Review Article   **Open Access**

Ret Oncogene and Thyroid Carcinoma

Elisei R, Molinaro E, Agate L, Bottici V, Viola D, Biagini A, Matrone A, Tacito A, Ciampi R, Vivaldi A and Romei C*

Endocrine Unit, Department of Clinical and Experimental Medicine, University of Pisa, Italy

Abstract

Thyroid cancer is a malignant neoplasm that originates from follicular or parafollicular thyroid cells and is categorized as papillary (PTC), follicular (FTC), anaplastic (ATC) or medullary thyroid carcinoma (MTC). The alteration of the Rearranged during trasfection (*RET*) (proto-oncogene, a gene coding for a tyrosine-kinase receptor involved in the control of cell differentiation and proliferation, has been found to cause PTC and MTC. In particular, *RET/PTC* rearrangements and *RET* point mutations are related to PTC and MTC, respectively. Although *RET/PTC* rearrangements have been identified in both spontaneous and radiation-induced PTC, they occur more frequently in radiation-associated tumors. *RET/PTC* rearrangements have also been reported in follicular adenomas. Although controversial, correlations between *RET/PTC* rearrangements, especially *RET/PTC3*, and a more aggressive phenotype and a more advanced stage have been identified.

Germline point mutations in the *RET* proto-oncogene are associated with nearly all cases of hereditary MTC, and a strict correlation between genotype and phenotype has been demonstrated. A large spectrum of *RET* point mutations has been reported to date. Somatic *RET* mutations, almost all of which are point mutations, have been described in approximately 40-50% of sporadic MTC cases. Although *RET* somatic mutations have been found in different codons, the M918T mutation in exon 16 is the most frequent. Detecting germline *RET* point mutations is a useful tool for identifying subjects who are clinically unaffected but who will develop the disease soon or late in their life, thus allowing presymptomatic treatment. From a clinical point of view, detecting *RET* somatic mutations in tumoral tissue is able to predict a poor outcome of the disease. In recent years, several drugs capable of inhibiting *RET* activity have been demonstrated to play an important role in the treatment of radiorefractory advanced thyroid carcinomas.

Keywords: Papillary thyroid cancer; Medullary thyroid cancer; *RET*; Point mutations; *RET/PTC* rearrangements

Introduction

Thyroid cancer is a malignant neoplasm that originates from follicular or parafollicular thyroid cells. Approximately 95% of malignant lesions are derived from thyroid follicular cells and are classified as papillary (PTC), follicular (FTC), or anaplastic (ATC) thyroid carcinoma [1]. A relatively small percentage of thyroid carcinomas (7.5-10%) [2] are derived from parafollicular C cells and are called medullary thyroid carcinomas (MTC). MTC can be sporadic (75% of cases) or familial (25% of cases), in which the disease is inherited in an autosomal dominant manner. In these cases, other organs (i.e., parathyroid and adrenal glands) can be involved, and three different syndromes, MEN 2A, MEN 2B and FMTC, are distinguished [3-5].

In the last 25 years, many studies have been conducted to identify the genetic alterations related to the pathogenesis of thyroid tumors. Most of these alterations affect genes coding for either some tyrosine kinases receptors, such as *RET* and *NTRK1* or other proteins such as RAS and *BRAF*. All mutated genes are involved in the activation of the mitogen-activated protein kinase (MAPK) pathway suggesting that activation of this signaling pathway is essential for tumor initiation and that the alteration of a single effector of the pathway is sufficient for cell transformation [6]. In the majority of cases these mutations are mutually exclusive and are present in about 70% of PTC and 65% of MTC [6-8] .

It is of interest that two different thyroid tumors such as PTC and MTC are characterized by the activation of the same *RET* gene with different type of alterations: *RET*/PTC rearrangements in PTC, and *RET* point mutations in MTC. Aim of this review is to describe and discuss the pathogenic and clinical roles of *RET* gene alterations in these thyroid tumors.

Ret Proto-Oncogene

Receptor tyrosine kinases (RTK) constitute a large family of receptors that, in response to ligand activation, are potent mediators of cell motility, proliferation, differentiation, and survival. Genetic alteration and impaired expression of RTK signaling are two of the most common molecular defects associated with malignancy.

The *RET* proto-oncogene, mapped to chromosome 10q11.2, was first identified in 1985 based on its ability to transform NIH3T3 cells [9]. The *RET* proto-oncogene encodes a transmembrane tyrosine-kinase receptor involved in the control of cell differentiation and proliferation. This receptor is characterized by an extracellular ligand-binding domain, including four cadherin-like repeats and a conserved cysteine-rich region; a transmembrane domain; and an intracellular domain containing two tyrosine-kinase subdomains that are involved in the dimerization of the receptor through disulfide bond formation [10].

RET gene is subjected to alternative splicing and is translated into three functional isoforms that contain the first 19 exons of the *RET* gene, *RET*51, *RET*9, and *RET*43; *RET* splice variants differ in the number of amino acids at their C terminal end [11]. These isoforms are highly conserved between humans and mice [12].

Four different ligands have been reported to date: glial-derived neurotrophic factors (GDN), neurturin (NRTN), artimin (ARTN), and persepin (PSPN) [13]. All of these ligands induce ret protein activation by binding to specific coreceptors. Ret receptor activation requires the formation of a multimeric complex with a ligand, a GDNF-family receptor-α (GDNFRα1-4) protein, and ret Figure 1.

The *RET* gene is expressed in tissues derived from the neural crest, including thyroid C cells and adrenal medulla; while, it is not, or only very moderately, expressed in normal thyroid follicular cells [14,15].

RET proto-oncogene genetic alterations cause both medullary thyroid carcinoma (MTC), which is derived from parafollicular C cells, and papillary thyroid carcinoma (PTC), which is derived from follicular cells. In MTC, point mutations are commonly found, whereas in PTC, the most frequent genetic alterations are gene rearrangements that carry the tyrosine kinase domain of ret under the control of the promoter of ubiquitously expressed genes. In addition to activating point mutations, several single-nucleotide polymorphisms have been described, of which several seem to play a role in the predisposition to MTC [16].

Ret/PTC Rearrangements in PTC

RET/PTC rearrangement was first identified in 1987 [17] by DNA transfection analysis using NIH3T3 cells and tumoral tissue derived from an irradiated PTC. This oncogene was a chimeric gene generated by the fusion of the *RET* tyrosine kinase domain with the 5' terminal region of the COILED-Coil Domain-Containing Protein 6 (CCD6) formerly named H4 [18]. Subsequently, several activating gene rearrangements with *RET* to form *RET/PTC* by juxtaposing the genomic region coding for the tyrosine kinase domain with the 5' terminal regions of several unrelated genes have been described [19] (Figure 2). The several genes reported to be rearranged with *RET* are ubiquitously present in all cells and harbour a coiled-coil domain which is responsible for the dimerization leading to the tyrosine hyperphosphorylation thus inducing the transforming activity of the *RET/PTC* oncogenes.

The presence of the rearrangement determines the constitutive activation of the intracellular domain of the receptor, which is followed by uncontrolled proliferation of the follicular cells and the development of malignancy. RET/PTC1 and RET/PTC3 are the most frequent chimeras identified in PTC [20]. The transforming ability of *RET/PTC* rearrangements has been unequivocally demonstrated with *in vitro* and *in vivo* studies: the *RET/PTC* oncogene is in fact capable of transforming normal rat thyroid PCCl3 cells into malignant cells [21] and transgenic mice that express *RET/PTC* oncogenes under the control of the thyroglobulin promoter, which is specific for follicular cells, develop classical PTC [22].

The reported *RET/PTC* prevalence in thyroid tumors varies greatly in different series [23-32]. This difference can be attributed to ethnic and geographic variations, as well as to the different sensitivities of the detection methods [33]. Although *RET/PTC* rearrangements have been identified in both spontaneous and radiation-induced PTC, they seem to occur more frequently in radiation-associated tumors: on this regard,

it is worth to note that the first *RET/PTC* rearrangement was discovered in an irradiated PTC [17]. As a matter of fact, ionizing radiation is a well-known risk factor for PTC [34,35]; in particular, the risk of thyroid cancer has a linear dose response for doses 0.1-2 Gy, with flattening of the dose-response curve at higher doses [1,35,36]. *RET/PTC*1 is more common after γ-radiation exposure, whereas *RET/PTC*3 was the predominant type found in patients exposed to I-131 who developed thyroid cancer 10 years after the Chernobyl accident (50–80%) [37-44]. *RET/PTC* rearrangements have also been found to be more prevalent in children than in adults for both irradiated and non-irradiated PTC [38,45]. According to our previous report, the *RET/PTC* prevalence is approximately 55% in post-Chernobyl tumors and 37% in spontaneous tumors (Figure 3). The relationship between the radiation exposure and the development of *RET/PTC* 1 and 3 rearrangements has been clearly demonstrated by in vitro studies, which showed that irradiated thyroid cells develop *RET/PTC* rearrangements, and in particular, radiation
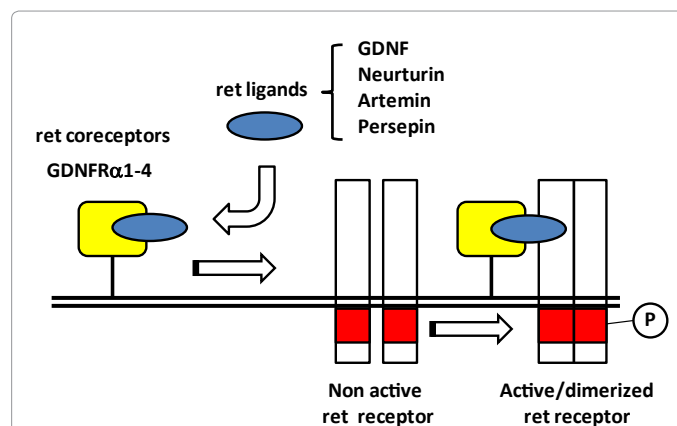

Figure 1: Mechanism of the ligand (GDNF, neurturin, artemin, persepin)-dependent activation of the ret receptor. The formation of a multimeric complex of the ret receptor with both a ligand and a GDNF-family co-receptor induces the dimerization of ret and its physiological activation. When an activating mutation (either point mutation or rearrangement) occurs the ret protein becomes constitutively activated undependently from the interaction with ligands and co-receptors.
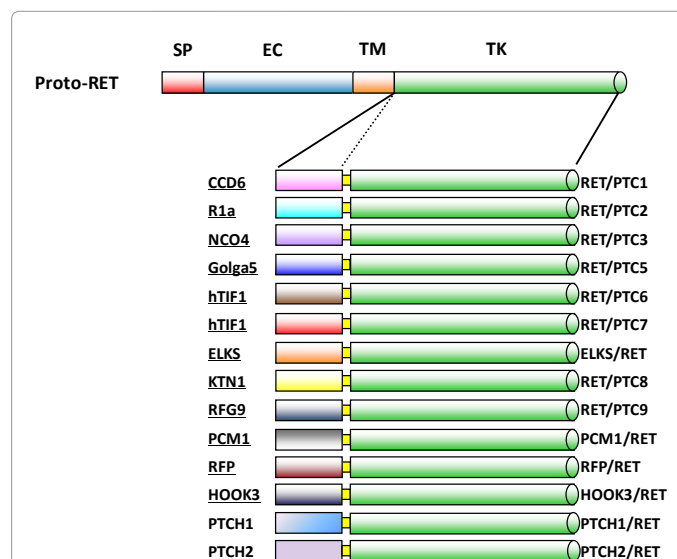

Figure 2: Schematic representation of *RET/PTC* rearrangements with different donor genes reported in the literature to date.
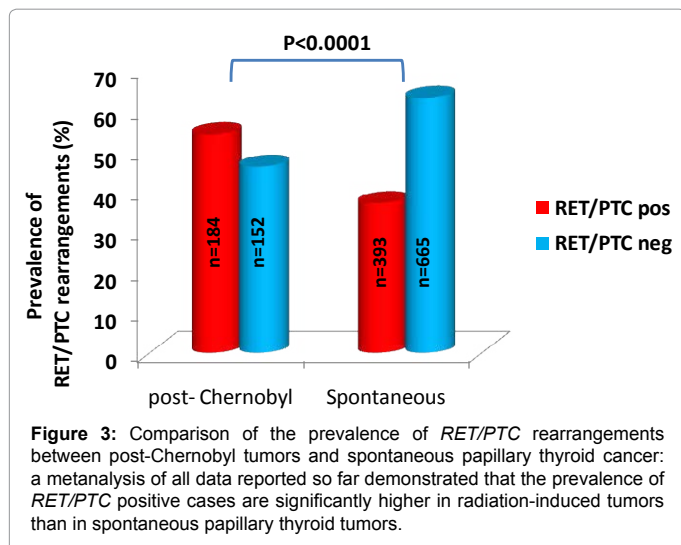
Figure 3: Comparison of the prevalence of *RET/PTC* rearrangements between post-Chernobyl tumors and spontaneous papillary thyroid cancer: a metanalysis of all data reported so far demonstrated that the prevalence of *RET/PTC* positive cases are significantly higher in radiation-induced tumors than in spontaneous papillary thyroid tumors.

exposure, under fragile site-inducing conditions, can generate breaks at fragile sites in the *RET* and CCDC6 genes, which could result in the formation of cancer-causing *RET*/PTC1 rearrangements [46-48]. The evidence that hydrogen peroxidase (H_2O_2) induces DNA single- and double-strand breaks in thyroid cells arose the question of whether H_2O_2 could be a potential mutagen for thyroid [49]. The link between radiation exposure, production of H_2O_2 and development of *RET/PTC1* rearrangement was recently proved by an elegant in vitro study [50].

As the latency period has increased, the prevalence of *RET/PTC* rearrangements in post-Chernobyl thyroid tumors has declined, and among the *RET/PTC*-positive tumors, the percentage of tumors with *RET/PTC*1 has increased, whereas the proportion of tumors with *RET/PTC*3 has decreased in parallel [40]. These findings confirm the relationship between the development of PTC and exposure to radiation, which has tended to decline over the years. A significant temporal decrease in the prevalence of *RET/PTC* rearrangements was also observed in spontaneous PTC [51,52], in parallel with an increase in the BRAF V600E point mutation, which represents the other most important genetic alteration in PTC [53]. These results suggest that the modification of the genetic profile of PTC can be related to the exposure to different environmental and etiological agents over time.

Despite an initial belief that *RET/PTC* rearrangements were exclusively associated with PTC, they were subsequently found in 10-45% of thyroid adenomas and other non-neoplastic thyroid lesions such as Hashimoto's thyroiditis [24]. *RET/PTC* rearrangements have also been reported in follicular adenomas in subjects previously exposed to therapeutic external radiation [43,45]. The clinical meaning of these genetic alterations in benign thyroid diseases is still undefined; however, the presence of a one or a few malignant cells within the follicular adenoma or in the context of Hashimoto's thyroiditis cannot be excluded [45,54-56]. Additionally, other *RET* oncogene rearrangements, different from those described to date in PTC, have recently been reported in a few cases of lung carcinoma and leukemia [57,58].

Clinical Relevance Of RET/PTC Rearrangements in Ptc

Prognosis

The biological and clinical significance of *RET* activation with regard to the outcome of PTC is controversial. Some studies have

reported higher rates of local extension and lymph node involvement in *RET/PTC*-positive cases than in *RET/PTC*-negative cases [59], whereas other studies were unable to find any relationship between *RET/PTC* rearrangements and classical prognostic factors [60-63]. Correlations with a more aggressive phenotype (i.e. rapidly, progressive disease) and a more advanced stage (i.e. T4/N1/M1) have been clearly identified for *RET/PTC*3 rearrangements [38,64] in cases of post-Chernobyl childhood thyroid cancer because *RET/PTC*3 positivity was more frequently associated with the more aggressive solid variant. This evidence was confirmed in a subsequent study on sporadic PTC, in which we demonstrated positive correlations between the presence of *RET/PTC*3 rearrangements and both a larger tumor size and a more advanced stage at the time of diagnosis [63]. Other studies have reported that PTC with *RET/PTC*3 rearrangements may be more prone to dedifferentiate, showing a more aggressive behavior [65,66], and others claimed that *RET/PTC*3 plays a role in metastatic spread [66,67]. In contrast, *RET/PTC*1 rearrangements seem to be correlated with a more favorable behavior [68].

In our experience, *RET/PTC* rearrangements do not seem to be correlated with any clinical or pathological feature of aggressiveness according to studies of both the expression of the *RET/PTC* protein by immunohistochemistry [62] or the mRNA expression of *RET/PTC* rearrangements by real-time RT-PCR [63].

Diagnosis

Fine needle aspiration cytology (FNAC) is the first-choice method to distinguish benign and malignant thyroid nodules [69-71]. Nevertheless, FNAC cannot make a definitive diagnosis for 25% of thyroid nodules [72,73], and the final diagnosis is made only by the histological examination of the surgically removed thyroid nodule. The progress recently made in the knowledge of the genetic alterations responsible for thyroid carcinoma has made possible the development of molecular methods for improving the presurgical diagnosis of PTC. The American Thyroid Association guidelines for the management of thyroid nodules recommend the use of molecular testing in the presurgical diagnosis of thyroid nodules [69]. Recent, large prospective studies have confirmed the ability of genetic markers to improve preoperative diagnostic accuracy for patients with indeterminate thyroid nodules. Many of these markers are available for commercial use in reference laboratories but have not yet been widely applied in clinical practice. However, the biggest diagnostic impact can be achieved only by testing FNA samples for a panel of mutations (i.e., *BRAF*, *RET/PTC*, *RAS*, *TRK*) rather than for a single mutation [74,75]. In particular, the detection of *RET/PTC* rearrangements does not provide a high positive predictive value because a low, but not negligible, percentage of nodules that were positive for *RET/PTC* rearrangements on FNA turned out to be benign according to histology [74,75].

Ret Point Mutations in Hereditary MTC (Men 2)

Multiple Endocrine Neoplasia type 2 (MEN 2) is an autosomal dominant inherited disease characterized by the presence of MTC plus other endocrine tumors (pheochromocytoma and/or parathyroid adenomas in MEN 2A, pheochromocytoma and mucosal neurinomas in MEN 2B). MTC alone, lacking an association with other endocrine tumors, may also be inherited (FMTC). In these syndromes, MTC develops in approximately 100% of the affected individuals, whereas pheochromocytoma and parathyroid adenoma develop in 50% and 20%, respectively [2,76].

In 1993, germline point mutations in the *RET* proto-oncogene were recognized to be associated with MEN 2 [77,78]. In the subsequent years, an International *RET* Consortium (IRC) [79] was established, and clinical and *RET* mutational data for 477 kindred affected by MEN 2A, MEN 2B and FMTC were collected. The causative role of the germline *RET* mutation in MEN 2 was clearly defined, and a strict correlation between genotype and phenotype was demonstrated. It was immediately observed that the classical MEN 2A phenotype was primarily associated with mutations in the *RET* cysteine codons 609, 611, 618, and 620 in exon 10 and, mostly, with the Cys634Arg mutation in exon 11, whereas MEN 2B was almost exclusively associated with the Met918Thr mutation in exon 16. Presently, the only alternative *RET* mutation to the classical Met918Thr is the Ala883Phe mutation in exon 15 that has been reported in a few cases of MEN 2B [80]. In contrast, a more widespread distribution of *RET* mutations was observed in FMTC families [81]. This correlation was confirmed in more recent studies in which several other *RET* mutations, not known at the time of the IRC, have been studied [2,76] (Figure 4). In fact, in the last 20 years, many other *RET* mutations have been discovered and reported. These mutations affect not only the classical cysteine codons but also non-cysteine codons, such as codon 804 in exon 14, codon 883 in exon 15 and others. These non-cysteine codon mutations are usually associated with FMTC [82-84]. Nearly all of the mutations reported to date are listed in public databases *(www.hgmd.cf.ac.uk; www.arup.utah.edu/database/MEN2; www.ensembl.org).*

Despite the well-established genotype-phenotype correlation, there is clinical evidence that the same *RET* mutation can manifest differently in different members of the same family, with some individuals affected by the complete spectrum of the MEN 2A syndrome and others by only one or two of the concurrent endocrine neoplasia. The most validated hypothesis to explain this phenomenon is that, the genetic background of each person can modulate the expression of the genetic alteration and this was clearly demonstrated in transgenic mice [85].

By analyzing the genotype-phenotype correlation it appears that not all mutations confer the same aggressiveness to MTC. It is well known that many cases of FMTC are rather indolent, and they are more frequently associated with non-cysteine *RET* mutated codons. In particular, mutations at codon 790 and 791 in exon 13 are associated with a non-aggressive form of MEN 2 [86,87] that is concentrated in a particular geographic area (i.e., Germany). Recently, it has also been postulated that mutations at codon 791 may more likely to be single-nucleotide polymorphisms rather than a real transforming mutations [88]. In this regard, it is noteworthy that several of these newly identified *RET* mutations are very rare, being present only in a few families and a few family members or even in a single affected member (i.e., private mutations), thus raising doubts as to whether they represent the driving force of the tumoral disease. In fact, many of these rare *RET* mutations are found as the result of very careful genetic screening in "apparently sporadic" MTC, with neither a familial history of MTC nor an association with other endocrine neoplasia [89,90]. These peculiar *RET* mutations have been defined as variants of unknown significance (VUS), and a comprehensive description is provided in Table 1.

Although *RET* germline mutations are generally unique and heterozygous, in few cases, tandem *RET* mutations of codons 805, 806, and 904 in a *cis* configuration with the Val804Met mutation have been reported in individuals with MEN 2B [91,92]. The likelihood of having a homozygous or compound heterozygous *RET* mutation is rare, but such mutations have nevertheless been described in the literature and can explain the acquired transforming role of *RET* mutations with very low or null transforming activity [93,94].
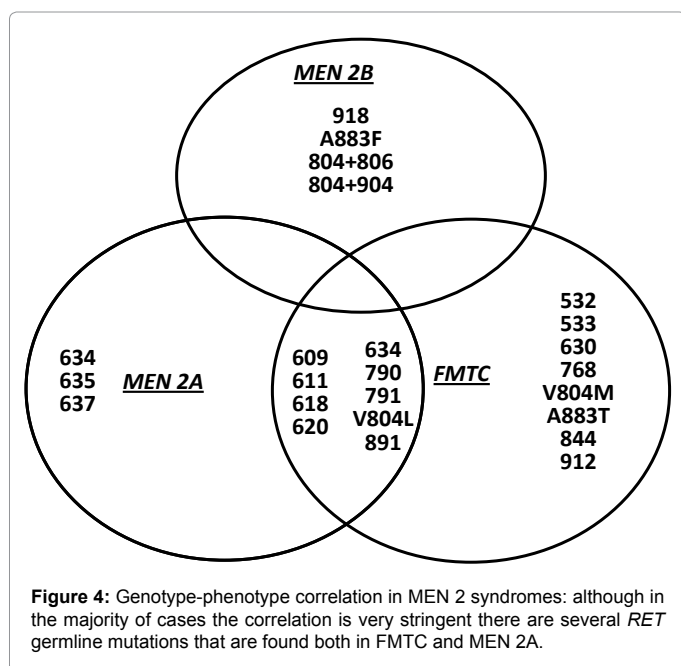
RET mutations have been found in more than 98% of individuals with MEN 2, and only few families affected by hereditary MTC are "orphans" of germline mutations. However, the prevalence and type distribution of *RET* germline mutations in MEN 2 families are clearly different in different countries [79,95,96], likely due to ethnic variations or to a founder effect. In particular, in a recent Italian series [96], the most frequent *RET* amino acid substitution was Val804Met. In other European series, although *RET* mutations at codon 634 were the most prevalent, a high frequency was found for the Leu790Phe and Tyr791Phe mutations [79,95].

Ret Point Mutations in Sporadic MTC

Approximately 7-10% of all apparently sporadic MTC patients harbor a germline *RET* mutation that is discovered at the time of genetic screening [82,97]. These cases are then reclassified as hereditary forms and the genetic screening of the first degree relatives is strongly suggested [98].

It is of great interest that the same *RET* mutations can be found at the somatic level in the tumoral tissue of true sporadic MTC cases. According to data reported in a public database (COSMIC), *RET* somatic mutations have been found in 518/1212 (43%) MTC tumoral tissues. As shown in Table 2, in the majority of cases, these mutations are point mutations (n=475), but deletions, insertions and complex mutations are also reported (n = 43). Although *RET* somatic mutations have been found at different codons, the Met918Thr mutation in exon 16 is the most frequent (361/475, 76%). As mentioned above for germline mutations, several uncommon somatic mutations have also been reported in isolated cases, but their transforming ability has not always been proven. In vitro and/or in silico analysis is mandatory for both newly discovered somatic and germline *RET* mutations to establish the transforming ability of these new genetic alteration [90,99]. A comprehensive catalog of all *RET* somatic mutations associated with MTC is provided on the website *http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/.*

Although it is well accepted that *RET* germline mutations are

Figure 4: Genotype-phenotype correlation in MEN 2 syndromes: although in the majority of cases the correlation is very stringent there are several *RET* germline mutations that are found both in FMTC and MEN 2A.

RET Mutation	*RET* exon	Families (n); GC (n)	*RET* Mutation	*RET* exon	Families (n); GC (n)
G321R	Exon 5	1; 4	K666N	Exon 11	1; 1
T338I	Exon 5	1;2	K666M	Exon 11	1; 8
A510V	Exon 8	1;1	S686N	Exon 11	1; 2
E511K	Exon 8	1;1	M700L	Exon 11	1; 1
C531R	Exon 8	1;1	R770Q	Exon 13	1; 3
G550E	Exon 9	1;1	N777S	Exon 13	1; 4
R600Q	Exon 10	1; 6	V778I	Exon 13	2; 6
K603Q	Exon 10	1; 3	Q781R	Exon 13	1; 1
Y606C	Exon 10	1;1	E805K	Exon 14	1; 1
E616Q	Exon 10	1;1	E818K	Exon 14	1; 1
F619F	Exon 10	1;1	S819I	Exon 14	1; 1
C620L	Exon 10	1;1	R833C	Exon 14	1; 1
E623K	Exon 10	1;1	P841L	Exon 14	1; 1
C630G	Exon 11	1;1	P841P	Exon 14	1; 1
C630S	Exon 11	1; 4	E843D	Exon 14	1; 1
D631V	Exon 11	2; 6	R844Q	Exon 14	1; 1
D631E	Exon 11	1; 1	R844L	Exon 14	1; 5
D631D	Exon 11	1; 3	A845A	Exon 14	1; 1
E632K	Exon 11	2; 2	M848T	Exon 14	1; 1
R635G	Exon 11	2; 2	L881V	Exon 15	1; 6
T636P	Exon 11	1; 3	R886W	Exon 15	1; 2
A640G	Exon 11	1; 1	S904C	Exon 15	1; 3
V648I	Exon 11	2; 5	S904F	Exon 15	1; 2
S649L	Exon 11	8; 15	R912Q	Exon 16	1; 10
H665Q	Exon 11	1; 4	M918V	Exon 16	1; 2

Table 1: Germline RET point mutations of unknown significance; data derived from "ARUP, MEN 2 RET database, PUBMED and The Human Gene Mutation Database.

Codon	Exon	Substitution	n	Codon	Exon	Substitution	n
M918	16	T	361	P766	13	S	1
C634	11	W/S/T/Y/A/F/C	42	V778	13	V	1
A883	15	T/F/S/P	18	Q781	13	R	1
C630	11	S/G/R/A	15	V804	14	M	1
C618	10	R/Y/S	7	K808	14	E	1
C620	10	W/R/S	5	K821	14	E	1
E768	13	D	4	A876	15	V	1
C611	10	R	2	E884	15	K	1
D631	11	N/G	2	G894	15	S	1
A513	8	G	1	E901	15	K	1
Q681	11	?	1	R908	15	K	1
V691	11	I	1	A919	16	V	1
E615	10	K	1	E921	16	K	1
V648	11	I	1	S922	16	P	1
V706	11	A	1	S922	16	I	1
G748	13	C	1	T930	16	M	1

Table 2: Somatic RET point mutations in sporadic MTC, data derived from "COSMIC, catalogue of somatic mutation in cancer" http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/

pathogenic for the hereditary forms, the role of somatic *RET* mutations in MTC is still not completely defined. *In vitro* and *in vivo* studies have demonstrated that *RET* mutations can transform NIH3T3 fibroblasts after transfection [100] and induce MTC in transgenic mice [101]. Recently, we analyzed the presence of *RET* somatic mutations in micro MTC (i.e., <2 cm), and we observed that the prevalence of the mutation was significantly lower than in larger tumors [102]. This finding can be interpreted as indicating that *RET* somatic mutation is a late event that, when it occurs, modifies the biological behavior of the tumor, thereby making it more aggressive and increasing the growth rate. As an alternative explanation, we can hypothesize that two different types of MTC, *RET*-negative small indolent tumors and *RET*-positive aggressive tumors, do exist from the beginning [102].

Clinical Relevance of Ret Point Mutations in MTC

Diagnosis

The primary clinical implication of *RET* mutations in MEN 2 is the ability to screen family members to find those who harbor the same germline mutation previously detected in an MTC index case. This screening allows for the identification of the "gene carriers" when they are clinically unaffected or at an early stage of the disease and the exclusion of "non-gene carriers" from further testing for the rest of their life. Several series have confirmed the effectiveness of this approach. Gene carriers have been detected with a frequency ranging from 15.5% to 69.0% [82,103-107] in nearly every decade of life, but more frequently at a very young age. A classification of the level of risk

for developing a more or less aggressive disease has been reported in the American Thyroid Association guidelines for the management of MTC patients. According to this classification, *RET* mutations are classified into 4 risk levels (i.e., A-B-C and D), and different indications for surgical treatment are proposed on the basis of the risk level [108].

RET genetic testing is also indicated in apparently sporadic MTC cases. Screening sporadic MTCs for germline *RET* mutations may help differentiate patients who exhibit truly sporadic disease from those with unrecognized hereditary disease. If an unexpected germline *RET* mutation is found, the physician will be alerted to the possible coexistence or future development of adrenal or parathyroid disease. This screening may be extended to the patient's first-degree relatives, thus detecting additional gene carriers, usually in the preclinical phase of the disease. Patients negative for germline *RET* mutations can be reassured on the sporadic nature of the disease, thus avoiding the need to look for other endocrine neoplasia and to screen his or her relatives. Very detailed guidelines for *RET* genetic screening and the management of subjects with *RET* mutation have been recently published by the European Thyroid Association [98].

Prognosis

Not all *RET* mutations confer the same aggressiveness to the tumor. It is well recognized that Met918Thr has a very high transforming activity [109] and, as expected, is associated with a very aggressive behavior of the MTC that develops in MEN 2B patients, who, especially in the past years, rarely survived to the pubertal age [110]. Although to a lesser extent, the Cys634Arg has also a very high transforming ability, and the MTC associated with MEN 2A with this mutation is rather aggressive [109]. All *RET* mutations are classified according to their level of risk [108]. The correlation between the presence of *RET* somatic mutations and the clinical outcome of MTC patients has been controversial for some time. Several years ago, a correlation between the presence of a somatic *RET* mutation and a more aggressive phenotype of the sporadic MTC was reported by several groups of researchers [111-113]. We recently provided evidence that MTC patients with a somatic *RET* mutation not only have a greater likelihood of unsuccessful treatment but also have a higher likelihood of dying from the disease, as demonstrated by their significantly worse 30-year survival rate with respect to that of patients without somatic *RET* mutations [114]. This evidence was confirmed in subsequent studies [115,116].

Ret Oncogene Mutations and Target Therapy

Recently, several new drugs that are capable of blocking activated tyrosine kinase receptors have been developed [117,118]. The majority of these drugs are inhibitors of several receptors, and nearly all are active against the vascular endothelial growth factors (VEGFR1, 2 and 3). There are evidences that RET enzymatic activity is refractory to several of these drugs as for example several tyrphostins [119]. At variance, there are drugs, such as motesanib (AMG706), vandetanib (ZD6474), lenvatinib (E7080), cabozantinib (XL184), sorafenib (BAY-439006) and sunitinib, that showed also a specific anti-RET activity when tested either in vitro or in vivo [120-124] (Table 3). These drugs can exert their antioncogenic activity not only as antiangiogenic drugs by blocking the VEGF receptors but also as antineoplastic drugs by inhibiting the signaling and tranforming ability of ret oncoproteins.

Dedifferentiated thyroid cancer that is not responsive to radioiodine and advanced MTC represent real clinical challenges because of the absence of any effective therapy among the conventional strategies, such as chemotherapy and radiotherapy [85]. The high prevalence of

DRUG	IC$_{50}$ (nM)				BRAF	OTHER TARGETS
	VEGFR1	VEGFR2	VEGFR3	RET		
Sunitinib	2	9	17	41		
Motesanib	2	3	6	59		PDGFR, cKIT
Sorafenib		90	20	49	6	
Vandetanib		40	110	100		EGFR
Lenvatinib	22	4	5	35		PDGFR, FGFR
Cabozantinib		0.035		5.2		MET, KIT

Table 3: Kinase inhibitor activities of drugs used in the treatment of advanced thyroid carcinomas.

RET genetic alterations, especially in advanced MTC, represents the rationale for treatment using the above mentioned drugs.

At the present, both vandetanib and cabozantinib have shown a statistically significant prolongation of progression-free survival (PFS) in MTC patients who are treated with either one or the other [125,126]. In particular, the ZETA trial showed a longer progression-free survival (PFS) for patients receiving vandetanib than for patients receiving the placebo (30.5 months versus 19.3 months; hazard ratio [HR], 0.46). Similarly, the EXAM trial demonstrated a significantly longer PFS for patients who received cabozantinib rather than the placebo (11.2 months versus 4.0 months; HR, 0.28). Presently, vandetanib (@ Caprelsa, AstraZeneca) is available in the USA and Europe following the approval by both the FDA and EMA, whereas cabozantinib (@ Cometriq, Exilixis) has been approved by the FDA and is under the EMA evaluation. Based on very promising results obtained in a phase 2 multicentric study, a phase 3 trial with vandetanib for the treatment of advanced radiorefractory differentiated thyroid cancer is now enrolling patients. Very interesting results in terms of PFS prolongation have been obtained in the DECISION study, which is a phase 3 study comparing the results for advanced radiorefractory differentiated thyroid cancer treated with sorafenib (@Nexavar, Bayer) or a placebo (10.8 months vs. 5.8 months; HR, 0.58) [data presented at the ASCO meeting, 2013]

In all of the studies, the correlation between the mutational status and the drug response has been analyzed. In the ZETA trial, MTC patients with *RET* somatic mutations showed better responses to the drug, but a good response was also obtained for *RET* negative and *RET* unknown cases [125,126]. Similar results have been obtained in the EXAM study. These results seem to confirm that the drug activity against the *RET* mutation is very important for the clinical benefit of the patients but also that other mechanisms are involved in the control of the disease, as likely happens in *RET*-negative cases.

Conclusions

The *RET* oncogene is of great relevance in the pathogenesis of both PTC and MTC, although different molecular mechanisms are responsible for its constitutive activation in these two thyroid tumors. Well-established diagnostic and prognostic roles of *RET* oncogene mutations have been globally recognized. Presently, the relevance of these molecular alterations in the development and use of new target therapies for the treatment of radiorefractory advanced thyroid cancer is under evaluation, with promising preliminary results.

References

1. Cardis E, Howe G, Ron E, Bebeshko V, Bogdanova T et al. (2006) Cancer consequences of the Chernobyl accident: 20 years on. J Radiol Prot 26: 127-140.

2. Romei C, Pardi E, Cetani F, Elisei R (2012) Genetic and clinical features of multiple endocrine neoplasia types 1 and 2. J Oncol 2012: 705036.

3. Keiser HR, Beaven MA, Doppman J, Wells S Jr, Buja LM (1973) Sipple's

syndrome: medullary thyroid carcinoma, pheochromocytoma, and parathyroid disease. Studies in a large family. NIH conference. Ann Intern Med 78: 561-579.

4. Cunliffe WJ, Hudgson P, Fulthorpe JJ, Black MM, Hall R, et al. (1970) A calcitonin-secreting medullary thyroid carcinoma associated with mucosal neuromas, marfanoid features, myopathy and pigmentation. Am J Med 48: 120-126.

5. Farndon JR, Leight GS, Dilley WG, Baylin SB, Smallridge RC, et al. (1986) Familial medullary thyroid carcinoma without associated endocrinopathies: a distinct clinical entity. Br J Surg 73: 278-281.

6. Ciampi R, Nikiforov YE (2007) RET/PTC rearrangements and BRAF mutations in thyroid tumorigenesis. Endocrinology 148: 936-941.

7. Ciampi R, Mian C, Fugazzola L, Cosci B, Romei C, et al. (2013) Evidence of a low prevalence of RAS mutations in a large medullary thyroid cancer series. Thyroid 23: 50-57.

8. Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE (2013) Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab 98: E1852-1860.

9. Takahashi M, Ritz J, Cooper GM (1985) Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell 42: 581-588.

10. de Groot JW, Links TP, Plukker JT, Lips CJ, Hofstra RM (2006) RET as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. Endocr Rev 27: 535-560.

11. Tahira T, Ishizaka Y, Itoh F, Sugimura T, Nagao M (1990) Characterization of ret proto-oncogene mRNAs encoding two isoforms of the protein product in a human neuroblastoma cell line. Oncogene 5: 97-102.

12. Carter MT, Yome JL, Marcil MN, Martin CA, Vanhorne JB, et al. (2001) Conservation of RET proto-oncogene splicing variants and implications for RET isoform function. Cytogenet Cell Genet 95: 169-176.

13. Arighi E, Borrello MG, Sariola H (2005) RET tyrosine kinase signaling in development and cancer. Cytokine Growth Factor Rev 16: 441-467.

14. Santoro M, Rosati R, Grieco M, Berlingieri MT, D'Amato GL, et al. (1990) The ret proto-oncogene is consistently expressed in human pheochromocytomas and thyroid medullary carcinomas. Oncogene 5: 1595-1598.

15. Fluge O, Haugen DR, Akslen LA, Marstad A, Santoro M, et al. (2001) Expression and alternative splicing of c-ret RNA in papillary thyroid carcinomas. Oncogene 20: 885-892.

16. Lantieri F, Caroli F, Ceccherini I, Griseri P (2013) The involvement of the RET variant G691S in medullary thyroid carcinoma enlightened by a meta-analysis study. Int J Cancer 132: 2808-2819.

17. Fusco A, Grieco M, Santoro M, Berlingieri MT, Pilotti S, et al. (1987) A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. Nature 328: 170-172.

18. Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, et al. (1990) PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 60: 557-563.

19. Greco A, Borrello MG, Miranda C, Degl'Innocenti D, Pierotti MA (2009) Molecular pathology of differentiated thyroid cancer. Q J Nucl Med Mol Imaging 53: 440-453.

20. Santoro M, Dathan NA, Berlingieri MT, Bongarzone I, Paulin C, et al. (1994) Molecular characterization of RET/PTC3; a novel rearranged version of the RETproto-oncogene in a human thyroid papillary carcinoma. Oncogene 9: 509-516.

21. Santoro M, Melillo RM, Fusco A (2006) RET/PTC activation in papillary thyroid carcinoma: European Journal of Endocrinology Prize Lecture. Eur J Endocrinol 155: 645-653.

22. Santoro M, Chiappetta G, Cerrato A, Salvatore D, Zhang L, et al. (1996) Development of thyroid papillary carcinomas secondary to tissue-specific expression of the RET/PTC1 oncogene in transgenic mice. Oncogene 12: 1821-1826.

23. Rhoden KJ, Johnson C, Brandao G, Howe JG, Smith BR, et al. (2004) Real-time quantitative RT-PCR identifies distinct c-RET, RET/PTC1 and RET/PTC3 expression patterns in papillary thyroid carcinoma. Lab Invest 84: 1557-1570.

24. Rhoden KJ, Unger K, Salvatore G, Yilmaz Y, Vovk V, et al. (2006) RET/papillary thyroid cancer rearrangement in nonneoplastic thyrocytes: follicular cells of Hashimoto's thyroiditis share low-level recombination events with a subset of papillary carcinoma. J Clin Endocrinol Metab 91: 2414-2423.

25. Puxeddu E, Moretti S, Giannico A, Martinelli M, Marino C, et al. (2003) Ret/PTC activation does not influence clinical and pathological features of adult papillary thyroid carcinomas. Eur J Endocrinol 148: 505-513.

26. Cinti R, Yin L, Ilc K, Berger N, Basolo F, et al. (2000) RET rearrangements in papillary thyroid carcinomas and adenomas detected by interphase FISH. Cytogenet Cell Genet 88: 56-61.

27. Fenton CL, Lukes Y, Nicholson D, Dinauer CA, Francis GL, et al. (2000) The ret/PTC mutations are common in sporadic papillary thyroid carcinoma of children and young adults. J Clin Endocrinol Metab 85: 1170-1175.

28. Chua EL, Wu WM, Tran KT, McCarthy SW, Lauer CS, et al. (2000) Prevalence and distribution of ret/ptc 1, 2, and 3 in papillary thyroid carcinoma in New Caledonia and Australia. J Clin Endocrinol Metab 85: 2733-2739.

29. Mayr B, Pötter E, Goretzki P, Rüschoff J, Dietmaier W, et al. (1998) Expression of Ret/PTC1, -2, -3, -delta3 and -4 in German papillary thyroid carcinoma. Br J Cancer 77: 903-906.

30. Lam AK, Montone KT, Nolan KA, Livolsi VA (1998) Ret oncogene activation in papillary thyroid carcinoma: prevalence and implication on the histological parameters. Hum Pathol 29: 565-568.

31. Zou M, Shi Y, Farid NR (1994) Low rate of ret proto-oncogene activation (PTC/ retTPC) in papillary thyroid carcinomas from Saudi Arabia. Cancer 73: 176-180.

32. Sheils OM, O'eary JJ, Uhlmann V, Lättich K, Sweeney EC (2000) ret/PTC-1 Activation in Hashimoto Thyroiditis. Int J Surg Pathol 8: 185-189.

33. Zhu Z, Ciampi R, Nikiforova MN, Gandhi M, Nikiforov YE (2006) Prevalence of RET/PTC rearrangements in thyroid papillary carcinomas: effects of the detection methods and genetic heterogeneity. J Clin Endocrinol Metab 91: 3603-3610.

34. Schneider AB (1990) Radiation-induced thyroid tumors. Endocrinol Metab Clin North Am 19: 495-508.

35. Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, et al. (1995) Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. Radiat Res 141: 259-277.

36. Cardis E, Kesminiene A, Ivanov V, Malakhova I, Shibata Y, et al. (2005) Risk of thyroid cancer after exposure to 131I in childhood. J Natl Cancer Inst 97: 724-732.

37. Fugazzola L, Pilotti S, Pinchera A, Vorontsova TV, Mondellini P, et al. (1995) Oncogenic rearrangements of the RET proto-oncogene in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear accident. Cancer Res 55: 5617-5620.

38. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA (1997) Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Res 57: 1690-1694.

39. Hieber L, Huber R, Bauer V, Schaffner Q, Braselmann H, et al. (2011) Chromosomal rearrangements in post-Chernobyl papillary thyroid carcinomas: evaluation by spectral karyotyping and automated interphase FISH. J Biomed Biotechnol 2011: 693691.

40. Unger K, Zurnadzhy L, Walch A, Mall M, Bogdanova T, et al. (2006) RET rearrangements in post-Chernobyl papillary thyroid carcinomas with a short latency analysed by interphase FISH. Br J Cancer 94: 1472-1477.

41. Di Cristofaro J, Vasko V, Savchenko V, Cherenko S, Larin A, et al. (2005) ret/ PTC1 and ret/PTC3 in thyroid tumors from Chernobyl liquidators: comparison with sporadic tumors from Ukrainian and French patients. Endocr Relat Cancer 12: 173-183.

42. Thomas GA, Bunnell H, Cook HA, Williams ED, Nerovnya A, et al. (1999) High prevalence of RET/PTC rearrangements in Ukrainian and Belarussian post-Chernobyl thyroid papillary carcinomas: a strong correlation between RET/ PTC3 and the solid-follicular variant. J Clin Endocrinol Metab 84: 4232-4238.

43. Bounacer A, Wicker R, Caillou B, Cailleux AF, Sarasin A, et al. (1997) High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external radiation. Oncogene 15: 1263-1273.

44. Bounacer A, Wicker R, Schlumberger M, Sarasin A, Suárez HG (1997)

Oncogenic rearrangements of the ret proto-oncogene in thyroid tumors induced after exposure to ionizing radiation. Biochimie 79: 619-623.

45. Elisei R, Romei C, Vorontsova T, Cosci B, Veremeychik V, et al. (2001) RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated, malignant and benign thyroid lesions in children and adults. J Clin Endocrinol Metab 86: 3211-3216.

46. Gandhi M, Dillon LW, Pramanik S, Nikiforov YE, Wang YH (2010) DNA breaks at fragile sites generate oncogenic RET/PTC rearrangements in human thyroid cells. Oncogene 29: 2272-2280.

47. Gandhi M, Evdokimova V, Nikiforov YE (2010) Mechanisms of chromosomal rearrangements in solid tumors: the model of papillary thyroid carcinoma. Mol Cell Endocrinol 321: 36-43.

48. Caudill CM, Zhu Z, Ciampi R, Stringer JR, Nikiforov YE (2005) Dose-dependent generation of RET/PTC in human thyroid cells after in vitro exposure to gamma-radiation: a model of carcinogenic chromosomal rearrangement induced by ionizing radiation. J Clin Endocrinol Metab 90: 2364-2369.

49. Driessens N, Versteyhe S, Ghaddhab C, Burniat A, De Deken X, et al. (2009) Hydrogen peroxide induces DNA single- and double-strand breaks in thyroid cells and is therefore a potential mutagen for this organ. Endocr Relat Cancer 16: 845-856.

50. Ameziane-El-Hassani R, Boufraqech M, Lagente-Chevallier O, Weyemi U, Talbot M, et al. (2010) Role of H2O2 in RET/PTC1 chromosomal rearrangement produced by ionizing radiation in human thyroid cells. Cancer Res 70: 4123-4132.

51. Smyth P, Finn S, Cahill S, O'Regan E, Flavin R, et al. (2005) Ret/PTC and BRAF act as distinct molecular, time-dependant triggers in a sporadic Irish cohort of papillary thyroid carcinoma. Int J Surg Pathol 13: 1-8.

52. Romei C, Fugazzola L, Puxeddu E, Frasca F, Viola D, et al. (2012) Modifications in the papillary thyroid cancer gene profile over the last 15 years. J Clin Endocrinol Metab 97: E1758-1765.

53. Xing M (2007) BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev 28: 742-762.

54. Ishizaka Y, Kobayashi S, Ushijima T, Hirohashi S, Sugimura T, et al. (1991) Detection of retTPC/PTC transcripts in thyroid adenomas and adenomatous goiter by an RT-PCR method. Oncogene 6: 1667-1672.

55. Chiappetta G, Toti P, Cetta F, Giuliano A, Pentimalli F, et al. (2002) The RET/PTC oncogene is frequently activated in oncocytic thyroid tumors (Hurthle cell adenomas and carcinomas), but not in oncocytic hyperplastic lesions. J Clin Endocrinol Metab 87: 364-369.

56. Sapio MR, Guerra A, Marotta V, Campanile E, Formisano R, et al. (2011) High growth rate of benign thyroid nodules bearing RET/PTC rearrangements. J Clin Endocrinol Metab 96: E916-919.

57. Gainor JF, Shaw AT (2013) Novel targets in non-small cell lung cancer: ROS1 and RET fusions. Oncologist 18: 865-875.

58. Ballerini P, Struski S, Cresson C, Prade N, Toujani S, et al. (2012) RET fusion genes are associated with chronic myelomonocytic leukemia and enhance monocytic differentiation. Leukemia 26: 2384-2389.

59. Adeniran AJ, Zhu Z, Gandhi M, Steward DL, Fidler JP, et al. (2006) Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol 30: 216-222.

60. Tallini G, Santoro M, Helie M, Carlomagno F, Salvatore G, et al. (1998) RET/PTC oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes. Clin Cancer Res 4: 287-294.

61. Musholt TJ, Musholt PB, Khaladj N, Schulz D, Scheumann GF, et al. (2000) Prognostic significance of RET and NTRK1 rearrangements in sporadic papillary thyroid carcinoma. Surgery 128: 984-993.

62. Basolo F, Molinaro E, Agate L, Pinchera A, Pollina L, et al. (2001) RET protein expression has no prognostic impact on the long-term outcome of papillary thyroid carcinoma. Eur J Endocrinol 145: 599-604.

63. Romei C, Ciampi R, Faviana P, Agate L, Molinaro E, et al. (2008) BRAFV600E mutation, but not RET/PTC rearrangements, is correlated with a lower expression of both thyroperoxidase and sodium iodide symporter genes in papillary thyroid cancer. Endocr Relat Cancer 15: 511-520.

64. Powell DJ Jr, Russell J, Nibu K, Li G, Rhee E, et al. (1998) The RET/PTC3 oncogene: metastatic solid-type papillary carcinomas in murine thyroids. Cancer Res 58: 5523-5528.

65. Mochizuki K, Kondo T, Nakazawa T, Iwashina M, Kawasaki T, et al. (2010) RET rearrangements and BRAF mutation in undifferentiated thyroid carcinomas having papillary carcinoma components. Histopathology 57: 444-450.

66. Sugg SL, Ezzat S, Zheng L, Freeman JL, Rosen IB, et al. (1999) Oncogene profile of papillary thyroid carcinoma. Surgery 125: 46-52.

67. Jhiang SM, Mazzaferri EL (1994) The ret/PTC oncogene in papillary thyroid carcinoma. J Lab Clin Med 123: 331-337.

68. Saad A, Falciglia M, Steward DL, Nikiforov YE (2004) Amiodarone-induced thyrotoxicosis and thyroid cancer: clinical, immunohistochemical, and molecular genetic studies of a case and review of the literature. Arch Pathol Lab Med 128: 807-810.

69. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, et al. (2009) Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19: 1167-1214.

70. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, et al. (2006) European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol 154: 787-803.

71. Baloch ZW, LiVolsi VA (2008) Fine-needle aspiration of the thyroid: today and tomorrow. Best Pract Res Clin Endocrinol Metab 22: 929-939.

72. Mazzaferri EL (1993) Management of a solitary thyroid nodule. N Engl J Med 328: 553-559.

73. Gharib H, Goellner JR, Zinsmeister AR, Grant CS, Van Heerden JA (1984) Fine-needle aspiration biopsy of the thyroid. The problem of suspicious cytologic findings. Ann Intern Med 101: 25-28.

74. Guerra A, Sapio MR, Marotta V, Campanile E, Moretti MI, et al. (2011) Prevalence of RET/PTC rearrangement in benign and malignant thyroid nodules and its clinical application. Endocr J 58: 31-38.

75. Cantara S, Capezzone M, Marchisotta S, Capuano S, Busonero G, et al. (2010) Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. J Clin Endocrinol Metab 95: 1365-1369.

76. Wells SA Jr, Pacini F, Robinson BG, Santoro M (2013) Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update. J Clin Endocrinol Metab 98: 3149-3164.

77. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, et al. (1993) Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 363: 458-460.

78. Eng C, Smith DP, Mulligan LM, Nagai MA, Healey CS, et al. (1994) Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours. Hum Mol Genet 3: 237-241.

79. Frank-Raue K, Höppner W, Frilling A, Kotzerke J, Dralle H, et al. (1996) Mutations of the ret protooncogene in German multiple endocrine neoplasia families: relation between genotype and phenotype. German Medullary Thyroid Carcinoma Study Group. J Clin Endocrinol Metab 81: 1780-1783.

80. Gimm O, Marsh DJ, Andrew SD, Frilling A, Dahia PL, et al. (1997) Germline dinucleotide mutation in codon 883 of the RET proto-oncogene in multiple endocrine neoplasia type 2B without codon 918 mutation. J Clin Endocrinol Metab 82: 3902-3904.

81. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, et al. (1996) The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA 276: 1575-1579.

82. Elisei R, Romei C, Cosci B, Agate L, Bottici V, et al. (2007) RET genetic screening in patients with medullary thyroid cancer and their relatives: experience with 807 individuals at one center. J Clin Endocrinol Metab 92: 4725-4729.

83. Niccoli-Sire P, Murat A, Rohmer V, Franc S, Chabrier G, et al. (2001) Familial medullary thyroid carcinoma with noncysteine ret mutations: phenotype-genotype relationship in a large series of patients. J Clin Endocrinol Metab 86: 3746-3753.

84. Romei C, Cosci B, Renzini G, Bottici V, Molinaro E, et al. (2011) RET genetic screening of sporadic medullary thyroid cancer (MTC) allows the preclinical diagnosis of unsuspected gene carriers and the identification of a relevant percentage of hidden familial MTC (FMTC). Clin Endocrinol (Oxf) 74: 241-247.

85. Orlandi F, Caraci P, Mussa A, Saggiorato E, Pancani G, et al. (2001) Treatment of medullary thyroid carcinoma: an update. Endocr Relat Cancer 8: 135-147.

86. Bihan H, Murat A, Fysekidis M, Al-Salameh A, Schwartz C, et al. (2013) The clinical spectrum of RET proto-oncogene mutations in codon 790. Eur J Endocrinol 169: 271-276.

87. Frank-Raue K, Machens A, Scheuba C, Niederle B, Dralle H, et al. (2008) Difference in development of medullary thyroid carcinoma among carriers of RET mutations in codons 790 and 791. Clin Endocrinol (Oxf) 69: 259-263.

88. Erlic Z, Hoffmann MM, Sullivan M, Franke G, Peczkowska M, et al. (2010) Pathogenicity of DNA variants and double mutations in multiple endocrine neoplasia type 2 and von Hippel-Lindau syndrome. J Clin Endocrinol Metab 95: 308-313.

89. Orgiana G, Pinna G, Camedda A, De Falco V, Santoro M, et al. (2004) A new germline RET mutation apparently devoid of transforming activity serendipitously discovered in a patient with atrophic autoimmune thyroiditis and primary ovarian failure. J Clin Endocrinol Metab 89: 4810-4816.

90. Cosci B, Vivaldi A, Romei C, Gemignani F, Landi S, et al. (2011) In silico and in vitro analysis of rare germline allelic variants of RET oncogene associated with medullary thyroid cancer. Endocr Relat Cancer 18: 603-612.

91. Miyauchi A, Futami H, Hai N, Yokozawa T, Kuma K, et al. (1999) Two germline missense mutations at codons 804 and 806 of the RET proto-oncogene in the same allele in a patient with multiple endocrine neoplasia type 2B without codon 918 mutation. Jpn J Cancer Res 90: 1-5.

92. Elisei R, Cosci B, Romei C, Agate L, Piampiani P, et al. (2004) Identification of a novel point mutation in the RET gene (Ala883Thr), which is associated with medullary thyroid carcinoma phenotype only in homozygous condition. J Clin Endocrinol Metab 89: 5823-5827.

93. Tessitore A, Sinisi AA, Pasquali D, Cardone M, Vitale D, et al. (1999) A novel case of multiple endocrine neoplasia type 2A associated with two de novo mutations of the RET protooncogene. J Clin Endocrinol Metab 84: 3522-3527.

94. Machens A, Niccoli-Sire P, Hoegel J, Frank-Raue K, van Vroonhoven TJ, et al. (2003) Early malignant progression of hereditary medullary thyroid cancer. N Engl J Med 349: 1517-1525.

95. Romei C, Mariotti S, Fugazzola L, Taccaliti A, Pacini F, et al. (2010) Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes. Eur J Endocrinol 163: 301-308.

96. Eng C, Mulligan LM, Smith DP, Healey CS, Frilling A, et al. (1995) Low frequency of germline mutations in the RET proto-oncogene in patients with apparently sporadic medullary thyroid carcinoma. Clin Endocrinol (Oxf) 43: 123-127.

97. Elisei R, Alevizaki M, Conte-Devolx B, Frank-Raue K, Lette V (2012) 2012 European Thyroid Association guidelines for genetic testing and its clinical consequences in medullary thyroid cancer. European Thyroid Journal 1: 216-231.

98. Ceccherini I, Pasini B, Pacini F, Gullo M, Bongarzone I, et al. (1997) Somatic in frame deletions not involving juxtamembranous cysteine residues strongly activate the RET proto-oncogene. Oncogene 14: 2609-2612.

99. Borrello MG, Smith DP, Pasini B, Bongarzone I, Greco A, et al. (1995) RET activation by germline MEN2A and MEN2B mutations. Oncogene 11: 2419-2427.

100. Michiels FM, Chappuis S, Caillou B, Pasini A, Talbot M, et al. (1997) Development of medullary thyroid carcinoma in transgenic mice expressing the RET protooncogene altered by a multiple endocrine neoplasia type 2A mutation. Proc Natl Acad Sci U S A 94: 3330-3335.

101. Romei C, Ugolini C, Cosci B, Torregrossa L, Vivaldi A, et al. (2012) Low prevalence of the somatic M918T RET mutation in micro-medullary thyroid cancer. Thyroid 22: 476-481.

102. Pacini F, Romei C, Miccoli P, Elisei R, Molinaro E, et al. (1995) Early treatment of hereditary medullary thyroid carcinoma after attribution of multiple endocrine neoplasia type 2 gene carrier status by screening for ret gene mutations. Surgery 118: 1031-1035.

103. Lips CJ, Landsvater RM, Höppener JW, Geerdink RA, Blijham G, et al. (1994) Clinical screening as compared with DNA analysis in families with multiple endocrine neoplasia type 2A. N Engl J Med 331: 828-835.

104. Frilling A, Dralle H, Eng C, Raue F, Broelsch CE (1995) Presymptomatic DNA screening in families with multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma. Surgery 118: 1099-1103.

105. Frilling A, Höppner W, Eng C, Mulligan L, Raue F, et al. (1995) Presymptomatic genetic screening in families with multiple endocrine neoplasia type 2. J Mol Med (Berl) 73: 229-233.

106. Skinner MA, Moley JA, Dilley WG, Owzar K, Debenedetti MK, et al. (2005) Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. N Engl J Med 353: 1105-1113.

107. Kloos RT, Eng C, Evans DB, Francis GL, et al. (2009) Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid 19: 565-612.

108. Wagner SM, Zhu S, Nicolescu AC, Mulligan LM (2012) Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2. Clinics (Sao Paulo) 67 Suppl 1: 77-84.

109. Russo R, Trippi D, Falaschi F, Orsitto E, Cioni R, et al. (1994) [MEN IIB. A case report]. Radiol Med 87: 168-171.

110. Romei C, Elisei R, Pinchera A, Ceccherini I, Molinaro E, et al. (1996) Somatic mutations of the ret protooncogene in sporadic medullary thyroid carcinoma are not restricted to exon 16 and are associated with tumor recurrence. J Clin Endocrinol Metab 81: 1619-1622.

111. Zedenius J, Dwight T, Robinson BG, Delbridge L, Bäckdahl M, et al. (1998) A rapid method for DNA extraction from fine-needle aspiration biopsies of thyroid tumors, and subsequent RET mutation analysis. Cancer Detect Prev 22: 544-548.

112. Schilling T, Bürck J, Sinn HP, Clemens A, Otto HF, et al. (2001) Prognostic value of codon 918 (ATG-->ACG) RET proto-oncogene mutations in sporadic medullary thyroid carcinoma. Int J Cancer 95: 62-66.

113. Elisei R, Cosci B, Romei C, Bottici V, Renzini G, et al. (2008) Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. J Clin Endocrinol Metab 93: 682-687.

114. Moura MM, Cavaco BM, Pinto AE, Domingues R, Santos JR, et al. (2009) Correlation of RET somatic mutations with clinicopathological features in sporadic medullary thyroid carcinomas. Br J Cancer 100: 1777-1783.

115. Mian C, Pennelli G, Barollo S, Cavedon E, Nacamulli D, et al. (2011) Combined RET and Ki-67 assessment in sporadic medullary thyroid carcinoma: a useful tool for patient risk stratification. Eur J Endocrinol 164: 971-976.

116. Sherman SI (2011) Targeted therapies for thyroid tumors. Mod Pathol 24 Suppl 2: S44-52.

117. Schlumberger M, Sherman SI (2012) Approach to the patient with advanced differentiated thyroid cancer. Eur J Endocrinol 166: 5-11.

118. Carlomagno F, Vitagliano D, Guida T, Napolitano M, Vecchio G, et al. (2002) The kinase inhibitor PP1 blocks tumorigenesis induced by RET oncogenes. Cancer Res 62: 1077-1082.

119. Okamoto K, Kodama K, Takase K, Sugi NH, Yamamoto Y, et al. (2013) Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models. Cancer Lett 340: 97-103.

120. Carlomagno F, Anaganti S, Guida T, Salvatore G, Troncone G, et al. (2006) BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst 98: 326-334.

121. Bentzien F, Zuzow M, Heald N, Gibson A, Shi Y, et al. (2013) In Vitro and In Vivo Activity of Cabozantinib (XL184), an Inhibitor of RET, MET, and VEGFR2, in a Model of Medullary Thyroid Cancer. Thyroid .

122. Coxon A, Bready J, Kaufman S, Estrada J, Osgood T, et al. (2012) Anti-tumor activity of motesanib in a medullary thyroid cancer model. J Endocrinol Invest 35: 181-190.

123. Jeong WJ, Mo JH, Park MW, Choi IJ, An SY, et al. (2011) Sunitinib inhibits papillary thyroid carcinoma with RET/PTC rearrangement but not BRAF mutation. Cancer Biol Ther 12: 458-465.

124. Elisei R, Schlumberger MJ, Müller SP, Schöffski P, Brose MS, et al. (2013)

Cabozantinib in progressive medullary thyroid cancer. J Clin Oncol 31: 3639-3646.

125. Leboulleux S, Bastholt L, Krause T, de la Fouchardiere C, Tennvall J, et al. (2012) Vandetanib in locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 2 trial. Lancet Oncol 13: 897-905.

126. Wells SA Jr, Robinson BG, Gagel RF, Dralle H, Fagin JA, et al. (2012) Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol 30: 134-141.