential of the C-cells left behind in the thyroid remnant. When calcitonin serum levels fail to normalize after an incomplete initial operation, systematic (i.e., compartment-oriented) lymph node dissection should be undertaken for the completion of occult metastatic disease even in the absence of clinically apparent recurrent or persistent disease (Tisell et al. 1986; Machens and Dralle 2013b). Fewer than 50 % of patients reach biochemical cure through systematic lymph node dissection when basal calcitonin levels are ≤1000 pg/mL after the removal of ≤5 lymph node metastases (Machens and Dralle 2013b). This fact needs to be detailed during the informed consent discussion.

Mediastinal re-exploration or lymph node dissection via the transsternal route is only warranted on clinical evidence of recurrent tumor at the cervical–mediastinal junction or within the upper anterior mediastinum (Machens et al. 1999, 2004; Dralle 2002). Subject to technical feasibility and operability of the patient, resection of the aerodigestive tract for tumor invasion hinges on (i) the absence of progressive distant disease and (ii) the unavailability of nonsurgical treatment modalities for control of advanced tracheobronchial or esophageal obstruction (Chen et al. 1998; Machens et al. 2001a, b). In the event of major tumor progression causing

symptoms locally, palliative nonsurgical therapies, such as external beam radiation, stent placement, or laser ablation, should be used preferentially.

It remains unclear to which extent reoperations for locoregional tumor lower the risk of subsequent recurrent disease, extending recurrence-free survival, or prolong overall survival. The small numbers of patients with recurrent disease together with the variable presentation of the condition, thwarting standardization, perhaps are the greatest impediment to address this question in a prospective clinical trial. Because clinical outcome correlates with the level of serum calcitonin after neck surgery (Miyauchi et al. 1988; Machens et al. 2013b), reoperations for locoregional recurrent disease are set to be beneficial, at least in the absence of gross distant disease.

6 Synopsis

Apart from being uncommon, MTC can vary much in tumor biology and progression. The most important determinant of distant metastases, shaping a patient's prognosis, is lymph node metastasis to the neck and mediastinum. Surgical cure, defined as normalization of postoperative calcitonin serum levels, is within reach in node-negative tumors or node-positive tumors with fewer than 10 lymph node metastases (Machens et al. 2000a; Scollo et al. 2003). It may be important to note that (i) routine calcitonin screening in nodular thyroid disease and (ii) DNA-based screening of the offspring of known RET families are paramount in improving the clinical outcome of patients with MTC. From a surgical point of view, compartment-oriented lymph node dissection, clearing gross and occult metastatic disease, has been instrumental in keeping recurrence rates low (Dralle et al. 1994; Machens et al. 2007b).

The discovery of missense germline mutations in the RET proto-oncogene and the close genotype–phenotype correlation in hereditary MTC promoted the worldwide breakthrough of the concept of prophylactic thyroidectomy. Previous recommendations regarding the optimal timing of prophylactic thyroidectomy used to be largely predicated on carrier age alone. The use of age thresholds for timing of thyroidectomy alone, based on '*worse case scenarios,*' precipitated overtreatment of gene carriers. Not heeding age-based thresholds conversely can cause undertreatment, necessitating more extensive surgery. These carriers then may also need systematic dissection of the central neck nodes at an incremental risk of postoperative hypoparathyroidism (Skinner et al. 2005). The most convincing approach to hereditary MTC at the moment affords the so-called DNA-based/biochemical concept, striving to limit prophylactic surgery to total thyroidectomy at minimal surgical morbidity before MTC can spread beyond the thyroid capsule.

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Long-Term Follow-up in Medullary Thyroid Carcinoma

Friedhelm Raue and Karin Frank-Raue

Abstract

After surgery, patients with medullary thyroid carcinoma (MTC) should be assessed regarding the presence of residual disease, the localization of metastases, and the identification of progressive disease. Postoperatively, patients with MTC are staged to separate those at low risk from those at high risk of recurrence. The TNM staging system is based on tumor size, extra-thyroidal invasion, nodal metastasis, and distant spread of cancer. In addition, the number of lymph-node metastases, the number of compartments involved, and the postoperative calcitonin (CTN) and carcinoembryonic antigen (CEA) levels should be documented. The postoperative normalization of the serum CTN level is associated with a favorable outcome. When patients have basal serum CTN levels less than 150 pg/ml after a thyroidectomy, any persistent or recurrent disease is nearly always confined to lymph nodes in the neck. When the postoperative serum CTN level exceeds 150 pg/ml, patients should be evaluated with imaging procedures, including computed tomography (CT) of the neck and chest, contrast-enhanced magnetic resonance imaging (MRI) and ultrasound (US) of the liver, bone scintigraphy, MRI of the bone, and positron emission tomography (PET)/CT. One can estimate the growth rate of MTC metastases by quantifying increases in tumor size over time from sequential imaging studies analyzed with response evaluation criteria in solid tumors (RECIST), and by determining the tumor marker doubling time from sequential measures of serum CTN or CEA levels over multiple time points. One of the main challenges remains to find effective adjuvant and palliative options for patients with metastatic disease. Patients with persistent or recurrent MTC localized to the neck following

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thyroidectomy are candidates for neck operations, depending on the tumor extension. Once metastases appear, the clinician must decide which patients require therapy. This requires a balance between the (often) slow rate of tumor progression, which is associated with a good quality of life, and the limited efficacy and potential toxicities of local and systemic therapies. Considering that metastatic MTC is incurable, the management goals are to provide loco-regional disease control, palliate symptoms of hormonal excess, such as diarrhea, palliate symptomatic metastases, like pain or bone fracture, and control metastases that threaten life, such as bronchial obstruction or spinal cord compression. This can be achieved with palliative surgery, external beam radiation therapy (EBRT), or systemic therapy with tyrosine kinase inhibitor (TKI).

Keywords

Medullary thyroid carcinoma · Multiple endocrine neoplasia · Calcitonin · Carcinoembryonic antigen · Somatic RET mutation

List of Abbreviations

MTC	Medullary thyroid carcinoma
MEN	Multiple endocrine neoplasia
CTN	Calcitonin
CEA	Carcinoembryonic antigen
EBRT	External beam radiation therapy
CT	Computed tomography
TKI	Tyrosine kinase inhibitor

Contents

1 Introduction

Medullary thyroid carcinoma (MTC) is a differentiated neuroendocrine tumor, mostly slowly growing, with a relatively good prognosis. Among individuals with MTC, the overall 10-year survival rate is 61–76 % (Raue 1998; de Groot et al. 2006). Surgery is the only curative therapy for MTC. When curative surgery is achieved, documented by non-measurable calcitonin (CTN) levels, patients have an excellent prognosis (10-year survival >95 %). Unfortunately, in most patients, MTC has metastasized to regional lymph nodes, and they also have systemic disease. This condition cannot be cured biochemically, despite aggressive surgery, including bilateral neck dissection (Machens and Dralle 2010, 2013). Patients with distant metastases at diagnosis have a poor prognosis, with only a 40 % of 10-year survival rate (Roman et al. 2006). Postoperative staging includes stratification with the TNM system, based on tumor size, extra-thyroidal invasion, nodal metastasis, and distant spread of cancer, and assessments of the number of lymph-node metastases involved and the number of compartments involved. However, in patients with MTC, the TNM classification lacks important prognostic factors, such as age and postoperative serum CTN levels. Postoperative CTN levels in patients with MTC are helpful for separating those at low risk from those at high risk of recurrence. The tumor burden can be estimated from imaging studies and measurements of tumor marker doubling times. Once definitive surgery has been performed, patients can be monitored in regular office visits, where clinicians can follow up on patient history, physical examinations, tumor marker determinations, and residual tumor imaging. The treatment goals depend on the postoperative tumor stage (Wells et al. 2015).

2 Postoperative Management: Long-Term Follow-up

Postoperatively, patients should be assessed to determine the absence or presence of residual disease, the localization of metastases, and the identification of progressive disease. These assessments can be achieved by measuring CTN and carcinoembryonic antigen (CEA) at defined intervals to calculate doubling time, and by imaging regions of interest to predict outcome and to plan the long-term follow-up strategy (Wells et al. 2015; Giraudet et al. 2007; Ong et al. 2007; Oudoux et al. 2007). A dynamic risk stratification system should be applied with the TNM/AJCC staging system. The nadirs of CTN and CEA should be measured within the first year after the initial treatment, and imaging studies should be conducted to identify local recurrences or distant metastases. This information will allow stratification of patients with MTC into three risk groups (Lindsey et al. 2015): (i) a complete biochemical and structural cure, defined as undetectable CTN levels and normal CEA levels without structural evidence of disease; (ii) a biochemically incomplete cure, defined as detectable CTN levels or abnormal CEA levels, but the absence of structural evidence of disease; and (iii) a structurally incomplete cure, defined as persistent/recurrent structural disease with elevated CTN and CEA levels. This

classification system provides more useful clinical prognostic information than provided by the static, initial anatomic staging system (TNM) (Lindsey et al. 2015). The growth rate of MTCs can be derived from sequential imaging studies analyzed with response evaluation criteria in solid tumors (RECIST) (Therasse et al. 2000), which facilitates the detection of specific increases or decreases in tumor size over time. These three groups of patients, with different risks and treatment goals, can be identified in the follow-up with the following procedures:

1. *Patients cured with surgery and no detectable CTN after surgery*. Patients with undetectable postoperative CTN levels are likely to be free of disease; thus, undetectable CTN was associated with a favorable outcome. This association was observed in 60–90 % of patients with a small tumor and no lymph-node involvement, but it was observed in only 20 % of patients with lymph-node metastases. In the long-term follow-up, it is typically sufficient to record observations without further treatment. In only 3 % of these patients, serum CTN became detectable during follow-up (Franc et al. 2001). In part, this phenomenon was attributed to the newer, more sensitive assay technology developed over the last two decades. In most cases, either the CTN was detected in a screening for thyroid nodules, or it was an incidental finding during a thyroid operation. In patients with hereditary MTC, a prophylactic thyroidectomy provides a cure in nearly all cases. In contrast, multiple endocrine neoplasia type 2 (MEN 2) and a palpable thyroid nodule and/or lymph-node metastases at the time of diagnosis (index cases) are often associated with persistent disease (Fialkowski et al. 2008).
2. *Patients with detectable CTN levels after initial treatment, but no initial evidence of disease in routine imaging*. After a total thyroidectomy, detectable serum CTN levels that are below 150 pg/ml are typically associated with loco-regional disease and occasionally (but rarely) with distant metastases (Yen et al. 2003; Laure Giraudet et al. 2008; Pellegriti et al. 2003). These patients might be the candidates for a second surgery with a curative intention (see Sect. 6, Surgery). All other asymptomatic cases should be followed up, primarily in a careful examination with neck ultrasound (US), but also watchful waiting (Kouvaraki et al. 2003). However, although there is a general correlation between the serum CTN levels and the extent of residual MTC postoperatively, there is substantial overlap among patients with locally recurrent disease and those with clinically evident distant metastases (Yen et al. 2003). In most cases, with comprehensive follow-up examinations, tumor markers increase slowly. In 40 % of patients, during a 10-year follow-up, imaging will detect either a local recurrence or a small, slowly growing or stable, distant metastasis without clinical symptoms. If any treatment is necessary, local treatment is typically sufficient. Active surveillance is appropriate for most cases. In some cases, during the follow-up, tumor markers start to increase rapidly, and distant metastases are documented.
3. *Patients in an advanced stage with distant metastases at diagnosis and high tumor marker levels*. These patients require documentation of the metastasis

sites, tumor volume, and progression rate. Different imaging procedures should be applied, including US and computed tomography (CT) of the neck and chest; contrast-enhanced magnetic resonance imaging (MRI) or three-phase contrast-enhanced CT of the liver; bone scintigraphy; and MRIs of the pelvis and axial skeleton (Giraudet et al. 2007; Koopmans et al. 2008; Rubello et al. 2008). The growth rate of selected metastases can be derived from sequential imaging studies conducted every 3–6 months, analyzed with RECIST criteria (Therasse et al. 2000). Measuring the tumor marker doubling time is helpful for estimating the total tumor burden and prognosis. Evaluation of symptomatic disease manifestation is crucial for informing the decision-making process for determining the appropriate therapy. An intensive discussion with the patient must be started with regard to the expectations of quality of life and the risks and benefits of therapy, and an agreement should be reached on a personalized treatment plan. Therefore, the treatment decision must strike a balance between the quality of life without treatment, based on the progression rate of the tumor, and the efficacy and side effects of therapy. Because currently, there are no available curative treatments, the goal of palliative therapy should be to improve the quality of life by relieving the symptoms (best supportive care).

3 Tumor Marker Doubling Time

Measurements of serum CTN and CEA levels are of paramount importance during the postoperative follow-up of individuals with MTC, because these biomarkers might indicate the presence and volume of residual disease (Engelbach et al. 2000). A postoperative, undetectable basal serum CTN level is a strong predictor of complete remission. Complete remission may be further confirmed when serum CTN remains undetectable, and also after a provocative (pentagastrin or calcium) test (Engelbach et al. 2000). In cases with very high preoperative CTN levels, a decrease in CTN might be delayed for 24 h to 12 weeks after surgery (Ismailov and Piulatova 2004). Therefore, 3-month postoperatively appears to be the optimal time point for determining serum CTN levels (Ismailov and Piulatova 2004; Elisei and Pinchera 2012). Persistently, high CTN levels might be found also in patients with kidney failure or liver cirrhosis, due to an increase in the hormone half-life caused by the decline in renal or hepatic function. Serum CTN determinations should be repeated every 3–6 months for the first 2–3 years. When CTN is unmeasurable, it should be determined annually thereafter.

Patients with biochemical remission after an initial treatment have only a 3 % chance of recurrence during a long-term follow-up (Franc et al. 2001). However, when basal serum CTN is detectable or becomes detectable during follow-up, it is an indication that the patient is not cured. The MTC growth rate can be determined by measuring serum levels of CTN and CEA over multiple time points (e.g., every 3–6 months), to determine the rate of doubling. The CTN and CEA doubling times were strongly related to disease progression, with very few overlaps. For example,

94 % of patients with doubling times shorter than 25 months had progressive disease, and 86 % of patients with doubling times longer than 24 months had stable disease (Laure Giraudet et al. 2008). When the CTN doubling time was less than 6 months, the 5- and 10-year survivals were 25 and 8 %, respectively; when the doubling time was between 6 months and 2 year, the 5- and 10-year survivals were 92 and 37 %, respectively; and all patients ($n = 41$) with CTN doubling times greater than 2 years were alive at the end of the study (Barbet et al. 2005). However, this method has the disadvantage that CTN levels often exhibit large fluctuations; consequently, serial measurements must be performed over a considerable time period to obtain an accurate determination of the doubling time. The American Thyroid Association has provided an online calculator for determining doubling times based on serial serum CTN and CEA measurements. This calculator can be accessed at the following Web address: (www.thyroid.org/thyroid-physiciansprofessionals/calculators/thyroid-cancer-carcinoma). Reliable estimates are obtained by including at least four data points acquired over a minimum of 2 years.

CEA is a less specific marker than CTN, but it is a useful marker of progressive disease. CEA levels are not always elevated in all patients, and CEA levels do not systematically correlate with CTN levels. The CEA levels might return to normal values after surgery, even in the presence of elevated CTN levels, which might be due to the presence of small, residual neoplastic foci. In contrast, in individuals with progressive disease, the CEA levels might increase without a corresponding elevation in the CTN levels. Increased serum CEA levels are taken to indicate a poor prognosis. When the CTN and CEA doubling times indicate rapidly progressing disease, a more thorough assessment that includes imaging tests should be considered, whenever possible, to localize the disease and indicate the proper treatment, if possible.

Most patients that are asymptomatic, but have elevated serum CTN levels and occult metastatic disease, are not candidates for aggressive surgical intervention or systemic treatment. Natural history studies have indicated that many of these patients have a relatively good prognosis, particularly when the CTN and CEA doubling times exceed 2 years (de Groot et al. 2006; Rendl et al. 2008).

4 RET-Mutation Analysis

When it was not performed preoperatively, a direct DNA analysis should be conducted for patients with MTC to probe for a mutated *RET* allele. About 1–7 % of patients with presumed sporadic MTC actually have hereditary disease (Elisei et al. 2007; Wohllk et al. 1996; Dvorakova et al. 2008; Eng et al. 1995). Patients that have a *RET* germline mutation should be evaluated, and their first-degree relatives should be offered genetic counseling and genetic testing. Germline mutations in the *RET* oncogene are found in nearly 100 % of familial forms of MTC (Frank-Raue et al. 2006). Patients with MEN 2A commonly experience better treatment outcomes than those with sporadic MTC, who are frequently diagnosed at a more

advanced tumor stage. The aggressiveness and age of onset differs between different types of MEN 2, and the related *RET* mutations have been stratified into three groups: moderate, high, and highest risk of disease (Wells et al. 2015).

5 Postoperative Imaging

Detection of persistent or recurrent MTC should begin with a neck US evaluation. When the postoperative serum CTN level exceeds 150 pg/ml, and metastatic MTC is expected, additional imaging procedures are indicated. The most frequent sites of distant metastasis are the liver (45 %), bones (54 %), and lungs (33 %) (Giraudet et al. 2007). Patients should be evaluated with imaging procedures, including US and CT of the neck and chest; contrast-enhanced MRI or three-phase contrast-enhanced CT of the liver; bone scintigraphy; and MRI of the pelvis and axial skeleton. The sensitivity of neck US for diagnosing loco-regional metastases is 97 %, compared to 72 % with CT, and 55 % with PET-CT (Giraudet et al. 2007). CT is the most sensitive imaging procedure for detecting lung and mediastinal lymph-node metastases. Three-phase contrast-enhanced multi-detector liver CT and contrast-enhanced MRIs are the most sensitive methods for detecting liver metastases. Axial MRIs and bone scintigraphy are complementary and the most sensitive procedures for detecting bone metastases. 2-[Fluorine-18] fluoro-2-deoxy-D-glucose (FDG) PET with CT (FDG-PET/CT) and ^{18}F-dihydroxyphenylalanine F-DOPA-PET/CT were proven to be superior to conventional imaging procedures for detecting metastases in patients with MTC (Ong et al. 2007; Rubello et al. 2008; Gourgiotis et al. 2003; Hoegerle et al. 2001; Santhanam and Taieb 2014). DOPA-PET is preferably used to assess the extent of the disease; FDG-PET seems to be preferable for identifying more progressive MTC (Verbeek et al. 2012). Unfortunately, no single procedure provides optimal whole-body imaging. The sensitivity of these tests in localizing metastatic disease ranges between 50 and 80 %, but the sensitivity is likely to be significantly lower in the setting of modestly elevated serum CTN values (Koopmans et al. 2008; Gourgiotis et al. 2003; Hoegerle et al. 2001; Mirallie et al. 2005). The frequency and use of different imaging methods depend on the growth rates of the metastases, the CTN and CEA doubling times, and the decision concerning systemic treatment. For example, in stable disease, it may be sufficient to perform a US every 6–12 months and a CT of the leading metastases every 12–14 months. When aggressive tumor behavior is present, it might be necessary to perform a US every 3 months and a CT every 6 months.

6 Postoperative Substitution of Thyroxine, Treatment for Hypoparathyroidism

After a thyroidectomy, patients require replacement therapy with levothyroxine to maintain serum thyroid stimulating hormone (TSH) levels in the euthyroid range. It is unnecessary to suppress TSH, because MTC is not TSH-dependent. When a total

thyroidectomy is performed, normal parathyroid glands should be preserved. However, they might be accidentally removed, particularly when a central compartment dissection is performed or when multiple operations are performed. Postoperatively, patients require careful monitoring for the development of hypocalcemia. Treatment with oral calcium and calcitriol is indicated in patients that become symptomatic and develop hypoparathyroidism.

7 Surgery for Residual Disease

Localized and limited loco-regional disease can be treated with resections, with the intention to cure. However, in more advanced localized or in a residual/recurrent disease, a multimodal approach is generally recommended to control local disease and to reduce tumor progression. The extent of surgery will depend on the types of surgical procedures performed previously and on the nature of the relapse. When the extent of initial surgery was incomplete, the preferred surgery protocol is resection. When a curative reoperation (to achieve undetectable serum CTN levels) is intended, the presence of distant metastases should be ruled out, before considering a cervical reoperation. After a cervical reoperation, the rates of CTN normalization vary from 16 to 44 %, depending on the preoperative CTN levels and the number of removed lymph-node metastases. In cases with preoperative CTN levels above 1000 pg/ml, and when more than 5 lymph-node metastases had been removed, a curative approach is no longer possible (Machens and Dralle 2013a, b; Gimm and Dralle 1997; Kebebew et al. 2000; Moley and Fialkowski 2007; Rowland et al. 2015). In this situation, recurrent local disease in the neck should only be resected for local control and for palliative indications, particularly in cases with high risk of compression or invasion of the trachea or the great vessels. The survival benefit of a repeat neck operation in patients with MTC remains contradictory (Fialkowski et al. 2008). It was previously shown that when patients with MTC received adequate primary surgery, which was performed in referral centers according to the ATA MTC guidelines, the local reoperation rates were reduced and the biochemical cure rates were increased (Verbeek et al. 2015).

8 Adjunctive External Beam Radiation Therapy

The role of regional external beam radiotherapy (EBRT) in the treatment of MTC remains controversial (Schwartz et al. 2008; Martinez et al. 2010; Terezakis and Lee 2010; Call et al. 2013). In patients with inoperable tumors, radiotherapy can offer prolonged palliation and achieve local tumor control. In individuals at a high risk of local recurrence (locally invasive tumors, microscopic residual disease, or neck lymph-node involvement), radiotherapy was shown to reduce the local recurrence rate in 86 % of patients at a 10-year follow-up (Brierley et al. 1996).

EBRT can also be used to treat hemoptysis or airway obstruction in individuals with extensive mediastinal and/or lung involvement. However, the potential benefits must be weighed against the acute and chronic toxicity associated with the therapy. Prior to initiating EBRT, surgeons should ensure that patients are not candidates for a repeat operation, because repeat procedures after EBRT are technically difficult and associated with significant complications.

The indications for radiotherapy in systemic metastatic disease are restricted to painful bone metastases that require palliative treatment, because they are not amenable to surgery, or a risk of fracture, which occurs in bone metastases secondary to other types of tumors. EBRT may lead to significant pain reduction that lasts for months (Brierley and Tsang 2008). In addition, patients with isolated brain metastases should be considered for EBRT (including stereotactic radiosurgery), when the lesions are not amenable to surgery. In contrast, although skin metastases respond to treatment with EBRT, the prognosis is poor, and most patients die of the disease within a year after diagnosis (Santarpia et al. 2008).

9 Treatment of Patients with Advanced MTC

In patients with lymph-node metastases, distant metastases begin to appear at basal serum CTN levels above 150 pg/ml; they appear in over 50 % of patients with CTN levels of 5000 pg/ml, and they are typically always present in patients with CTN levels that exceed 20,000 pg/ml (Machens et al. 2005). Distant metastases are the main cause of death in patients with MTC. Half of MTC cases present metastases in the initial assessment. They can simultaneously affect multiple organs, such as the liver, lungs, and bones. Survival after the discovery of distant metastases is 40 % after 10 years (Roman et al. 2006). Long-term survival has been observed in a few patients with metastatic disease, even without any systemic treatment, and particularly when the metastases are discovered at an early stage.

Once metastases appear, the clinician must decide which patients require therapy. They must balance the often slow rate of tumor progression, which is associated with a good quality of life, against the limited efficacy and potential toxicities of local and systemic therapies. Considering that metastatic MTC is incurable, the goals of management are to provide loco-regional disease control, palliate symptoms of excess hormone (such as diarrhea or Cushing's syndrome), palliate the symptoms of metastases (such as pain or bone fracture), and control metastases that threaten life (such as bronchial obstruction or spinal cord compression). Systemic therapy should not be administered to patients that exhibit increasing serum CTN and CEA levels, but have no documented metastatic disease. Moreover, systemic therapy should not be administered to patients with stable, low volume metastatic disease, determined with imaging studies, and serum CTN and CEA levels with doubling times greater than 2 years (Wells et al. 2015).

10 Local Treatment Modalities for Distant Metastases

Liver metastases occur in 45 % of patients with advanced MTC (Giraudet et al. 2007). In most cases, liver metastases grow slowly, and patients are asymptomatic. Treatment is indicated when liver metastases are large, progressive, or associated with severe symptoms, such as diarrhea or pain. Single, large, isolated metastases should be resected, when possible. However, liver metastases are often numerous and disseminated throughout the parenchyma. Also, they are typically not amenable to surgery, percutaneous ethanol ablation, or radiofrequency ablation. When rapid growth or severe symptoms occur, treatment options are either systemic therapy or chemoembolization (Isozaki et al. 1999; Lorenz et al. 2005; Fromigue et al. 2006; Wertenbroek et al. 2008).

Lung metastases occur in 33 % of patients with MTC (Giraudet et al. 2007). They are typically numerous, and they are often associated with mediastinal lymph-node metastases. Surgical resection of the mediastinal lesions may be considered, particularly when they cause airway compression or bleeding. Other therapeutic options are EBRT or radiofrequency ablation. Systemic therapy is indicated in patients with widespread metastases that are progressing.

Bone metastases occur in 54 % of patients with advanced MTC (Giraudet et al. 2007). The management of patients with symptomatic bone metastases includes vertebroplasty, surgical excision, thermoablation (radiofrequency or cryoablation), cement injection, or EBRT (Wexler 2011; Quan et al. 2012). The therapy selection depends on whether the clinical presentation is pain, fracture, or spinal cord compression. EBRT may considerably reduce pain in 80 % of patients, and this amelioration may last for months. Intravenous bisphosphonates or denosumab have been applied, but there is no substantial experience in MTC (Vitale et al. 2001).

11 Systemic Therapy

Once metastases appear, the clinician must decide which patients require therapy. They must balance the often slow rate of tumor progression, which is associated with a good quality of life, against the limited efficacy and potential toxicities of local and systemic therapies (Wells et al. 2015). Only patients with significant tumor burden and those with symptomatic or progressive disease are candidates for systemic therapy. One can estimate the growth rate of MTC from sequential imaging studies and RECIST (Therasse et al. 2000). Patients are candidates for systemic treatment when they have measurable lesions and progression documented with imaging. Progression is defined as ≥20 % increase in the sum of the longest lesion diameters or the appearance of one or more new lesions within a given time interval (e.g., 12 months).

11.1 Radiolabeled Molecules

There is limited experience with radiolabeled molecules, such as [^{90}Yttrium-DOTA]-TOC, which can deliver high radiation doses to cancers. A phase II clinical trial on patients with advanced MTC evaluated response, survival, and long-term safety of DOTA-TOC therapy, and tumor uptake of ^{111}In-octreoscan. The median survival from the start of treatment was significantly longer among responders (39 %) than among non-responders. Thirteen percent of patients developed hematologic toxicities and 23 % developed renal toxicities (Iten et al. 2007). The efficacy of pretargeted radio-immunotherapy with a bispecific monoclonal anti-CEA antibody and ^{131}I-labeled bivalent hapten showed promising results in early studies. However, no prospective, randomized trials have compared this therapy to other therapies or a placebo (Chatal et al. 2006; Kraeber-Bodere et al. 2006). At present, treatment with radioisotope-based therapy should only be considered in the context of a clinical trial.

11.2 Diarrhea

Diarrhea occurs most frequently in patients with advanced disease and hepatic metastases. The diarrhea may be due to gastrointestinal hypersecretion or to enhanced gastrointestinal motility, or a combination of these conditions (Rambaud et al. 1988). Diarrhea can be debilitating, both in terms of quality of life and nutrition. Patients with advanced MTC and diarrhea should be advised to avoid alcohol intake and to maintain a diet that limits high-fiber foods. The anti-motility agents, loperamide, codeine, or tincture of 0.1 % opium have minimal side effects and should be used as first-line therapy. Other treatments include somatostatin analogs and the debulking of large tumors. Reports of somatostatin analogs therapy for diarrhea have been small nonrandomized, cohort studies with variable results, which suggested modestly improved symptoms in some patients (Mahler et al. 1990; Vainas et al. 2004), but no real effect on tumor mass. The currently available somatostatin analogs have a high affinity for SSTR2 and SSTR5 (octreotide and lanreotide). They did not seem to have an effect on survival, but in some patients, they reduced flushing and diarrhea (Vitale et al. 2000). Therefore, somatostatin analogs can be considered for symptomatic treatment of diarrhea, when other drugs are ineffective.

Other nonrandomized studies showed that local treatment of large hepatic metastases with selective arterial chemoembolization could improve diarrhea. This treatment provided some symptomatic benefit and partial reduction of the tumor mass. In one study, 2 of 5 patients with advanced MTC and diarrhea had an objective response to chemoembolization. In a second study, all 6 patients with diarrhea improved with chemoembolization (Isozaki et al. 1999; Lorenz et al. 2005).

11.3 Chemotherapy

MTC is relatively insensitive to chemotherapy; accordingly, the results from most studies have been poor. Chemotherapy might be indicated when the tumor mass has escaped local control and has entered a more aggressive growth phase. Monotherapy with Adriamycin (60 mg/m^2 every 3 weeks) or combinations of Adriamycin and various other drugs have been used in some trials, but the response rates were below 20 % (Scherubl et al. 1990; Orlandi et al. 1994; Schlumberger et al. 1995; Wu et al. 1994). A recommendation of chemotherapy must take into account the quality of life and toxic side effects. Therefore, chemotherapy should not be considered a first-line therapy for patients with advanced MTC; instead, it must be individualized based on clinical grounds.

11.4 Tyrosine Kinase Inhibitors

In recent years, several tyrosine kinase inhibitors (TKIs) (axitinib, cabozantinib, gefitinib, imatinib, motesanib, sorafenib, sunitinib, and vandetanib) have been evaluated in phase I, II, and III clinical trials of patients with advanced MTC (Schlumberger et al. 2009; de Groot et al. 2007; Frank-Raue et al. 2007; Kurzrock et al. 2011; Lam et al. 2010; Wells et al. 2010; Robinson et al. 2010; Abraham et al. 2010; Elisei et al. 2013; Wells and Santoro 2014). The partial response rate ranged from 20 to 50 %, and a large number of patients (up to 87 %) demonstrated prolonged, stable disease. On the basis of recently completed phase III clinical trials, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved two orally administered TKIs, vandetanib (2011) and cabozantinib (2012), for the treatment of patients with advanced progressive MTC. Both vandetanib and cabozantinib improved the quality of life, reduced pain, and reduced diarrhea to the extent that a number of patients could resume a normal social life. When patients were evaluated in subgroups of different tumor burdens, progression rates, or symptoms, all subgroups showed benefits from treatment. Adverse events were mainly grade 1 or 2. Currently, TKI therapy appears to be the most effective treatment modality for patients with symptomatic and progressive MTC.

12 Prognostic Factors

The natural history of sporadic MTC is variable. The spectrum ranges from years of dormant residual disease after surgery to rapidly progressive disseminated disease and death, related to either metastatic thyroid tumor or complications of pheochromocytoma in MEN 2. The 10-year survival rates for all patients with MTC range from approximately 61–76 % (Raue 1998; de Groot et al. 2006; Roman et al. 2006; Kebebew et al. 2000; Cupisti et al. 2007). It is generally agreed that early diagnosis and improvements in surgical therapy have had a favorable influence on the clinical course of the disease. With early detection, surgical treatment of MTC is

likely to be curative; over 98 % of patients with MTC that was detected at an early disease stage remained disease-free after treatment (normal or undetectable CTN values) (Frank-Raue et al. 2006; Machens et al. 2003; Modigliani et al. 1998). The excellent prognosis associated with MTC identified in the earliest stages underscores the importance of prospective screening (CTN screening) (Elisei et al. 2004) and early diagnosis (RET-mutation analysis in hereditary MTC), followed by adequate therapy. Patients without detectable recurrence after an initial treatment had a life expectancy similar to the general population (de Groot et al. 2006).

At clinical presentation, about 50 % of patients with MTC show lymph-node metastases. Distant metastases are detected in 10 % of newly diagnosed patients, and more than 20 % of patients will die from progressive metastatic disease. Although the prognosis is generally favorable, when diagnosed and treated at an early, localized stage, the prognosis strongly depends on disease stage. The 10-year overall survival rates are 95 % in patients with localized disease, 75 % in patients with regional disease, and 40 % in those with metastasized disease (Roman et al. 2006). The strongest independent clinical predictor of survival was the stage at diagnosis (tumor size, lymph node, distant metastases, extra-thyroid invasion, and vascular invasion). Distant metastatic disease is the main cause of death in patients with MTC, and it often involves multiple organs, such as liver, lungs, and bone (Pacini et al. 2010). The other factors that influence survival are the type of tumor (sporadic versus familial) and the age and sex of the patient. Patients with MEN 2A have a better survival rate than patients with sporadic disease. Patients with familial MTC can be detected with screening at a relatively young age. Thus, because these patients can be diagnosed at an early stage of disease, they are mostly cured, and their life expectancy is similar to that of the general population (de Groot et al. 2006). When a multivariate analysis was adjusted for tumor stage, the significant difference in survival disappeared between patients with sporadic disease and patients with familial disease (Raue 1998; de Groot et al. 2006; Saltiki et al. 2014). The same was true for gender and age. In a standardized survival analysis, corrected for basal mortality in the general population, a univariate analysis showed that women and younger individuals had higher survival rates than men and older individuals. However, those differences disappeared when the multivariate analysis was adjusted for sex and age (de Groot et al. 2006).

Preoperative CTN levels, disease stage at diagnosis (Yip et al. 2011), and postoperative CTN levels (Saltiki et al. 2014) were independent predictors for prognosis. Tumor size was correlated with the preoperative CTN level. Also, because detectable postoperative CTN is indicative of disease persistence, it is probably a more significant prognostic factor than tumor size alone; indeed, detectable postoperative CTN includes distant metastases or local microscopic lymph-node metastases that were not detected during the operation. In the follow-up, CTN and CEA doubling times were strongly correlated with disease progression (Laure Giraudet et al. 2008), and they significantly predicted survival in a multivariate analysis (Barbet et al. 2005; Meijer et al. 2010). When the doubling time of CTN was <6 months, the 5- and 10-year survival rates were 25 and 8 %, respectively; when the doubling time was >2 years, all patients were alive at the end of follow-up (Barbet et al. 2005).

Somatic *RET* mutations can be identified in about 30–50 % of sporadic cases of MTC. The most common *RET* mutation is an M918T substitution in exon 16, the same mutation that occurs in MEN 2B, in the germline (Marsh et al. 1996a, b; Schilling et al. 2001). Other *RET* somatic mutations, and some small deletions, have been reported in 'hot spot' regions in exons 10 and 11 (Romei et al. 1996). The latter include a RET fusion gene (Grubbs et al. 2015). The presence of the somatic *RET* mutation, M918T, is a strong negative prognostic factor for metastasis-free survival and for overall survival in patients with sporadic MTC (Elisei et al. 2008). Several studies have indicated that patients with MTC due to somatic *RET* mutations are typically in an advanced stage at diagnosis, and they have a worse prognosis than those with no evidence of a *RET* mutation (Schilling et al. 2001; Elisei et al. 2008; Zedenius et al. 1995; Moura et al. 2009; Mian et al. 2011). The 10-year survival rates were approximately 45 and 90 % in those with and without the M918T mutation, respectively (Schilling et al. 2001). Also, the presence of a somatic *RET* mutation was positively correlated with an increased percentage of cells with elevated mitotic activity (Mian et al. 2011). A more aggressive sporadic MTC, identified with immunohistochemical findings of the somatic *RET* M918T mutation combined with high Ki-67 expression, was indicative of a more advanced stage and low overall survival (Mian et al. 2011). A summarization of these findings in a meta-analysis demonstrated that patients with the somatic *RET* M918T mutation had a 5.82-fold higher risk of death from thyroid cancer than those without a *RET* mutation (Pak et al. 2015).

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Use of Tyrosine Kinase Inhibitors for Treatment of Medullary Thyroid Carcinoma

Ramona Dadu, Mimi N. Hu, Elizabeth G. Grubbs and Robert F. Gagel

Abstract

Two independent events—the identification of activating mutations of the RET proto-oncogene, a receptor tyrosine kinase, in medullary thyroid carcinoma, and the recognition that small organic molecules could bind to and inhibit phosphorylation of signaling molecules, thereby inactivating the pathway—led to the recognition that kinase inhibitors could be used to treat medullary thyroid carcinoma (MTC). The introduction of these compounds into clinical practice has transformed the treatment of metastatic MTC and provided insight into the mechanisms by which RET causes C-cell transformation. This chapter will review the progress in this field over the past 7 years.

Keywords

Multiple endocrine neoplasia type 2 · Calcitonin · Tyrosine kinase inhibitor · Vandetanib · Cabozantinib · RET proto-oncogene · Medullary thyroid carcinoma · Metastasis

Contents

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1 Introduction

The past few decades have been a remarkable period of discovery for cancer biology. Research on specific tumor types and the use of high-throughput DNA sequencing has identified a broad spectrum of signaling abnormalities in cancer. These defects fall into two broad categories: those that activate signaling molecules and regulate growth or prevent death, and a second category where a molecule that normally inhibits growth or promotes cell death is lost or inactivated.

Another concept that has emerged, particularly over the past decade, is the identification of mutant genes that drive accelerated growth, transformation, and metastasis—so-called driver mutations—that occur in the germ line or are acquired somatically. These are to be differentiated from so-called passenger mutations that are acquired during the transformation process and may contribute to transformation, but are not essential (Stratton et al. 2009).

Medullary thyroid carcinoma (MTC) is a tumor derived from the calcitonin (CT)-producing parafollicular or C cells of the thyroid gland. This neuroendocrine cell type is thought to derive from the pharyngeal endoderm or neural crest (or both) and coalesces to form a structure called the ultimobranchial body, which subsequently migrates into the developing thyroid gland (see Chapter: "Thyroid C-cell biology and oncogenic transformation"). The parafollicular cell is the major production site of CT, a hormone that functions to modulate osteoclast-mediated bone resorption, and is used as a tumor marker to diagnose and monitor progression of MTC (Wells et al. 2015).

In 1961, the observation was made that MTC (along with parathyroid neoplasia and pheochromocytoma) is transmitted as an autosomal dominant trait in families as a part of a syndrome designated multiple endocrine neoplasia type 2 (MEN2) (Sipple 1961). This observation led subsequently to the identification of large multi-generational families with MEN2, mapping of the causative gene in the 1980s and the identification of the RET proto-oncogene mutations in 1993 (Donis-Keller et al. 1993; Mulligan et al. 1993).

RET is a member of the receptor tyrosine kinase family. It is a single-pass receptor that partners with one of the 4 extracellular proteins (GFRα1-4) to function as a receptor for one of the 4 ligands, artemin, glial-cell line-derived neurotrophic factor, neurturin, and persephin (PSPN). In the C or parafollicular cell, GFRα-4 partners with RET to form a receptor for PSPN. Activation of RET by PSPN leads to dimerization of the receptor, autophosphorylation of specific tyrosine residues within the receptor and activation of downstream signaling pathways (Ibanez 2013). The phosphorylation occurs by interaction of adenosine triphosphate (ATP) with the RET intracellular kinase domain and the subsequent transfer of phosphate to the receptor and downstream signaling molecules.

The mutations of RET associated with MEN2 fall into two broad categories: extracellular and intracellular. The extracellular mutations (codon 634 is the most common) promote dimerization of the receptor, autophosphorylation, and activation of downstream signaling pathways. The most common intracellular mutation (codon M918T) causes phosphorylation in the absence of dimerization and subsequent activation of downstream signaling pathways (Santoro and Carlomagno 2013). It was demonstrated subsequently that more than 50 % of sporadic MTCs have a RET activating mutation (Wohllk et al. 1996) and, more recently, HRAS and KRAS mutations have been identified in 15–30 % and BRAF mutations in 0–7 % of sporadic MTCs without identifiable RET mutations (Goutas et al. 2008; Boichard et al. 2012; Moura et al. 2011; Agrawal et al. 2013). That RET, RAS, and BRAF mutations are the likely "driver mutations" in MTC is evidenced by the fact that exomic sequencing of MTCs, both hereditary and sporadic, has identified RET, HRAS, KRAS, and BRAF mutations but few other consistent mutations. That is not to say that other mutations do not occur. In the largest reported study of exomic sequencing in sporadic MTC, 18 nonidentical mutations were identified; none were considered to be driver mutations (Agrawal et al. 2013). It also does not exclude the possibility of loss of genomic material or rearrangements.

2 How Does the RET Proto-Oncogene Cause Transformation of the C Cell—the Role of ATF4

The cancer genome atlas project has provided much insight into the molecular abnormalities identified in a broad spectrum of cancer. Included in the first 34 malignancies to be sequenced was papillary thyroid carcinoma. Perhaps the most remarkable finding of this analysis was the low frequency of gene mutation that occurs in papillary thyroid carcinoma. This contrasts with other malignancy types

such as melanoma and lung cancer where there are a large number of molecular abnormalities observed. Preliminary exome sequence analysis suggests MTC will fall into the same category as papillary thyroid carcinoma with few mutations other than RET, RAS, and BRAF. While this does not exclude the possibility that there are other genetic abnormalities that contribute to transformation not yet been identified, we are left with the reality that mutation of a single gene (RET or RAS or BRAF) may drive transformation of the C cell.

Studies over the past 2 decades since RET was first identified as the cause for hereditary MTC have provided some insight into molecular pathogenesis. We know with some certainty that phosphorylation of RET residue tyrosine 1062 and others, through ligand activation or mutation of the receptor, results in activation of RAS/MAPK and JNK pathways. However, it is not clear how activation of these pathways leads to transformation. Is continuous activation of RET, as would be seen with an activating mutation of RET, sufficient for transformation or are additional molecular events (loss of genetic material or activation of other oncogenes through mutation) required for transformation to occur? There is currently no clear answer to these questions, although we know from several studies of MTC tumors that additional molecular events (loss of all or part of chromosomes 1p, 22q, 7q36.1, 12p13.31, 13q12.11, and 19p13.3–19p13.11) occur with some frequency. A question that has challenged the field is why a period of 4–10 years is required for C cells to evolve from normal to hyperplasia to MTC. Recent studies that implicate the transcription factor ATF4 in the downstream RET cascade may provide some insight into why C cells accumulate.

ATF4 is a member of the CREB family of transcription factors and also plays a central role in the regulation of the integrated stress response, a process that activates survival or death pathways when the cell is exposed to stress. Increased ATF4 expression causes increased NOXA and PUMA expression. These two proteins activate caspase pathways and promote entry into the apoptotic pathway. Recent studies have shown that upon activation (by ligand or mutation), RET is transported intact from the cell membrane to the nucleus where it is available to interact with nuclear proteins (Bagheri-Yarmand et al. 2015). RET joins a growing group of receptor tyrosine kinases capable of exerting direct nuclear effects (Hsu and Hung 2007; Lemmon et al. 2014). In the nucleus, RET interacts with and downregulates expression of ATF4 (Fig. 1). In another surprise, RET, which is known as a receptor tyrosine kinase, also phosphorylates ATF4 on threonine residues, thus functioning as a dual-specificity kinase (Bagheri-Yarmand et al. 2015). This interaction causes two effects: transcriptional downregulation of ATF4, and its ubiquitination and subsequent degradation. This causes downregulation of NOXA and PUMA leading to decreased apoptosis. The attendant reduction of cell death may be causative for the accumulation of C cells (C-cell hyperplasia) that is a precursor to MTC and occurs in the thyroid glands of children with germline-activating mutations of RET.

While much work is needed to place these recent observations in proper perspective, they are likely to have relevance to the mechanism by which RET-specific tyrosine kinases cause MTC cell death.

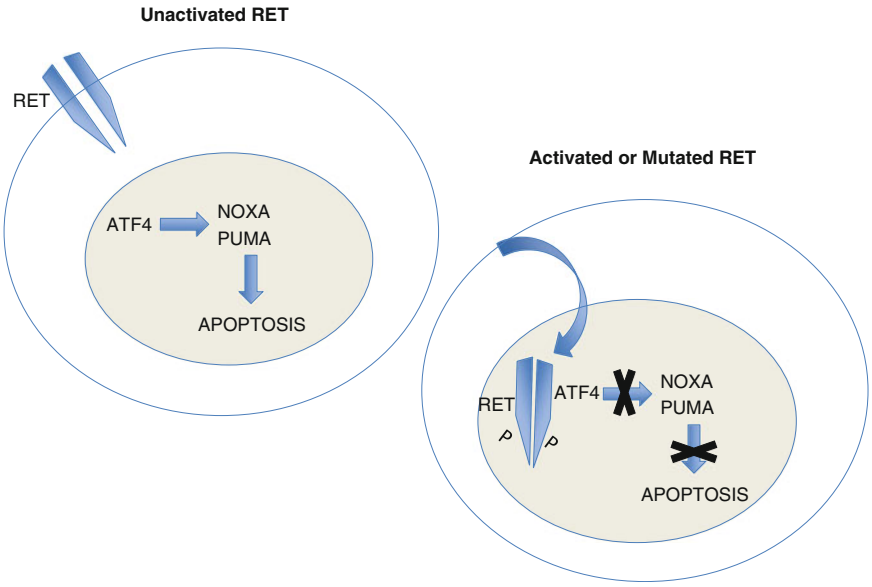

Fig. 1 Role of ATF4 in regulation of apoptosis. Activation of RET by ligand activation or mutation results in its translocation to the nucleus, where it reduces transcription and promotes ubiquitination of the transcription factor ATF4. Reduced expression of ATF4 lowers expression of NOXA and PUMA, two factors that promote apoptosis. As a result, C-cell death is inhibited by RET activation and may be a factor in the development of C-cell hyperplasia

3 The Recognition that Tyrosine Kinase Inhibitors Have Efficacy in the Treatment of MTC—the Gastrointestinal Stromal Tumor Precedent

The dawn of tyrosine kinase inhibitor use in oncology occurred with the recognition that imatinib, a small organic ATP analogue that preferentially interacts with the ATP binding pocket of a kinase domain and inhibits ATP binding, was capable of reversing the hematologic abnormalities associated with chronic myelogenous leukemia. This led to its examination in solid tumors. In 2001, a case report documented a dramatic response of a gastrointestinal stromal tumor (GIST) to imatinib, a small organic molecule that inhibited ATP binding to the *kit* receptor (Joensuu et al. 2001). What is remarkable about this observation and the subsequent experience was the recognition that inhibition of signaling caused by a "driver mutation" not only prevented growth, but also triggered massive apoptotic death of tumor cells, thereby leading to substantial reductive effects on tumor mass. Indeed the term, "oncogene addiction" was coined in an attempt to explain why a small organic ATP analogue that targeted the kinase domain of a receptor could have such profound effects on a solid tumor in the context of a multitude of other genetic abnormalities (Pagliarini et al. 2015).

4 Approved Tyrosine Kinase Inhibitors for Treatment of MTC

It was a report from Carlomagno and colleagues in 2002 that first brought this into focus for MTC (Carlomagno et al. 2002). They demonstrated vandetanib, a tyrosine kinase inhibitor with known activity against the vascular endothelial growth factor receptor 2 (KDR) and the epidermal growth factor receptor (EGFR), also targeted the RET receptor and inhibited RET-mediated MTC transformation and growth. Although these observations prompted excitement in the MEN2 community, convincing the pharmaceutical manufacturer to invest in a clinical trial for a rare thyroid cancer required perseverance. A phase II trial led by Wells et al. in patients with metastatic MTC demonstrated that 20 % of the 30 treated patients experienced a 30 % or greater reduction of tumor size (Wells et al. 2010) and a second phase II trial with a lower dose performed by Robinson and colleagues showed substantial activity (Robinson et al. 2010). The results of these trials convinced the manufacturer to support the first phase III trial ever performed for MTC. This trial of vandetanib (300-mg starting dose) demonstrated a prolongation of progression-free survival of 11 months with a hazard ratio of 0.46 and an objective response rate of 45 % (Wells et al. 2012). The design of the study (patients on placebo were permitted to cross over to active drug if they had disease progression) will make a determination of overall survival difficult, although an analysis is planned. Serum CT and carcinoembryonic antigen (CEA) response rates (decline in serum CT and CEA) were 62 and 59 %, respectively, and were durable (Wells et al. 2012). Vandetanib was approved for treatment for metastatic MTC in April 2011 by the Food and Drug Administration (FDA) in the USA and in February 2012 by the European Medicine Agency (EMA). The speed of evaluation and approval was remarkable. From the time of treatment of the first patient in the phase II trial to approval was less than 7 years.

After the identification of vandetanib activity, additional kinase inhibitors were found to have activity against RET. Among these was cabozantinib, a multi-kinase agent with greater affinity for RET and KDR than vandetanib and activity against the MET oncogene (Table 1). In a phase I/II trial, 10 of 35 patients (29 %) with measureable MTC had a partial response (>30 % reduction in tumor diameter) and an additional 15 of 37 patients (41 %) had stable disease for an overall response rate of 68 % (Kurzrock et al. 2011). These findings led to a phase III study of cabozantinib that differed in several significant ways from the earlier phase III study for vandetanib. The first was the requirement that patients have evidence of progression during a 14-month period prior to entry (the vandetanib phase III study did not require disease progression). The second was a study design in which crossover from placebo to active drug was not permitted, making it possible to compare overall survival of the treatment and placebo groups. Thus, patients were randomized to either cabozantinib 140 mg or placebo. The estimated progression-free survival for cabozantinib was 11.2 months compared with 4 months for the placebo, a highly significant difference. The overall response rate was 28 % for cabozantinib

Table 1 Tyrosine kinase inhibitors with activity in medullary thyroid carcinoma

Compound	Receptor tyrosine kinase activity IC_{50} (nM)			Efficacy in MTC Partial response rate
	VEGFR2	RET	Other	
Vandetanib	40	100	EGFR 500	Phase III 47 %
Cabozantinib	0.035	4.5	C-MET 1.8	Phase III 28 %
Axitinib	0.25	–	PDGFR 1.7	Phase II 18 %
Lenvatinib	4	35	FGFR 46	Phase II 36 %
Motesanib	3	59	PDGFR 84	Placebo controlled phase II 2 %
Pazopanib	30	2800	PDGFR 74	Phase II 14 %
Sorafenib	90	49	PDGFR 58	Phase II 25 %, 6 %
Sunitinib	9	41	–	Phase II 50 %

and 0 % for the placebo group (Elisei et al. 2013). A recent analysis (a mean of 52.4 months after study initiation) showed a 5.5-month overall survival advantage of cabozantinib over placebo, although this did not reach statistical significance in the intention to treat analysis (Schlumberger et al. 2015). However, there was evidence of a 24.5-month survival advantage for patients with codon M918T mutations compared to placebo (44.3 vs. 18.9 months), a statistically significant improvement. Cabozantinib was approved for marketing November 2012 by the FDA and March 2014 by the EMA.

It is important to recognize that both US and European regulatory agencies have cautioned physicians that these agents should be used only in patients with progressive metastatic MTC. There is no evidence that these agents are curative and at the approved doses toxicity occurs commonly. Despite these concerns, there is the sense that these agents are altering clinical outcomes in a subset of patients with metastatic MTC as will be discussed later in this chapter.

At this time, there is no basis for choosing one of the approved therapies over another—there have been no direct comparison of these two agents. There may be legitimate reasons to consider one or the other. For example, it may be appropriate to consider cabozantinib over vandetanib in a patient who is on a pharmacological agent(s) that prolongs the QT interval; similarly, in a patient who develops palmar-plantar erythrodysesthesia on cabozantinib, it may be appropriate to consider switching the patient to vandetanib.

One of the remarkable but not highlighted findings in the phase II and III studies of both vandetanib and cabozantinib is the subset of the patients who had very substantial tumor responses within the first several months of treatment, suggesting

a phenomenon similar to that observed for GIST tumors treated with imatinib. The rapidity of response (as evidenced by reductions of tumor size and rapid and sustained decreases in tumor markers) suggests a triggering of apoptotic cell death similar to that observed earlier in treatment of GIST tumors with imatinib. Alternatively, this could result from an inhibition of vascular flow. The finding that tyrosine kinase inhibition of RET activity downregulates ATF4 and through it enhances apoptosis (Bagheri-Yarmand et al. 2015), provides a plausible regulatory mechanism for the rapid effect of kinase inhibitors on tumor size, the so-called phenomenon of oncogene addiction. Another potential mechanism is the effects of vandetanib and cabozantinib on VEGFR2. There is clear evidence of increased VEGF and VEGFR2 expression by MTC and tumor vasculature. Some of the effects to cause rapid MTC death may be related to a reduction in blood flow to the tumor. The fact that each of the tyrosine kinase inhibitors with activity in MTC targets not only RET, but also VEGFR2 provides credence to this thought process.

5 What Constitutes an Appropriate Trial of Therapy?

How long should one continue therapy before determining that a particular therapy is or is not effective? There seem to be at least two different patterns of response. The first is characterized by rapid (within the first month or two) decreases in serum CT and CEA and radiographic evidence at first imaging (usually 3 months after initiation of therapy) of response. In this situation, continuation of therapy is axiomatic. Inevitably, the rate of response slows. A question for which there is no answer at present when this happens is whether to continue the therapeutic agent at the initial dose, with potential development of toxicity, or to consider a dose reduction. In most cases, if there is no toxicity, it is appropriate to continue the initial dose. If toxicity develops, a dose reduction will become mandatory. Dose reduction is usually associated with a plateauing or reversal of the response. A second pattern of positive response is defined by a slow but continuous decline in tumor markers and a reduction in the size of the lesion over a 6-, 9-, and 12-month period with continued declines over a 1- to 2-year period. Therapy should not be discontinued if there is no response at time of first radiographic assessment. This type of response is often durable for a number of years.

In a substantial minority of patients, after an initial response, there will be progression of disease, either at the site(s) of initial response or other sites. Unless there is a specific explanation for the progression, such as a drug holiday because of side effects, in most cases it will be appropriate to discontinue the therapeutic agent and consider the options discussed below.

6 Other Tyrosine Kinase Inhibitors with Activity in Medullary Thyroid Carcinoma

The recognition that multi-kinase inhibitors, particularly those that target RET and VEGFR2, have activity in MTC led to an examination of kinase inhibitors with similar target specificity in phase II MTC trials. None has been examined in a phase III trial, but the results of phase I/II trials for several are promising. These agents are summarized in Table 1 and are also highlighted in several recent reviews. Some of these agents, particularly sunitinib (Carr et al. 2010) and lenvatinib (Schlumberger et al. 2012), have significant activity and could be considered as potential third-line therapy in MTC. Other agents including axitinib, pazopanib (Bible et al. 2014), and sorafenib have lower levels of activity (Table 1). Another of these agents, motesanib (Sherman et al. 2008), despite comparable activity against RET and VEGFR2 in vitro, had little activity in a phase II MTC trial, presumably because of diarrhea-induced malabsorption of the agent leading to inadequate plasma concentrations (Schlumberger et al. 2009).

6.1 When Is It Appropriate to Consider second- or third-Line or Salvage Therapy?

There is no formal literature on the question of third-line or salvage therapy for MTC. However, a small experience exists for papillary thyroid carcinoma where about 40 % of patients treated with a second-line therapy will have a subsequent response (Dadu et al. 2014). This combined with anecdotal experience in clinical trials, demonstrating that a small but significant percentage of patients, after failing one therapy, will respond to a second therapy makes it reasonable to consider a second- or even third-line therapy. The choice of a second-line therapy would most commonly include an approved therapy (either vandetanib or cabozantinib); a third-line therapy could include sunitinib or lenvatinib, each approved for other indications and available. Several other kinase inhibitors, such as pazopanib, sorafenib, or axitinib, have lower activity, or there is less experience; therefore, these agents would fall to a lower level of consideration.

6.2 Other Agents or Combinatorial Therapy

The recognition that RET activates MAP kinase and JNK pathways has raised the question of whether agents that target PI3 K pathways (such as mTOR inhibitors) might have utility in the treatment of MTC. Preclinical literature indicates that mTOR inhibitors, either alone (Lyra et al. 2014) or in combination with tyrosine kinase inhibitors that target RET (Gild et al. 2013), have considerable activity in MTC model systems. There is a limited experience with the mTOR inhibitor, everolimus, in humans that shows activity (Faggiano et al. 2012). Clinical trials that

combine RET-specific kinase inhibitors and mTOR inhibitors will undoubtedly be developed over the next several years. One issue that has emerged in combinatorial trials of targeted agents is the potential for not only greater activity but also accentuation of toxicity. For therapies taken for extended time periods, this is a significant issue that will have to be addressed in clinical trials.

Similarly, the aforementioned studies defining the role of ATF4 in the regulation of C-cell apoptosis provides another potential therapeutic target (Bagheri-Yarmand et al. 2015). Although there are preliminary in vitro data to suggest the utility of stabilizing ATF4 in MTC as a therapeutic strategy, either alone or in combination with RET-targeted agents, these studies have not yet reached the clinical arena.

7 Side Effects of Approved Tyrosine Kinase Inhibitors

The signaling molecules targeted with high affinity (RET, VEGFR, EGFR, and MET) by the two approved tyrosine kinase inhibitors have broad importance in human biology and regulate the neurologic, gastrointestinal, vascular, dermatologic and hepatic systems, and among others. It is therefore not surprising that multi-kinase inhibitors that target several of these receptor systems should have toxicity. Indeed, the term "targeted therapy" refers to an agent that targets a specific signaling pathway in a neoplastic process, but conveniently ignores that the fact that these same signaling systems are important for a broad spectrum of normal biologic processes. The challenge for the clinician is to balance the substantial positive effects of these agents (on-target effects) with their off-target toxicity.

Tables 2 and 3 list the most common side effects and laboratory abnormalities observed with the two approved agents from their phase III studies (Elisei et al. 2013; Wells et al. 2012). The range of toxicity for both agents is significant, particularly when prescribed at the approved starting dose of 300 mg for vandetanib and 140 mg for cabozantinib. There have been no trials reported that directly compare the approved starting doses to lower dose therapy, although an FDA-mandated trial comparing vandetanib 300–150 mg for treatment of MTC is currently underway. There have been no trials that directly compare vandetanib to cabozantinib.

Management of toxicity can be challenging, particularly for individuals who have trained in endocrinology and have less experience with the types of toxicity observed with cancer treatment. In contrast, oncologists are very familiar with toxicity, but have less experience managing other medical problems (hypothyroidism, hypoparathyroidism, and adrenal insufficiency following bilateral adrenalectomy) that occur in MEN2. In some oncologic centers, endocrinologists have become comfortable with management of toxicity associated with these agents and directly manage these patients; in others, close partnerships have developed between oncologists and endocrinologists to provide optimal management for these patients.

Table 2 Side effects of tyrosine kinase inhibitors approved for treatment of medullary thyroid carcinoma from phase III studies

Side effect	Vandetanib (%)	Cabozantinib (%)
Diarrhea	57	63
Rash	53	*
Stomatitis	*	51
Hand–foot reaction	*	50
Weight loss	*	48
Decreased appetite	21	48
Nausea	33	43
Headache	26	18
Fatigue	24	41
Dysgeusia	*	34
Dermatitis acneiform/acne	35	*
Hair color changes/graying	*	34
Hypertension	33	33
Constipation	*	27
Abdominal pain	21	27
Vomiting	15	24
Dysphonia	*	20
Dry skin	15	*
QT prolongation	14	*
Photosensitivity	13	*
Dysphagia	*	13
Pruritus	11	*
Dyspepsia	11	11
Proteinuria	10	*
Depression	10	*

*Implies that these side effects either were not captured by the monitoring system or were infrequent. It is also important to keep in mind that these data were collected from a composite of clinical trials and are not intended as a head to head comparison of these two agents

There are several issues related to toxicity that are important to address. The first relates to the "black box warning" and the risk evaluation mitigation strategy (REMS) program mandated by the FDA for vandetanib. This agent caused prolongation of the QT interval in approximately 14 % of treated patients in the phase III trial (Wells et al. 2012), and there was unexplained death in several patients who participated in the phase II and phase III trials. Physicians who prescribe vandetanib are required to participate in a risk evaluation and management program (REMS) that mandates periodic electrocardiographic evaluation and avoidance of other drugs that may cause prolongation of the QT interval. In practice, routine evaluation of electrocardiograms for QT prolongation before and during therapy and

Table 3 Common laboratory abnormalities associated with approved tyrosine kinase inhibitor use from phase II and II trials

Test	Vandetanib (%)	Cabozantinib (%)
Increased AST	*	86
Increased ALT	51	86
Hypocalcemia	57	52
Neutropenia	10	35
Thrombocytopenia	9	35
Increased TSH	*	57
Increased alkaline phosphatase	*	52
Decreased phosphorus	*	28
Increased bilirubin	13	25
Decreased glucose	24	*
Decreased magnesium	7	19
Decreased potassium	6	18
Increased potassium	6	*
Decreased sodium	*	10

*Implies that these side effects either were not captured by the monitoring system or were infrequent. It is also important to keep in mind that these data were collected from a composite of clinical trials and are not intended as a head to head comparison of these two agents
Abbreviations: *ALT* alanine aminotransferase; *AST* aspartate aminotransferase

avoidance of other medications that prolong the QT interval will permit safe use of this compound. Other vandetanib effects that may contribute to QT prolongation include hypokalemia, hypocalcemia, hypothyroidism, and hypomagnesemia (Wells et al. 2012). These should be addressed promptly when recognized.

The risks of hypocalcemia, hypomagnesemia, and hypothyroidism are much greater in patients with MTC who have undergone thyroidectomy and who may have compromised parathyroid function. Both vandetanib and cabozantinib, and most other compounds described in Table 1 that target RET and VEGFR2, cause malabsorption and diarrhea. In patients who have compromised parathyroid function (common in patients with extensive surgical neck dissections), malabsorption of vitamin D, calcium, and magnesium can lead to symptomatic hypocalcemia. Malabsorption of thyroid hormone occurs with high frequency. Adjustment of calcium, vitamin D, and thyroid hormone dosages is required in most patients and many require magnesium supplementation. In a subset of these patients, the dose adjustments can be substantial.

The most troubling side effect of cabozantinib is hand–foot reaction or palmar-plantar erythrodysesthesia, a disabling, desquamating condition of the palms of the hands and the plantar surface of the feet that may be associated with considerable pain. Inevitably, these patients will require dermatologic evaluation and treatment with local and occasionally systemic corticosteroids and a likely dose reduction of the cabozantinib. The frequency of hand–foot reaction with

cabozantinib is approximately 50 % (Elisei et al. 2013), and if a patient needs to continue use of hands or feet during work, this may be a dose-limiting toxicity.

As illustrated in Table 2, hypertension occurs in approximately one-third of patients treated with either cabozantinib or vandetanib. It is important to be vigilant and measure blood pressure regularly (or have it measured by the patient or patient's primary physician) and to treat hypertension aggressively. Most commonly, the hypertension will respond to some combination of ACE inhibitor, angiotensin receptor blockade, α- or β-antagonists, or calcium channel inhibitors. Uncontrolled hypertension can necessitate stoppage of the drug related to development of intractable headache or renal or cardiac dysfunction.

8 The Decision to Initiate Therapy with a Tyrosine Kinase Inhibitor—When to Move from Watchful Waiting to Active Therapy—a Case-Based Approach

8.1 Watchful Waiting

The question of when to consider initiation of therapy is often a difficult one. MTC is frequently a slowly progressive malignancy, even when there is metastatic disease. As neither of the 2 approved agents is curative, there is broad consensus that "watchful waiting" is an appropriate course of action in many patients. It is not uncommon for MTC to progress slowly over years or decades. A helpful tool to understand the rate of progression is to plot serum CT and CEA, an indicator of tumor mass, over years/decades and to calculate a doubling time for these tumor markers (Laure Giraudet et al. 2008). Patients with a doubling time of greater than 2 years have a lower probability of dying from metastatic MTC, whereas those with a shorter doubling time and significant soft tissue metastasis are at risk for meaningful progression of metastatic disease and death and should be considered for therapy. However, even patients with a doubling time over 2 years may eventually develop bulky metastatic disease that causes hepatic dysfunction, compromise of pulmonary function, bone pain, or troublesome diarrhea. In patients in this category with a substantial "tumor burden," intervention with tyrosine kinase inhibitor therapy may be appropriate. Indeed, in the phase II and phase III studies of vandetanib, there were patients in whom treatment of slowly progressive disease resulted in a reduction of tumor mass and a decrease of CT and CEA to a lower level with a concordant reduction of symptoms such as diarrhea and flushing. Thus, use of vandetanib or cabozantinib in a symptomatic patient with assessable metastasis is reasonable.

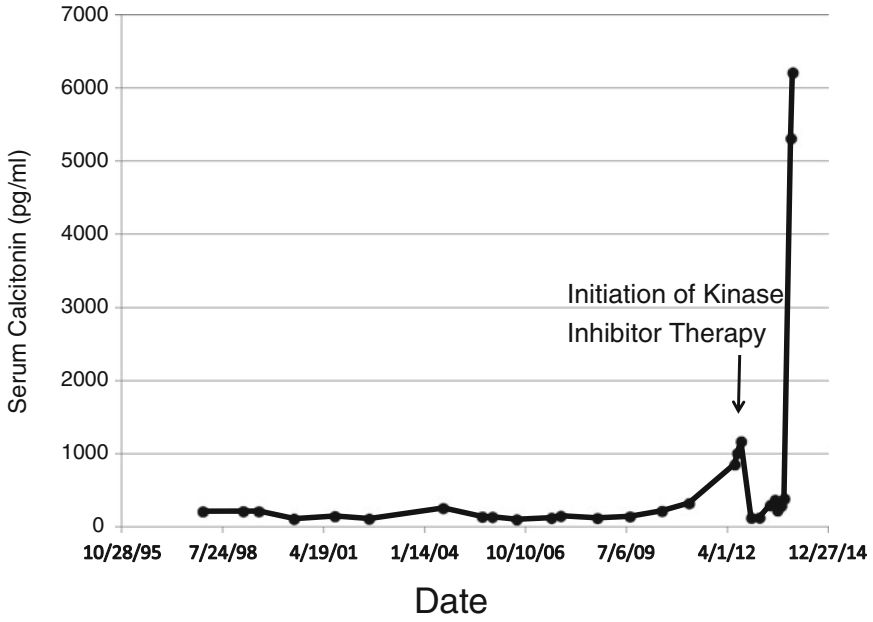

Fig. 2 Watchful waiting. This patient with MEN2 underwent a total thyroidectomy and neck dissection for MTC. Serum CT and CEA (not shown) measurements remained stable over almost a 15-year period before increasing rapidly. This was associated with development of hepatic and pulmonary metastasis. Treatment with tyrosine kinase inhibitor therapy resulted in a return of serum CT and CEA to pretreatment levels with stability for slightly more than a year and resolution of pulmonary metastasis. The patient subsequently progressed rapidly while on therapy and died

8.2 Rapid Escalation of Tumor Growth in a Patient with Prior Stable Disease

Rapid escalation of tumor markers and tumor growth following a prior period of stability is another situation in which kinase inhibitor therapy is appropriate. Figure 2 shows such an example. This patient had a 15-year period following thyroidectomy for MEN2B-associated MTC where there was no clinical evidence of disease other than minimal elevations of the serum CT and CEA where "watchful waiting" was the most appropriate approach. A rapid rise in serum CT and CEA was associated with development of hepatic and pulmonary metastasis. Although the patient had a substantial response to tyrosine kinase inhibitor therapy with marked reduction of pulmonary metastasis and had an additional year of quality life, tumor growth subsequently escalated on therapy and the patient succumbed a year and a half later. While the question can be asked of whether earlier low-dose therapy might have impacted the subsequent rate of tumor growth, there is, in fact, no current evidence that earlier therapy would be beneficial.

8.3 Location of Metastatic Medullary Thyroid Carcinoma

Another feature that could drive a decision to initiate therapy is the location of the lesion. It is possible to have substantial metastases in the liver and lungs that grow slowly over time with little functional impairment. Figure 3 shows an example of patient with hepatic metastasis who had a prolonged and durable response to tyrosine kinase therapy over an extended period. In contrast, lesions that about the upper airway or sizeable lesions in the mediastinum can lead to significant impairment of respiratory or esophageal function. In a patient with such an inoperable lesion, tyrosine kinase therapy could be considered. One complication that has been observed is fistula development or bleeding associated with a lesion adjacent to the trachea or esophagus, particularly if there is a rapid response to therapy. As both vandetanib and cabozantinib target VEGFR2 receptors, their impact on tumor vascularity can cause hemorrhage in a patient with a lesion adjacent to and invading the trachea. Figure 4 shows an example of a patient with long-standing MTC who developed tracheal invasion that was not considered

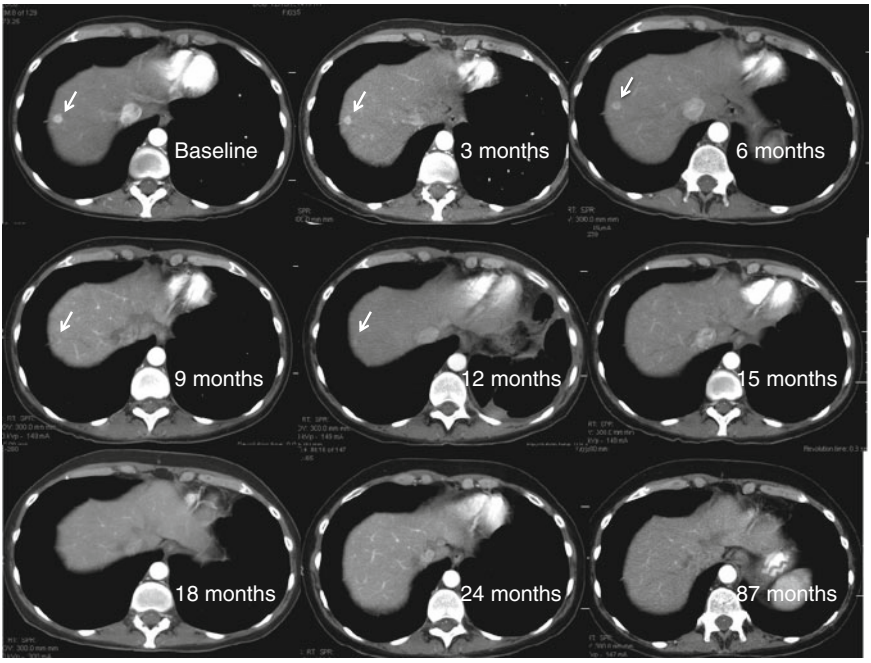

Fig. 3 Prolonged response to tyrosine kinase therapy. This patient with MEN2A and hepatic metastasis responded to therapy within 12 months; after 7 years of continuous tyrosine kinase inhibitor therapy, there is no evidence of recurrence

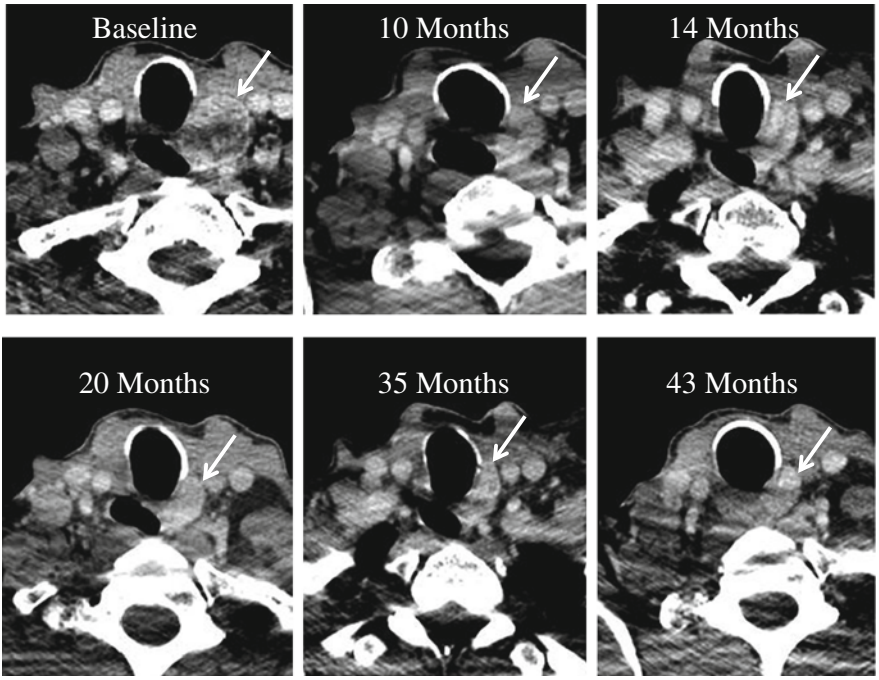

Fig. 4 Response of paratracheal mass to tyrosine kinase therapy. This patient with MTC presented with a paratracheal mass that was unresectable without a concurrent laryngectomy, which he declined. Treatment with tyrosine kinase inhibitor therapy resulted in a significant reduction in tumor size. The response has been maintained for a period of almost 4 year, but without further reduction in size after the first 20 months

resectable without performance of a laryngectomy, which the patient declined. A decision was made, with full discussion with the patient of the potential risks of fistula development and/or hemorrhage, to initiate therapy with a tyrosine kinase inhibitor. The patient has tolerated the starting dose without toxicity for a period of almost 4 years with reduction in the size of the paratracheal mass.

8.4 Bone Metastasis

Bone metastasis occurs commonly in patients with metastatic MTC. As metastasis to bone is excluded from analysis using RECIST criteria for evaluation of tumor response, there is no formal analysis of response in the phase II or III trials of either vandetanib or cabozantinib (Wells et al. 2012; Elisei et al. 2013). Figure 5 shows a bone response to tyrosine kinase inhibitor therapy at 13 months with a reduction in size of multiple lesions and remineralization of lytic lesions, indicating potential efficacy.

9 Prediction of Response

The only criterion that predicts response is the presence of a somatic or germline RET codon M918T mutation as was demonstrated for both vandetanib and cabozantinib in the phase III trials (Wells et al. 2012; Elisei et al. 2013). In addition, there is evidence of an overall survival benefit for patients with RET codon M918T mutations treated with cabozantinib in recent results from the phase III trial (Schlumberger et al. 2015).

10 Duration of Response

There are no predictors of duration of response in the phase III trials of either vandetanib or cabozantinib. That durable responses occur has been documented. Figure 4 shows a durable response of almost 4 years for a paratracheal mass and Fig. 3 shows an example of an MEN2 patient with hepatic metastasis treated with a tyrosine kinase inhibitor who responded within a 12-month period and had no

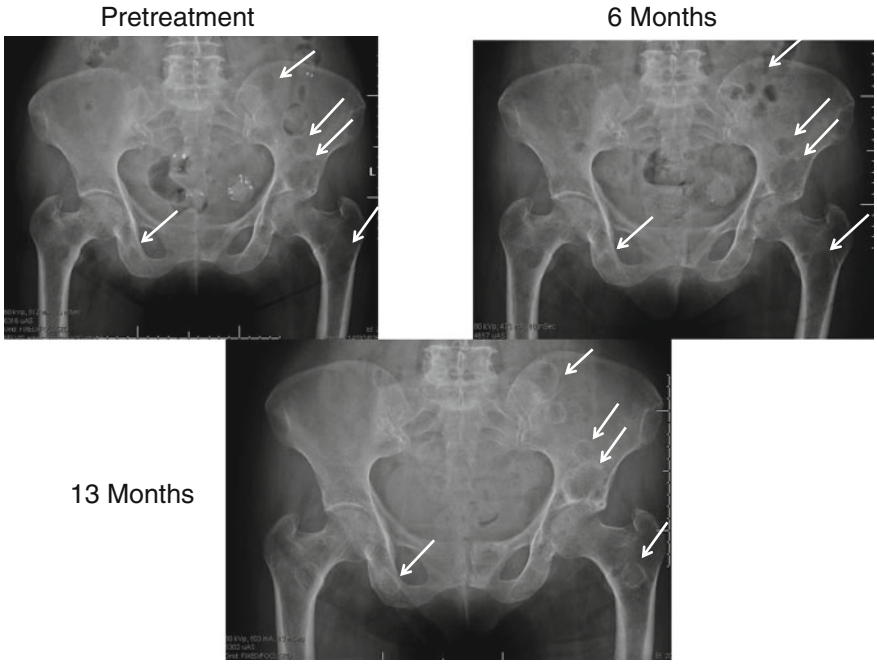

Fig. 5 Reduction in size of bone metastasis and partial healing of lytic lesions in a patient with metastatic MTC. This elderly patient had widespread metastatic MTC with extensive bone metastasis. This figure shows reduction in the size of pelvic bone metastasis and remineralization of lytic lesions over a 13-month period of treatment with tyrosine kinase inhibitor therapy. The patient subsequently developed recurrence while on therapy and died

evidence of recurrence over a 7-year period. What is not clear is the frequency of such responses. Available evidence from the phase III trial of cabozantinib indicates that overall survival, a surrogate for response, is enhanced only in patients with a somatic or germline RET codon M918T mutation (Schlumberger et al. 2015). There are no similar data available for vandetanib at this time. Anecdotal experience suggests that responses lasting greater than 3–4 years will occur in a minority of patients.

11 Tyrosine Kinase Inhibitor Use in Pediatric Patients

A phase I/II trial of vandetanib in children and adolescents at a starting dose of 100 mg/m^2 with a potential dose escalation to 150 mg/m^2 after two 28-day cycles showed an objective partial response rate of 47 %, with a mean treatment duration of 27 cycles (Fox et al. 2013). Eleven of 16 patients entering the trial remained on therapy at the conclusion of the study. There have been no comparable trials of cabozantinib in the pediatric or adolescent populations.

12 Dose Reductions of Tyrosine Kinase Inhibitor

There is a long-standing tenet in the oncology community that starting at the highest effective and tolerable dose is preferred over starting at a lower dose and escalating the dose over time. This is based on the fact that some patients will have a substantial reduction in tumor size and vascularity during the first 3 months after initiation of therapy and there is concern that starting at a lower dose will impact negatively on this initial response. A significant percentage of patients will not tolerate the approved starting doses of vandetanib or cabozantinib because of toxicity and will require a dose reduction.

There have been no published studies directly comparing lower initial doses of either approved agent, although a comparative trial of vandetanib 150/day versus 300 mg/day is currently nearing completion. A prior phase II trial of vandetanib (100 mg/day) in hereditary MTC indicates that a lower starting dose has activity (Robinson et al. 2010). It therefore may be reasonable to consider a lower starting dosage with subsequent dose escalation in an individual with lower than normal body weight, poor performance status, or some other disease process (cardiac, pulmonary, dermatologic, or hepatic) that might predict toxicity.

The decision to lower the dose of either cabozantinib or vandetanib should be based on the development of significant toxicity. In the case of cabozantinib or vandetanib, this could include the development of hypertension not controlled by antihypertensives, deterioration in renal function or development of significant proteinuria, liver function abnormalities, unmanageable vomiting or diarrhea with profound weight loss, and severe dermatologic manifestations or other less common but significant side effects (Tables 2 and 3). Unique to cabozantinib is the

development of palmar-plantar erythrodysesthesia (Cho and Chan 2013), whereas significant prolongation of the QT interval that is not remedied by the drug or supplement modifications mentioned earlier is an indication for vandetanib dose reduction (Shah et al. 2013). In general, when severe side effects occur, cessation of drug for a period of time to allow for normalization is mandatory followed by a dose reduction of 25–30 %.

Generally, the development of primary hypothyroidism, hypocalcemia caused by calcium, vitamin D malabsorption, or hypomagnesemia can be managed without dose reduction of the kinase inhibitor by dose escalation of thyroid hormone in thyroidectomized patients or dose increases or supplementation with calcium, vitamin D, or magnesium preparations. Symptomatic hypocalcemia may require a temporary cessation of kinase inhibitor therapy. In a patient who had a bilateral adrenalectomy for pheochromocytoma in the context of MEN2 and is on corticosteroid and mineralocorticoid replacement, it may be challenging to differentiate between adrenal insufficiency and symptoms attributable to kinase inhibitor therapy. A dose increase of corticosteroid or mineralocorticoid may be needed. It is clear from published reports that vandetanib has profound effects to inhibit ectopic production of ACTH by MTC (Nella et al. 2014), thereby reversing Cushing's syndrome. A similar effect was seen in a patient with MTC treated with sorafenib (Barroso-Sousa et al. 2014). It is less clear whether vandetanib has similar effects on the normal pituitary gland. One published report suggests that plasma ACTH concentrations are increased in vandetanib-treated patients (Brassard et al. 2011). The finding that vandetanib reverses ectopic ACTH production in MTC may have relevance to management of ectopic ACTH syndrome caused by lung cancer; earlier studies showed activity of this agent in the treatment of lung cancer (Tsao et al. 2013). One of the authors (RFG) has a patient with MEN2A and bilateral adrenalectomy who had two episodes of adrenal insufficiency while on cabozantinib, necessitating a dose reduction of the kinase inhibitor.

13 Combining Tyrosine Kinase Inhibitor Therapy with Other Treatment Modalities

The availability of agents that are capable of reducing tumor mass opens up the possibility of combining tyrosine kinase inhibitor therapy with other proven treatment modalities. In the example shown in Fig. 4, the question asked was whether treatment with a kinase inhibitor might make this patient's tumor resectable at some future date. A similar logic could be applied to other neck or mediastinal lesions that threaten the airway. At present, this is uncharted territory, but is being actively considered. A potential concern is that treatment with these agents may make clean surgical margins more difficult to obtain. In addition, it is important to recognize the anti-VEGFR2 effects of these agents. Stoppage of these agents a suitable time prior to surgery is mandatory to facilitate wound healing. Similarly, treatment of a neck or mediastinal mass with kinase inhibitor therapy prior to

radiation might reduce the size of the radiation field and hence reduce collateral radiation damage. Such strategies are being considered and will undoubtedly be pursued in the future.

14 The Future

One of the realities facing the MTC research community is the rarity of MTC and hence the lack of enthusiasm for pharmaceutical manufacturers to make a major commitment to new drug development for this tumor. However, that should not stop investigators in the field from pursuing the growing number of agents with known activity against RET, RAS, or BRAF, known to be mutated in MTC, or other potential targets such as mTOR, ATF4, or other factors that interact with RET-mediated signaling pathways. In addition, there is the possibility that other kinase inhibitors that target receptor tyrosine kinases may prove more effective than current agents. One such candidate is ponatinib, a multi-kinase inhibitor with substantial activity against RET and VEGFR2, which is currently under investigation. Finally, the potential for combining tyrosine kinase therapy with immunotherapy is largely unexplored. It is clear that the future is bright for progress in the treatment of this malignancy.

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