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Review

Genetics of medullary thyroid cancer: An overview

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HIGHLIGHTS

- Medullary thyroid carcinoma (MTC) represents 3–5% of thyroid cancers.
- 75% is sporadic and 25% is the dominant component of the hereditary multiple endocrine neoplasia (MEN) type 2 syndromes.
- In MEN2A, Codon 634 in exon 11 is the most commonly altered codon.
- V109G polymorphism is associated with sporadic MTCs negative for RET mutations, and might influence the clinical course of the patients affected by MTC.
- Interesting results come from two large phase III clinical trials with two targeted tyrosine kinase inhibitors (TKIs), vandetanib and cabozantinib.

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ABSTRACT

Medullary thyroid carcinoma (MTC) represents 3–5% of thyroid cancers. 75% is sporadic and 25% is the dominant component of the hereditary multiple endocrine neoplasia (MEN) type 2 syndromes. Three different subtypes of MEN2, such as MEN2A, MEN2B, and Familial MTC (FMTC) have been defined, based on presence or absence of hyperparathyroidism, pheocromocytoma and characteristic clinical features. Mutations of the RET proto-oncogene are implicated in the pathogenesis of MTC, but there are many other mutational patterns involved. In MEN2A, Codon 634 in exon 11 (Cys634Arg), corresponding to a cysteine in the extracellular cysteine-rich domain, is the most commonly altered codon. Many other mutations include codons 611, 618, 620. In the genetical testing of RET mutations in MTCs, Next-Generation Sequencing (NGS) is taking an increasingly important role. One of the most important benefit is the comprehensive analysis of molecular alterations in MTC, which allows rapidly to select patients with different risk levels. There is a difference in miRNA expression pathway between sporadic and hereditary MTCs. Among sporadic cases, expression of miR-127 was significantly lower in those who harbor somatic RET mutations than those with wild-type RET. CDKN1B mutations are associated with many clinical pictures of cancers, such as MEN4. V109G polymorphism is associated with sporadic MTCs negative for RET mutations, and might influence the clinical course of the patients affected by MTC. Although surgery (i.e. total thyroidectomy with neck lymph node dissection) is the elective treatment for MTCs, about 80% of patients have distant metastases at diagnosis and in this cases surgery is not enough and an additional treatment is needed. Interesting results come from two large phase III clinical trials with two targeted tyrosine kinase inhibitors (TKIs), vandetanib and cabozantinib. *Conclusions:* New genetical testings and therapeutical approaches open new perspectives in MTC management.

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1. Introduction

Medullary thyroid carcinoma (MTC) originated from the neural crest derived by parafollicular C-cells of the thyroid gland, which secrete the polypeptide calcitonin. MTC occurs in 3–5% of thyroid cancers. 75% is sporadic and 25% is the dominant component of the hereditary multiple endocrine neoplasia (MEN) type 2 syndromes [1]. Three different subtypes of MEN2, such as MEN2A, MEN2B, also named MEN3, and Familial MTC (FMTC) have been defined, based on presence or absence of hyperparathyroidism, pheocromocytoma and characteristic clinical features [2]. MEN 2A is characterized by the association between MTC and pheochromocytoma with parathyroid hyperplasia or adenoma, Hirschprung disease (rare), cutaneus lichen, amyloidosis. MEN 2B is instead identified by the presence MTC and pheochromocytoma, marfanoid habitus, mucosal neuromas, medullated corneal fibers and intestinal autonomic ganglion dysfunction, leading to mega colon [3]. In this phenotype hyperparathyroidism is absent. In other cases MTC is isolated (familial MTC). The age of onset of sporadic MTC is between fourth and sixth decade of life, while MTC typically occurs in early childhood in MEN 2B, early adulthood in MEN 2A, and middle age in FMTC [4]. Mutations of the RET proto-oncogene are implicated in the pathogenesis of MTC, but there are many other mutational patterns involved in pathogenesis and correlation genotype-phenotype of MTC.

2. RET mutations

RET proto-oncogene alterations are crucial events for thyroid cancer development [5]. Different mechanisms of RET activation, point mutations and gene rearrangements characterize medullary and papillary thyroid carcinoma and they are a very strong factor for poor prognosis in MTC [6]. RET is located on chromosomal band 10q11.2 and it encodes a receptor tyrosine kinase involved in many signaling pathway, which plays a key role in the development of parathyroids, urogenital system, and neural crest, including brain, para- and sympathetic ganglia, adrenal medulla, enteric ganglia, and thyroid C cells [7]. Not surprisingly, many of these organ systems are involved in the tumors which characterize MEN syndromes [8]. More than 100 gain-of-function RET mutations have been reported in patients with medullary thyroid carcinoma, including germline mutations in patients with hereditary disease and somatic mutations in patients with sporadic disease [9]. Specific germline mutations have been associated with the different subtypes of MEN2: several RET mutations give rise to MEN2A syndrome, the majority of which are located in cysteines within the extracellular cysteine-rich domain (corresponding to exons 10 and 11). The mutational pattern less frequenty involves exons 13 e 14. These mutations allow for constitutive dimerization and activation, in the absence of ligand [10,11]. In MEN2A, Codon 634 in exon 11 (Cys634Arg), corresponding to a cysteine in the extracellular cysteine-rich domain, is the most commonly altered codon. Many other mutations include codons 611, 618, 620. We reported [12] a novel case of multiple endocrine neoplasia type 2A (MEN 2A) associated with two mutations of the protooncogene RET. This patient showed one mutation at codon 634 and the second at codon 640 causes an alanine to glycine substitution in the transmembrane region. The two mutations were present on the same RET allele and were detected in germline and tumor DNA. The patient presented with MTC and pheochromocytoma without parathyroid gland involvement, thus we speculate that this clinical picture could be correlated with the two RET mutations and to the unusual calcitonin production. Five years after surgical treatment, the patient developed right and left Pheo recurrence and to reduce the persistently high calcitonin levels, two subsequent cervical lymphectomies were performed showing multiple MTC nodal metastases. Serum calcitonin levels, however, remained elevated. Lastly, multiple MTC liver metastases were identified. So, we carried out a new genetic test on DNA extracted from peripheral blood. In addition to the two germline mutations previously reported, the analysis revealed a novel A to T transversion, at nucleotide c.2097 in RET exon 11, that causes a methionine to leucine substitution at codon p.700. The new genetic variant was not a DNA polymorphism, as it was not detected in the DNA of at least 100 healthy individuals [13].

The specific codon mutated correlates with the age of presentation of MTC and the probability of developing pheochromocytoma or hyperparathyroidism. 95% of patients with MEN2B have a single mutation in exon 16 (Met918Thyr, M918T) that causes a conformational change in the intracellular TK2 binding pocket and allows for constitutive kinase activation in the absence of dimerization, as well as altered substrate binding [14].

The most important mutations in FMTC have been located in the cysteine rich domain or TK 1 domain, and it's the same mutation of MEN 2A; for this reason there is a partial overlapping between MEN2A and FMTC, and FMTC is considered a variant of MEN 2A.

RET mutational heterogeneity has been reported in sporadic tumors harboring somatic mutations. This means that a subset of tumor cells may harbor a *RET* mutation that is not present throughout the entire tumor and may be present in some but not all metastases. In sporadic MTC, RET mutations don't drive tumorigenesis, but they are important for tumor progression [15]. The most common mutation in sporadic MTC is M918T: this is the same mutation of MEN 2B. Another mutation, with a high prevalence, is Ala883Phe: both mutations are related with lymph-node metastases and postoperative persistence of disease. Much more recently, activating RAS mutations have also been identified in 68% of sporadic MTCs with wild-type RET. Thus, we consider two distinct pathways for sporadic MTCs. These mutations may be associated with clinically less aggressive tumors [16,17]. Mutations occur within known hotspots in exons 2, 3, and 4 and predominantly involve HRAS and KRAS; mutations more rarely involve NRAS. RAS mutations are always mutually exclusive with RET mutations and are present in approximately 10%–45% of RET wild-type sporadic tumors [16–18]. This leaves a small group of RET and RAS mutation-negative tumors for which the main genetic changes are undefined. It is important to note that the RAS signaling pathway is activated by the RET tyrosine kinase receptor. Therefore, activation of this pathway, either by RET or RAS mutation, appears important in both hereditary and sporadic tumors [19]. RET could also have an epigenetic role in pathogenesis of MTC. Recent studies have demonstrated a lower degree of methylation of RET prot-oncogene in MTC cells compared with normale thyroid tissues, suggesting a pathogenetic role in MTC development [20].

RET genetic screening is the key tool for the diagnosis of patients with MTC. Molecular diagnostic testing has been a mainstay of the clinical management of MTC for many years. Patients are routinely referred for RET mutation analysis of constitutional DNA to identify hereditary cases so that they can be monitored for other associated endocrine abnormalities, and at-risk family members can be screened and treated prior to the development of MTC with prophylactic thyroidectomy [21]. Somatic mutations in either RET or RAS also occur in most sporadic MTCs.

The original ATA Guidelines used A, B, C, and D designations to define categories of RET mutations associated with increasing aggressiveness (from A to D) of the MTC [22]. Therefore, the Task Force recommends that category D be changed to a new category, “highest risk” (HST); category C be changed to a new category, “high risk” (H); and the A and B categories be combined into a new category, “moderate risk” (MOD). The ATA-HST category includes

patients with MEN2B and the RET codon M918T mutation, the ATA-H category includes patients with RET codon C634 mutations and the RET codon A883F mutation, and the ATAMOD category includes patients with RET codon mutations other than M918T, C634, and A883F [23]. A and B categories of RET germline mutations have a similar aggressiveness and level of risk; for this reason, they were combined into a new category, "moderate risk" (MOD) (see Table 1).

In the genetical testing of RET mutations in MTCs, Next-generation sequencing (NGS) in taking an increasingly important role. One of the most important benefit is the comprehensive analysis of molecular alterations in MTC, which allows rapidly to select patients with different risk levels [24].

3. Role of miRNAs

Micro-RNAs (miRNAs) are post-transcriptional regulators of genetic expression [10]. They have a pro-oncogenic role in MTCs. In fact, increased expression of different miRNA, such as miR-21, miR-183, and miR-375 was related with worse clinical outcome and persistent and metastatic disease. miR-224 is conversely associated with non metastatic disease and biochemical cure [25,26]. There is a difference in miRNA expression pathway between sporadic and hereditary MTCs. Among sporadic cases, expression of miR-127 was significantly lower in those who harbor somatic RET mutations than those with wild-type RET [27]. One recent study has noted the overexpression of three genes involved in microRNA biogenesis (DICER, DGCR8, and XPO5) in RET-mutated tumors but no difference associated with RAS mutation status [28]. Recent findings have shown that the silencing of miR-200b and miR-200c in MTC cell lines induced transition to mesenchymal phenotypes, and enhanced in vitro invasiveness. The increased expression of TGFβ-1, as well as the silencing of miR-183 in an MTC cell line led to decreased cell vitality and upregulation of microtubule-associated protein 1 light chain 3B, an essential protein for autophagy [29]. This data suggest that oncogenic and tumor suppressor functions of microRNAs may play important roles in the pathogenesis and progression of MTC.

4. p27 polymorphism as a prognostic factor in MTCs

The CDKN1B gene, encodes the cyclin-dependent kinase (Cdk) inhibitor p27Kip1, a regulatory nuclear protein that is a component of ***Cip/Kip family proteins*** involved in the regulation of cell cicle, in particular is an inhibitor of cell division. Progression from the G1 to the S phase of the cell cycle by interacting with cyclinE/Cdk2 and cyclinD1/Cdk4 complexes. It has been demonstrated that low levels are related with an increase of cell proliferation and loss-of-function mutations contribute to tumorigenesis so much so that CDKN1B mutations in rats are associated with development of MENX, with a phenotype that overlaps MEN 1 and MEN 2. This clinical picture has the same features of MEN 4 in the humans [30]. In addition to the mutational status, 21 single nucleotide polymorphism of this gene have been described. The most important and more studied is the substitution of a valine with a glycine residue at codon 109 (V109G polimorphysm) caused by a single nucleotide polymorphism (T/G) at position 326. The association of this polymorphism with cancer development is controversial: in prostate cancer and squamous cell oral cancer it has been associated with cancer risk and progression of the disease [31,32], while it has a decreased risk role in breast cancer [33]. In MEN1, it has been demonstrated that this polymorphism is associated with the development of more aggressive tumors [34]. We have studied the association between CDKN1B mutations and/or polymorphism and sporadic MTCs negative for RET mutation, and we have found a better prognosis (normal post-operative calcitonin levels and

Table 1
Summarize relationship of common RET mutations to risk of aggressive MTC in MEN2A and MEN2B, and to the Incidence of pheochromocytoma(PHEO), hyperparathyroidism (HPTH), cutaneus lichen amyloidosis (CLA), and Hirschsprung's disease (HD) in MEN2A.

RET mutation	Exon	MTC risk level	Incidence of PHEO	Incidence of HPTH	CLA	HD	Age of prophylactic thyroidectomy
G533C	8	MOD	+	–	N	N	When calcitonin raise
							After 5 years
C609F/G/R/S/Y	10	MOD	+/++	+	N	Y	When calcitonin raise
							After 5 years
C611F/G/S/Y/W	10	MOD	+/++	+	N	Y	When calcitonin raise
							After 5 years
C618F/R/S	10	MOD	+/++	+	N	Y	When calcitonin raise
							After 5 years
C620F/R/S	10	MOD	+/++	+	N	Y	When calcitonin raise
							After 5 years
C630R/Y	11	MOD	+/++	+	N	N	When calcitonin raise
							After 5 years
D631Y	11	MOD	+++	–	N	N	When calcitonin raise
							After 5 years
K666E	11	MOD	+	–	N	N	When calcitonin raise
							After 5 years
E768D	13	MOD	–	–	N	N	When calcitonin raise
							After 5 years
L790F	13	MOD	+	–	N	N	When calcitonin raise
							After 5 years
V804L	14	MOD	+	+	N	N	When calcitonin raise
							After 5 years
V804M	14	MOD	+	+	Y	N	When calcitonin raise
							After 5 years
S891A	15	MOD	+	+	N	N	When calcitonin raise
							After 5 years
R912P	16	MOD	–	–	N	N	When calcitonin raise
							After 5 years
A883F	15	H	+++	–	N	N	<5 years
C634F/G/R/S/W/Y	11	H	+++	++	Y	N	<5 years
M918T	16	HST	+++	–	N	N	As soon as possible

biochemical remission) in polymorphism versus wild type allele-bearing patients [35]. These findings suggest that CDKN1B V109G polymorphism might influence the clinical course of the patients affected by MTC.

5. Molecular targeted therapy: present and future of therapeutical approach to MTC

The knowledge of tumor biology is crucial for the identification of drugs able to block tumor development. Although surgery (i.e. total thyroidectomy with neck lymph node dissection) [36–38] is the elective treatment for MTCs, about 80% of patients have distant metastases at diagnosis and in this cases surgery is not enough and an additional treatment is needed [39–41]. Conventional chemotherapy has been shown low response and many side effects. For this reason, increased interest have been demonstrated the results of two large phase III clinical trials with two targeted tyrosine kinase inhibitors (TKIs), vandetanib and cabozantinib, which have shown increased progression-free survival compared with placebo [42,43]. Thus, they have been approved by the US Food and Drug Administration for the treatment of symptomatic, advanced, or progressive MTC. The efficacy of these drugs results in part from their ability to target multiple kinases. Unfortunately, both TKIs induce partial responses with resistance eventually developing, and an increase in overall survival has not yet been shown [42,43]. The most significant side effect associated with vandetanib is QT interval prolongation with rare torsades de pointes and sudden death; with cabozantinib, the side effects are rare fistula formation, gastrointestinal perforation, and hemorrhage. Nevertheless, these TKIs represent a significant advance in the treatment of MTC, and it seems likely that in the future, they will be a part of multidrug therapy. Another open question that has been raised by the advent of TKIs for the treatment of MTC is the influence of RET and RAS mutation status on tumor response to the drug. . For example, it has been demonstrated that M918T-mutated tumors (seen in MEN2B and sporadic tumors) have increased sensitivity to the drug. Other RET mutations conferred resistance to vandetanib [42]. All RET mutation subgroups, including RET mutation-negative tumors, showed benefit in the cabozantinib, but the benefit was marginal in the RET-negative group and best in the M918T-positive tumors. TKI response by RAS mutation status was not assessed initially in either clinical trial, likely because the discovery of RAS mutations in MTC was relatively recent, but it was newly demonstrated a good response in the cabozantinib treatment [43], in contrast with the results of this TKI therapy in other types of tumors, such as colon and lung cancer. These findings suggests that TKI response varies by mutational status, so additional information are needed.

Ethical approval

Ethical approval is not applicable.

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Author contribution

Giacomo Accardo, Giovanni Conzo, Daniela Esposito, Claudio Gambardella, Marco Mazzella, Filomena Castaldo, Carlo Di Donna, Andrea Polistena, Nicola Avenia, Vittorio Colantuoni, Dario Giugliano and Daniela Pasquali have contributed to data collection.

Giacomo Accardo and Daniela Pasquali have contributed to data collection and writing.

Conflicts of interest

We haven't any conflict of interest.

Guarantor

Daniela Pasquali is the guarantor.

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