REVIEW

Molecular genetics of medullary thyroid carcinoma: the quest for novel therapeutic targets

Aniello Cerrato, Valentina De Falco and Massimo Santoro

Istituto di Endocrinologia ed Oncologia Sperimentale CNR c/o Dipartimento di Biologia e Patologia Cellulare e Molecolare, 'L. Califano', Università Federico II di Napoli, via S. Pansini 5, 80131 Naples, Italy

(Correspondence should be addressed to M Santoro; Email: masantor@unina.it)

Abstract

Medullary thyroid carcinoma (MTC) is a rare tumour arising from neural crest-derived parafollicular C-cells. Metastatic MTC patients are incurable because the cancer does not respond to radiotherapy or chemotherapy. The REarranged during Transfection (*RET*) proto-oncogene plays a key role in the development of MTC. However, one-half of the sporadic MTC do not carry *RET* mutations. Mice models and early evidence obtained in human samples suggest that other genes, including those encoding components of the *RB1* (retinoblastoma) and *TP53* tumour-suppressor pathways, may be involved in MTC formation. Here, we review the data on the involvement of genes acting in the *RET* and *RB1/TP53* pathways in MTC. Understanding genetic lesions that occur in MTC is a prerequisite to identifying molecular therapeutic targets in MTC and in improving the efficacy of *RET*-targeted therapies.

Journal of Molecular Endocrinology (2009) **43**, 143–155

Medullary thyroid carcinoma: a genetic overview

Medullary thyroid carcinoma (MTC) arises from calcitonin-producing neural crest-derived parafollicular (C) cells of the thyroid. MTC accounts for 5–8% of all thyroid cancers (Matias-Guiu *et al.* 2004, Schlumberger *et al.* 2008). MTC is sporadic in about 75% of the cases; in the remaining cases, it occurs as a component of the autosomal dominant familial multiple endocrine neoplasia type 2 (MEN 2) syndrome. MEN 2, first described by Sipple (1961), includes three disorders: MEN 2A; MEN 2B; and familial MTC (FMTC; Marx 2005, Elisei *et al.* 2007, Zbuk & Eng 2007). MEN 2-associated MTC is bilateral and multicentric, and it is usually preceded by multifocal C-cell hyperplasia (CCH; Gagel & Marx 2003). REarranged during Transfection (*RET*) is mutated in roughly 50% of sporadic MTC and in more than 95% of MEN 2 families.

In FMTC, although the germ line *RET* mutation is present in all somatic cells of the affected individual, tumours are monoclonal, which suggests that other genetic alterations must occur at the somatic level and act in concert with *RET* mutations for the tumour to develop (Gagel & Marx 2003). Moreover, a few MEN 2 families negative for *RET* mutations have been described, suggesting the existence of additional loci predisposing to MEN 2 (Montero-Conde *et al.* 2007).

Finally, about 50% of sporadic MTC do not carry *RET* mutations. Whether another frequently mutated gene or multiple low-frequency mutated genes occur in *RET* wild-type MTC samples is unknown.

In MEN 2, MTC is associated in about 50% of cases to pheochromocytoma (MEN 2A and 2B), and in 10–35% of cases to parathyroid hyperplasia or adenoma (MEN 2A; Gagel & Marx 2003). This suggests that MTC shares pathogenetic mechanisms with pheochromocytomas and parathyroid tumours. However, MTC is not a phenotype of non-MEN 2 familial forms of parathyroid tumours or pheochromocytomas (Gagel & Marx 2003, Marx 2005, Zbuk & Eng 2007). Accordingly, *Prad1/Ccnd1*, *Men1* and *Hprt2* genes (associated to non-MEN 2 forms of parathyroid tumours; Ferris & Simental 2004) and *Vhl*, *Nf1* and succinate dehydrogenase subunits B, C and D (*SdhB*, *SdhC* and *SdhD*; associated to non-MEN 2 forms of pheochromocytomas; Maher & Eng 2002, Kaelin 2008) do not seem to be mutated in MTC. *SdhB*, C and D were not found to be mutated in sporadic MTC (Lima *et al.* 2003, Cascon *et al.* 2005, Montani *et al.* 2005), although germ line *SdhB* and *SdhD* variants were over-represented in MTC samples with respect to healthy individuals (Sobrinho-Simões *et al.* 2008). One mutation and three monoallelic deletions were found in the *Vhl* gene in five familial *RET* mutant MTC samples, suggesting cooperation of *Ret* gain with *Vhl* loss in MTC formation (Koch *et al.* 2006). Intriguingly, *Vhl*,

Nf1 and *Sdh* gene products collaborate with RET in a common signalling pathway involved in controlling EglN3 prolyl hydroxylase-mediated neuronal cell apoptosis. In this pathway, *RET* (gain-of-function) and *Nf1* and *VHL* (loss-of-function) mutations lead to increased JunB transcription factor, which, in turn, blunts the expression of EglN3, thereby leading to inappropriate cell survival and tumourigenesis (Kaelin 2008). Loss of SDH activity results in higher succinate levels; this in turn triggers the survival pathway because EglN3 is feedback-inhibited by succinate. Finally, the recently discovered *KIF1B* tumour suppressor, which maps in a chromosomal region (1p36) frequently deleted in MTC (see below), is required for EglN3 pro-apoptotic activity (Kaelin 2008). Thus, even if not frequently mutated, these proteins should be functionally analyzed in relation to MTC formation.

In the following sections we focus on genes acting in the *RET* (Fig. 1) and *RB/TP53* (Fig. 2) pathways.

RET signalling pathway in MTC

The *RET* gene in FMTC and sporadic MTC

The *RET* gene has been extensively reviewed elsewhere (Kodama *et al.* 2005, Kondo *et al.* 2006, Santoro & Carlomagno 2006, Asai *et al.* 2006b). Therefore, here we

Figure 1 Schematic representation of the RTK (receptor tyrosine kinase) signalling pathways. Potential oncoproteins are in white, whereas tumour suppressors are in grey, $\hexagon$ = kinases. It should be noted that only some signalling effectors are represented. RAS and AKT proteins have several effectors (like RALGDS for RAS) in addition to those represented in the figure. In addition, only some of the interactions that occur among the various proteins are represented. For instance, RTKs like RET are known to directly phosphorylate beta-catenin and STAT, besides activating them through RAS and AKT. Moreover, AKT may directly phosphorylate IKK proteins.

Figure 2 Schematic representation of RB and p53 signalling pathways leading to cell proliferation arrest and apoptosis. Potential oncoproteins are in white, whereas tumour suppressors are in grey, $\hexagon$ = kinases.

will summarize a few key points. The *RET* protein product is a single pass transmembrane receptor with an intracellular tyrosine kinase domain (RTK= receptor tyrosine kinase) that binds glial-derived neurotrophic factor (GDNF) ligands. *Ret* was initially described as a *bona fide* proto-oncogene because it is activated by chromosomal aberrations in papillary thyroid carcinoma (PTC; Kondo *et al.* 2006, Santoro & Carlomagno 2006). Subsequently, it was found that the germ line point mutations in *Ret* causes MEN 2 syndrome, and similar mutations at the somatic level are the most common genetic alterations identified so far in sporadic MTC (Gagel & Marx 2003, Kouvaraki *et al.* 2005, Marx 2005, Zbuk & Eng 2007). Most MEN 2B patients (95% of cases) carry the M918T-mutation in *RET*; the remaining fraction harbours the A883F substitution or other rare mutations. In 98% of MEN 2A, the mutations affect one of the five cysteines in the extracellular cysteine-rich domain of *RET*. In FMTC, mutations affect either the extracellular cysteines or the intracellular domain of *RET* (Niccoli-Sire *et al.* 2001, Gagel & Marx 2003, Kouvaraki *et al.* 2005, Marx 2005, Elisei *et al.* 2007, Zbuk & Eng 2007). The genotype-phenotype correlation between the type of *Ret* mutation and penetrance and expressivity of the disease further supports the prime role exerted by *Ret* mutations in FMTC (Machens & Dralle 2007). Thanks to this close correlation between a specific genetic lesion and cancer occurrence, MEN 2 is the best example in oncology of the efficacy of molecular diagnosis in mainstream clinical management. In fact, early thyroidectomy in *Ret* mutation carriers significantly improved their prognosis (Brandi *et al.* 2001, Gagel & Marx 2003, Skinner *et al.* 2005, Machens & Dralle 2007). Sporadic MTC, particularly more aggressive cases, also frequently (30–50% of cases) feature the M918T-*RET* mutation (Elisei *et al.* 2007, 2008). Finally, susceptibility to

sporadic MTC could be influenced by the *RET* polymorphisms G691S/S904S (Robledo *et al.* 2003, Elisei *et al.* 2004, Cebrian *et al.* 2005, Lesueur *et al.* 2006), however, these findings require confirmation on larger casistics (Weber & Eng 2005).

As discussed above, secondary genetic alterations at somatic level must act in concert with mutations in *Ret* for MTC to develop (Gagel & Marx 2003). Probably, only a small number of secondary genetic events are required in MEN 2B mutation carriers because, in these patients, the disease develops in the first few months of their life. A secondary genetic hit may target the *Ret* gene itself, either through duplication of the mutant allele or loss of the wild-type allele (Huang *et al.* 2003). Additional hits may involve chromosome deletion and amplification events, such as the deletion in chromosome 1p (Mathew *et al.* 1987, Khosla *et al.* 1991, Mulligan *et al.* 1993, Marsh *et al.* 2003, Ye *et al.* 2008).

MTC-associated *Ret*-mutations convert *Ret* into a dominantly transforming oncogene. Extracellular cysteine MEN 2A/FMTC *RET*-mutants exert constitutive kinase activity consequent to ligand-independent homodimerization. In the case of mutation M918T, constitutive *RET* activation probably results from disruption of an auto-inhibited head-to-tail *RET* TK homodimer (Knowles *et al.* 2006).

Transgenic mouse models demonstrated that *RET* oncogenes are able to drive MTC formation. Mice expressing *RET-C634R* or *RET-M918T*, but not wild-type *RET*, under the control of the calcitonin gene promoter developed MTC (Michiels *et al.* 1997, Acton *et al.* 2000). Also transgenic mice carrying *RET-C634R* under the control of a ubiquitous viral promoter developed MTC, suggesting that murine C-cells are highly susceptible to *RET*-mediated transformation (Kawai *et al.* 2000). However, the knock-in of the M918T mutation into mouse endogenous *Ret* gene caused CCH but not MTC, suggesting that, in the background of a normally expressed *Ret*-mutant allele, the accumulation of secondary genetic alterations is required for development of MTC (Smith-Hicks *et al.* 2000). Genetic background strongly affected the MTC phenotype in transgenic mice, with tumour penetrance varying from 0% in FVB/N to 98% in CBA/ca mice, which suggests that the genetic modifiers greatly affect *RET*-driven MTC risk (Cranston & Ponder 2003).

RET knock-down by dominant-negative mutants, ribozymes or RNAi impaired proliferation of *RET*-mutant MTC cell lines (Parthasarathy *et al.* 1999, Drosten *et al.* 2004). Taken together, these studies strongly implicated *RET* in the formation and maintenance of a subset of MTC, and provided the conceptual framework for the use of *RET* kinase inhibitory compounds in MTC clinical trials (Wells & Nevins 2004, Castellone *et al.* 2008, Schlumberger *et al.* 2008, Sherman 2008).

RET signalling cascade

Genetic screenings in model organisms have shown that the same phenotype can arise from alterations in any of several genes acting epistatically in common signalling cascades. Similarly, although the number of potential cancer driver genes is large, this probably reflects changes in only a few pathways. For instance, a systematic cancer genome analysis recently revealed that many mutations in colon and breast cancer cluster in genes acting in few signalling cascades (Wood *et al.* 2007, Sjöblom 2008). Thus, if gain-of-function *RET*-mutations are associated with human MTC, it is equally plausible that mutations in the genes encoding co-receptors/ligands that trigger *RET* activation or signalling effectors that mediate *RET* intracellular effects play a role in MTC. An important proof of this concept was provided by genetic analysis of PTC, another thyroid tumour type, in which *RET* is implicated. Indeed, it was found that most PTC cases that are negative for *RET* (*RET*/PTC) rearrangements harbour either mutations of *BRAF*, an effector of the *RET*-initiated extracellular-signal regulated kinase (ERK) signalling cascade or, less frequently, in *NTRK1*, another growth factor receptor (Fig. 1; Fagin 2005, Kondo *et al.* 2006, Pierotti & Greco 2006).

RET is activated through the binding of four GDNF family ligands (GFL) GDNF, neurturin (NRTN), artemin (ARTN), persephin (PSPN) together with the four corresponding membrane co-receptors (GFR α 1, 2, 3 and 4; Airaksinen & Saarma 2002). GFR α 4, in particular, is expressed in normal C-cells and the corresponding ligand, PSPN, is required for calcitonin production by C-cells (Lindahl *et al.* 2001, Lindfors *et al.* 2006). No somatic mutation in any of the *GFL/GFRA* encoding genes has been reported in MTC (Marsh *et al.* 1997, Borrego *et al.* 1998), although *GFL/GFRA* genes map in chromosomal regions where allelic imbalances were detected in MTC (Marsh *et al.* 2003). Polymorphic variants of *GFL/GFRA* genes, particularly *GFRA1* in familial (Gimm *et al.* 2001a, Lesueur *et al.* 2006) and *GFRA4* in sporadic (Cebrian *et al.* 2005, Vanhorne *et al.* 2005, Ruiz-Llorente *et al.* 2007) MTC cases, have been reported.

Once activated, *RET* transmits mitogenic, survival and motogenic signals (Kodama *et al.* 2005, Asai *et al.* 2006b, Santoro & Carlomagno 2006). Two major signalling cascades, namely RAS and phosphatidylinositol 3-kinase (PI3K), are triggered by *RET* (Fig. 1). In turn, RAS and PI3K contribute to the activation of many signalling effectors and, as described below, they concur to the activation of nuclear factor- κ B (NF- κ B), signal transducer and activator of transcription (STAT) and beta-catenin. Other signalling effectors, namely SRC (Encinas *et al.* 2004, Iavarone *et al.* 2006), phospholipase

C γ (Borrello *et al.* 1996, Jain *et al.* 2006), and RAC1/JUN NH(2)-terminal kinase (JNK; Chiariello *et al.* 1998, Fukuda *et al.* 2002, Asai *et al.* 2006a) are activated by RET (Fig. 1). In principle, gain-of-function of these pathways may contribute to MTC. Moreover, negative regulators of RET signalling have also been identified and, in principle, their loss-of-function may contribute to MTC formation (Fig. 1).

Hereafter, we focus on the RET pathways that have been more extensively studied in MTC. Components of these pathways may be exploited as molecular targets for MTC treatment.

RAS pathway

Growth-factor binding to cell surface RTKs creates docking sites for adaptor molecules that activate guanine nucleotide-exchange factors, which in turn favours GTP binding to RAS small G-proteins (KRAS, HRAS and NRAS; Schubbert *et al.* 2007). Intrinsic RAS-GTPase activity terminates signalling, a reaction that is accelerated thousands of fold by GTPase-activating proteins such as neurofibromin (NF1; Fig. 1; Schubbert *et al.* 2007). Once activated, RAS stimulates numerous intracellular transducers, including RAF, PI3K and Ral guanine nucleotide-dissociation stimulator, to regulate proliferation, survival and differentiation (Fig. 1; Halilovic & Solit 2008). The RAS–RAF–MEK–ERK cascade is the best characterized RAS-effector pathway. There are three RAF serine/threonine kinases (ARAF, BRAF and CRAF) that activate the MEK (MAP2K1/MAP2K2)–ERK (MAPK3/MAPK1) kinase cascade. ERK, in turn, stimulates gene transcription by directly phosphorylating transcription factors or by targeting intracellular kinases like p90RSK (Fig. 1; Schubbert *et al.* 2007). Negative regulators attenuate RAS signalling at various levels of the signalling cascade (Fig. 1; see below).

RAS genes are most commonly activated by point mutations in cancer. Alternatively, the RAS pathway can be triggered indirectly by loss of the negative regulator NF1, by upstream activation of cell surface RTKs or *PTPN11* (which encodes the Src homology-2-containing protein tyrosine phosphatases-2 (SHP-2)) or by downstream activation of RAS signalling effectors (Fig. 1; Wellbrock *et al.* 2004, Halilovic & Solit 2008). This paradigm applies to thyroid carcinoma of follicular cell lineage, where RET gene rearrangements are prevalent in PTC, RAS-mutations in follicular carcinoma and in follicular-variant PTC, and BRAF-mutations in PTC and anaplastic carcinoma (Kondo *et al.* 2006). Sequencing analysis of all three RAS family members did not reveal any mutation in about 30 MTC samples (Moley *et al.* 1991, Horie *et al.* 1995, Bockhorn *et al.* 2000). Similarly, no BRAF-mutation was found in 65 MTC samples (Xing 2005). Taken together, these

findings excluded that RAS/BRAF gene mutations exert a prominent role in MTC formation. However, a recent study led to a different conclusion by showing 41% KRAS-mutations and 68% BRAF-mutations in MTC samples (Goutas *et al.* 2008).

The degree and duration of activation dictate the final biological outcome of RAS signalling. For example, in PC12 pheochromocytoma cells, transient RAS activation stimulates proliferation, whereas sustained RAS activation induces differentiation (Schubbert *et al.* 2007). Similarly, oncogenic HRAS and CRAF or RAF1 alleles decreased MTC cell proliferation and increased calcitonin gene expression (Nakagawa *et al.* 1987, Carson-Walter *et al.* 1998). Such a pro-differentiating effect of constitutive RAS–RAF signalling may explain why mutations in these genes are unlikely to occur in MTC. In this context, NRAS exerted a protective effect against MTC formation as shown by the finding that NRAS deletion increased MTC formation in *RBI*-knock-out mice (see also below; Takahashi *et al.* 2006). However, the role of RAS signalling in MTC cells is probably complex and different components of the RAS family may exert different effects. In fact, when targeted to C-cells, an oncogenic HRAS mutant caused MTC in transgenic mice (Johnston *et al.* 1998). Similarly, Moloney murine sarcoma virus oncogene (*MOS*) another oncogene that potently activates ERK induced MTC and pheochromocytoma in transgenic mice (Schulz *et al.* 1992). With the caveat that findings obtained in artificial animal models should be interpreted with caution, it is conceivable that RAS signalling along the ERK cascade is involved in mitogenic signalling in MTC cells. In this context, it is noteworthy that inhibition of the ERK pathway reduced proliferation of a RET-mutant MTC cell line (Zatelli *et al.* 2005). Inhibitors of MEK are currently undergoing clinical experimentation in thyroid cancer patients (Sherman 2008).

PI3K pathway

Class I PI3K are constituted by a regulatory (p85 α , p55 α , p50 α , p85 β , p55 γ) and a catalytic (p110 α , p110 β , p110 δ) subunit. Upon recruitment to the plasma membrane by activated RTK or RAS, class I PI3K phosphorylates phosphatidylinositol-4, 5-bisphosphate to generate phosphatidylinositol-3, 4, 5-triphosphate (PIP3; Fig. 1). PIP3, in turn, activates downstream molecules such as the RAC small GTPase, 3-phosphoinositide-dependent protein kinase 1 (PDK1), and the AKT (also known as PKB) serine/threonine kinase (Yuan & Cantley 2008). The lipid phosphatase, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) antagonizes this cascade by dephosphorylating PIP3 (Salmena *et al.* 2008). Besides, buffering the PI3K pathway, PTEN also

exerts phosphatase-independent nuclear functions that may contribute to the potent oncogenic effect resulting from its inactivation in tumours (Salmena *et al.* 2008). AKT phosphorylates and inactivates pro-apoptotic transcription factors of the forkhead-box class O (FOXO) family, the cell-cycle inhibitor CDKN1B (p27Kip1), and the GSK3B kinase, thereby releasing beta-catenin from the inhibitory effects of GSK3B (see below; Fig. 1; Yuan & Cantley 2008). In addition, AKT stimulates the serine/threonine kinase mammalian target of rapamycin (mTOR; Fig. 1). mTOR is associated with two complexes: the rapamycin-sensitive TORC1 complex (that phosphorylates S6K to regulate protein translation); and the rapamycin-insensitive TORC2 (which is the PDK2 activity that controls serine 473 phosphorylation of AKT itself; Bjornsti & Houghton 2004). TORC1 also contributes to NF- κ B activation (see below; Fig. 1).

The PI3K–AKT–mTOR cascade is important in tumorigenesis because of its ability to promote growth (cell size) and proliferation (cell number) and to prevent cell death. Mutations in major nodes of this cascade are prevalent in human cancer and include gain-of-function mutations and amplification of the genes encoding the catalytic subunit p110 α of PI3K (*PIK3CA*) and AKT (Zbuk & Eng 2007, Yuan & Cantley 2008). Mutations in this pathway are very frequent, for instance, in breast and colon cancer (Wood *et al.* 2007). Germ line inactivating mutations of *PTEN* cause autosomal dominant hamartoma syndromes, and somatic *PTEN* inactivation by deletion is very frequent (up to 30–50%) in sporadic tumours (Zbuk & Eng 2007, Paes & Ringel 2008). Many studies have demonstrated that the PI3K–AKT system plays a key role in RET signalling (Segouffin-Cariou & Billaud 2000, Kodama *et al.* 2005, Asai *et al.* 2006b). However, no systematic genetic analysis of PI3K pathway components has been reported so far in MTC. *PIK3CA* gene amplification, which frequently occurs in aggressive tumours of thyroid cells of follicular lineage, was not detected in 13 MTC samples (Wu *et al.* 2005). *PTEN* analysis in MTC has so far been limited to promoter methylation assessment, and no methylation was detected in a small MTC set (Schagdarsurengin *et al.* 2006). However, CCH and MTC occur in *PTEN* heterozygous mice, particularly when crossed with mice knocked-out for *CDKN2C* (encoding the p18Ink4c cell-cycle inhibitor; see below; Bai *et al.* 2006). Thus, as discussed for RAS, the PI3K–AKT cascade, even though infrequently mutated, may play a role in MTC. Accordingly, *in vitro* chemical PI3K inhibition reduced MTC cell proliferation and survival, which indicates that this pathway could be a molecular target in MTC treatment (Kunnimalaiyaan *et al.* 2006a). Given its central role in PI3K–AKT signalling, and the availability of potent and selective inhibitors (everolimus, temsirolimus) derived from rapamycin

(sirolimus), mTOR is one of the most appealing therapeutic targets in this pathway (Bjornsti & Houghton 2004).

NF- κ B

The NF- κ B family includes five transcription factors named NF- κ B1 (p50), NF- κ B2 (p52), Rel, RelA (p65) and RelB. NF- κ B activates transcription of genes associated with cell proliferation, angiogenesis, metastasis, and inflammation and suppression of apoptosis (Baud & Karin 2009). NF- κ B proteins are rendered inactive in non-stimulated cells through binding to inhibitors, known as the I κ B (I κ B α , β , ϵ) proteins. Activation of most forms of NF- κ B, especially the most common form (the p50/RelA dimer), depends on phosphorylation-induced ubiquitination of I κ B that is mediated by the I κ B kinase (IKK) complex (CHUK, I κ BKB, I κ BKG or NEMO; Baud & Karin 2009). Thus, NF- κ B is activated by different membrane receptors as well as by BRAF that directly associates with IKK (Encinas *et al.* 2008) and by PI3K/AKT that mediates an mTOR/IKK interaction (Dan *et al.* 2008; Fig. 1). RET stimulates IKK phosphorylation and NF- κ B activation, thus contributing to MTC cell survival (Ludwig *et al.* 2001, Encinas *et al.* 2008).

Recent studies have found mutations that directly target NF- κ B pathway components in human cancer (Wood *et al.* 2007). A genetic analysis of the NF- κ B pathway in MTC has not yet been reported. Histochemical analysis of MTC tissue samples revealed that many proteins of the NF- κ B family, particularly p65, p52 and c-Rel, are localized in the nucleus (Gallel *et al.* 2008). NF- κ B inhibitors, particularly IKK inhibitors, are being exploited in cancer therapy (Baud & Karin 2009). Moreover, inhibitors of the 26S proteasome, such as bortezomib (Velcade), that prevent I κ B degradation and NF- κ B nuclear translocation, exerted cytotoxic effects in MTC cells (Mitsiades *et al.* 2006b).

Beta-catenin (CTNNB1)

Beta-catenin, which is encoded by the *CTNNB1* gene, plays an important role in cellular adhesion by associating with E-cadherin and alpha-catenin. Upon disassembling of the membrane complex, beta-catenin migrates into the nucleus where it acts as a co-activator of TCF/LEF (T-cell factor/lymphoid-enhancing factor) transcriptional factors (Brembeck *et al.* 2006). RET stimulates beta-catenin activation via direct phosphorylation on Y654 and via PI3K/AKT- and RAS/ERK-mediated inhibition of GSK3B (Fig. 1; Cassinelli *et al.* 2009, Gujral *et al.* 2008, Castellone *et al.* 2009). Although an analysis of the *CTNNB1* gene in MTC has not yet

been reported, MTC samples from human patients and RET(M918T) transgenic mice showed nuclear beta-catenin accumulation (Gujral *et al.* 2008).

STAT

STAT transcription factors are activated in response to cytokines and growth factors. Cytokines activate STAT through JAK tyrosine kinases, whereas RTKs can phosphorylate STAT directly. The JAK–STAT pathway has been implicated in several neoplastic diseases, particularly myeloproliferative disorders (Levine & Gilliland 2008). Oncogenic *RET* mutants induce serine phosphorylation through the RAS pathway, and tyrosine phosphorylation of STAT3 (Fig. 1; Plaza Menacho *et al.* 2005, 2007). Moreover, activated STAT3 was identified in the nucleus of cells from MTC samples (Plaza Menacho *et al.* 2005).

Negative regulators of RET signalling

Several proteins function as feedback regulators to attenuate RTK signalling and, intriguingly, the corresponding genes are often downregulated in diverse tumour types (van Staveren *et al.* 2006, Amit *et al.* 2007). Negative regulators of RET signalling have been identified. However, also in this case, no systematic analysis of genetic alterations in MTC has yet been reported. Below, we briefly discuss the effects exerted by tyrosine phosphatases (LAR, PTPRJ, SHP-1), ERK dual-specificity phosphatases (DUSP) and RAS–BRAFF signalling inhibitors (SPRY) on RET signalling.

Tyrosine phosphatases dephosphorylate RET and attenuate RET signalling; theoretically, their loss could promote MTC formation (Fig. 1). Leukocyte common antigen-related (LAR) phosphatase (also called ‘PTPRF’, protein tyrosine phosphatase, receptor type, F) is a receptor tyrosine phosphatase that maps on a region of chromosome 1 (1p) that is frequently lost in MTC (Mathew *et al.* 1987, Mulligan *et al.* 1993). LAR forms stable complexes with RET and dephosphorylates RET cysteine mutants (but not RET-M918T) thereby blunting cell proliferation (Qiao *et al.* 2001). Similarly, the receptor-protein tyrosine phosphatase J (PTPRJ) binds and de-phosphorylates RET cysteine mutants and thus impairs their transforming effect (Iervolino *et al.* 2006). The SHP-1, SHP-2 are non-transmembrane phosphotyrosine phosphatases (PTPN6 and PTPN11 respectively). While SHP-2 functions as a positive RTK signal transducer and stimulates downstream RET signalling along the RAS cascade (D’Alessio *et al.* 2003), SHP-1 serves as a negative regulator of signalling systems. SHP-1 associates with RET, restrains RET autophosphorylation, and inhibits MTC cell proliferation (Hennige *et al.* 2001,

Incoronato *et al.* 2004, Zatelli *et al.* 2005). Intriguingly, SHP-1 is involved in the cytostatic effects of somatostatin in MTC cells (Zatelli *et al.* 2005).

Activated ERKs are inactivated through dephosphorylation of threonine and/or tyrosine residues within the activation loop. The DUSP, also called ‘MAP kinase phosphatases’ (MKP), carries out this function (Fig. 1; Kondoh & Nishida 2007). Intriguingly, MKPs/DUSPs are rapidly induced upon growth factor signalling, and functions as feedback regulators of the pathway (Amit *et al.* 2007). RET-mediated signalling increased MKP-3 levels (Colucci-D’Amato *et al.* 2000). In principle, a loss-of-function of MKPs may favour RET signalling along the ERK cascade. However, it should be noted that the pro-mitogenic and anti-mitogenic effects of MKPs/DUSPs may vary depending on the specific complement of MAPK family members they dephosphorylate. For instance, DUSP4/MKP-2, which dephosphorylates not only p42/44 MAPK (ERK) but also p38MAPK and JNK, exerts a positive (rather than a negative) role in RET-mediated tumourigenesis and it is upregulated in MTC samples (Hasegawa *et al.* 2008).

Sprouty (SPRY) and Spred proteins are evolutionarily conserved inhibitors of signalling that act by blocking RAS–RAF interaction and ERK activation. The expression of SPRY family members is induced by RET, and SPRY2 blunted RET–ERK signalling (Ishida *et al.* 2007). Intriguingly, genetic ablation of SPRY2 led to enteric neuronal hyperplasia by promoting RET signalling (Taketomi *et al.* 2005). Similarly, SPRY1-deficient mice had kidney defects because of RET hypersignalling (Basson *et al.* 2005). SPRY/SPRED downregulation has been reported in several human cancers (Lo *et al.* 2006). Germ line loss-of-function mutations in *SPRED1* caused a neurofibromatosis 1-like syndrome (Brems *et al.* 2007). Thus, a loss of *SPRY/SPRED* family members in C-cells can, in principle, favour MTC formation.

Other growth factor receptors

It is conceivable that other RTKs, besides RET, are involved in MTC. This point is of great topical interest because tyrosine kinase inhibitors are now being tested in MTC patients (Castellone *et al.* 2008, Sherman 2008). Proliferation of cultured MTC cells is stimulated by insulin-like growth factor 1 (IGF1) and inhibited by compounds targeting IGF1-R (Yang *et al.* 1992, Mitsiades *et al.* 2004). NTRKs, which are tyrosine kinase receptors for growth factors of the nerve growth factor family, have been studied in MTC because, like RET, they exert neurotrophic effects and are involved in PTC (*NTRK1* rearrangements; Pierotti & Greco 2006). Moreover, there is functional evidence that *NTRK1*–RET signalling is involved in neuronal cell survival (Tsui-Pierchala *et al.* 2002, Luo *et al.* 2007, Pierchala *et al.* 2007). Although no

mutations have been found in *NTRK1*, 2 and 3 (Gimm *et al.* 1999, 2001b), *NTRK2* expression was reduced, whereas *NTRK3* expression was increased in MTC (McGregor *et al.* 1999). Moreover, *NTRK2* expression impaired the tumourigenicity of MTC cells (McGregor *et al.* 1999). Interaction between epidermal growth factor receptor (EGFR) and RET was recently found to mediate EGFR-dependent RET activation (Croyle *et al.* 2008). Phosphorylated EGFR has been identified in MTC cells (Gorla *et al.* 2008). It is noteworthy that Vandetanib, a RET kinase inhibitor currently being investigated in MTC patients, is also an EGFR inhibitor (Carlomagno *et al.* 2002). No mutation in *EGFR* was found in small MTC sample sets (Mitsiades *et al.* 2006a, Cerrato & Santoro unpublished). Fibroblast growth factor receptor-4 (FGFR4) is expressed in aggressive thyroid tumour types and MTC cells. Molecular targeting of FGFR-4 with an ATP-competitive inhibitor prevented the growth and reduced the tumourigenesis of MTC cells (Ezzat *et al.* 2005).

Finally, membrane receptors of families other than the RTK family have been implicated in MTC. NOTCH1 is a multifunctional transmembrane receptor that regulates cell differentiation, development, proliferation and survival. Binding of several ligands promotes proteolytic cleavage events, which result in the release of the NOTCH1 intracellular domain that, in turn, translocates to the nucleus and activates transcription of various target genes. NOTCH1 is a negative regulator of ASH1 (achaete-scute homolog-1, called 'MASH1' in rodents), which is a highly conserved basic helix-loop-helix transcription factor that is critical for C-cell development (Lanigan *et al.* 1998). Interestingly, MTC expresses ASH1 but not NOTCH1, and NOTCH1 expression arrested proliferation of MTC cells (Kunnimalaiyaan *et al.* 2006b). The prolactin receptor (PRLR) belongs to the cytokine receptor family and activates the JAK-STAT pathway. Unexpectedly, PRLR-null mice developed MTC at a high frequency, thereby suggesting that PRLR suppresses MTC formation at least in mice (Kedzia *et al.* 2005).

Tumour suppressors of the *RB1* and *TP53* pathways in MTC

The tumour-suppressor genes *RB1* (retinoblastoma: pRB protein) and *TP53* (p53 protein) are frequently mutated in human cancer, and several lines of evidence indicate that both pathways must be inactivated in cancer to overcome senescence or apoptosis (Hahn & Weinberg 2002). *RB1* is the prototypic member of the class of tumour suppressors known as 'gatekeepers', which control tumour growth in a cell-autonomous manner. This mainly depends on pRB's ability to repress the effect exerted by the E2F/DP family of transcription

factors, namely, stimulation of cell-cycle progression or apoptosis (Fig. 2; Hahn & Weinberg 2002). Binding of the pRB protein to E2F/DP transcription factors is high when pRB is hypophosphorylated in G1, and low when pRB is hyperphosphorylated in S and G2 phases. pRB is phosphorylated sequentially by D-, E- and A-type cyclin-mediated CDK activity. In turn, CDKs are negatively regulated by CDK inhibitors (CKI) of the INK4 (p16INK4A, p15INK4B, p18INK4C, p19INK4D) and CIP/KIP (p21CIP1, p27KIP1, p57KIP2) families (Fig. 2). Tethering of pRB to E2F target genes results in cell-cycle arrest (Trimarchi & Lees 2002). There are multiple interactions between the pRB and the p53 pathways (Fig. 2). On one hand, by stimulating transcription of the p21CIP1 (*CDKN1A*) cell-cycle inhibitor, p53 obstructs the activity of cyclin E/CDK complexes, thereby reducing pRB phosphorylation and, consequently, E2F activity. On the other hand, loss-of-function of pRB releases not only the pro-mitogenic but also pro-apoptotic activity of E2F transcription factors. The final outcome may depend on *TP53* genetic status because E2F-mediated apoptosis is dependent on the upregulation of p14ARF (*CDKN2B*) that in turn stabilizes p53. Therefore, in cancer, *RB1* and *TP53* are often concurrently mutated (Hahn & Weinberg 2002).

There is extensive genetic evidence in rodents that the pRB and p53 pathways are involved in MTC. *RB1*-deficient mice developed MTC (Harrison *et al.* 1995). Conditional *RB1* inactivation also induced highly aggressive MTC in mice (Kucherlapati *et al.* 2006). Loss of *TP53* further increased MTC formation in *RB1*-deficient mice (Williams *et al.* 1994, Harvey *et al.* 1995). E2F family transcription factors exerted a dual role in MTC formation. Genetic deletion of *E2F1* or *E2F4* reduced MTC formation in *RB1*-deficient mice (Yamasaki *et al.* 1998, Lee *et al.* 2002). Instead, deletion of *E2F3* further increased the incidence and aggressiveness of MTC (Ziebold *et al.* 2003).

Interestingly, MTC from *RB1/TP53*-deficient mice acquired somatic cysteine mutations in *RET* that closely resemble activating mutations observed in human MTC. This suggested that murine MTC requires mutational dysregulation within both the RET and nuclear tumour suppressor gene pathways (Coxon *et al.* 1998). High grade MTC were observed in mice simultaneously lacking *RB1* and *CDKN1B* (that codes for the p27Kip1 cell-cycle inhibitor; Park *et al.* 1999). Interestingly, germ line mutation in *CDKN1B* predisposed rats to a multiple endocrine neoplasia syndrome featuring MTC formation (Pellegata *et al.* 2006). In transgenic mice, the loss of two CDKIs, *CDKN1B* and *CDKN2C* (coding for the p18INK4C cell-cycle inhibitor), led to accelerated MTC formation (Franklin *et al.* 2000, Joshi *et al.* 2007). *CDKN2C* deficiency also accelerated MTC formation in *PTEN*-deficient mice (Bai *et al.* 2006). Finally, transgenic mice expressing

oncogenic *RET* crossed with mice lacking *CDKN2C* developed MTC at a higher incidence and sooner than their single mutant littermates (van Veelen *et al.* 2008).

Taken together, these studies provide robust evidence that, in rodents, disruption of the *RB1* and *TP53* pathways predisposes to MTC formation. However, mice models may not faithfully mimic the human situation, and the tumour spectrum may significantly differ in the two species. A prominent example of this concept is provided by the phenotype of *RB1*-deficient mice. In humans, loss of the *RB1* gene is associated with the development of retinoblastoma and osteosarcoma and, later in life, small-cell lung carcinoma, whereas *RB1*-deleted mice do not develop these types of tumours, and develop retinoblastoma only when the *RB1*-related *RBL1* gene is concurrently deleted (Rangarajan & Weinberg 2003). Previous studies did not find *TP53*-mutations in sets of 9 (Yoshimoto *et al.* 1992) and 22 (Herfarth *et al.* 1997) MTC samples. More recent studies identified a high prevalence of *TP53* mutations (Pavelić *et al.* 2006) and deletions in MTC (Sheikh *et al.* 2004). Very recently, about 10% of MTCs were found to carry loss-of-function mutations in *CDKN2C* (van Veelen *et al.* 2009); however, we did not find any *CDKN2C*-mutation in 15 MTC samples (Cerrato & Santoro unpublished). A systematic analysis of the genes in the *RB1* and *TP53* pathways in human samples will help to clarify their role in MTC formation. Given the role played by these tumour-suppressor pathways in the response of tumours to therapy, this information might be important for the analysis of data from the ongoing MTC trials involving the use of targeted agents.

Conclusions

The identification of *RET* mutations has revolutionized the medical treatment of patients with FMTC. Twenty-five years after this seminal discovery, no other genetic lesion has been consistently associated with MTC formation. Studies of the *RET* pathway and mouse models of MTC formation are generating an ever-growing list of genes, including the recently described *CDKN2C* gene (p18INK4C cell-cycle inhibitor), that could play a role in MTC. Biochemical data also indicate that these pathways play a role in MTC formation. A thorough analysis of these genes has not yet been performed, and the results of the few studies available, conducted, moreover, on a limited number of samples, are often conflicting. An unbiased genome-wide analysis of sequence variations, copy gains and losses will probably provide groundbreaking information as has occurred for various tumour types (Sjöblom 2008). It is expected that identification of lesions in genes other than *RET* will clarify the biology of MTC and foster the development of targeted

therapeutic approaches. In any event, the data acquired in recent years about the signalling mechanisms operating in MTC show that molecular targeting of pathways like the RAS/ERK, PI3K/AKT and NF- κ B pathways is a plausible therapeutic approach for this cancer.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC, 2008–2010), the Italian Ministero della Salute (2008–2009), and Ministero dell'Università e della Ricerca (MiUR) (2008–2009).

Acknowledgements

We gratefully acknowledge members of our laboratory for their continuous support. We are grateful to Jean Ann Gilder for text editing and StudioCiotola for art-work.

References

- Acton DS, Velthuyzen D, Lips CJ & Höppener JW 2000 Multiple endocrine neoplasia type 2B mutation in human RET oncogene induces medullary thyroid carcinoma in transgenic mice. *Oncogene* **19** 3121–3125.
- Airaksinen MS & Saarma M 2002 The GDNF family: signalling, biological functions and therapeutic value. *Nature Reviews. Neuroscience* **3** 383–394.
- Amit I, Citri A, Shay T, Lu Y, Katz M, Zhang F, Tarcic G, Siwak D, Lahad J, Jacob-Hirsch J *et al.* 2007 A module of negative feedback regulators defines growth factor signaling. *Nature Genetics* **39** 503–512.
- Asai N, Fukuda T, Wu Z, Enomoto A, Pachnis V, Takahashi M & Costantini F 2006a Targeted mutation of serine 697 in the Ret tyrosine kinase causes migration defect of enteric neural crest cells. *Development* **133** 4507–4516.
- Asai N, Jijiwa M, Enomoto A, Kawai K, Maeda K, Ichihara M, Murakumo Y & Takahashi M 2006b RET receptor signaling: dysfunction in thyroid cancer and Hirschsprung's disease. *Pathology International* **56** 164–172.
- Bai F, Pei XH, Pandolfi PP & Xiong Y 2006 p18 Ink4c and Pten constrain a positive regulatory loop between cell growth and cell cycle control. *Molecular and Cellular Biology* **26** 4564–4576.
- Basson MA, Akbulut S, Watson-Johnson J, Simon R, Carroll TJ, Shakya R, Gross I, Martin GR, Lufkin T, McMahon AP *et al.* 2005 Sprouty1 is a critical regulator of GDNF/RET-mediated kidney induction. *Developmental Cell* **8** 229–239.
- Baud V & Karin M 2009 Is NF- κ B a good target for cancer therapy? Hopes and pitfalls *Nature Reviews. Drug Discovery* **8** 33–40.
- Bjornsti MA & Houghton PJ 2004 The TOR pathway: a target for cancer therapy. *Nature Reviews. Cancer* **4** 335–348.
- Bockhorn M, Frilling A, Kalinin V, Schröder S & Broelsch CE 2000 Absence of H- and K-ras oncogene mutations in sporadic medullary thyroid carcinoma. *Experimental and Clinical Endocrinology and Diabetes* **108** 49–53.

- Borrego S, Eng C, Sánchez B, Sáez ME, Navarro E & Antiñolo G 1998 Molecular analysis of the ret and GDNF genes in a family with multiple endocrine neoplasia type 2A and Hirschsprung disease. *Journal of Clinical Endocrinology and Metabolism* **83** 3361–3364.
- Borrello MG, Alberti L, Arighi E, Bongarzoni I, Battistini C, Bardelli A, Pasini B, Piutti C, Rizzetti MG, Mondellini P *et al.* 1996 The full oncogenic activity of Ret/ptc2 depends on tyrosine 539, a docking site for phospholipase Cgamma. *Molecular and Cellular Biology* **16** 2151–2163.
- Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordini C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia A *et al.* 2001 Guidelines for diagnosis and therapy of MEN type 1 and type 2. *Journal of Clinical Endocrinology and Metabolism* **86** 5658–5671.
- Brembeck FH, Rosário M & Birchmeier W 2006 Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. *Current Opinion in Genetics and Development* **16** 51–59.
- Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R, Somers R, Messiaen L, De Schepper S, Fryns JP *et al.* 2007 Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. *Nature Genetics* **39** 1120–1126.
- Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, Ryan AJ, Fontanini G, Fusco A & Santoro M 2002 ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. *Cancer Research* **15** 7284–7290.
- Carson-Walter EB, Smith DP, Ponder BA, Baylin SB & Nelkin BD 1998 Post-transcriptional silencing of RET occurs, but is not required, during raf-1 mediated differentiation of medullary thyroid carcinoma cells. *Oncogene* **17** 367–376.
- Cascon A, Cebrian A, Pollan M, Ruiz-Llorente S, Montero-Conde C, Leton R, Gutierrez R, Lesueur F, Milne RL, Gonzalez-Albarran O *et al.* 2005 Succinate dehydrogenase D variants do not constitute a risk factor for developing C cell hyperplasia or sporadic medullary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* **90** 2127–2130.
- Cassinelli G, Favini E, Degl'Innocenti D, Salvi A, De Petro G, Pierotti MA, Zunino F, Borrello MG & Lanzi C 2009 RET/PTC1-driven neoplastic transformation and proinvasive phenotype of human thyrocytes involve Met induction and beta-catenin nuclear translocation. *Neoplasia* **11** 10–21.
- Castellone MD, Carlomagno F, Salvatore G & Santoro M 2008 Receptor tyrosine kinase inhibitors in thyroid cancer. *Best Practice and Research. Clinical Endocrinology and Metabolism* **22** 1023–1038.
- Castellone MD, De Falco V, Rao DM, Bellelli R, Muthu M, Basolo F, Fusco A, Gutkind JS & Santoro M 2009 The β -catenin axis integrates multiple signals downstream from RET/PTC and leads to cell proliferation. *Cancer Research* **69** 1867–1876.
- Cebrian A, Lesueur F, Martin S, Leyland J, Ahmed S, Luccarini C, Smith PL, Luben R, Whittaker J, Pharoah PD *et al.* 2005 Polymorphisms in the initiators of RET (rearranged during transfection) signaling pathway and susceptibility to sporadic medullary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* **90** 6268–6274.
- Chiariello M, Visconti R, Carlomagno F, Melillo RM, Bucci C, de Francis V, Fox GM, Jing S, Coso OA, Gutkind JS *et al.* 1998 Signalling of the Ret receptor tyrosine kinase through the c-Jun NH2-terminal protein kinases (JNKs): evidence for a divergence of the ERKs and JNKs pathways induced by Ret. *Oncogene* **16** 2435–2445.
- Colucci-D'Amato GL, D'Alessio A, Califano D, Cali G, Rizzo C, Nitsch L, Santelli G & de Francis V 2000 Abrogation of nerve growth factor-induced terminal differentiation by ret oncogene involves perturbation of nuclear translocation of ERK. *Journal of Biological Chemistry* **275** 19306–19314.
- Coxon AB, Ward JM, Geradts J, Otterson GA, Zajac-Kaye M & Kaye FJ 1998 RET cooperates with RB/p53 inactivation in a somatic multi-step model for murine thyroid cancer. *Oncogene* **17** 1625–1628.
- Cranston AN & Ponder BA 2003 Modulation of medullary thyroid carcinoma penetrance suggests the presence of modifier genes in a RET transgenic mouse model. *Cancer Research* **63** 4777–4780.
- Croyle M, Akeno N, Knauf JA, Fabbro D, Chen X, Baumgartner JE, Lane H & Fagin JA 2008 RET/PTC-induced cell growth is mediated in part by epidermal growth factor receptor (EGFR) activation: evidence for molecular and functional interactions between RET and EGFR. *Cancer Research* **68** 4183–4191.
- D'Alessio A, Califano D, Incoronato M, Santelli G, Florio T, Schettini G, Carlomagno MS, Cerchia L & de Francis V 2003 The tyrosine phosphatase Shp-2 mediates intracellular signaling initiated by Ret mutants. *Endocrinology* **144** 4298–4305.
- Dan HC, Cooper MJ, Cogswell PC, Duncan JA, Ting JP & Baldwin AS 2008 Akt-dependent regulation of NF- κ B is controlled by mTOR and Raptor in association with IKK. *Genes and Development* **22** 1490–1500.
- Drosten M, Hilken G, Böckmann M, Rödicker F, Mise N, Cranston AN, Dahmen U, Ponder BA & Pützer BM 2004 Role of MEN2A-derived RET in maintenance and proliferation of medullary thyroid carcinoma. *Journal of the National Cancer Institute* **96** 1231–1239.
- Elisei R, Cosci B, Romei C, Bottici V, Sculli M, Lari R, Barale R, Pacini F & Pinchera A 2004 RET exon 11 (G691S) polymorphism is significantly more frequent in sporadic medullary thyroid carcinoma than in the general population. *Journal of Clinical Endocrinology and Metabolism* **89** 3579–3584.
- Elisei R, Romei C, Cosci B, Agate L, Bottici V, Molinaro E, Sculli M, Miccoli P, Basolo F, Grasso L *et al.* 2007 RET genetic screening in patients with medullary thyroid cancer and their relatives: experience with 807 individuals at one center. *Journal of Clinical Endocrinology and Metabolism* **92** 4725–4729.
- Elisei R, Cosci B, Romei C, Bottici V, Renzini G, Molinaro E, Agate L, Vivaldi A, Faviana P, Basolo F *et al.* 2008 Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. *Journal of Clinical Endocrinology and Metabolism* **93** 682–687.
- Encinas M, Crowder RJ, Milbrandt J & Johnson EM Jr 2004 Tyrosine 981, a novel ret autophosphorylation site, binds c-Src to mediate neuronal survival. *Journal of Biological Chemistry* **279** 18262–18269.
- Encinas M, Rozen EJ, Dolcet X, Jain S, Comella JX, Milbrandt J & Johnson EM Jr 2008 Analysis of Ret knockin mice reveals a critical role for IKKs, but not PI 3-K, in neurotrophic factor-induced survival of sympathetic neurons. *Cell Death and Differentiation* **15** 1510–1521.
- Ezzat S, Huang P, Dackiw A & Asa SL 2005 Dual inhibition of RET and FGFR4 restrains medullary thyroid cancer cell growth. *Clinical Cancer Research* **11** 1336–1341.
- Fagin JA 2005 Genetics of papillary thyroid cancer initiation: implications for therapy. *Transactions of the American Clinical and Climatological Association* **116** 259–269.
- Ferris RL & Simental AA Jr 2004 Molecular biology of primary hyperparathyroidism. *Otolaryngologic Clinics of North America* **37** 819–831.
- Franklin DS, Godfrey VL, O'Brien DA, Deng C & Xiong Y 2000 Functional collaboration between different cyclin-dependent kinase inhibitors suppresses tumor growth with distinct tissue specificity. *Molecular and Cellular Biology* **20** 6147–6158.
- Fukuda T, Kiuchi K & Takahashi M 2002 Novel mechanism of regulation of Rac activity and lamellipodia formation by RET tyrosine kinase. *Journal of Biological Chemistry* **277** 19114–19121.
- Gagel FR & Marx SJ 2003 Multiple endocrine neoplasia. In *Williams Textbook of Endocrinology*, 10 edn, pp 1717–1762. Eds PR Larsen, H Kronenberg, S Melmed & K Polonsky. Philadelphia: Saunders, Elsevier Science.
- Galle P, Pallares J, Dolcet X, Llobet D, Eritja N, Santacana M, Yeramian A, Palomar-Asenjo V, Lagarda H, Mauricio D *et al.* 2008 Nuclear factor-kappaB activation is associated with somatic and germ line RET mutations in medullary thyroid carcinoma. *Human Pathology* **39** 994–1001.

- Gimm O, Greco A, Hoang-Vu C, Dralle H, Pierotti MA & Eng C 1999 Mutation analysis reveals novel sequence variants in NTRK1 in sporadic human medullary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* **84** 2784–2787.
- Gimm O, Dziema H, Brown J, Hoang-Vu C, Hinz R, Dralle H, Mulligan LM & Eng C 2001a Over-representation of a germline variant in the gene encoding RET co-receptor GFRalpha-1 but not GFRalpha-2 or GFRalpha-3 in cases with sporadic medullary thyroid carcinoma. *Oncogene* **20** 2161–2170.
- Gimm O, Dziema H, Brown J, de la Puente A, Hoang-Vu C, Dralle H, Plass C & Eng C 2001b Mutation analysis of NTRK2 and NTRK3, encoding 2 tyrosine kinase receptors, in sporadic human medullary thyroid carcinoma reveals novel sequence variants. *International Journal of Cancer* **92** 70–74.
- Goila L, Mondellini P, Cucurru G, Micciché F, Cassinelli G, Cremona M, Pierotti MA, Lanzi C & Bongarzone I 2009 Proteomics study of medullary thyroid carcinomas expressing RET germ-line mutations: identification of new signaling elements. *Molecular Carcinogenesis* **48** 220–231.
- Goutas N, Vlachodimitropoulos D, Bouka M, Lazaris AC, Nasioulas G & Gazouli M 2008 BRAF and K-RAS mutation in a Greek papillary and medullary thyroid carcinoma cohort. *Anticancer Research* **28** 305–308.
- Gujral TS, van Veelen W, Richardson DS, Myers SM, Meens JA, Acton DS, Duñach M, Elliott BE, Höppener JW & Mulligan LM 2008 A novel RET kinase-beta-catenin signaling pathway contributes to tumorigenesis in thyroid carcinoma. *Cancer Research* **68** 1338–1346.
- Hahn WC & Weinberg RA 2002 Modelling the molecular circuitry of cancer. *Nature Reviews. Cancer* **2** 331–341.
- Halilovic E & Solit DB 2008 Therapeutic strategies for inhibiting oncogenic BRAF signaling. *Current Opinion in Pharmacology* **8** 419–426.
- Harrison DJ, Hooper ML, Armstrong JF & Clarke AR 1995 Effects of heterozygosity for the Rb-t19neo allele in the mouse. *Oncogene* **10** 1615–1620.
- Harvey M, Vogel H, Lee EY, Bradley A & Donehower LA 1995 Mice deficient in both p53 and Rb develop tumors primarily of endocrine origin. *Cancer Research* **55** 1146–1151.
- Hasegawa T, Enomoto A, Kato T, Kawai K, Miyamoto R, Jijiwa M, Ichihara M, Ishida M, Asai N, Murakumo Y *et al.* 2008 Roles of induced expression of MAPK phosphatase-2 in tumor development in RET-MEN2A transgenic mice. *Oncogene* **27** 5684–5695.
- Hennige AM, Lammers R, Höppner W, Arlt D, Strack V, Teichmann R, Machicao F, Ullrich A, Häring HU & Kellerer M 2001 Inhibition of Ret oncogene activity by the protein tyrosine phosphatase SHP1. *Endocrinology* **142** 4441–4447.
- Herfarth KK, Wick MR, Marshall HN, Gartner E, Lum S & Moley JF 1997 Absence of TP53 alterations in pheochromocytomas and medullary thyroid carcinomas. *Genes, Chromosomes and Cancer* **20** 24–29.
- Horie H, Yokogoshi Y, Tsuyuguchi M & Saito S 1995 Point mutations of ras and Gs alpha subunit genes in thyroid tumors. *Japanese Journal of Cancer Research* **86** 737–742.
- Huang SC, Torres-Cruz J, Pack SD, Koch CA, Vortmeyer AO, Mannan P, Lubensky IA, Gagel RF & Zhuang Z 2003 Amplification and overexpression of mutant RET in multiple endocrine neoplasia type 2-associated medullary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* **88** 459–463.
- Iavarone C, Acunzo M, Carlomagno F, Catania A, Melillo RM, Carlomagno SM, Santoro M & Chiariello M 2006 Activation of the Erk8 mitogen-activated protein (MAP) kinase by RET/PTC3, a constitutively active form of the RET proto-oncogene. *Journal of Biological Chemistry* **281** 10567–10576.
- Iervolino A, Iuliano R, Trapasso F, Viglietto G, Melillo RM, Carlomagno F, Santoro M & Fusco A 2006 The receptor-type protein tyrosine phosphatase J antagonizes the biochemical and biological effects of RET-derived oncoproteins. *Cancer Research* **66** 6280–6287.
- Incoronato M, D'Alessio A, Paladino S, Zurzolo C, Carlomagno MS, Cerchia L & de Franciscis V 2004 The Shp-1 and Shp-2, tyrosine phosphatases, are recruited on cell membrane in two distinct molecular complexes including Ret oncogenes. *Cellular Signalling* **16** 847–856.
- Ishida M, Ichihara M, Mii S, Jijiwa M, Asai N, Enomoto A, Kato T, Majima A, Ping J, Murakumo Y *et al.* 2007 Sprouty2 regulates growth and differentiation of human neuroblastoma cells through RET tyrosine kinase. *Cancer Research* **67** 815–821.
- Jain S, Encinas M, Johnson EM Jr & Milbrandt J 2006 Critical and distinct roles for key RET tyrosine docking sites in renal development. *Genes and Development* **20** 321–333.
- Johnston D, Hatzis D & Sunday ME 1998 Expression of v-Ha-ras driven by the calcitonin/calcitonin gene-related peptide promoter: a novel transgenic murine model for medullary thyroid carcinoma. *Oncogene* **16** 167–177.
- Joshi PP, Kulkarni MV, Yu BK, Smith KR, Norton DL, Veelen W, Höppener JW & Franklin DS 2007 Simultaneous downregulation of CDK inhibitors p18(Ink4c) and p27(Kip1) is required for MEN2A-RET-mediated mitogenesis. *Oncogene* **26** 554–570.
- Kaelin WG Jr 2008 The von Hippel–Lindau tumour suppressor protein: O₂ sensing and cancer. *Nature Reviews. Cancer* **8** 865–873.
- Kawai K, Iwashita T, Murakami H, Hiraiwa N, Yoshiki A, Kusakabe M, Ono K, Iida K, Nakayama A & Takahashi M 2000 Tissue-specific carcinogenesis in transgenic mice expressing the RET proto-oncogene with a multiple endocrine neoplasia type 2A mutation. *Cancer Research* **60** 5254–5260.
- Kedzia C, Lacroix L, Ameer N, Ragot T, Kelly PA, Caillou B & Binart N 2005 Medullary thyroid carcinoma arises in the absence of prolactin signaling. *Cancer Research* **65** 8497–8503.
- Khosla S, Patel VM, Hay ID, Schaid DJ, Grant CS, van Heerden JA & Thibodeau SN 1991 Loss of heterozygosity suggests multiple genetic alterations in pheochromocytomas and medullary thyroid carcinomas. *Journal of Clinical Investigation* **87** 1691–1699.
- Knowles PP, Murray-Rust J, Kjaer S, Scott RP, Hanrahan S, Santoro M, Ibáñez CF & McDonald NQ 2006 Structure and chemical inhibition of the RET tyrosine kinase domain. *Journal of Biological Chemistry* **281** 33577–33587.
- Koch CA, Brouwers FM, Vortmeyer AO, Tannapfel A, Libutti SK, Zhuang Z, Pacak K, Neumann HP & Paschke R 2006 Somatic VHL gene alterations in MEN2-associated medullary thyroid carcinoma. *BMC Cancer* **6** 131.
- Kodama Y, Asai N, Kawai K, Jijiwa M, Murakumo Y, Ichihara M & Takahashi M 2005 The RET proto-oncogene: a molecular therapeutic target in thyroid cancer. *Cancer Research* **65** 143–148.
- Kondo T, Ezzat S & Asa SL 2006 Pathogenetic mechanisms in thyroid follicular-cell neoplasia. *Nature Reviews. Cancer* **6** 292–306.
- Kondoh K & Nishida E 2007 Regulation of MAP kinases by MAP kinase phosphatases. *Biochimica et Biophysica Acta* **1773** 1227–1237.
- Kouvaraki MA, Shapiro SE, Perrier ND, Cote GJ, Gagel RF, Hoff AO, Sherman SI, Lee JE & Evans DB 2005 RET proto-oncogene: a review and update of genotype-phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors. *Thyroid* **15** 531–544.
- Kucherlapati MH, Nguyen AA, Bronson RT & Kucherlapati RS 2006 Inactivation of conditional Rb by Villin-Cre leads to aggressive tumors outside the gastrointestinal tract. *Cancer Research* **66** 3576–3583.
- Kunnimalaiyaan M, Ndiaye M & Chen H 2006a Apoptosis-mediated medullary thyroid cancer growth suppression by the PI3K inhibitor LY294002. *Surgery* **140** 1009–1014.
- Kunnimalaiyaan M, Vaccaro AM, Ndiaye MA & Chen H 2006b Overexpression of the NOTCH1 intracellular domain inhibits cell proliferation and alters the neuroendocrine phenotype of medullary thyroid cancer cells. *Journal of Biological Chemistry* **281** 39819–39830.

- Lanigan TM, DeRaad SK & Russo AF 1998 Requirement of the MASH-1 transcription factor for neuroendocrine differentiation of thyroid C cells. *Journal of Neurobiology* **34** 126–134.
- Lee EY, Cam H, Ziebold U, Rayman JB, Lees JA & Dynlacht BD 2002 E2F4 loss suppresses tumorigenesis in Rb mutant mice. *Cancer Cell* **2** 463–472.
- Lesueur F, Cebrian A, Robledo M, Niccoli-Sire P, Svensson KA, Pinson S, Leyland J, Whittaker J, Pharoah PD & Ponder BA 2006 Polymorphisms in RET and its coreceptors and ligands as genetic modifiers of multiple endocrine neoplasia type 2A. *Cancer Research* **66** 1177–1180.
- Levine RL & Gilliland DG 2008 Myeloproliferative disorders. *Blood* **112** 2190–2198.
- Lima J, Teixeira-Gomes J, Soares P, Máximo V, Honavar M, Williams D & Sobrinho-Simões M 2003 Germline succinate dehydrogenase subunit D mutation segregating with familial non-RET C cell hyperplasia. *Journal of Clinical Endocrinology and Metabolism* **88** 4932–4937.
- Lindahl M, Poteryaev D, Yu L, Arumae U, Timmusk T, Bongarzone I, Aiello A, Pierotti MA, Airaksinen MS & Saarma M 2001 Human glial cell line-derived neurotrophic factor receptor alpha 4 is the receptor for persephin and is predominantly expressed in normal and malignant thyroid medullary cells. *Journal of Biological Chemistry* **276** 9344–9351.
- Lindfors PH, Lindahl M, Rossi J, Saarma M & Airaksinen MS 2006 Ablation of persephin receptor glial cell line-derived neurotrophic factor family receptor alpha4 impairs thyroid calcitonin production in young mice. *Endocrinology* **147** 2237–2244.
- Lo TL, Fong CW, Yusoff P, McKie AB, Chua MS, Leung HY & Guy GR 2006 Sprouty and cancer: the first terms report. *Cancer Letters* **242** 141–150.
- Ludwig L, Kessler H, Wagner M, Hoang-Vu C, Dralle H, Adler G, Böhm BO & Schmid RM 2001 Nuclear factor-kappaB is constitutively active in C-cell carcinoma and required for RET-induced transformation. *Cancer Research* **61** 4526–4535.
- Luo W, Wickramasinghe SR, Savitt JM, Griffin JW, Dawson TM & Ginty DD 2007 A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. *Neuron* **54** 739–754.
- Machens A & Dralle H 2007 Genotype–phenotype based surgical concept of hereditary medullary thyroid carcinoma. *World Journal of Surgery* **31** 957–968.
- Maher ER & Eng C 2002 The pressure rises: update on the genetics of pheochromocytoma. *Human Molecular Genetics* **11** 2347–2354.
- Marsh DJ, Zheng Z, Arnold A, Andrew SD, Learoyd D, Frilling A, Komminoth P, Neumann HP, Ponder BA, Rollins BJ *et al.* 1997 Mutation analysis of glial cell line-derived neurotrophic factor, a ligand for an RET/coreceptor complex, in multiple endocrine neoplasia type 2 and sporadic neuroendocrine tumors. *Journal of Clinical Endocrinology and Metabolism* **82** 3025–3028.
- Marsh DJ, Theodosopoulos G, Martin-Schulte K, Richardson AL, Phillips J, Röher HD, Delbridge L & Robinson BG 2003 Genome-wide copy number imbalances identified in familial and sporadic medullary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* **88** 1866–1872.
- Marx SJ 2005 Molecular genetics of multiple endocrine neoplasia types 1 and 2. *Nature Reviews. Cancer* **5** 367–375.
- Mathew CG, Smith BA, Thorpe K, Wong Z, Royle NJ, Jeffreys AJ & Ponder BA 1987 Deletion of genes on chromosome 1 in endocrine neoplasia. *Nature* **328** 524–526.
- Matias-Guiu X, Bussolati G, DeLellis R, Kaserer K, Moley JF, Williams ED, Gagel RF, Baloch Z & Arbores-Saavedra J 2004 Medullary thyroid carcinoma. In *World Health Organization Classification of Tumours. Pathology and Genetics. Tumours of Endocrine Organs*, pp 86–91. Ed RA DeLellis *et al.*. Geneva: WHO Press.
- McGregor LM, McCune BK, Graff JR, McDowell PR, Romans KE, Yancopoulos GD, Ball DW, Baylin SB & Nelkin BD 1999 Roles of trk family neurotrophin receptors in medullary thyroid carcinoma development and progression. *PNAS* **96** 4540–4545.
- Michiels FM, Chappuis S, Caillou B, Pasini A, Talbot M, Monier R, Lenoir GM, Feunteun J & Billaud M 1997 Development of medullary thyroid carcinoma in transgenic mice expressing the RET protooncogene altered by a multiple endocrine neoplasia type 2A mutation. *PNAS* **94** 3330–3335.
- Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, Hideshima T, Chauhan D, Joseph M, Libermann TA *et al.* 2004 Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. *Cancer Cell* **5** 221–230.
- Mitsiades CS, Kotoula V, Poulaki V, Sozopoulos E, Negri J, Charalambous E, Fanourakis G, Voutsinas G, Tseleni-Balafouta S & Mitsiades N 2006a Epidermal growth factor receptor as a therapeutic target in human thyroid carcinoma: mutational and functional analysis. *Journal of Clinical Endocrinology and Metabolism* **91** 3662–3666.
- Mitsiades CS, McMillin D, Kotoula V, Poulaki V, McMullan C, Negri J, Fanourakis G, Tseleni-Balafouta S, Ain KB & Mitsiades N 2006b Antitumor effects of the proteasome inhibitor bortezomib in medullary and anaplastic thyroid carcinoma cells *in vitro*. *Journal of Clinical Endocrinology and Metabolism* **91** 4013–4021.
- Moley JF, Brother MB, Wells SA, Spengler BA, Biedler JL & Brodeur GM 1991 Low frequency of ras gene mutations in neuroblastomas, pheochromocytomas, and medullary thyroid cancers. *Cancer Research* **51** 1596–1599.
- Montani M, Schmitt AM, Schmid S, Locher T, Saremaslani P, Heitz PU, Komminoth P & Perren A 2005 No mutations but an increased frequency of SDHx polymorphisms in patients with sporadic and familial medullary thyroid carcinoma. *Endocrine-Related Cancer* **12** 1011–1016.
- Montero-Conde C, Ruiz-Llorente S, González-Albarran O, Landa I, Pita G, Milne R, Leskelä S, López-Jiménez E, Urioste M, Letón R *et al.* 2007 Identification of a candidate chromosomal region using a SNP linkage panel suggests a second locus responsible for non-RET MEN2 families. *Hormone Research* **68** 6–7.
- Mulligan LM, Gardner E, Smith BA, Mathew CG & Ponder BA 1993 Genetic events in tumour initiation and progression in multiple endocrine neoplasia type 2. *Genes, Chromosomes and Cancer* **6** 166–177.
- Nakagawa T, Mabry M, de Bustros A, Ihle JN, Nelkin BD & Baylin SB 1987 Introduction of v-Ha-ras oncogene induces differentiation of cultured human medullary thyroid carcinoma cells. *PNAS* **84** 5923–5927.
- Niccoli-Sire P, Murat A, Rohmer V, Franc S, Chabrier G, Baldet L, Maes B, Savagner F, Giraud S, Bezieau S *et al.* 2001 Familial medullary thyroid carcinoma with noncysteine ret mutations: phenotype–genotype relationship in a large series of patients. *Journal of Clinical Endocrinology and Metabolism* **86** 3746–3753.
- Paes JE & Ringel MD 2008 Dysregulation of the phosphatidylinositol 3-kinase pathway in thyroid neoplasia. *Endocrinology and Metabolism Clinics of North America* **37** 375–387 (viii–ix).
- Park MS, Rosai J, Nguyen HT, Capodiceci P, Cordon-Cardo C & Koff A 1999 p27 and Rb are on overlapping pathways suppressing tumorigenesis in mice. *PNAS* **96** 6382–6387.
- Parthasarathy R, Cote GJ & Gagel RF 1999 Hammerhead ribozyme-mediated inactivation of mutant RET in medullary thyroid carcinoma. *Cancer Research* **59** 3911–3914.
- Pavličić K, Dedivitis RA, Kapitanović S, Cacev T, Guirado CR, Daničić D, Radošević S, Brkić K, Pegan B, Krizanac S *et al.* 2006 Molecular genetic alterations of FHIT and p53 genes in benign and malignant thyroid gland lesions. *Mutation Research* **599** 45–57.

- Pellegata NS, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, Höfler H, Fend F, Graw J & Atkinson MJ 2006 Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *PNAS* **103** 15558–15563.
- Pierchala BA, Tsui CC, Milbrandt J & Johnson EM 2007 NGF augments the autophosphorylation of Ret via inhibition of ubiquitin-dependent degradation. *Journal of Neurochemistry* **100** 1169–1176.
- Pierotti MA & Greco A 2006 Oncogenic rearrangements of the NTRK1/NGF receptor. *Cancer Letters* **232** 90–98.
- Plaza Menacho I, Koster R, van der Sloot AM, Quax WJ, Osinga J, van der Sluis T, Hollema H, Burzynski GM, Gimm O, Buys CH *et al.* 2005 RET-familial medullary thyroid carcinoma mutants Y791F and S891A activate a Src/JAK/STAT3 pathway, independent of glial cell line-derived neurotrophic factor. *Cancer Research* **65** 1729–1737.
- Plaza-Menacho I, van der Sluis T, Hollema H, Gimm O, Buys CH, Magee AI, Isacke CM, Hofstra RM & Eggen BJ 2007 Ras/ERK1/2-mediated STAT3 Ser727 phosphorylation by familial medullary thyroid carcinoma-associated RET mutants induces full activation of STAT3 and is required for c-fos promoter activation, cell mitogenicity, and transformation. *Journal of Biological Chemistry* **282** 6415–6424.
- Qiao S, Iwashita T, Furukawa T, Yamamoto M, Sobue G & Takahashi M 2001 Differential effects of leukocyte common antigen-related protein on biochemical and biological activities of RET-MEN2A and RET-MEN2B mutant proteins. *Journal of Biological Chemistry* **276** 9460–9467.
- Rangarajan A & Weinberg RA 2003 Opinion: comparative biology of mouse versus human cells: modelling human cancer in mice. *Nature Reviews. Cancer* **3** 952–959.
- Robledo M, Gil L, Pollán M, Cebrián A, Ruíz S, Azañedo M, Benítez J, Menárguez J & Rojas JM 2003 Polymorphisms G691S/S904S of RET as genetic modifiers of MEN 2A. *Cancer Research* **63** 1814–1817.
- Ruiz-Llorente S, Montero-Conde C, Milne RL, Moya CM, Cebrián A, Letón R, Cascón A, Mercadillo F, Landa I, Borrego S *et al.* 2007 Medullary Thyroid Carcinoma Clinical Group. Association study of 69 genes in the ret pathway identifies low-penetrance loci in sporadic medullary thyroid carcinoma. *Cancer Research* **67** 9561–9567.
- Salmena L, Carracedo A & Pandolfi PP 2008 Tenets of PTEN tumor suppression. *Cell* **133** 403–414.
- Santoro M & Carlomagno F 2006 Drug insight: small-molecule inhibitors of protein kinases in the treatment of thyroid cancer. *Nature Clinical Practice. Endocrinology and Metabolism* **2** 42–52.
- Schagdarsurengin U, Gimm O, Dralle H, Hoang-Vu C & Dammann R 2006 CpG island methylation of tumor-related promoters occurs preferentially in undifferentiated carcinoma. *Thyroid* **16** 633–642.
- Schlumberger M, Carlomagno F, Baudin E, Bidart JM & Santoro M 2008 New therapeutic approaches to treat medullary thyroid carcinoma. *Nature Clinical Practice. Endocrinology and Metabolism* **4** 22–32.
- Schubbert S, Shannon K & Bollag G 2007 Hyperactive Ras in developmental disorders and cancer. *Nature Reviews. Cancer* **7** 295–308.
- Schulz N, Probst F, Rosenberg MP, Linnoila RI, Paules RS, Kovatch R, Ogiso Y & Vande Woude G 1992 Pheochromocytomas and C-cell thyroid neoplasms in transgenic c-mos mice: a model for the human multiple endocrine neoplasia type 2 syndrome. *Cancer Research* **52** 450–455.
- Segouffin-Cariou C & Billaud M 2000 Transforming ability of MEN2A-RET requires activation of the phosphatidylinositol 3-kinase/AKT signaling pathway. *Journal of Biological Chemistry* **275** 3568–3576.
- Sheikh HA, Tometsko M, Niehouse L, Aldeeb D, Swalsky P, Finkelstein S, Barnes EL & Hunt JL 2004 Molecular genotyping of medullary thyroid carcinoma can predict tumor recurrence. *American Journal of Surgical Pathology* **28** 101–106.
- Sherman SI 2008 Early clinical studies of novel therapies for thyroid cancers. *Endocrinology and Metabolism Clinics of North America* **37** 511–524 (xi).
- Sipple JH 1961 The association of pheochromocytoma with carcinoma of the thyroid gland. *American Journal of Medicine* **31** 163–166.
- Sjöblom T 2008 Systematic analyses of the cancer genome: lessons learned from sequencing most of the annotated human protein-coding genes. *Current Opinion in Oncology* **20** 66–71.
- Skinner MA, Moley JA, Dilley WG, Owzar K, Debenedetti MK & Wells SA Jr 2005 Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. *New England Journal of Medicine* **353** 1105–1113.
- Smith-Hicks CL, Sizer KC, Powers JF, Tischler AS & Costantini F 2000 C-cell hyperplasia, pheochromocytoma and sympathoadrenal malformation in a mouse model of multiple endocrine neoplasia type 2B. *EMBO Journal* **19** 612–622.
- Sobrinho-Simões M, Máximo V, Rocha AS, Trovisco V, Castro P, Preto A, Lima J & Soares P 2008 Intragenic mutations in thyroid cancer. *Endocrinology and Metabolism Clinics of North America* **37** 333–362 (viii).
- van Staveren WC, Solís DW, Delys L, Venet D, Cappello M, Andry G, Dumont JE, Libert F, Detours V & Maenhaut C 2006 Gene expression in human thyrocytes and autonomous adenomas reveals suppression of negative feedbacks in tumorigenesis. *PNAS* **103** 413–418.
- Takahashi C, Contreras B, Iwanaga T, Takegami Y, Bakker A, Bronson RT, Noda M, Loda M, Hunt JL & Ewen ME 2006 Nras loss induces metastatic conversion of Rb1-deficient neuroendocrine thyroid tumor. *Nature Genetics* **38** 118–123.
- Taketomi T, Yoshiga D, Taniguchi K, Kobayashi T, Nonami A, Kato R, Sasaki M, Sasaki A, Ishibashi H, Moriyama M *et al.* 2005 Loss of mammalian Sprouty2 leads to enteric neuronal hyperplasia and esophageal achalasia. *Nature Neuroscience* **8** 855–857.
- Trimarchi JM & Lees JA 2002 Sibling rivalry in the E2F family. *Nature Reviews. Molecular Cell Biology* **3** 11–20.
- Tsui-Pierchala BA, Milbrandt J & Johnson EM Jr 2002 NGF utilizes c-Ret via a novel GFL-independent, inter-RTK signaling mechanism to maintain the trophic status of mature sympathetic neurons. *Neuron* **33** 261–273.
- Vanhorne JB, Andrew SD, Harrison KJ, Taylor SA, Thomas B, McDonald TJ, Ainsworth PJ & Mulligan LM 2005 A model for GFR alpha 4 function and a potential modifying role in multiple endocrine neoplasia 2. *Oncogene* **24** 1091–1097.
- van Veelen W, van Gasteren CJ, Acton DS, Franklin DS, Berger R, Lips CJ & Höppener JW 2008 Synergistic effect of oncogenic RET and loss of p18 on medullary thyroid carcinoma development. *Cancer Research* **68** 1329–1337.
- van Veelen W, Klompmaaker R, Gloerich M, van Gasteren CJ, Kalkhoven E, Berger R, Lips CJ, Medema RH, Höppener JW & Acton DS 2009 P18 is a tumor suppressor gene involved in human medullary thyroid carcinoma and pheochromocytoma development. *International Journal of Cancer* **124** 339–345.
- Weber F & Eng C 2005 Editorial: germline variants within RET: clinical utility or scientific playtoy? *Journal of Clinical Endocrinology and Metabolism* **90** 6334–6336.
- Wellbrock C, Karasides M & Marais R 2004 The RAF proteins take centre stage. *Nature Reviews. Molecular Cell Biology* **5** 875–885.
- Wells SA & Nevins JR 2004 Evolving strategies for targeted cancer therapy – past, present, and future. *Journal of the National Cancer Institute* **96** 980–981.
- Williams BO, Remington L, Albert DM, Mukai S, Bronson RT & Jacks T 1994 Cooperative tumorigenic effects of germline mutations in Rb and p53. *Nature Genetics* **7** 480–484.
- Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J *et al.* 2007 The genomic landscapes of human breast and colorectal cancers. *Science* **318** 1108–1113.

- Wu G, Mambo E, Guo Z, Hu S, Huang X, Gollin SM, Trink B, Ladenson PW, Sidransky D & Xing M 2005 Uncommon mutation, but common amplifications, of the PIK3CA gene in thyroid tumors. *Journal of Clinical Endocrinology and Metabolism* **90** 4688–4693.
- Xing M 2005 BRAF mutation in thyroid cancer. *Endocrine-Related Cancer* **12** 245–262.
- Yamasaki L, Bronson R, Williams BO, Dyson NJ, Harlow E & Jacks T 1998 Loss of E2F-1 reduces tumorigenesis and extends the lifespan of Rb1(+/-) mice. *Nature Genetics* **18** 360–364.
- Yang KP, Samaan NA, Liang YF & Castillo SG 1992 Role of insulin-like growth factor-I in the autocrine regulation of cell growth in TT human medullary thyroid carcinoma cells. *Henry Ford Hospital Medical Journal* **40** 293–295.
- Ye L, Santarpia L, Cote GJ, El-Naggar AK & Gagel RF 2008 High resolution array-comparative genomic hybridization profiling reveals deoxyribonucleic acid copy number alterations associated with medullary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* **93** 4367–4372.
- Yoshimoto K, Iwahana H, Fukuda A, Sano T, Saito S & Itakura M 1992 Role of p53 mutations in endocrine tumorigenesis: mutation detection by polymerase chain reaction-single strand conformation polymorphism. *Cancer Research* **52** 5061–5064.
- Yuan TL & Cantley LC 2008 PI3K pathway alterations in cancer: variations on a theme. *Oncogene* **27** 5497–5510.
- Zatelli MC, Piccin D, Tagliati F, Bottoni A, Luchin A & degli Uberti EC 2005 SRC homology-2-containing protein tyrosine phosphatase-1 restrains cell proliferation in human medullary thyroid carcinoma. *Endocrinology* **146** 2692–2698.
- Zbuk KM & Eng C 2007 Cancer phenomics: RET and PTEN as illustrative models. *Nature Reviews. Cancer* **7** 35–45.
- Ziebold U, Lee EY, Bronson RT & Lees JA 2003 E2F3 loss has opposing effects on different pRB-deficient tumors, resulting in suppression of pituitary tumors but metastasis of medullary thyroid carcinomas. *Molecular and Cellular Biology* **23** 6542–6552.

Received in final form 9 April 2009

Accepted 21 April 2009

Made available online as an Accepted Preprint 21 April 2009