ISSN 2472-1972

Update on Multiple Endocrine Neoplasia Type 2: Focus on Medullary Thyroid Carcinoma

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Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant hereditary cancer syndrome caused by missense gain-of-function mutations in the *RET* proto-oncogene on chromosome 10. Specific *RET* mutations can predispose toward a particular phenotype and clinical course, with strong genotype–phenotype correlations. MEN2 is highly penetrant in medullary thyroid carcinoma (MTC), and it can be associated with bilateral pheochromocytoma and primary hyperparathyroidism. Two different clinical variants of MEN2 are known: MEN2A, which includes the familial subtype, and MEN2B. Treatment includes early thyroidectomy. Recommendations on the timing and extent of surgery are based on the *RET* mutation risk categories (moderate-, high-, or highest-risk) regarding the age of MTC onset. Early identification of patients with hereditary MTC has improved treatment outcomes. Previously, MTC was diagnosed based on clinical tumors; in contrast, with genetic screening, MTC can be diagnosed at preclinical disease states. This approach has resulted in a high cure rate and a much better prognosis for MTC. However, classification into one of the three *RET* mutation risk groups for predicting aggressiveness and prognosis has had limited impact. Increasing evidence has shown that patients with *RET* mutations in different risk classifications exhibit a broad spectrum of MTC aggressiveness during follow-up, with no relevant difference in survival. The specific germline activating mutation of the *RET* proto-oncogene appears to be the first determinant of the age of MTC onset, but, presumably, different regulatory events determine long-term tumor behavior.

Freeform/Key Words: calcitonin, medullary thyroid carcinoma, multiple endocrine neoplasia type 2, RET mutation

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant hereditary cancer syndrome caused by missense gain-of-function mutations in the *RET* proto-oncogene. MEN2 is highly penetrant in medullary thyroid carcinoma (MTC), and it can be associated with bilateral pheochromocytoma and primary hyperparathyroidism [1]. Two different clinical variants of MEN2 are known: MEN2A, which includes the familial subtype, and MEN2B. Over 100 *RET* point mutations, duplications, insertions, deletions, and fusions have been identified in patients with MEN2A. In contrast, only two *RET* mutations (918 and 883) have been identified in patients with MEN2B [2]. With molecular testing, *RET* mutations can be identified in 98% to 100% of evaluated cases. Specific *RET* mutations predispose the individual to a particular phenotype, with strong genotype–phenotype correlations. *RET* mutations are classified into three *RET* mutation risk levels: moderate-, high-, and highest-risk, determined by age of onset and the potential aggressiveness of MTC. These genotype-phenotype correlations have become the basis for clinical decision-making when managing patients with MEN2. Although different guidelines might vary, all include assigning

Abbreviations: ATA, American Thyroid Association; ATF, activating transcription factor; CEA, carcinoembryonic antigen; Ctn, calcitonin; MEN2, multiple endocrine neoplasia type 2; miRNA, microRNA; MTC, medullary thyroid carcinoma; TNM, tumor/node/metastasis.

Received 11 June 2018
Accepted 10 July 2018
First Published Online 13 July 2018

patients to risk groups and management based on the specific mutation [3–7]. The American Thyroid Association (ATA) categories are: ATA moderate-risk mutations (exon 10, exon 11 mutations other than codon 634, and exons 13 through 15), which confer the lowest risk of developing MTC and a relatively late MTC onset; ATA high-risk mutations (exon 11, codon 634, the classical MEN2A mutation; and exon 15, codon 883), which confer intermediate risk; and ATA highest-risk mutations, including the typical MEN2B mutation (exon 16, codon 918), which confer the highest risk of early MTC development and growth.

In addition to the *RET* mutation, a tool for clinical decision-making in MEN2 is the detection of elevated calcitonin (Ctn), the primary secretory product of MTC. Ctn is elevated in nearly all patients with MTC; the prevalence of nonsecretory MTC is 0.83% [8]. Basal Ctn concentrations typically correlate with tumor mass; Ctn is almost always elevated in patients with palpable tumors [9]. Moreover, elevated plasma Ctn after surgical tumor removal indicates persistent or recurrent disease. The reference ranges of serum Ctn levels are sex and age specific. Normal Ctn concentrations are <9.6 pg/mL in men and <6.4 pg/mL in women. Higher values have been reported in children, depending on the assay used [10–12].

Several lines of evidence have suggested that, although the age of onset in hereditary MTC is clearly different in the different *RET*-mutation risk groups, there is no clear difference in the marked variations of MTC aggressiveness, progression, or outcome between risk groups. Consequently, in the last few years, several study groups have attempted to refine and revise the current risk classifications. Some aimed to break down the risk groups into individual codon-based categories. Other ongoing studies have aimed to improve Ctn determinations with more sensitive and/or more specific assays to facilitate clinical decision-making in early thyroidectomy. Here, we review the current approaches for performing genotype-phenotype correlations, with a particular focus on the timing of early thyroidectomy and the prognosis of MTC, in different MEN2 risk groups.

1. Search Strategies

We conducted a PubMed search of the international literature from the last 10 years with a cutoff of 30 April 2018. The key words included “multiple endocrine neoplasia type 2,” “medullary thyroid cancer/carcinoma,” “calcitonin,” and “RET mutation.” We also included the guidelines of international societies. Topics for further discussion were based on clinical impact.

2. Origin of C Cells: Clearing Up an “Old Story”

Thyroid C cells were previously thought to be derived from the neural crest. This hypothesis was based largely on chick-quail xenotransplantation studies by Le Douarin *et al.* (1970) [13], which revealed that avian Ctn-producing ultimobranchial bodies were derived from the neural crest. Recently, Johansson *et al.* (2015) [14] used an elegant, lineage-tracing scheme to confirm that mouse ultimobranchial bodies and thyroid C cells were not derived from neural crest cells but rather from pharyngeal endoderm. Nevertheless, C-cell development is significantly influenced by neural crest–derived cells [15].

3. Timing of Prophylactic Thyroidectomy in Patients With MEN2: “Window of Opportunity”

A. Risk Classification

The only cure MTC is surgery; however, the timing of surgery is crucial in MEN2. Ideally, a “true prophylactic thyroidectomy” or an “early thyroidectomy” should be performed in a patient with a *RET* mutation that is clinically asymptomatic before the development of MTC or at least when the MTC is confined to the thyroid and before the disease spreads beyond the gland [3, 16]. The multicentric and bilateral nature of hereditary MTC implies that a total thyroidectomy is necessary. The majority of patients who undergo surgeries at early ages

have C-cell hyperplasia or small MTC foci. C-cell hyperplasia follows an age-related progression to lymph node–negative MTC and then to lymph node–positive MTC. These stages exhibit steep gradients among patients with highest-risk, high-risk, and moderate-risk *RET* mutations [17]. The youngest patients with moderate-risk MTC were 9 years (mutation 891) and 5 years (mutation 618) of age; the youngest patient with high-risk MTC was 16 months (mutation 634) of age, and the youngest patient with highest-risk MTC was 9 weeks (mutation 918) of age. The ages of the youngest patients with lymph node metastases were 16 years (moderate-risk, mutation 620), 11 years (high-risk, mutation 634), and 2 years (highest-risk mutation 918) [18].

To obtain the best outcomes, it is critical to identify *RET* mutation carriers as early as possible [*e.g.*, after birth in the highest-risk group (*RET* mutation 918, MEN2B), by age 3 in the high-risk group (*RET* mutation 634), and by age 5 in the moderate-risk group (*RET* mutations in exons 10 and 13 through 15]. After identifying a mutation carrier, children in the highest-risk group with a *RET* codon M918T mutation should have a thyroidectomy as soon as possible in the first year of life. Children in the high-risk group (*RET* mutation 634) should have a thyroidectomy at 5 years of age or earlier, based on the detection of elevated serum Ctn level measured every 6 months. In children in the moderate-risk group, the timing of thyroidectomy should be based on the detection of an elevated serum Ctn level. Measurement of serum Ctn levels should be started around 5 years of age and repeated every 6 to 12 months [3]. Malignant transformation occurs when Ctn levels begin to exceed the age- and assay-dependent upper reference limit of the Ctn assay (*e.g.*, >10 pg/mL). However, lymph node metastases have not been reported for Ctn serum levels ≤30 pg/mL [18].

B. Surgical Procedure

Early identification and risk classification of asymptomatic infants and young children as *RET* carriers is crucial. Early identification permits management within the "window of opportunity," which represents the time that a total thyroidectomy alone can provide adequate therapy. This timing can obviate the need for lymph node dissections, which increase surgical morbidity [19]. Indeed, when the window of opportunity is missed and neck lymph node dissection is necessary, the chances of an immediate biochemical cure are reduced: cure rates are only 57% to 31% with 1 to 10 node metastases and merely 0% to 4% with >10 node metastases [20]. These associations were confirmed in a recent study in 79 children who received late genetic testing. Those surgeries were performed after the ages recommended by the guidelines, and they required cervical lymph node dissections, which resulted in more permanent hypoparathyroidism and reduced cure rates [21]. When an additional central node dissection is necessary, it raises the frequencies of transient and permanent hypoparathyroidism and transient recurrent laryngeal nerve palsy [22, 23]. Moreover, children aged 3 years and younger have higher vulnerability and more often develop transient and permanent hypoparathyroidism, particularly when they carry *RET* 918. To reduce the risk of surgery-associated morbidity, it may be prudent to enlist a high-volume surgeon to apply optical magnification, bipolar forceps coagulation, and nerve-monitoring devices and to preserve the parathyroid glands *in situ* as much as possible. Managing these patients is challenging, and decision-making is often not straightforward; it involves balancing the risks and benefits, particularly in younger patients.

C. Genotype–Phenotype Correlation

The strong genotype–phenotype correlation defines the onset of MTC development in MEN2. Children with codon 918 mutations (found in >95% of patients with MEN2B) are in the highest-risk group. Surgery is recommended as early as possible, preferably within the first year after birth, despite the high operative morbidity. A surgical cure is achievable in expert hands before the age of 4 years, but it becomes exceptional thereafter [24, 25]. In contrast, these recommendations do not apply to patients with MEN2B who have codon 883 mutations. The codon 883 mutation appears to confer lower risk than the codon 918 mutation [26, 27].

Based on current data, patients with codon 883 mutations have been moved from the ATA highest-risk category to the high-risk category [3]. For patients in the moderate-risk group, MTC penetrance is lower and age-related onset later than in the other risk groups; thus, the timing of thyroidectomy is challenging. In this risk group, the age of MTC onset shows substantial variability, even among patients with the same codon mutation [28]. For example, with a codon 611 mutation, the likelihood of developing MTC by age 70 is estimated to be 89%, and at age 20 it is only 10%. Moreover, even within exon 10 mutations, studies have shown differences in both the age-related penetrance and aggressiveness of MTC, depending on the mutated codon. For example, an earlier onset of MTC was linked to mutations located in the extracellular cysteine-rich domain, which is close to the cell membrane, compared with other mutations (*i.e.*, codon 618/620 vs codon 609/611) [29, 30].

Within the last 20 years, routine sequencing of *RET* mutations in all patients with MTC has led to a shift in the detected frequency of *RET* mutations. Previously, the most common mutations were in the classical 634, high-risk codon. Later, more patients were found to harbor the moderate-risk mutations in exons 13 through 15 [31]. In addition, in 7.3% of apparently sporadic MTC cases, a germline *RET* mutation was found. Therefore, it is important that each patient with sporadic MTC be tested for a germline RET mutation. Most of the latter cases were moderate-risk level mutations with a late onset and a lack of family history [32]. These data supported findings that MTC onset was later for lower-risk *RET* mutations compared with the more "classic" descriptions of MEN2. However, the age of MTC onset remains highly variable, even among patients with the same codon mutation.

D. Importance of Ctn Determination

Within each risk group defined by the *RET* mutation, the individual risk of developing C-cell hyperplasia/micro-MTC can be assessed by determining the Ctn level; when the Ctn rises, a thyroidectomy should be planned. All patients with preoperative basal CT levels <30 ng/L were cured with a thyroidectomy, regardless of the genotype risk level. Therefore, first screenings should be performed at ages <1 year (highest-risk group), 1 to 5 years (high-risk group), or 5 years (moderate-risk group) [18]. This recommendation was consistent with results from both an Italian group, who showed that the timing of the thyroidectomy in gene carriers could be personalized and safely planned when Ctn was below 60 pg/mL [24], and a Norwegian group, who showed that preoperative basal Ctn alone could indicate the optimal timing and extent of thyroid surgery for patients with MEN2A [33].

Therefore, the optimum procedure for scheduling a thyroidectomy seems to be (1) perform early genetic testing and identification of the *RET* mutation, (2) classify the risk to determine the age-related MTC penetrance based on the *RET* mutation group, and (3) determine the serum Ctn level.

4. Follow-up and Progression of MTC in Patients With MEN2

A. Follow-up and Risk Stratification

A dynamic system for stratifying risk in patients treated for MTC uses a combination of the tumor/node/metastasis (TNM)/American Joint Committee on Cancer staging system, the postoperative nadir of Ctn and carcinoembryonic antigen (CEA), and imaging studies to identify local recurrences or distant metastases. With this system, patients can be stratified into three postoperative categories: cured, biochemically incomplete response, and structurally incomplete response [34–36].

B. Postoperative Ctn

Patients with MEN2 who receive an early thyroidectomy should be cured with no measurable postoperative serum Ctn. A detectable basal serum CTN after surgery, even within

the normal range, represents a persistent state with the new assays. MTC progression is variable; years might pass before a clinical recurrence develops, if ever. In one study, 50 consecutive youngsters with a germline *RET* mutation were evaluated at least 5 years after a prophylactic thyroidectomy; 44 had no measurable Ctn and no evidence of persistent or recurrent MTC [37]. The six patients with elevated Ctn were older at surgery and received prophylactic surgery after the age recommended based on risk level. Schreinemarkers *et al.* [38] reported similar findings in 18% of patients with MEN2 who received prophylactic thyroidectomies. In a French multicenter study on hereditary MTC, which included 170 patients <21 years old, the only independent factors associated with disease-free survival after MTC surgery were based on the TNM stage: <10 mm tumor diameter and N0 lymph node rating. That study supported the notion that TNM staging is more reliable than genotype for scheduling follow-ups for these patients. Moreover, all patients with persistent disease had preoperative Ctn levels >30 pg/mL [18]. Those results were consistent with the Norwegian MEN2A study, which showed that biochemical cure was achieved in patients with preoperative Ctn levels <40 pg/mL [33]. The best results were reported by Machens *et al.* [16], who observed postoperative Ctn normalization in 114 of 115 children within an 84-month observation period. Unfortunately, no published data are available on long-term (>40 years) outcomes to reinforce the concept that early thyroidectomy provides a cure. Not surprisingly, when index patients present with Ctn levels >30 to 60 pg/mL and clinical disease with lymph node metastases before the genetic diagnosis is performed, they exhibit a worse outcome due to the more advanced stage at presentation. When patients diagnosed with C-cell hyperplasia are screened before clinically apparent MTC has developed, they uniformly experience good outcomes. Thus, the importance of the three diagnostic steps cannot be overemphasized: (1) confirm a *RET* mutation, (2) classify the *RET* risk group, and (3) determine the preoperative Ctn to ensure a timely thyroidectomy in an asymptomatic carrier.

C. Ctn Doubling Time

Lifelong follow-up is indicated for MTC, beginning every 3 months postoperatively and at longer intervals when there is no evidence of persistent or recurrent disease in the first year after thyroidectomy. If Ctn is undectable, it should be measured every 6 months for 1 year and then yearly thereafter, especially in patients with CCH at primary surgery. Ctn measurement can be stopped after a period of years. Several measurements of serum Ctn and CEA levels are useful in documenting disease progression. In particular, doubling time should be calculated for these two markers [39, 40]. Ctn and/or CEA doubling times <6 to 12 months are associated with rapid structural disease progression, decreased survival, and decreased progression-free survival. Conversely, doubling times >2 years are associated with disease stability and excellent survival. In most cases, with comprehensive follow-up examinations, tumor markers increase slowly; small, slow-growing local recurrences or stable distant metastases without clinical symptoms are typically detected during a 10-year follow-up. When necessary, local treatment may be sufficient, but active surveillance is appropriate in most cases. Ctn levels parallel the overall tumor mass [11], but they can vary considerably, even among comparable tumors and the same tumor burden. One single Ctn serum measurement provides only a rough outline of the actual tumor growth/recurrence [9].

D. MTC Tumor Growth and Aggressiveness: Dependent on RET-Genotype or the Risk Group?

The onset of MTC is significantly age related across the *RET* mutation risk categories [17]. This graduation suggested that both MTC development and progression might be age related. Compared with moderate-risk *RET* mutations, high-risk *RET* mutations are associated with an MTC onset nearly two decades earlier and presumably with greater MTC aggressiveness. This observation raises the question: Does the age of MTC onset predict its long-term behavior? Only a few studies have focused on the aggressiveness of MTC in different risk groups during follow-up.

The natural course or growth rate of MTC cannot be measured directly in patients with *RET* mutations. Tumor growth is approximated at the surgical pathology level. Thus, tumor diameter

and the age of MTC diagnosis can be compared between asymptomatic carriers with smaller tumors (identified through family screening) and index patients with larger, clinically apparent tumors. With this procedure, primary tumor growth rate for a lymph node–negative MTC was 0.4 to 0.5 mm/y, with no difference between ATA high-risk (*RET* 634) and moderate-risk (exon 13 through 15 mutations) groups [41]. The annual primary tumor growth rates for node-positive MTC were 2.6 mm (ATA high-risk mutations) and 1.2 mm (ATA moderate-risk, exon 1 through 15 mutations). When patients with node-negative and node-positive MTC were combined, primary tumor growth rates were 0.72 mm/y for ATA moderate-risk and 0.97 mm/y for ATA high-risk groups, but the difference between groups was not significant. Moreover, for node-positive MTC, the annual lymph node metastasis rate was 0.6 to 0.7 nodes, independent of ATA risk group. These results did not support the assumption that MTC aggressiveness depended on risk group. In an additional study of hereditary MTC grouped by *RET* mutational risk, the progression of MTC within histopathological groups (normal/C-cell hyperplasia, node-negative, and node-positive MTC) was significantly age related [17], but the development of lymph node–negative into lymph node–positive MTC occurred at similar time intervals in all risk groups (8 to 12 years). These results challenged the perception that clinical aggressiveness was increased in the high-risk group compared with the moderate-risk group. Conversely, the results supported the hypothesis that the different risk-related *RET* mutations, after the initial onset of MTC, give rise to equally aggressive tumors. In other words, before MTC manifests, disease onset is largely driven by the respective *RET* mutation. Then, after MTC manifests, equally aggressive tumors become apparent across all *RET* mutation risk levels.

That hypothesis is consistent with the observation that, when the time of MTC diagnosis was taken as the time of origin, patients with high- and moderate-risk *RET* mutations had similar overall survival rates and showed a similar rate of developing distant metastatic disease [42]. Thus, once MTC developed, the clinical course was statistically equivalent in terms of distant metastases and survival. In fact, the only (and most important) prognostic factor in patients with MEN2 is tumor stage at diagnosis. Thus, because the percentage of advanced tumors at diagnosis is higher in the higher-risk groups, the survival rates are lower in the higher-risk groups. In a study that included a group with the highest-risk RET mutation, among 73 patients with MEN2B, 20% were cured, and, astonishingly, the 5-, 10-, and 20-year cancer-specific survival rates were 85%, 74%, and 58%, respectively [43]. Those findings were comparable to those from the recent SEER Study, which included 2400 patients with MTC who were not differentiated between sporadic and hereditary disease. These patients showed 5- and 10-year survival rates of 83% and 72%, respectively [44]. Survival data for patients with MEN2B showed similar disease course and tumor stage–dependent survival compared with those of patients with MEN2A or sporadic MTC. A multivariate analysis showed that the most important prognostic factor of life expectancy in 120 patients with sporadic and hereditary MTC was disease stage at diagnosis [45–47]. Thus, the different risk groups showed comparable MTC tumor growth rates, similar times to development from node-negative to node-positive MTC, comparable disease-free survival rates, and similar times to develop distant metastases. These findings supported the hypothesis that all hereditary MTC progressed with comparable aggressiveness after the initial signs of MTC, independent of risk classification.

These observations have important clinical implications: the follow-up for a patient with a moderate-risk mutation must be as intensive as the follow-up for an individual with a high-risk mutation. Both develop equally aggressive tumors but at different times in life. Therefore, surgical treatment of these tumors should be appropriate for the extent of disease. However, these findings have raised important questions about the molecular events, aside from the *RET* mutation, that occur in the different risk groups after MTC manifestation.

5. Progression of MTC: The *RET* Mutation and Beyond

Germline-dominant, activating mutations in the *RET* proto-oncogene, which encodes a receptor tyrosine kinase, have been identified as primary initiating events that cause MTC and other components of MEN2. The role of *RET* in MTC was further supported by the finding

that somatic point mutations in the *RET* proto-oncogene were associated with sporadic MTC (mostly the *RET* M918T mutation) [48, 49]. Several researchers have shown that *RET* somatic *M918T* mutations played a prognostic role in the clinical outcomes of patients with MTC, demonstrating a significant correlation between the presence of a somatic *RET* mutation and an aggressive phenotype of sporadic MTC [50–52].

Oncogenic *RET* mutations induce a ligand-independent, constitutive, *trans*-autophosphorylation of the RET receptor, which stimulates multiple downstream pathways that promote cell growth, proliferation, survival, and differentiation. These activation events appear to be prerequisite for cellular transformation. The activated signaling cascades involve MAPK, phosphoinositide 3-kinase, protein kinase B, signal transducer and activator of transcription 3, proto-oncogene tyrosine-protein kinase Src1, and focal adhesion kinase. Independent of the type of activating *RET* mutation, the final effect is uncontrolled activation of MAPK and phosphoinositide 3-kinase pathways, which results in uncontrolled growth and cell dedifferentiation. Sporadic MTC cases are typically associated with few mutations aside from the *RET* mutation. The only other mutations identified in sporadic MTC are mutations in *NRAS*, *KRAS*, and *HRAS*, and those are nearly mutually exclusive with *RET* mutations. This exclusivity supports the hypothesis that *RET*-mediated oncogenic transformation occurs separately from *RAS* [53]. *RAS* mutations showed little prognostic value in predicting tumor aggressiveness [54]. However, because MTC occurs in the absence of *RET* mutations in sporadic MTC, other pathways to C-cell oncogenesis must exist. Moreover, tumor progression and aggressiveness might be caused by simultaneous hits to different pathways.

The aberrant loss of retinoblastoma (RB) proteins has been associated with the development and progression of numerous cancer types. Thus, *RB* genes serve as tumor suppressor genes. Consequently, RB pathway disruption could potentially mediate C-cell transformation. Although no loss or mutation of the *RB1* gene has been found in MTC, inactivation of the Rb1 regulatory pathway was found in MTC tissue, and this finding was associated with reduced patient survival [55].

When oncogenic *RET* activity is disrupted, cell viability and cell proliferation decrease; this result indicates that oncogenic *RET* stimulates proliferation and blocks apoptosis. This activity could involve the tyrosine kinase activity of RET, which is a membrane-bound receptor that, once activated, can transfer into the nucleus. In the nucleus, RET inhibits the proapoptotic transcription factor activating transcription factor (ATF)4, which might be the mechanism underlying the antiapoptotic function of RET. MTC tumors have lower ATF4 levels than normal thyroid follicle cells, and RET expression is negatively correlated with ATF4 protein levels in MTC tumors. Low ATF4 expression levels were associated with a more aggressive form of MTC and poor overall survival. This feedback loop between RET and ATF4 might partly explain the variable aggressiveness of tumors among patients with MTC.

Several microRNAs (miRNAs) were shown to be dysregulated in MTC. Some of these miRNAs participate in tumor progression [56–58]. Some miRNAs, like miR-183, miR-375, and miR-21, were strongly correlated with more aggressive disease phenotypes, and this correlation was not significantly different in sporadic and hereditary MTC [58]. A significant upregulation of miR-182 (a member of the miR-183 ~96 ~182 cluster) was observed in MTC tissues that harbored *RET* mutations in codons 918 and 634 compared with normal thyroid tissue. Mutated *RET* activates NF-κB, which induces miR-182 expression in the nucleus. A direct target of miR-182 is HES1; the binding of miR-182 to the 3′ untranslated region of HES1 mRNA caused significant downregulation of HES1 activity *in vitro* and in MTC tissues. HES1 is a key player in the Notch signaling pathway. The loss of HES1 expression, through a negative regulatory loop, leads to the reduction of Notch1, which could explain the loss of the Notch pathway in MTC. Consequently, Notch1 cannot exhibit its tumor suppressor function in MTC, which leads to a more malignant phenotype [59]. Thus, another mechanism for promoting MTC aggressiveness starts with mutated *RET*, which stimulates NF-κB, which drives expression of miR-182, which impedes HES1 activation, which reduces Notch 1 tumor suppression.

When the entire MTC gene expression profile was classified by the type of *RET* gene mutation and the cancer genetic background (hereditary vs sporadic), no distinct differences were found in the gene expression profiles of hereditary and sporadic MTCs [60].

Taken together, these studies highlighted the complexity of RET (oncogenic) signaling. The localization of the receptor in specific subcellular compartments and its interactions with other intracellular pathways are important elements in understanding RET function and its oncogenic deregulation. This information may lead to a better understanding of the different malignant phenotypes of MEN2.

Acknowledgments

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Disclosure Summary: The authors have nothing to disclose.

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