

Thyroid Cancer: Role of RET and Beyond

Francesca Carlomagno

Dipartimento di Biologia e Patologia Cellulare e Molecolare L. Califano, Università degli Studi di Napoli Federico II, Napoli, Italia

Key Words

RET · Receptor tyrosine kinase · Thyroid cancer · Chromosome rearrangements · Tyrosine kinase inhibitors

Abstract

Specific thyroid cancer histotypes, such as papillary and medullary thyroid carcinoma, display genetic rearrangements or point mutations of the RET gene, resulting in its oncogenic conversion. The molecular mechanisms mediating RET rearrangement with other genes and the role of partner genes in tumorigenesis have been described. In addition, the RET protein has become a molecular target for medullary thyroid carcinoma treatment.

Copyright © 2012 European Thyroid Association
Published by S. Karger AG, Basel

Introduction

The RET (rearranged during transfection) gene is located on chromosome 10q11.2 straddling a region of around 55,000 bp and is composed of 21 exons. The gene codes for a transmembrane protein belonging to the receptor tyrosine kinases family [1].

The RET protein is composed of three different domains: the extracellular domain, transmembrane domain and intracellular domain, and varies in length due

to a 3' end alternative splicing that generates three different isoforms containing 9 (RET9), 43 (RET43) or 51 (RET51) amino acids behind the RET G1063 residue [1, 2]. RET9 and RET51 are the most represented isoforms in vivo. The extracellular segment of RET protein is N-glycosylated and contains four Ca²⁺ dependent cell adhesion (cadherin)-like domains followed by a cysteine-rich domain [3, 4]. N-glycosylation of the extracellular domain occurs in the endoplasmic reticulum and Golgi apparatus and is necessary for transport to the plasma membrane. Indeed, only the fully mature glycosylated 170-kDa isoform of RET is exposed on the cell surface while the highly mannose-rich 150-kDa isoform is confined to the Golgi apparatus [5]. The transmembrane segment is composed of 22 amino acids. The intracellular portion contains the tyrosine kinase domain, divided into two subdomains by the insertion of 27 amino acids, and a COOH terminal tail.

RET functions as the receptor of the four members of the glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs) which include GDNF itself, neurturin, artemin and persephin [4]. GFLs are presented to RET by GPI (glycosylphosphatidylinositol)-anchored coreceptor molecules, named GFR α (GDNF family receptor- α) and comprising four different members sequentially numbered GFR α 1–4, each binding preferentially one of the four different GFLs.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2012 European Thyroid Association
Published by S. Karger AG, Basel
2235-0640/12/0011-0015\$38.00/0
Accessible online at:
www.karger.com/etj

Francesca Carlomagno
Dipartimento di Biologia e Patologia Cellulare e Molecolare L. Califano
Università degli Studi di Napoli Federico II
Via Sergio Pansini 5, IT-80123 Napoli (Italy)
Tel. +39 081 746 3603, E-Mail francesca.carlomagno@unina.it

RET binding to any GFL-GFR α complex induces receptor dimerization, which in turn triggers transphosphorylation of specific tyrosine (Y) residues located in the tyrosine kinase domain and in the COOH-terminal tail of each RET monomer. Phosphorylation of such tyrosines initiates an intracellular signaling cascade that culminates in the activation of several cellular programs such as motility, survival and proliferation [6].

Several tyrosines have been identified as RET autophosphorylation sites [7, 8]. In particular, Y900 and Y905 are located in the activation loop of the kinase and their phosphorylation is necessary for stabilization of RET in an active conformation, while RET Y1062 is located in the COOH-tail and is a multidocking site for many signaling molecules containing a phosphotyrosine-binding domain such as SHC, N-SHC (RAI), FRS2 and IRS1/2 [9–15]. Binding of these proteins contributes to activate the two main RET signaling pathways, i.e. the RAS/MAPK and the PI3K/AKT ones.

RET is expressed mostly in tissues of neuroectodermal origin, such as the enteric ganglia, the adrenal chromaffin cells and thyroid C cells; in sensory and autonomic ganglia of the peripheral nervous system; in a subset of central nervous system nuclei; in the kidney during embryonic and fetal stage; and in testes germ cells [6, 16]. RET and its ligands play a significant role in the development of all these structures as shown by the RET-null mice phenotype featuring absence of the superior cervical ganglia and enteric nervous system, kidney agenesis and reduction of thyroid C cells, as well as impaired spermatogenesis due to lack of spermatogonial stem cell renewal and differentiation [6, 16]. In accordance, individuals with germline loss-of-function mutations of RET are affected by congenital megacolon or Hirschsprung disease characterized by intestinal aganglionosis [17].

Gain-of-function mutations of the RET gene have been identified in two different types of thyroid neoplasia: medullary thyroid carcinoma (MTC) and papillary thyroid carcinoma.

RET Mutations in MTC: A New Target for Cancer Treatment

MTC arises from neural crest-derived calcitonin-producing thyroid parafollicular C cells and represents 5–10% of all thyroid cancers. Although most MTCs are sporadic and affect adult patients, around 25% of cases are familial and occur in the frame of inherited cancer

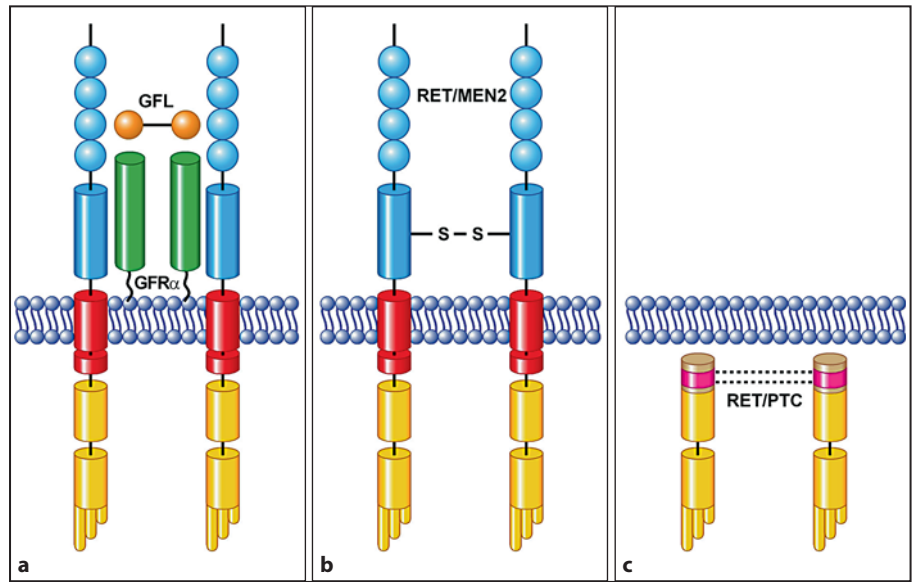
syndromes called multiple endocrine neoplasia type 2 (MEN2) syndromes (OMIM: No. 171400) and may have an early onset [18].

MEN2 syndromes comprise three different kinds: MEN2A, MEN2B and familial MTC (FMTC). MEN2A and B phenotypes are depicted by a plethora of tumors that, with different combinations, arise from tissues of neuroectodermal origins. All patients display MTC, associated in 50% of cases to pheochromocytoma arising from chromaffin cells of adrenal medulla. Additional features are parathyroid hyperplasia and hereditary localized pruritus in MEN2A, ganglioneuromatosis of the intestine, thickening of corneal nerves and marfanoid habitus in MEN2B. FMTC consists in the isolated occurrence of MTC and is nowadays regarded as a phenotypic variant of MEN2A with decreased penetrance and/or delay of the other neoplastic manifestation [19, 20].

In over 90% of cases, MEN2 syndromes are due to germline missense mutations of the RET gene. There is partial overlap between the type of mutation and the kind of phenotype displayed by patients. Thus, the majority of MEN2A and FMTC carriers bear mutations of one of the cysteines in the extracellular cysteine-rich domain of RET (most frequently, C634). FMTC is also associated with changes in the N-terminal (E768D, L790F, Y791F, V804L, V804M) or C-terminal (S891A) lobes of RET kinase. The vast majority of MEN2B patients display the M918T mutation in RET kinase domain, whereas only a small fraction harbors the A883F substitution. In addition, somatic mutations of RET E768, V804 and mainly M918 are found in approximately 40% of sporadic MTC cases and correlate with a bad prognosis [20–23]. Over the years many new germline RET mutations have been identified due to the systematic screening of MTC patients; however, the pathological meaning of such variants is not always clear due to lack of information about mutation cosegregation with disease within families and of functional studies. A comprehensive database annotating all RET variants has been generated [24].

The mechanism through which MEN2-associated mutations switch on RET oncogenic activity is strictly dependent on the location of the amino acid change. Extracellular cysteine mutants display constitutive kinase activity consequent to disulfide bond-mediated homodimerization (fig. 1). Mutations associated with FMTC, which in cases target cysteine residues other than C634, are less potently transforming than MEN2A-associated mutations, possibly because of their lower activity to induce RET S-S linked dimers [25, 26]. Constitutive activation as well as a change in substrate specificity has

Fig. 1. Schematic representation of the different mechanisms of wild-type RET and RET-derived oncoproteins activation. **a** Wild-type RET activation is mediated by ligand-induced dimerization. **b** RET/MEN2A-FMTC activation is mediated by disulfide bond-mediated dimerization. **c** RET/PTC activation is mediated by coiled-coil-induced dimerization.



been implicated in the MEN2B-associated substitution [27]. In line with this model, MEN2B mutants differ from MEN2A mutants in the stoichiometry of phosphorylation of RET tyrosines and of various intracellular proteins [28, 29]. Moreover, MEN2B-expressing tumors have different gene expression profiles as compared to MEN2A-expressing tumors [30]. The mechanisms through which RET intracellular mutations (other than M918T) constitutively activate RET enzymatic function has not been clearly elucidated.

In 2009, the American Thyroid Association created specific MTC clinical guidelines consisting of 122 evidence-based recommendations to assist in the clinical care of MTC patients [31]. The guidelines included germline genetic testing in all MTC patients to distinguish sporadic from familial cases and to classify patients in different disease risk levels ranging from A (most severe) to D (less severe) on the basis of RET mutation. The risk level classification is relevant for making decisions with respect to prophylactic thyroidectomy and intraoperative amputation of the parathyroid glands.

Although identification of RET mutations in MEN2 carriers has drastically improved clinical management of patients for preventive or early surgery, most MTC cases come to diagnosis when the disease is already metastatic and little can be done because of the resistance that MTC displays towards conventional chemotherapy and radiotherapy [32]. Therefore, identification of novel treatments for MTC patients appears to be mandatory. The past 10

years have witnessed the advent of new classes of antineoplastic drugs whose mechanism of action is based on targeting of tyrosine kinases causally involved in cancer [33]. These drugs include monoclonal antibodies such as Herceptin (trastuzumab) or Erbitux (cetuximab) directed against HER2 and HER1 receptor tyrosine kinases and used in breast and colon cancer treatment, respectively.

Another important class of targeted therapies is represented by small molecule tyrosine kinase inhibitors, which compete with ATP, thereby obstructing autophosphorylation and signal transduction downstream from the targeted kinase. Prominent examples are STI571 [Gleevec (imatinib)] against BCR-ABL in chronic myeloid leukemia and against c-KIT and PDGFR in gastrointestinal stromal tumors [33], and ZD1839 [Iressa (gefitinib)] against epithelial growth factor receptor (EGFR) in non-small cell lung carcinoma [34]. RET is a critical target for medullary thyroid cancer treatment [32]. Several small molecules are known to exert RET inhibition. Virtually all of them are believed to block the kinase by competing with ATP.

Two pyrazolopyrimidines (PP1 and PP2) showed half-maximal RET inhibitory concentrations in the nM range (≤ 100 nM) and were able to block RET's oncogenic effects in cell cultures [35, 36]. PP1 also induced RET protein destruction through proteosomal degradation [37]. The 2-indolinone RPI-1 was effective against RET, but only at high doses ($IC_{50} = 3.6 \mu M$ for cell proliferation) and ex-

Table 1. RET/PTC rearrangements

Fusion name	RET partner	Function	Chromosome rearrangement
RET/PTC1	H4/D10S170	apoptotic factor	inv10(q11.2;q21)
RET/PTC2	PKA RI α	regulatory subunit of protein kinase A	t(10;17)(q11.2;q23)
RET/PTC3	RFG/ELE1/NcoA4	coactivator of nuclear receptor	inv10(q11.2;q10)
RET/PTC4	RFG/ELE1/NcoA4	coactivator of nuclear receptor	inv10(q11.2;q10)
RET/PTC5	RFG5/Golgin 84	Golgi structure maintenance	t(14;10)(q11.2;q32)
RET/PTC6	HTIF1	coactivator of nuclear receptor	t(7;10)(q32-34;q11.2)
RET/PTC7	RFG7/HTIF γ /TRIMM33	coactivator of androgen receptor and SMAD4-E3 ligase	t(1;10)(p13;q11.2)
RET/PTC8	KNT1/kinectin	regulator of intracellular motility	t(1;10)(q11.2;q22.1)
RET/PTC9	RFG9	unknown	t(10;18)(q11.2;q21-22)
RET/ELKS	ELKS	NF- κ B regulatory protein	t(10;12)(q11.2;p13.3)
RET/PCM1	PCM1	centrosome assembly protein	t(8;10)(p21-22;q11.2)
RET/RFP	RFP/TRIMM27	PML interactor and RB modulator	t(6;10)(p21;q11.2)
RET/HOOK3	HOOK3	microtubule-binding protein	t(8;10)(p11.21;q11.2)

erted *in vivo* antitumor effects [38]. Two indolocarbazole derivatives, CEP-701 and CEP-751, inhibited RET-MEN 2A oncoproteins at nM concentrations. Importantly, these compounds also inhibited tumor growth in MTC cell xenografts [39]. Nevertheless, none of these compounds has been evaluated in clinical studies due to their toxicity or limited bioavailability.

Another group of anti-RET compounds that had been tested in phase I clinical trials has been described. Such molecules comprise vandetanib, sorafenib, sunitinib, cabozantinib and lenvatinib [40–43]. All these compounds have been evaluated in clinical trials for efficacy in MTC treatment. The anilinoquinazoline vandetanib, also known as ZD6474, inhibits RET with an IC₅₀ of 100 nM and has been the first anti-RET agent to be FDA approved for MTC treatment on the basis of the results exerted in clinical trials for MTC treatment [44]. ZD6474 is also a potent inhibitor of KDR, the vascular endothelial growth factor receptor and EGFR. Inhibition of KDR potentiates the antineoplastic activity through an antiangiogenic effect, whereas EGFR inhibition might be important to impede the complementation of RET inhibition via EGFR hyperactivation [40, 45]. Mutations in codons 804 and 806 in the ATP-binding pocket have been shown to confer resistance to vandetanib, which may be a concern for secondary resistance to the drug [46, 47]. In a phase III clinical trial, vandetanib was able to increase progression-free survival, inducing a partial response in around 20% of patients and a stabilization of disease in 50% of treated cases compared to control [44].

RET in Papillary Thyroid Carcinoma: A New Role for Partner Genes

Papillary thyroid carcinoma (PTC) is the most frequent thyroid cancer and consists in a well-differentiated carcinoma, originating from thyroid follicular cells and associated to exposure to ionizing radiation [48, 49]. Consistently, typical molecular features of PTCs are chromosomal aberrations generated as a consequence of ionizing radiation-induced double-strand breaks and unfaithful repair. In particular, PTCs display chromosomal rearrangements of chr. 10q, causing the rupture of the RET gene and its fusion to heterologous genes due to unfaithful repair [50]. The partner genes encode heterogeneous proteins all containing protein-protein interaction domains such as coiled-coil motifs [51]. RET/PTC1 and RET/PTC3 represent over 90% of all RET/PTC rearrangements identified so far. In both cases, the chromosomal aberration consists in a paracentric inversion of the long arm of chromosome 10 where, together with RET the corresponding fusion partner of RET/PTC1, CCDC6 (H4) and of RET/PTC3, NCOA4 (RFG, ELE1, ARA70) map [50, 52]. RET/PTC3 is mainly associated with radiation-induced carcinomas and is frequently found in more aggressive PTC variants such as the solid-follicular or the tall cell histotypes. The other 11 RET/PTC isoforms are very rare and have been found only in few cases of radiation-induced PTCs (table 1).

RET/PTCs behave as oncogenes and are able to induce thyroid cell transformation in cell culture and in transgenic mice, strongly supporting their oncogenic function

and causal contribution to PTC development [53–56]. The best described mechanism for RET/PTC oncogenic conversion resides in the ligand-independent activation of RET kinase as a consequence of its fusion to heterologous proteins, such as CCDC6 and NCOA4. The independence from ligand binding is achieved through removal of negatively regulating sequence from wild-type RET proteins such as the extracellular and juxtamembrane domains as well as its joining to protein homodimerization motives contained in the fusion partners, namely coiled-coil domains. Constitutive dimerization and transphosphorylation of RET mediates the continuous activation of downstream signaling pathways. Moreover, as a consequence of gene fusion, the RET tyrosine kinase domain comes under the control of the new gene promoters. Differently from RET, which is normally expressed in a restricted subset of neuronal and neuroectoderm-derived cells, such partner gene promoters are ubiquitously active and drive RET expression in thyroid follicular cells [16, 57] (fig. 1).

RET oncogenic signaling sustains the acquisition of several hallmarks of cancer cells including cell autonomy and independence from growth factors (like thyroid stimulating hormone), resistance to proapoptotic stimuli and motility [53, 55]. Such programs are principally switched on by the activation of the RAS/MAPK and PI3K/AKT signaling pathways through RET Y1062 autophosphorylation [55, 58]. Nevertheless, the low penetrance of the disease in transgenic animals, as well as the presence of such rearrangements in papillary microcarcinomas that do not progress to invasive cancer, suggests that additional oncogenic events should occur to generate a clinically relevant disease [59]. In line with these observations, RET/PTC1 and RET/PTC3 oncogenes are unable to induce a fully transformed phenotype in normal rat thyroid cells in vitro [53, 55]. Notably, the acute expression of RET/PTC1 and RET/PTC3 in thyroid cells in vitro has been shown to activate a proapoptotic response due to unscheduled activation of the RAS/MAPK pathway [60, 61]. It is conceivable that RET/PTC rearrangements are not sufficient to induce a symptomatically evident cancer, unless cells activate antiapoptotic or antisenesence programs, through further mutational events or epigenetic modifications.

RET/PTC rearrangements are confined to the papillary histotype of thyroid carcinoma and have never been described in other tumor types [51]. The thyroid gland is exposed to ionizing radiation more than other tissues due to its ability to concentrate iodide radioactive isotopes. On the other hand, a large set of data have shown

that nuclear chromatin architecture in thyroid cells bring in close proximity the CCDC6, NCOA4 and RET genetic loci, facilitating the assembly of RET/PTC1 (CCDC6-RET) and RET/PTC3 (NCOA4-RET) fusions [62–64]. Importantly, in other cancers, also displaying chromosomal rearrangements such as prostate carcinoma, the spatial organization of chromatin has been shown to be dictated by the action of transcriptional factors that somehow guide the relevant genes in the same nuclear neighborhood, promoting their fusion [65]. Not all RET/PTC rearrangements seem to be linked to radiation exposure, especially those displaying RET/PTC1 rearrangement [48]. In these cases, the presence of the fragile sites FRA10C and FRA10G located on chromosome 10 next to CCDC6 and RET genes, respectively, might provide an alternative mechanism for the generation of RET/PTC fusion [66]. Thus, under specific insults such as ethanol, caffeine and hypoxia, fragile sites are hotspots of spontaneous chromosome breakage and translocation [67]. In addition, H₂O₂, a potent DNA damaging agent produced in large amounts by the thyroid gland during the process of thyroid hormone synthesis, is able to induce RET/PTC1 rearrangement as well, suggesting that oxidative stress might be an additional mechanism through which RET/PTC1 rearrangement might occur [68].

Many types of cancers, such as hepatocarcinoma and gastric and colon cancers, are promoted by inflammation. Hashimoto's thyroiditis, the most common organ-specific autoimmune disease in humans, is often associated to papillary thyroid cancer [69]. Several reports have shown that RET/PTC signaling via the RAS/MAPK cascade in thyroid follicular cells endorses an inflammatory-like response through the production of several cytokines and chemokines that act in an autocrine as well as paracrine fashion, recruiting macrophages, lymphocytes and mast cells within the tumor mass and promoting cell survival, invasion and angiogenesis [55, 58, 70, 71]. More studies need to be done to clarify whether the oncogene-mediated proinflammatory response supports the development of a chronic inflammation or whether the latter facilitates the process of neoplastic transformation.

Several studies suggest that the genes most frequently rearranged to RET, CCDC6 and NCOA4 might display a tumor suppressor-like activity and their loss of function could be involved in tumorigenesis. CCDC6 (coiled-coil domain containing sequence 6, also known as H4/D10S170) gene product is a ubiquitously expressed 65-kDa nuclear and cytosolic protein that has been shown to display proapoptotic activity and to be involved in the

ATM-mediated cellular response to DNA damage [72, 73]. More recently, a direct role of *CCDC6* in the repression of *CREB1*, a transcriptional factor essential for thyroid cell growth and differentiation, has been described [74]. The *CCDC6* gene has been found fused to the *PDGFR β* gene in atypical chronic myeloid leukemia and to *PTEN* tumor suppressor phosphatase in PTCs, indicating its high susceptibility to gene fusion [75, 76]. The *NCOA4* (nuclear receptor coactivator 4, also known as *RFG/ELE1/ARA70*) gene encodes a 70-kDa protein functioning as a coactivator of androgen receptor and *PPAR γ* (peroxisome-proliferator activated receptor- γ) [77]. *PPAR γ -PAX8* gene fusion has been found in around 30% of follicular thyroid carcinoma [78]. Functional studies have demonstrated that the *PAX8-PPAR γ* chimeric protein functions as a dominant negative inhibitor of the parental wild-type *PPAR γ* protein which physiologically exerts antineoplastic signaling in thyroid follicular cells. *NCOA4* is a *PPAR γ* and might help this tumor suppressor function [78]. In addition, ectopic overexpression of *NCOA4* in the prostate cancer cell line LNCAP reduces cell proliferation, and *NCOA4* protein expression has been shown to be reduced in aggressive prostate and breast cancers with respect to normal tissue or well-differentiated carcinomas [79–81]. Finally, the chromosomal region containing the *NCOA4* gene has been identified as a prostate cancer risk locus [82, 83]. A *RET* partner with a well-known tumor suppressor function is *PRKARIA*, encoding the regulatory subunit of protein kinase A, *RI α* and fused to *RET* in *RET/PTC2* rearrangement. Germline inactivating mutations of *PRKARIA* gene have been found in patients affected by

a rare autosomal dominant cancer-prone syndrome, Carney Complex, characterized by lentiginosis, atrial and cutaneous myxoma, pituitary adenoma, testicular tumors, ovarian cystis, schwannoma, and thyroid adenoma and carcinoma [84, 85]. Somatic LOH at the gene locus on chr. 17q22–24 in Carney Complex patient tumor DNA confirms the tumor suppressor function of *PRKARIA* [84].

In conclusion, all these data strongly support the concept that *RET* partner genes' inactivation in the context of *RET/PTC* rearrangements might be involved in thyroid carcinogenesis. In addition, since the rearrangement affects only one allele, it might be envisaged that chimeric *RET/PTC* oncoproteins might function as dominant negative mutants for their ability to interact and phosphorylate the wild-type partner proteins, as shown for *CCDC6* and *NCOA4* proteins [72, 86]. In this light, the function of *RET* gene fusion partners, possibly representing new thyroid cancer genes, should be studied.

Acknowledgements

We thank Rosa Marina Melillo and Massimo Santoro for useful discussion and Ciotola presentation for art work. This study was supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC).

Disclosure Statement

The author declares no conflict of interest.

References

- Pasini B, Hofstra RM, Yin L, Boccardi R, Santamaria G, Grootscholten PM, Ceccherini I, Patrone G, Priolo M, Buys CH, et al: The physical map of the human *RET* proto-oncogene. *Oncogene* 1995;11:1737–1743.
- Myers SM, Eng C, Ponder BA, Mulligan LM: Characterization of *RET* proto-oncogene 3' splicing variants and polyadenylation sites: a novel C-terminus for *RET*. *Oncogene* 1995; 11:2039–2045.
- Anders J, Kjar S, Ibáñez CF: Molecular modeling of the extracellular domain of the *RET* receptor tyrosine kinase reveals multiple cadherin-like domains and a calcium-binding site. *J Biol Chem* 2001;276:35808–35817.
- Airaksinen MS, Saarma M: The GDNF family: signalling, biological functions and therapeutic value. *Nat Rev Neurosci* 2002;3:383–394.
- Takahashi M, Asai N, Iwashita T, Isomura T, Miyazaki K, Matsuyama M: Characterization of the *ret* proto-oncogene products expressed in mouse L cells. *Oncogene* 1993;8: 2925–2929.
- Arighi E, Borrello MG, Sariola H: *RET* tyrosine kinase signaling in development and cancer. *Cytokine Growth Factor Rev* 2005; 16:441–467.
- Liu X, Vega QC, Decker RA, Pandey A, Worthy CA, Dixon JE: Oncogenic *RET* receptors display different autophosphorylation sites and substrate binding specificities. *J Biol Chem* 1996;271:5309–5312.
- Kawamoto Y, Takeda K, Okuno Y, Yamakawa Y, Ito Y, Taguchi R, Kato M, Suzuki H, Takahashi M, Nakashima I: Identification of *RET* autophosphorylation sites by mass spectrometry. *J Biol Chem* 2004;279:14213–14224.
- Asai N, Murakami H, Iwashita T, Takahashi M: A mutation at tyrosine 1,062 in *MEN2A-Ret* and *MEN2B-Ret* impairs their transforming activity and association with *shc* adaptor proteins. *J Biol Chem* 1996;271: 17644–17649.

- 10 Alberti L, Borrello MG, Ghizzoni S, Torriti F, Rizzetti MG, Pierotti MA: Grb2 binding to the different isoforms of Ret tyrosine kinase. *Oncogene* 1998;17:1079–1087.
- 11 Murakami H, Iwashita T, Asai N, Shimono Y, Iwata Y, Kawai K, Takahashi M: Enhanced phosphatidylinositol 3-kinase activity and high phosphorylation state of its downstream signalling molecules mediated by ret with the MEN 2B mutation. *Biochem Biophys Res Commun* 1999;262:68–75.
- 12 Segouffin-Cariou C, Billaud M: Transforming ability of MEN2A-RET requires activation of the phosphatidylinositol 3-kinase/AKT signaling pathway. *J Biol Chem* 2000;275:3568–3576.
- 13 Melillo RM, Santoro M, Ong SH, Billaud M, Fusco A, Hadari YR, Schlessinger J, Lax I: Docking protein FRS2 links the protein tyrosine kinase RET and its oncogenic forms with the mitogen-activated protein kinase signaling cascade. *Mol Cell Biol* 2001;21:4177–4187.
- 14 Melillo RM, Carlomagno F, De Vita G, Formisano P, Vecchio G, Fusco A, Billaud M, Santoro M: The insulin receptor substrate (IRS)-1 recruits phosphatidylinositol 3-kinase to Ret: evidence for a competition between Shc and IRS-1 for the binding to Ret. *Oncogene* 2001;20:209–218.
- 15 Takahashi M: The GDNF/RET signaling pathway and human diseases. *Cytokine Growth Factor Rev* 2001;12:361–373.
- 16 Manié S, Santoro M, Fusco A, Billaud M: The RET receptor: function in development and dysfunction in congenital malformation. *Trends Genet* 2001;17:580–589.
- 17 Brooks AS, Oostra BA, Hofstra RM: Studying the genetics of Hirschsprung's disease: unraveling an oligogenic disorder. *Clin Genet* 2005;67:6–14.
- 18 de Groot JW, Links TP, Plukker JT, Lips CJ, Hofstra RM: RET as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. *Endocr Rev* 2006;27:535–560.
- 19 Cote GJ, Gagel RF: Lessons learned from the management of a rare genetic cancer. *N Engl J Med* 2003;349:1566–1568.
- 20 Marx SJ: Molecular genetics of multiple endocrine neoplasia types 1 and 2. *Nat Rev Cancer* 2005;5:367–375.
- 21 Eng C, Smith DP, Mulligan LM, Healey CS, Zvelebil MJ, Stonehouse TJ, Ponder MA, Jackson CE, Waterfield MD, Ponder BA: A novel point mutation in the tyrosine kinase domain of the RET proto-oncogene in sporadic medullary thyroid carcinoma and in a family with FMTc. *Oncogene* 1995;10:509–513.
- 22 Ponder BA: The phenotypes associated with ret mutations in the multiple endocrine neoplasia type 2 syndrome. *Cancer Res* 1999;59:1736s–1741s.
- 23 Elisei R, Cosci B, Romei C, Bottici V, Renzini G, Molinaro E, Agate L, Vivaldi A, Faviana P, Basolo F, Miccoli P, Berti P, Pacini F, Pinchera A: Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. *J Clin Endocrinol Metab* 2008;93:682–687.
- 24 Margraf RL, Crockett DK, Krautscheid PM, Seamons R, Calderon FR, Wittwer CT, Mao R: Multiple endocrine neoplasia type 2 RET protooncogene database: repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. *Hum Mutat* 2009;30:548–556.
- 25 Carlomagno F, Salvatore G, Cirafici AM, De Vita G, Melillo RM, de Franciscis V, Billaud M, Fusco A, Santoro M: The different RET-activating capability of mutations of cysteine 620 or cysteine 634 correlates with the multiple endocrine neoplasia type 2 disease phenotype. *Cancer Res* 1997;57:391–395.
- 26 Ito S, Iwashita T, Asai N, Murakami H, Iwata Y, Sobue G, Takahashi M: Biological properties of Ret with cysteine mutations correlate with multiple endocrine neoplasia type 2A, familial medullary thyroid carcinoma, and Hirschsprung's disease phenotype. *Cancer Res* 1997;57:2870–2872.
- 27 Santoro M, Melillo RM, Carlomagno F, Vecchio G, Fusco A: Minireview: RET: normal and abnormal functions. *Endocrinology* 2004;145:5448–5451.
- 28 Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, Fusco A, Vecchio G, Matoskova B, Kraus MH, et al: Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. *Science* 1995;267:381–383.
- 29 Salvatore D, Melillo RM, Monaco C, Visconti R, Fenzi G, Vecchio G, Fusco A, Santoro M: Increased in vivo phosphorylation of ret tyrosine 1,062 is a potential pathogenetic mechanism of multiple endocrine neoplasia type 2B. *Cancer Res* 2001;61:1426–1431.
- 30 Jain S, Watson MA, DeBenedetti MK, Hiraki Y, Moley JF, Milbrandt J: Expression profiles provide insights into early malignant potential and skeletal abnormalities in multiple endocrine neoplasia type 2B syndrome tumors. *Cancer Res* 2004;64:3907–3913.
- 31 Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD, Schlumberger M, Wells SA Jr, American Thyroid Association Guidelines Task Force: Medullary thyroid cancer: management guidelines of the American Thyroid Association *Thyroid* 2009;19:565–612.
- 32 Schlumberger M, Carlomagno F, Baudin E, Bidart JM, Santoro M: New therapeutic approaches to treat medullary thyroid carcinoma. *Nat Clin Pract Endocrinol Metab* 2008;4:22–32.
- 33 Gschwind A, Fischer OM, Ullrich A: The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer* 2004;4:361–370.
- 34 Minna JD, Gazdar AF, Sprang SR, Herz J: Cancer. A bull's eye for targeted lung cancer therapy. *Science* 2004;304:1458–1461.
- 35 Carlomagno F, Vitagliano D, Guida T, Napolitano M, Vecchio G, Fusco A, Gazit A, Levitzki A, Santoro M: The kinase inhibitor PP1 blocks tumorigenesis induced by RET oncogenes. *Cancer Res* 2002;62:1077–1082.
- 36 Carlomagno F, Vitagliano D, Guida T, Basolo F, Castellone MD, Melillo RM, Fusco A, Santoro M: Efficient inhibition of RET/papillary thyroid carcinoma oncogenic kinases by 4-amino-5-(4-chloro-phenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP2). *J Clin Endocrinol Metab* 2003;88:1897–1902.
- 37 Carniti C, Perego C, Mondellini P, Pierotti MA, Bongarzone I: PP1 inhibitor induces degradation of RETMEN2A and RET-MEN2B oncoproteins through proteosomal targeting. *Cancer Res* 2003;63:2234–2243.
- 38 Cuccuru G, Lanzi C, Cassinelli G, Pratesi G, Tortoreto M, Petrangolini G, Seregni E, Martinetti A, Laccabue D, Zanchi C, Zunino F: Cellular effects and antitumor activity of RET inhibitor RPI-1 on MEN2A-associated medullary thyroid carcinoma. *J Natl Cancer Inst* 2004;96:1006–1014.
- 39 Strock CJ, Park JJ, Rosen M, Dionne C, Ruggeri B, Jones-Bolin S, Denmeade SR, Ball DW, Nelkin BD: CEP-701 and CEP-751 inhibit constitutively activated RET tyrosine kinase activity and block medullary thyroid carcinoma cell growth. *Cancer Res* 2003;63:5559–5563.
- 40 Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, Ryan AJ, Fontanini G, Fusco A, Santoro M: ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. *Cancer Res* 2002;62:7284–7290.
- 41 Carlomagno F, Anaganti S, Guida T, Salvatore G, Troncone G, Wilhelm SM, Santoro M: BAY 43–9006 inhibition of oncogenic RET mutants. *J Natl Cancer Inst* 2006;98:326–334.
- 42 Kim DW, Jo YS, Jung HS, Chung HK, Song JH, Park KC, Park SH, Hwang JH, Rha SY, Kweon GR, Lee SJ, Jo KW, Shong M: An orally administered multitarget tyrosine kinase inhibitor, SU11248, is a novel potent inhibitor of thyroid oncogenic RET/papillary thyroid cancer kinases. *J Clin Endocrinol Metab* 2006;91:4070–4076.
- 43 Verbeek HH, Alves MM, de Groot JW, Osinga J, Plukker JT, Links TP, Hofstra RM: The effects of four different tyrosine kinase inhibitors on medullary and papillary thyroid cancer cells. *J Clin Endocrinol Metab* 2011;96:E991–E995.

- 44 Wells SA Jr, Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, Baudin E, Elisei R, Jarzab B, Vasselli JR, Read J, Langmuir P, Ryan AJ, Schlumberger MJ: Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J Clin Oncol* 2012;30:134–141.
- 45 Vitagliano D, De Falco V, Tamburrino A, Coluzzi S, Troncone G, Chiappetta G, Ciardiello F, Tortora G, Fagin JA, Ryan AJ, Carlomagno F, Santoro M: The tyrosine kinase inhibitor ZD6474 blocks proliferation of RET mutant medullary thyroid carcinoma cells. *Endocr Relat Cancer* 2010; 18:1–11.
- 46 Carlomagno F, Guida T, Anaganti S, Vecchio G, Fusco A, Ryan AJ, Billaud M, Santoro M: Disease associated mutations at valine 804 in the RET receptor tyrosine kinase confer resistance to selective kinase inhibitors. *Oncogene* 2004;23:6056–6063.
- 47 Carlomagno F, Guida T, Anaganti S, Provi-tera L, Kjaer S, McDonald NQ, Ryan AJ, Santoro M: Identification of tyrosine 806 as a molecular determinant of RET kinase sensitivity to ZD6474. *Endocr Relat Cancer* 2009; 16:233–241.
- 48 Sherman SI: Thyroid carcinoma. *Lancet* 2003;361:501–511.
- 49 Williams D: Radiation carcinogenesis: lessons from Chernobyl. *Oncogene* 2008; 27(suppl 2):S9–S18.
- 50 Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della Porta G, Fusco A, Vecchio G: PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. *Cell* 1990;60:557–563.
- 51 Nikiforov YE, Nikiforova MN: Molecular genetics and diagnosis of thyroid cancer. *Nat Rev Endocrinol* 2011;7:569–580.
- 52 Santoro M, Dathan NA, Berlingieri MT, Bongarzone I, Paulin C, Grieco M, Pierotti MA, Vecchio G, Fusco A: Molecular characterization of RET/PTC3; a novel rearranged version of the RET proto-oncogene in a human thyroid papillary carcinoma. *Oncogene* 1994;9:509–516.
- 53 Santoro M, Melillo RM, Grieco M, Berlingieri MT, Vecchio G, Fusco A: The TRK and RET tyrosine kinase oncogenes cooperate with ras in the neoplastic transformation of a rat thyroid epithelial cell line. *Cell Growth Differ* 1993;4:77–84.
- 54 Powell DJ Jr, Russell J, Nibu K, Li G, Rhee E, Liao M, Goldstein M, Keane WM, Santoro M, Fusco A, Rothstein JL: The RET/PTC3 oncogene: metastatic solid-type papillary carcinomas in murine thyroids. *Cancer Res* 1998;58:5523–5528.
- 55 Melillo RM, Castellone MD, Guarino V, De Falco V, Cirafici AM, Salvatore G, Caiazzo F, Basolo F, Giannini R, Kruhoffer M, Orntoft T, Fusco A, Santoro M: The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and fitogeni phenotype of thyroid cancer cells. *J Clin Invest* 2005;115:1068–1081.
- 56 Santoro M, Chiappetta G, Cerrato A, Salvatore D, Zhang L, Manzo G, Picone A, Portella G, Santelli G, Vecchio G, Fusco A: Development of thyroid papillary carcinomas secondary to tissue-specific expression of the RET/PTC1 oncogene in transgenic mice. *Oncogene* 1996;12:1821–1826.
- 57 Santoro M, Carlomagno F: Drug insight: small-molecule inhibitors of protein kinases in the treatment of thyroid cancer. *Nat Clin Pract Endocrinol Metab* 2006;2:42–52.
- 58 Borrello MG, Alberti L, Fischer A, Degl'innocenti D, Ferrario C, Gariboldi M, Marchesi F, Allavena P, Greco A, Collini P, Pilotti S, Cassinelli G, Bressan P, Fugazzola L, Mantovani A, Pierotti MA: Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. *Proc Natl Acad Sci USA* 2005;102:14825–14830.
- 59 Viglietto G, Chiappetta G, Martinez-Tello FJ, Fukunaga FH, Tallini G, Rigopoulou D, Visconti R, Mastro A, Santoro M, Fusco A: RET/PTC oncogene activation is an early event in thyroid carcinogenesis. *Oncogene* 1995;11:1207–1210.
- 60 Castellone MD, Cirafici AM, De Vita G, De Falco V, Malorni L, Tallini G, Fagin JA, Fusco A, Melillo RM, Santoro M: Ras-mediated apoptosis of PC CL 3 rat thyroid cells induced by RET/PTC oncogenes. *Oncogene* 2003;22:246–255.
- 61 Wang J, Knauf JA, Basu S, Puxeddu E, Kuroda H, Santoro M, Fusco A, Fagin JA: Conditional expression of RET/PTC induces a weak oncogenic drive in thyroid PCCL3 cells and inhibits thyrotropin action at multiple levels. *Mol Endocrinol* 2003;17:1425–1436.
- 62 Nikiforova MN, Stringer JR, Blough R, Medvedovic M, Fagin JA, Nikiforov YE: Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. *Science* 2000;290:138–141.
- 63 Gandhi M, Medvedovic M, Stringer JR, Nikiforov YE: Interphase chromosome folding determines spatial proximity of genes participating in carcinogenic RET/PTC rearrangements. *Oncogene* 2006;25:2360–2366.
- 64 Gandhi MS, Stringer JR, Nikiforova MN, Medvedovic M, Nikiforov YE: Gene position within chromosome territories correlates with their involvement in distinct rearrangement types in thyroid cancer cells. *Genes Chromosomes Cancer* 2009;48:222–228.
- 65 Mani RS, Tomlins SA, Callahan K, Ghosh A, Nyati MK, Varambally S, Palanisamy N, Chinnaiyan AM: Induced chromosomal proximity and gene fusions in prostate cancer. *Science* 2009;326:1230–1232.
- 66 Gandhi M, Dillon LW, Pramanik S, Nikiforov YE, Wang YH: DNA breaks at fragile sites generate oncogenic RET/PTC rearrangements in human thyroid cells. *Oncogene* 2010;29:2272–2280.
- 67 Durkin SG, Glover TW: Chromosome fragile sites. *Annu Rev Genet* 2007;41:169–192.
- 68 Ameziane-El-Hassani R, Boufrajech M, Lagente-Chevallier O, Weyemi U, Talbot M, Métivier D, Courtin F, Bidart JM, El Mzibri M, Schlumberger M, Dupuy C: Role of H₂O₂ in RET/PTC1 chromosomal rearrangement produced by ionizing radiation in human thyroid cells. *Cancer Res* 2010;70:4123–4132.
- 69 Rhoden KJ, Unger K, Salvatore G, Yilmaz Y, Vovk V, Chiappetta G, Qumsiyeh MB, Rothstein JL, Fusco A, Santoro M, Zitzelsberger H, Tallini G: 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* 2006;91:2414–2423.
- 70 Russell JP, Shinohara S, Melillo RM, Castellone MD, Santoro M, Rothstein JL: Tyrosine kinase oncoprotein, RET/PTC3, induces the secretion of myeloid growth and chemotactic factors. *Oncogene* 2003;22:4569–4577.
- 71 Melillo RM, Guarino V, Avilla E, Galdiero MR, Liotti F, Prevete N, Rossi FW, Basolo F, Ugolini C, de Paulis A, Santoro M, Marone G: Mast cells have a protumorigenic role in human thyroid cancer. *Oncogene* 2010;29: 6203–6215.
- 72 Celetti A, Cerrato A, Merolla F, Vitagliano D, Vecchio G, Grieco M: H4(D10S170), a gene frequently rearranged with RET in papillary thyroid carcinomas: functional characterization. *Oncogene* 2004;23:109–121.
- 73 Merolla F, Pentimalli F, Pacelli R, Vecchio G, Fusco A, Grieco M, Celetti A: Involvement of H4(D10S170) protein in ATM-dependent response to DNA damage. *Oncogene* 2007;26: 6167–6175.
- 74 Leone V, Mansueto G, Pierantoni GM, Tornincasa M, Merolla F, Cerrato A, Santoro M, Grieco M, Scaloni A, Celetti A, Fusco A: CCDC6 represses CREB1 activity by recruiting histone deacetylase 1 and protein phosphatase 1. *Oncogene* 2010;29:4341–4351.
- 75 Schwaller J, Anastasiadou E, Cain D, Kutok J, Wojciski S, Williams IR, LaStarza R, Crescenzi B, Sternberg DW, Andreasson P, Schiavo R, Siena S, Mecucci G, Gilliland DG: H4(D10S170), a gene frequently rearranged in papillary thyroid carcinoma, is fused to the platelet-derived growth factor receptor beta gene in atypical chronic myeloid leukemia with t(5;10)(q33;q22). *Blood* 2001;97: 3910–3918.
- 76 Puxeddu E, Zhao G, Stringer JR, Medvedovic M, Moretti S, Fagin JA: Characterization of novel non-clonal intrachromosomal rearrangements between the H4 and PTEN genes (H4/PTEN) in human thyroid cell lines and papillary thyroid cancer specimens. *Mutat Res* 2005;570:17–32.

- 77 Heinlein CA, Ting HJ, Yeh S, Chang C: Identification of ARA70 as a ligand-enhanced co-activator for the peroxisome proliferator-activated receptor gamma. *J Biol Chem* 1999; 274:16147–16152.
- 78 Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, Fletcher JA: PAX8-PPAR γ fusion oncogene in human thyroid carcinoma. *Science* 2000; 289:1357–1360.
- 79 Li P, Yu X, Ge K, Melamed J, Roeder RG, Wang Z: Heterogeneous expression and functions of androgen receptor co-factors in primary prostate cancer. *Am J Pathol* 2002; 161:1467–1474.
- 80 Ligr M, Li Y, Zou X, Daniels G, Melamed J, Peng Y, Wang W, Wang J, Ostrer H, Pagano M, Wang Z, Garabedian MJ, Lee P: Tumor suppressor function of androgen receptor coactivator ARA70 α in prostate cancer. *Am J Pathol* 2010;176:1891–1900.
- 81 Kollara A, Kahn HJ, Marks A, Brown TJ: Loss of androgen receptor associated protein 70 (ARA70) expression in a subset of HER2-positive breast cancers. *Breast Cancer Res Treat* 2001;67:245–253.
- 82 Chang BL, Cramer SD, Wiklund F, Isaacs SD, Stevens VL, Sun J, Smith S, Pruettt K, Romero LM, Wiley KE, Kim ST, Zhu Y, Zhang Z, Hsu FC, Turner AR, Adolfsson J, Liu W, Kim JW, Duggan D, Carpten J, Zheng SL, Rodriguez C, Isaacs WB, Grönberg H, Xu J: Fine mapping association study and functional analysis implicate a SNP in MSMB at 10q11 as a causal variant for prostate cancer risk. *Hum Mol Genet* 2009;18:1368–1375.
- 83 Pomerantz MM, Shrestha Y, Flavin RJ, Regan MM, Penney KL, Mucci LA, Stampfer MJ, Hunter DJ, Chanock SJ, Schafer EJ, Chan JA, Taberero J, Baselga J, Richardson AL, Loda M, Oh WK, Kantoff PW, Hahn WC, Freedman ML: Analysis of the 10q11 cancer risk locus implicates MSMB and NCOA4 in human prostate tumorigenesis. *PLoS Genet* 2010;6:e1001204.
- 84 Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA: Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. *Nat Genet* 2000;26:89–92.
- 85 Boikos SA, Stratakis CA: Carney complex: pathology and molecular genetics. *Neuroendocrinology* 2006;83:189–199.
- 86 Monaco C, Visconti R, Barone MV, Pierantoni GM, Berlingieri MT, De Lorenzo C, Mineo A, Vecchio G, Fusco A, Santoro M: The RFG oligomerization domain mediates kinase activation and re-localization of the RET/PTC3 oncoprotein to the plasma membrane. *Oncogene* 2001;20:599–608.