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Thyroid C-Cell Biology and Oncogenic Transformation

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Abstract

The thyroid parafollicular cell, or commonly named “C-cell,” functions in serum calcium homeostasis. Elevations in serum calcium trigger release of calcitonin from the C-cell, which in turn functions to inhibit absorption of calcium by the intestine, resorption of bone by the osteoclast, and reabsorption of calcium by renal tubular cells. Oncogenic transformation of the thyroid C-cell is thought to progress through a hyperplastic process prior to malignancy with increasing levels of serum calcitonin serving as a biomarker for tumor burden. The discovery that Multiple Endocrine Neoplasia, type 2 is caused by activating mutations of the *RET* gene serves to highlight the RET-RAS-MAPK signaling pathway in both initiation and progression of medullary thyroid carcinoma. Thyroid C-cells are known to express RET at high levels relative to most cell types, therefore aberrant activation of this receptor is targeted primarily to the C-cell, providing one possible cause of tissue-specific oncogenesis. The role of RET signaling in normal C-cell function is unknown though calcitonin gene transcription appears to be sensitive to RET activation. Beyond RET the modeling of oncogenesis in animals and screening of human tumors for candidate gene mutations has uncovered mutation of *RAS* family members and inactivation of Rb1 regulatory pathway as potential mediators of C-cell transformation. A growing understanding of how RET interacts with these pathways, both in normal C-cell function and during oncogenic transformation will help in the development of novel molecular targeted therapies.

1. Introduction

The expression of calcitonin by medullary thyroid carcinoma (MTC) defines the C-cell (parafollicular cell) origin of this tumor and serves a valuable role in monitoring the progression of disease. C-cells comprise a minor population of the thyroid, representing approximately 2–4% of the organ’s cells, which also represents the relative frequency of MTC among all thyroid cancer types. The remaining majority of thyroid cells are follicular in origin. Despite a shared anatomical location, initiation and progression of MTC tumors from C-cells differ from follicular cell-derived tumors, of which papillary thyroid carcinoma is the most common. Immunofluorescent detection of calcitonin performed initially by

Bussolati and Pearce localized expression to the thyroid C-cell and ultimately MTC cells [1,2]. Our understanding of the initiating and driving events involved in the oncogenic transformation of the C-cell has been greatly facilitated through the discovery that activating germline mutations of the *RET* gene are responsible for hereditary forms of MTC [3–5]. However, it remains unclear as to what drives thyroid C-cell sensitivity to RET activation, which in turn sets in motion the development of MTC. Predicting the occurrence of MTC in patients with the germline RET mutation is important for clinicians attempting to treat the disease at its earliest onset. A RET mutation is also thought to play a role in 40% of sporadic MTCs [6]. How an alteration in *RET* initiates C-cell transformation remains unclear in this setting as well. There exists a need to understand RET's mechanism of action further and to uncover other mediators of C-cell transformation and tumor progression. Expanding our understanding of the biology of the normal C-cell and of MTC will aid in the future treatment of this malignancy, by refining the timing of resection of the thyroid in hereditary cases and allowing the further development of novel, molecular-targeted therapies of advanced cases.

2. Defining the C-cell

The thyroid gland consists of two endocrine cell types, the follicular cells that produce the thyroid hormones thyroxine (T4) and triiodothyronine (T3), and the parafollicular C-cells that synthesize calcitonin. It is well accepted that these two thyroid cell types have unique embryonic origins, although details of their differentiation and development during organogenesis remain somewhat controversial. However, tumors arising from the two cell types are clearly distinct entities with different treatment and prognosis.

The identification of the C-cell pre-dates the discovery of calcitonin by nearly 90 years. As early as 1876, Baber had pointed out the presence in dog thyroid gland of a distinct group of “parenchymatous cells” which he described as markedly different from follicular cells in that they were large, rounded, frequently solitary cells with oval nuclei [7]. These cells have since been redescribed by numerous authors using various names such as “*ovoid* cells” [8], “interfollicular cells” [9] or “mitochondria-rich cells” [10]. Nonidez is perhaps most frequently credited with [11,12] redefining these cells based on the intuition that they might be argyrophilic. Silver staining led him to visualize their sparse distribution throughout the thyroid gland and to describe these cells as parafollicular [11]. Still, the role for these parafollicular cells remained unclear for decades, postulated to be either non-secretory [13] or a stage in the life-cycle of the follicular cells [14,15]. However, with the application of electron microscopy, a more detailed examination of the parafollicular cell from several species uncovered a common abundance of granules believed to be secretory in nature [16,17]. Their ultrastructural and cytochemical characteristics lead to the suggestion by Pearce [18,19] that parafollicular cells belong to a group of endocrine cells he termed APUD (amine precursor uptake and decarboxylation), all of endodermal origin [20–24], and each producing polypeptide hormones. Ultimately, immunohistological studies [2,25,26] provided the evidence that the hormone calcitonin was specifically secreted by the parafollicular cells and not by follicular cells. This localization also fits well a previously proposed label of C-cells for calcitonin, which was introduced as a functional substitute for the existing misleading descriptive terms [27]. As such parafollicular cells are now commonly referred to

as thyroid C-cells given their primary functional role in calcitonin production. The hormone acts to reduce the serum calcium level, counteracting the effects of parathyroid hormone.

3. Tracing the Developmental Origin of the Thyroid C-cell

A detailed discussion of the embryonic origins of the parafollicular C-cell and thyroid development is beyond the scope of this chapter. Readers are referred to several excellent reviews on thyroid development [28,29]. The now classic chick-quail chimera studies by Le Douarin and Le Lievre continue to serve as the basis for defining the neural crest origin of C-cells [30,31]. Quail neural crest cells transplanted to chick embryos and identified by their characteristic chromatin structure, were specifically demonstrated to invade the chick ultimobranchial gland and differentiate into C-cells based on calcitonin immunostaining. However, 2 major caveats of these studies need to be considered in the context of MTC. First, is the fact that in lower vertebrates, including fish, reptiles and birds, the C-cells comprise a separate ultimobranchial gland that exists separate from the thyroid (reviewed in [29]). Thus, a major difference in cellular migration exists beginning with mammals. While the relevance of this divergence to oncogenesis is not clear, it is interesting to note that nonthyroidal C-cell carcinomas have only been reported for zebrafish among the various lower vertebrates [32,33]. Second, it is important to remember the limited availability of lineage-specific markers along with the capability to employ engineered genetic models to aid in cell lineage tracing at the time these studies were performed. However, the clear demonstration of calcitonin-producing cells within the avian ultimobranchial gland, coupled with the species-specific (chick-quail) cell tracking studies, defined avian C-cells as of neural crest origin. These observations, in turn, provided the original basis to postulate a similar neuroectodermal origin for thyroid C-cells.

The evolutionary mechanism behind C-cell migration into the thyroid, as well as its potential functional roles beyond calcitonin production, remains unclear. In mammals it is believed that a specific subpopulation of ectodermal-derived neural crest cells migrate to the most inferior pharyngeal arch to invade/merge with a pair of endodermal-derived ultimobranchial bodies (also referred to as the lateral thyroid anlage). The ultimobranchial bodies then serve to specifically carry the C-cell precursors into the developing thyroid gland. The ultimobranchial body origin of thyroid C-cells was originally suggested from light microscopic observations in dog thyroid [34], and first experimentally documented by the specific uptake of fluorescent amine [22]; subsequently this role was supported by demonstration of characteristics consistent with defining amine precursor uptake and decarboxylation (APUD) cell types [35]. Electron microscopy studies further identified cells with ultrastructural features of C-cells in the ultimobranchial glands prior to fusion with the thyroid gland [36]. Despite these biochemical and structural features, the precursor cells within ultimobranchial bodies do not stain positive for calcitonin [37,38]. Therefore, unlike lower vertebrates ultimobranchial bodies do not serve as a site of C cell differentiation, but merely to ensure specific migration into the developing thyroid. This specific role is supported by several mouse knockout models demonstrating that failed or abnormal development of the ultimobranchial body causes aberrant localization or even loss of calcitonin-producing C-cells [39–42]. Once within the thyroid gland, Mash1 and perhaps RET signaling pathways, are associated with differentiation and the induction of neuronal

traits in the cells. In two independent studies, Mash1 null mice are severely impaired in their ability to generate mature C-cells [43,44]. The near or complete absence of C-cells in the Mash1 null mutants suggests progenitors may degenerate before they become differentiated. Available evidence suggests Mash1 enhances survival of C-cell progenitors by inhibiting apoptosis while promoting differentiation [43]. Surprisingly the impact of loss of RET function is less severe. Unlike the complete loss of the neural crest derived enteric nervous system associated with loss of Mash1, RET null mice reportedly demonstrate only a 37% loss of C cell number [45]. This observation suggests that C-cell development may not be dependent on an intact RET neural crest cell lineage (see below).

The actual mechanisms involved in the direction of C-cell precursors to the ultimobranchial body and dissemination of these maturing C-cells within the thyroid remain unknown. Perhaps an even greater area of controversy is the true origin of C-cell precursors. Circumstantial evidence based on genetic fate mapping studies in mice suggests that mammalian thyroid C-cells might have an endoderm origin [46]. This challenges the prevailing concept of MTC being a neuroectodermal tumor. Lineage-tracing studies have invalidated a neural crest origin of gut endocrine cells, proving that endoderm stem cells can differentiate into both exocrine and endocrine phenotypes (reviewed in [47]). Similar fate mapping studies have also challenged C-cell origin in mice. In mice, using either Connexin43 or Wnt1 as neural crest cell markers failed to localize staining to the pharyngeal pouches or developing ultimobranchial bodies [46]. Unexpectedly, these areas were found to stain intensely for E-cadherin, and to co-localize with calcitonin-positive cells following birth suggesting an endodermal epithelial origin. Support for an endodermal origin of neural crest cells is also provided by a recent reexamination of the sonic hedgehog *Shh*^{-/-} mouse [38]. *Shh* is required for both migration and survival of neural crest cells [48–50]. In the absence of *Shh*, mature C-cells instead appear in ectopic locations. Calcitonin-positive C-cells are found to populate the regions deriving pharyngeal pouches that remain in the absence of ultimobranchial body formation. These findings indicate that *Shh* determines the endoderm territory for C-cell differentiation and guides the migration of C-cell precursors into the thyroid, presumably by regulating the separation of glandular domains in the pharyngeal pouch endoderm.

In summary, the embryonic origin of thyroid C-cells, whether it is neural crest cell, endoderm, or perhaps both, remains a controversy. What is clear is that to date there are no reports of C-cells in the thyroid of mice lacking ultimobranchial bodies, indicating that thyroid progenitors from the midline anlage are not capable of generation of C-cell lineage, and there are no examples of a normally formed thyroid completely devoid of C-cells, suggesting a yet to be defined functional interaction of C-cells and follicular cells. However, while under normal circumstances C-cell progenitors differentiate within the thyroid, differentiation is not uniquely dependent on thyroid localization. Whether these developmental findings in mice can be translated to humans is unclear. A strict dependence on the ultimobranchial body for C-cell development may not exist. First, thyroid C-cells are not ablated in patients with DiGeorge syndrome [51] in which not only do the thymus and parathyroid fail to develop but also the ultimobranchial body is assumed to be missing due to defective development of all posterior pharyngeal arches and pouches [40]. More recently,

ectopic lingual thyroids located far away from the origin of the ultimobranchial body were also found to contain C-cells [52,53].

4. C-Cell Function

It is universally accepted that a primary function of the thyroid C-cell is to secrete calcitonin, and to a lesser degree smaller quantities of several neuroendocrine peptides such as somatostatin, calcitonin gene-related peptide (CGRP) and serotonin [54]. In animals that have been examined to date, the secretion of calcitonin by C-cells is evolutionarily conserved beginning from teleost fish through mammals. Furthermore, despite only 50% conservation at the amino acid level, salmon calcitonin is functionally active in humans [55]. In all vertebrate species, calcitonin, along with parathyroid hormone and vitamin D play key roles in serum calcium homeostasis (for details [56,57]). It is interesting to note evolutionarily that parathyroid gland development from gills occurs with tetrapods [58], and that integration of ultimobranchial C-cells in the thyroid coincides with that of mammals [29].

Calcitonin release from the C-cell is triggered primarily in response to elevated serum calcium, though other hormones including gastrin have been reported to stimulate release. Indeed the sensitivity of C-cells to gastrin led to the pentagastrin stimulation test, which measures calcitonin elevations in MTC. This test has been replaced by calcium testing where pentagastrin is not available [59,60]. While the precise mechanism controlling calcitonin release has not clearly been elucidated, both the calcium-sensing receptor (CASR) and cholecystokinin-B receptor (gastrin receptor) are expressed in MTC cells [61–63]. In MTC cells, the functionally of CASR has recently been demonstrated to be modulated through interactions with RAMP1, which act to regulate trafficking of the receptor to the cell surface [62]. Unfortunately, these studies are complicated by the reliance on MTC tumor cell line models rather than normal C-cells. Much of the focus on C-cell function is a direct result from the development of calcitonin as a marker of tumor progression and the study of MTC tumor cell biology rather than normal cells.

In mice, calcitonin production begins around embryonic day 15 as the progenitor C-cells enter the thyroid and follicular organization becomes apparent [37,38]. In humans, calcitonin positivity is noted by the end of the first trimester [64]. In humans C-cells are differentially distributed with the thyroid tending to be concentrated at a junction approximating the upper two-thirds of the thyroid [65]. Overall C-cells represent minority cell population typically situated basally in the epithelium, without direct contact with the follicular lumen. The mean C-cell densities in mouse ($\sim 220/\text{mm}^2$) and rat ($\sim 450/\text{mm}^2$) is considerably greater than the numbers observed in human thyroid glands ($0\text{--}20/\text{mm}^2$) [66,67,65]. Importantly, calcitonin and C-cells are not essential for normal development. Genetic knockout of calcitonin in zebrafish [68] or mice [69] does not affect animal viability or present an overt phenotype. However, challenges to calcium homeostasis do induce notable differences in bone formation. In humans, while it is clear that early prophylactic thyroidectomy in MEN2 patients is not associated with calcium metabolism or bone problems, other sources of calcitonin may compensate for this C-cell loss. Population studies have also demonstrated no effect on fracture rate in men having undergone thyroidectomy [70]. Also, patients with

calcitonin deficiency caused by thyroid dysgenesis do not present with alterations of bone development or bone mass [71]. Thus the precise normal physiological role of both calcitonin and the thyroid C cell remain to be completely elucidated.

5. Defining the C-Cell as the origin of MTC

The earliest descriptions of MTC predate the characterization of calcitonin including descriptions of argyrophilic tumors in dog [12], ‘microadenomas’ or ‘light cell’ tumors in rat [72,73], and ultimobranchial carcinomas in bulls [74]. The first detailed description in humans along with their specific classification as ‘medullary’ thyroid carcinomas is generally credited to Hazard et al. in 1959 [75]. However, the recognition that MTC derived from thyroidal C-cells was not made until several years later. Through a comparison of thyroid tumors derived from rats, dogs and humans, a histological class of tumors that was distinct from papillary, follicular and anaplastic tumors was apparent [76]. The linking of tumor formation to C-cell hyperplasia (CCH) in rats led to the postulation that all tumors of this classification are C-cell derived. It was the identification that thyroidal calcitonin was derived from the C-cell [2], that ultimately led to this cell being identified as the definitive origin of MTC through immunohistochemical localization [1] and that elevated plasma calcitonin levels served as a sensitive tumor marker [77,78]. These findings represented a paradigm shift in the treatment of familial MTC, the presence of elevated calcitonin allowing early diagnosis of CCH and MTC in affected family members [79] often prior to clinical or radiographic methods of detection.

The availability of calcitonin antibodies provided a tool to confirm the C-cell origin of tumors in several animal species. Interestingly, despite serving as an early model for the purification of calcitonin, ultimobranchial tumors have not been reported in salmon, though are known to occur in zebrafish [32,33]. A recent examination found ultimobranchial tumors to occur at a frequency of ~1% in a population of 702 fish or ~5% of all tumors observed [33]. Among all species evaluated for MTC, several rat strains are noted to have the highest incidence of spontaneously occurring tumors in old age: 10–20% of Wistar rats [80,81], approximately 20% of Long-Evans rats [82], 50–70% of Wag/Rij rats [83,84] and 78% of Sprague Dawley rats [85]. This compares to a reported frequency of less than 1 in 2,500 for mice [86] and 1 in 6,700 in humans where sporadic MTC accounts for approximately 5% of the reported 298 cases per 100,000 thyroid cancer cases observed in 2012 [87]. These differences in MTC incidence provide strong evidence for the role of genetic drivers in C cell tumorigenesis.

6. Molecular Pathway Dysregulations in Human C-cell Oncogenic Transformation

Dysregulation of pleiotropic growth factors and their receptors is a common feature of carcinogenesis, and contributes to proliferation, anti-apoptosis and invasive behavior of the tumor cells. Relevant mutations observed in C-cell neoplasia mainly involve the RET receptor and the RAS downstream effector, but mutations in the components of other pathways are also encountered, albeit less frequently. Indeed these molecules are instrumental for progenitor cell survival, normal C-cell development, and migration from the

neural crest. The pathways they trigger are normally tightly regulated. Dysregulation of these factors and their pathway components is therefore connected with essential MTC properties such as tumor cell proliferation, cell cycle check points, and invasive behavior.

6.1. RET signaling

The RET receptor is encoded by the *RET* proto-oncogene localized on human chromosome 10q11.2. This gene encodes a single-pass transmembrane protein that belongs to the receptor tyrosine kinase (RTK) family [88]. The extracellular region of the protein contains cadherin-like domains, while the intracellular region contains the active tyrosine kinase domain. RET is activated through extracellular binding of the glial cell line-derived neurotrophic factor (GDNF) family of ligands, which include GDNF, Neurturin (NRTN), Persephin (PSPN) and Artemin (ARTN)(Figure 1). In order to stimulate RET tyrosine kinase activity, these growth factors need first to form a complex with glycosylphosphatidylinositol (GPI)-anchored co-receptors belonging to the GDNF receptor- α family (GFRA1–4). The growth factor–GFRA complex then recruits RET, which dimerizes and is activated by trans-autophosphorylation. The RET receptor contains multiple tyrosine sites within its intracellular domain that upon phosphorylation mediate differential downstream signaling [89,90](Figure 2). Located within the activation loop of the RET kinase adjacent to the ATP binding pocket, the Y905 site serves a key role in RET receptor kinase activation [91]. Its phosphorylation serves as the initiating event for additional phosphorylation. Tyrosine 1062 (Y1062) is a multi-docking site for proteins containing a phosphotyrosine-binding (PTB) domain [92]. Once recruited, these docking proteins will activate the RAS-MAPK and PI3K-AKT pathways. The Y1096 site, which exists only on the long isoform of RET, also couples to the RAS-MAPK and the PI3K-AKT pathways. Finally, Y981 and Y1015 are coupled to other important signaling molecules such as SRC and PLC- γ , respectively [93,94]. For a more extensive discussion of RET signaling and its role in disease the reader is referred to the following excellent recent reviews [95,96].

6.1.1 Attenuated RET signaling—RET signaling is crucial for mammalian embryonic development as mouse models lacking RET function die before birth. The mutant embryos exhibit abnormal kidney development, and aberrant ureter, ovary, muscle, and intestine morphology. RET signaling seems also essential for neurogenesis and establishment of neuronal populations of the central nervous system. In addition, RET activation is necessary for the development of neural crest derived lineages, in particular the enteric nervous system, adrenal medulla chromaffin cells and thyroid C-cells [96]. Notably, loss of RET, but not GFRA4 or PSPN is associated with C-cell reductions in adult mice [45,97]. Within the nervous system GDNF/RET signaling is crucial to the maintenance of the nigrostriatal pathway by preventing cell death and onset of Parkinson disease [98]. It is also essential for the survival of motor neurons; alterations in the pattern of phosphorylation lead to amyotrophic lateral sclerosis [99]. RET signaling also maintains the enteric nervous system, and loss of RET function in these neurons leads to Hirschsprung's disease, a condition characterized by absence of enteric ganglia [99]. Importantly, this role of RET is reproduced in both RET knockout [100] and RET knockin mice with RET C620R mutations (mixed Hirschsprung's/MTC phenotype)[101,102]. Finally it is important to remember that GDNF/RET signaling has a clearly established role in driving spermatogonial stem cell self-

renewal in the testis, and is therefore essential to male fertility [103–106]. Thus, in hereditary RETopathies or in therapies targeting RET activity, cell types beyond the thyroid C-cell must be considered.

6.1.2 Oncogenic RET signaling—Several known mutations can convert the RET receptor into a dominant transforming oncogene resulting in neoplasms affecting multiple cell types and organs. While RET mutation has been primarily associated with thyroid cancer, somatic structural RET alterations or changes of its expression have been described in lung, breast and pancreatic cancers [107–114]. Germline activating mutations of RET are the cause of multiple endocrine neoplasia type 2 (MEN2), which is discussed in detail within other chapters of this book. Activating mutations of the RET oncogene fall into two major classes, extracellular and intracellular (Figure 2). The extracellular cysteine-rich domain of RET is most frequently mutated at cysteine 634 (85% of the cases) but mutations also occur distributed among all cysteines (C609, C611, C618, C620, C630) [115]. Intracellular RET mutations are observed in the RET kinase domain and have a variable impact on RET activity. The most frequent mutation observed is M918T, which is found in patients with MEN2B and somatically in sporadic MTC. The molecular mechanism by which *RET* mutations trigger transformation have been elucidated [116–118]. As discussed, the normal route to activation involves binding of GFRA1–4/ligand complex to RET, which promotes dimerization and in turn auto-phosphorylation of the receptor. Mutations present in the extracellular domain are known to target highly conserved cysteine residues. Because these residues are normally involved in intramolecular disulfide bond formation, mutation generates a free sulfhydryl group. Mutant RET receptors in close proximity will then form intermolecular disulfide bonds, which function to stabilize dimerization even in absence of the ligand (Figure 2). This will therefore constitutively activate auto-phosphorylation and the intracellular pathways downstream of the receptor [116,119,120]. In contrast, mutations in the intracellular tyrosine-kinase domain of RET, are known to target regions associated with the ATP-binding pocket, activation loop, and substrate binding pocket [116,121,122]. Depending on their specific location, they induce structural changes and alter RET substrate specificity. This results in phosphorylation of alternative intracellular proteins. Therefore, the mutated receptor no longer needs dimerization to become active. RET mutations are often an initial step in the oncogenic process.

6.2 RAS signaling

The RAS family of proteins consists of small GTPases that act as binary molecular switches downstream of ligand-receptor interactions [123,124]. They are mainly activated through growth factors binding to RTKs, such as RET. RAS proteins are anchored to the plasma membrane through prenylation, which is indispensable to their function [125–127]. RTK autophosphorylation enables interaction with the adaptor protein GRB2 (Figure 3). GRB2 is bound to SOS, a guanosine nucleotide exchange factor (GEF) that activates RAS by stimulating the release of guanosine diphosphate (GDP) to allow binding of guanosine triphosphate (GTP)[128]. Therefore, upon stimulation by an activated receptor, RAS switches from the inactive “off” form (GDP-bound) to the active “on” form (GTP-bound). Binding of GTP induces a conformational change leading to an increase in RAS affinity and binding to downstream mediators such as RAF or PI3K family members depending on the

cell status. RAF is a serine-threonine kinase. RAS acts to recruit it to the plasma membrane and promotes dimerization and activation [129]. Activated RAF phosphorylates and activates the kinase MEK, which in turn phosphorylates and activates ERK kinase. Activated ERK phosphorylates a number of substrates, including other kinases and transcription factors, which will induce cell cycle progression [130]. In the other main pathway, activation of PI3K will induce phosphorylation of AKT, leading to inhibition of several tumor suppressors (eg. p21, BAD, etc.) or activation of the mTORC1 complex (Figure 3)[131–135] (Figure 3). Note that activation of the PI3K/AKT pathway also occurs independently of RAS through direct and indirect binding of RTKs [136]. The ultimate outcome is regulated induction of cell survival, growth and migration. Components of these main signaling cascades are often mutated in cancer, with approximately one third of human tumors expressing a constitutively activated mutant form of the RAS protein [137]. For a more extensive discussion of RAS signaling and targeting in cancer the reader is referred to the following excellent recent reviews [138–141,137].

6.2.1. Attenuated RAS signaling—There are approximately 30 members of the RAS family, but the most clinically relevant are H-RAS, N-RAS and K-RAS [124]. In mice, *Kras* knockout is embryonic lethal, whereas *Hras*, *Nras* and *Hras-Nras* double knockouts survive normally [142–145]. A thyroid C-cell phenotype has not been reported in any of these mouse models. Surprisingly, knockout of *Nras*, but not *Hras* or *Kras*, in combination with *Rb1* haploinsufficiency is associated with a pronounced MTC in mice (see below). In human cell lines somatic cell knockout of oncogenic *KRAS* has been accomplished in human colon cancer cells [146]. Upon *KRAS* gene disruption, these cells lost anchorage-independence and grew slower than the parental cells in vitro and after injection into the flank of nude mice. They showed reduced expression of *C-MYC*, a transcription factor normally stabilized by phosphorylation in response to activation of the RAS/ERK pathway [147]. To date there have been no reports examining the impact of RAS loss in human MTC cell lines.

6.2.2. Activated and oncogenic RAS signaling—The RAS signal transduction pathway has been extensively studied in the context of tumorigenesis since the recognition that somatic gain-of-function mutations were responsible for cellular transformation. It is interesting to note that germline RAS gain-of-function mutations are observed in two separate genetic syndromes, both without a predominant cancer phenotype. Noonan syndrome, which is associated with heterozygous mutation of *KRAS* or *NRAS*, is characterized by learning disabilities, skin lesions, heart and facial abnormalities, cryptorchidism, and of potential relevance short stature with craniofacial anomalies [148,149]. A role for the C-cell in any of these phenotypic features has not been reported. Costello syndrome is caused by heterozygous germline mutations at amino acid positions 12 and 13 in *HRAS* [150]. The result is a net increase in the active GTP-bound form of RAS. Interestingly, these positions are also the most frequently mutated positions in oncogenic RAS, and patients with Costello syndrome are at increased risks of neural crest tumors, although there have been no reports to date of MTC [151,112]. Those interested in RASopathies are referred to exhaustive reviews [152,153].

Somatic gain-of-function mutations in *RAS* genes that promote cancer were identified more than three decades ago (for a review, refer to [141]). Mutations are found in each of the three major isoforms and nearly all occur in the highly conserved codons 12, 13, and 61 [139,138]. Mutation in these codons alters both nucleotide-binding properties and GTPase properties of the resulting *RAS* proteins. The primary outcome is a constitutive activation because *RAS* remains in the active, GTP-bound form [154]. Driver mutations involving *RAS* codons 12, 13, and 61 have been reported in a broad spectrum of cancer types, including MTC. The widespread involvement of *RAS* in tumorigenesis prompted the first MTC screen for somatic driver mutations in 1989 [155]. The discovery of a single *HRAS* codon 61 mutation found in one of 18 tumors suggested *RAS* mutation was a rare of low frequency event. Three subsequent studies examining all three *RAS* family members in 30 MTC failed to uncover a single somatic mutation [156–158]. This lack of *RAS* involvement in C-cell oncogenesis fit well with in vitro studies performed during the same period demonstrating that oncogenic *HRAS* and *CRAF* decreased proliferation through induction of neuroendocrine differentiation rather than enhancing tumorigenicity [159,160]. However, these results were in conflict with transgenic mouse model data demonstrating that C-cells were sensitive to activated MAPK-induced tumorigenesis [161,162]. More recently the widespread availability of tools for somatic mutation analysis has led to a re-examination of *RAS* involvement in C-cell tumorigenesis. By focusing on identifying driver mutations in MTC tumors lacking somatic *RET* mutation Moura *et al.* uncovered an unexpectedly high fraction of *RAS* mutation [163]. Subsequent to these findings several other studies have reported the presence of somatic *RAS* mutation [164–167]. As seen in other tumor types mutations are nearly exclusive to *RAS* codons 12, 13, and 61. The currently reported distribution of mutations seen in MTC differs from what is seen in other thyroid cancer with targeting of the *HRAS* gene, and specifically codon 61 mutations, occurring most frequently. In nearly all cases reported to date *RET* and *RAS* mutations are mutually exclusive supporting the belief that *RET*-mediated oncogenic transformation occurs separately from *RAS*.

6.3 Other pathways

RET and *RAS* pathways have emerged as predominant oncogenic pathways for the C-cell, however mutation of these genes currently only explains only about 60% of MTC. The search for other major drivers of transformation has been largely unsuccessful. This failure may be explained in part by the lack of large unbiased search for new drivers. To date only a single study reporting exomic sequencing was performed, which included only 25 MTC tumors [164]. An alternative explanation is that a significant proportion of C-cell oncogenesis is driven by gene copy number changes rather than gene mutation. A role for copy number changes is supported by high-density comparative genomic hybridization (CGH) array studies [168,169] and the unexpected MTC phenotype observed in numerous mouse models with allelic loss (see Table 1). From these studies the retinoblastoma tumor suppressor (*RB1*) pathway has emerged as a pathway to C-cell oncogenesis in mice and should be considered a potentially important pathway in humans. Indeed a recent association study specifically identified cell cycle genes in MTC development [170]. The pathway primarily is made up of cyclin dependent protein kinases (CDKs), cyclin dependent kinase inhibitors (CDKNs), Cyclins (A, D and E), *RB* proteins, and the E2F – family of

transcription factors [171](Figure 4). The pathway plays a critical role in cell cycle checkpoint regulation, which safeguards appropriate cell division; alterations in one or more of various components may lead to aberrant cell proliferation and development of cancer. Cell cycle progression is positively regulated by Cyclins activated by CDKs and negatively regulated by CDKNs. The activity of both is in turn regulated by environmental and cellular signals. For example MAPK signaling leads to increased Cyclin D expression while p53 activation enhances p21 function. The CDKN family members thus play a central role in preventing inappropriate cell division through negative CDK regulation and G1 cell cycle arrest. The CDKNs are made up two major families - the INK4/CDKN2 family, which includes p15, p16, p18, and p19, and the Cip/Kip/CDKN1 family that includes p21, p27, and p57. The absence of CDKN suppression leads to increased phosphorylation of RB, which disrupts an inhibitory RB-E2F interaction. Once E2F is free of RB suppression it functions to transcribe genes requires for progression through S phase. The aberrant loss of CDKNs and RB proteins has been associated with the development and progression of numerous cancer types, and thus serve roles as tumor suppressor genes [172–174,171]. Given the fundamental role that the *RB1* gene played in the establishment of “two-hit” hypothesis of retinoblastoma [175] it was surprising that knockout of *Rb1* in mice was initially associated with the development of pituitary tumors, and subsequently C-cell hyperplasia [176–178]. Indeed, it is clear from several studies that Rb1 plays a central role in mouse C-cell tumorigenesis (see next section). Despite this observation a direct role for RB1 loss in human C-cells has not been found. Studies that including *RB1* sequence analysis failed to find mutation in 46 MTC tumors examined [164,179,180]. A separate study found positive RB1 staining in 46 MTC tumors [181]. When considered together all these studies suggest that RB1 inactivation specifically induces C-cell oncogenesis but that mutation of the *RB1* gene is a low frequency event.

In a similar manner, tumor modeling in mice has implicated roles for the RB1 regulatory CDKNs: p18 (CDKN2C), p21 (CDKN1A) and p27 (CDKN1B) in C-cell oncogenesis (see below). A role for CDKNs in MTC is supported by the observation that genetic loss of chromosome 1p (p18) is a frequent event in MTC [182], and that reproducible losses targeting other CDKN genes are observed in recent CGH array studies [168,169]. Indeed, loss of the p18 region has been seen in 20% of MTC tumors examined by CGH. A direct role for specific p18 loss in human MTC is also supported by a single study that found p18 mutations in 4 of 30 tumors examined [183]. A caveat of these studies is that mutations were observed in patients with RET driver mutations, two of which were germline C634W mutations arguing against a driver role in human tumorigenesis. Additionally, an earlier study that included 50 MTC tumors found no p18 mutations [181] while a recent exomic study that included 17 tumors found no mutations in any of the CDKN genes [164]. The incongruity that exists between CGH and mutation studies suggests that as observed in mice CDKN haploinsufficiency is sufficient to promote C-cell oncogenesis, or the inactivation of the remaining allele by mutation is a rare event. In support of a haploinsufficiency model, reduced p27 expression is significantly correlated with larger MTC tumor size and elevated serum calcitonin [184]. Further investigation is needed to elucidate the role of the Rb pathway in MTC oncogenesis; a worthwhile endeavor given that targeted therapies, which

function through direct CDK inhibition, are being developed to exploit these paths across many human cancers.

7. Modeling MTC in Animals

The first widespread animal models of MTC were the Long-Evan, Wag/Rij and Sprague Dawley rat strains. The high incidence of spontaneous MTC tumors in these mice provided the tools for development of transplantable tumors and establishment of MTC cell lines [185–187]. However, despite the high frequency of MTC in these rat strains, the underlying genetic cause has only been identified in Sprague Dawley rats as mutation of the p27 gene (*Cdkn1b*) [188]. For the Long-Evans and Wag/Rij rat strains we can only say that germline mutations of the *RET* gene do not play a role [189,85]. With the development of tools to manipulate the mouse genome, rats were largely replaced as disease models for many disorders including MTC. This section focuses on engineered mouse models of MTC. A summary of mice with a reported MTC phenotype is provided in Table 1.

7.1 RET

Activating mutations within the *RET* gene were identified as a genetic cause of MTC in human in 1993 [3,5]. With this discovery began the work to generate genetic mouse models to better understand the pathogenesis of the disease. Transgenic mice employing the CT/CGRP promoter for cell specificity predominate early models, which focused on the common *RET* mutations of C634R and M918T [190–192]. A single transgenic line has been created using nonspecific Moloney murine leukemia virus LTR [193]. A direct comparison of these models is difficult given that in addition to the variable number of copies integrated, different expression promoters were used along with expression of either long or short *RET* isoforms. Despite the variations, all mice were found to develop C-cell hyperplasia (CCH) as a precursor to MTC (Table 1). Curiously, unlike humans, the progression of MTC in mice expressing *RET* M918T is less aggressive than that observed in C634R models; this finding may relate to the use of the short *RET* isoform. Genetic knockin models, which are clearly a more accurate representation of human disease, actually fail to develop MTC [101,194,102]. However, similar to human disease, the M919T (in mice *RET* M919T is equivalent to the human MEN2B M918T) model develops earlier onset of CCH and pheochromocytoma compared to MEN2A models. Furthermore, MEN2A models display a pathology consistent with Hirschsprung's disease, which is associated with the specific *RET* C620R mutation.

The differential onset of MTC in *RET* transgenic mice and failure to develop tumors in knockin models argues for a role beyond aberrant *RET* activation in C-cell oncogenesis. Indeed in comparing the models, the site of transgene integration may be more telling than expression levels. Two studies also support that *RET*-mediated oncogenesis requires a cooperative genetic background. First, Cranston et al. demonstrated that the development of MTC was highly strain specific. Using mice with a single *RET* C634R insertion driven from the CT/CGRP promoter, tumor production varied from zero in FVB/n mice to nearly 100% in CBA/ca mice (Table 1) [195]. Of note the *RET* MEN2B knockin mouse model exists in an FVB/n mixed background. The second study is an extensive comparison of *RET* M918T-mediated tumorigenesis in mice with copy number variations of the cyclin-dependent kinase

inhibitors p18 (cdkn2c) and p27 (cdkn1b)[196]. This work demonstrates clear synergism between RET activation of MAPK pathways and deregulation of cell cycle progression (Table 1). These findings further correlate with the high frequency of allelic loss observed in chromosome 1p, where p18 maps, in human MTC tumors [168,169] and the more aggressive phenotype observed in patients with coincident somatic M918T mutation and p18 loss (unpublished observations).

7.2 RAS

The first description of HRAS activation as an oncogenic driver of C-cells actually predates the discovery that RET germline mutations cause MEN2 [155]. The development of a transgenic mouse with C-cell specific expression of activated v-ha-ras occurred much later [161]. The v-ha-ras transgenic mouse most closely resembles the RET line generated by Michiels *et al.* in that both employ the rat CT/CGRP promoter and SV40 poly adenylation sites [191]. Tumor formation in v-ha-ras mice is similar to that observed in RET transgenic mice (Table 1). Tumors were preceded by C-cell hyperplasia, were calcitonin positive, and found to be invasive in older animals. Importantly these findings are consistent with RET tumorigenesis being mediated through a ras-dependent pathway, but are in opposition to the neuroendocrine differentiation observed with ectopic expression in TT cells [160]. However, despite the high frequency of MTC formation observed in this model a comparable genetic knockin, *hras* G12V, has no overt tumor phenotype in animals up to 18 months of age [197,198]. Furthermore, Costello and Noonan syndromes, which are associated with germline *HRAS* and *KRAS/NRAS* mutation respectively, have no reported incidence of MTC [152,153]. Therefore, a clear understanding of the role of RAS activation in C-cell oncogenesis remains to be elucidated.

7.3 Other mouse models with a MTC phenotype

Three engineered mouse models with MTC actually predate the first reported RET transgenic mouse [191]. These include 2 transgenic models, one overexpressing *c-Mos* [162], the other SV40 T-antigen [199], and the earliest genetic knockouts of the *Rb1* gene [178,200,177]. Although the mechanism of *Mos*-mediated tumorigenesis was unclear, the authors speculated that *Mos* “may function in the same pathway that gives rise to MEN2.” The subsequent finding that MOS, like RET, activates the MAPK pathway serves to underscore the importance of MAPK activation in C-cell oncogenesis [201]. For the remaining models, given that one of actions of SV40 T-antigen is to inhibit Rb1 function [202], these early findings served to identify regulation of cell cycle progression as a second key pathway of C-cell oncogenesis.

Since these initial studies more than 20 engineered mouse models have been reported to have MTC as part of their phenotypic presentation (Table 1). These models nearly universally share aberrant upregulation of the MAPK pathway (Spry1) or targeted inhibition of RB1-mediated cell cycle control. A single exception is the observation that loss of prolactin receptor is associated with MTC formation [203]. How loss of prolactin receptor causes C-cell transformation remains unclear though it is interesting that, like *Rb1* loss, animals develop pituitary hyperplasia [204]. In looking at the remaining mouse models some generalized observations seem to emerge. First, the targeting of *p18* (Cdkn2c) or *p27*

(Cdkn1b), which indirectly inactivate Rb1 through enhanced phosphorylation, reproduces the *Rb1*^{+/-} MTC phenotype albeit at a reduced level. Furthermore, as predicted the simultaneous targeting of these Cdkn's with Rb1 enhances C-cell oncogenicity; combination deletions with *p53* or *Men1*, which act further upstream of Rb1, have only minor impact on tumorigenesis; and inactivation of *E2f1*, which is a downstream target of Rb1, inhibits tumorigenesis (Figure 4, Table 1). Finally, targeted inactivation of Rb1 protein, mediated through its phosphorylation by hyperactivated Cdk5, results in early onset of aggressive MTC with 100% penetrance [205]. Despite the above observations, two additional Rb1 mouse models raise questions regarding its role in C-cell oncogenesis. Similar to the actions of Cdk5, *Rb1* haploinsufficiency combined with either *E2f3* or *Nras* loss, results in the formation of highly penetrance aggressive MTC [206,207]. The counterintuitive nature of these two observations serves to underscore the need for further investigation of pathways leading to C-cell oncogenesis.

8. Considering Mechanisms of C-Cell Tumor Initiation and Progression

Unlike some cancers, a clear step-wise route to C-cell oncogenesis has not emerged. Hereditary MTC demonstrates that activating mutations of RET serve as a primary initiating event. This role of RET is further supported by the observed high prevalence (~40%) of RET mutations in sporadic MTC [208]. Several models have been proposed for how RET might function in initiation of C-cell oncogenesis. First, inactivation of RET in MTC cell culture models causes a marked reduction in tumor cell growth supporting a mechanism of oncogene addiction [209,210]. This model is in part supported by patient responses to targeted therapies [211,212]. A second proposal is that RET increases genetic instability. In patient tumors, RET mutation is associated with increases in copy number variants and somatic mutations [168,169,164]. In one report examining sporadic MTC tumors using exomic sequencing the mutation number was more than doubled in tumors with RET mutation (21.9 in RET⁺ versus 8.4 in RET⁻) [164]. In hereditary tumors somatic mutation identified by exomic sequencing appears to be less frequent [213]. Finally, a role for RET in altering the stem cell population needs to be considered in both hereditary and sporadic disease. RET is known to play a central role in regulation of human spermatogonial stem cell self-renewal [214] and neural crest stem cell lineage derivation [215]. In fact, somatic *RET*^{M918T} mutation in male germ cells is associated with enhanced stem cell proliferation and provides a plausible mechanism for the paternal origin of MEN2B [216]. While it remains to be formally examined, germline mutation of *RET* is also predicted to increase the neural crest progenitor cell population, thereby providing a larger pool of tumor initiating or the so call "cancer stem cells." A thyroid cancer stem cell model has been proposed for both medullary and nonmedullary thyroid cancer [217–219]. Support for a role in MTC derives from the observations that C-cells or their progenitors play a role in adult thyroid regeneration [220], and that *RET* mutation is associated with EGF/FGF independent [221], as well as, 5-fluorouracil resistant MTC tumorsphere growth [222].

Because MTC occurs in the absence of *RET* mutation, other pathways to C-cell oncogenesis must exist. Activation of RAS gene family members has emerged as a second oncogenic pathway with mutations observed in approximately 15% of sporadic MTCs examined [164–166,163,167]. A specific role for RAS as an initiator derives from the mouse models

previously discussed, as patients with germline *HRAS* mutations (Costello syndrome) or *KRAS/NRAS* mutations (Noonan syndrome) have not been reported to develop MTC [152]. Finally, no single gene has emerged as a common driver responsible for MTC without *RET* or *RAS* mutation. However, a focused examination remains to be performed. First, a recent comparison of Sanger-based sequencing with Ion Torrent AmpliSeq found a 30% increase in the number of RET mutations detected [180]. Furthermore, we have recently identified a case of RET gene fusion as a driver of tumorigenesis [223]. RET fusions would go undetected by most DNA sequencing approaches. Regardless a focused search for other mediator of tumorigenesis remains to be performed. At the time of writing, only 5 such MTC tumors had been examined by exome sequencing [164]. The number of reported mutations in these 5 tumors ranged from 7–10, with only the *TDG* gene found mutated in more than one tumor. Unfortunately, Sanger sequencing could not confirm these specific *TDG* mutations, and *TDG* mutation was not in a separate larger validation set. While the small sample size may have prevented discovery of other MTC genetic drivers it is also possible that noncoding RNAs or gene copy number changes went undetected because of the limitations associated with the sequencing approach [224–227]. Indeed, in mice heterozygous loss of *Rb1* or *p27* in the absence of a second mutation of the remaining allele is sufficient to induce MTC [177,200,228,229]. Although clear links establishing a role for cell cycle regulators in human C-cell oncogenesis do not exist there is supportive evidence. Copy number changes which would not be detected by exome sequencing have been observed using CGH arrays [168,169]. There have also been association studies linking increased MTC risk to genes associated with cell cycle regulation [170,230,231,184], although they are not universally positive [231]. These observations fit well with a newer model suggesting heterozygous gene loss is sufficient to drive cancer [227,226]. The observation that MTC tumorigenesis in mice is significantly enhanced when targeting multiple Rb1 pathway members, particularly in combination with the RET/RAS pathway, suggests that progression and aggressive cancer might be caused by simultaneous hits to both pathways.

9. Summary

The production and secretion of calcitonin in response to increased serum calcium remains as the only well-defined role of the thyroid C-cell. The specific linking of the C-cell as the origin of MTC through calcitonin production has served as the primary motivator for understanding the biology of this cell. Ironically, this role has proven to be physiologically redundant, as genetic ablation of calcitonin is relatively inconsequential [232]. Unfortunately, little is known about other possible physiologic roles of the C-cell and the mechanisms behind its unique sensitivity to RET-mediated oncogenesis. Genetic modeling in mice is beginning to gain insight into both processes. Studies of thyroidal development have suggested a possible interactive relationship between C-cells and follicular cells within the thyroid microenvironment [233]. Tumor modeling in mice has demonstrated that site-directed mutation of MEN2B-equivalent M919T alone is insufficient to cause MTC, but does induce pheochromocytoma. Models with unexpected MTC phenotypes have also linked oncogenesis to the Rb1-mediated pathway of cell cycle regulation. Together these pathways appear to synergize in a manner that appears to preferentially target the C-cell. Whether it is

these cellular pathways that provide the C-cell with specific sensitivity to oncogenesis remains an unanswered question. Indeed, a role for RET signaling in normal C-cell function remains to be elucidated. In the nervous system RET plays an essential role during development and in neuronal survival. A parallel role may exist for the C-cell, or perhaps like for the spermatogonia stem cell, RET functions as a C-cell signal for stem cell maintenance [95]. Roles for C-cells and calcitonin in thyroid regeneration and goiter formation have been proposed [220,234]. Such a role would certainly fit with dysregulation of these pathways being associated with oncogenesis. Thus, while we have an ever-increasing knowledge base linked to the cellular drivers of C-cell oncogenesis, the primary role of these genes in normal C-cell physiology remains unclear. A greater understanding of the normal function of these genes is likely to contribute to defining the differences observed in the biology of MTC between mouse models and human, as well as offering greater insight into pathways of tumorigenesis beyond aberrant RET activation.

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References

1. Bussolati G, Foster GV, Clark MB, Pearse AG. Immunofluorescent localisation of calcitonin in medullary C-cell thyroid carcinoma, using antibody to the pure porcine hormone. *Virchows Archiv B: Cell pathology*. 1969; 2(3):234–238. [PubMed: 4978695]
2. Bussolati G, Pearse AG. Immunofluorescent localization of calcitonin in the ‘C’ cells of pig and dog thyroid. *The Journal of endocrinology*. 1967; 37(2):205–209. [PubMed: 5334670]
3. Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells SA Jr. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. *Human molecular genetics*. 1993; 2(7):851–856. [PubMed: 8103403]
4. Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, Pasini B, Hoppener JW, van Amstel HK, Romeo G, et al. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature*. 1994; 367(6461):375–376. DOI: 10.1038/367375a0 [PubMed: 7906866]
5. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature*. 1993; 363(6428):458–460. DOI: 10.1038/363458a0 [PubMed: 8099202]
6. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, Flanagan A, Teague J, Futreal PA, Stratton MR, Wooster R. The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *British journal of cancer*. 2004; 91(2):355–358. <http://www.sanger.ac.uk/cosmic>. DOI: 10.1038/sj.bjc.6601894 [PubMed: 15188009]
7. Baber EC. Contributions to the minute anatomy of the thyroid gland of the dog. *Phil Trans R Soc*. 1876; 166:557–568.
8. Bensley RR. The thyroid gland of the opossum. *Anatomical Record*. 1914; 8:431.
9. Takagi K. A cytological study on the dog’s thyroid gland. *Folia Antomica Japonica*. 1922–23; 1:69.
10. Seecof DP. Studies on mitochondria: II. The occurrence of mitochondria-rich and mitochondria-poor cells in the thyroid gland of man and animals. *The American journal of pathology*. 1927; 3(4):365–384. 369. [PubMed: 19969754]
11. Nonidez FJ. The origin of the “parafollicular” cell, a second epithelial component of the thyroid of the dog. *Am J Anat*. 1932; 49:479–495.
12. Nonidez FJ. Further observations on the parafollicular cells of the mammalian thyroid. *Anat Rec*. 1932; 53:339–347.

13. Saito K, Shibata K. Parafollicular cell in the rat thyroid. *Endocrinologia japonica*. 1957; 4(4):253–261. [PubMed: 13537860]
14. Gabe M. Donnees histochimiques sur les cellules parafolliculaires de la glande thyrospacede du chien. *Acta Anatomica*. 1959; 38:332–344. [PubMed: 13825800]
15. Gabe M. Donnees histologiques sur les macrothyreocytes (cellules parafolliculaires) de quelques sauropsides et anamniotes. *Acta Anatomica*. 1961; 47:34–54. [PubMed: 13896053]
16. Ekholm R, Ericson LE. The ultrastructure of the parafollicular cells of the thyroid gland in the rat. *Journal of ultrastructure research*. 1968; 23(5):378–402. [PubMed: 5692296]
17. Young BA, Leblond CP. The light cell as compared to the follicular cell in the thyroid gland of the rat. *Endocrinology*. 1963; 73:669–686. DOI: 10.1210/endo-73-6-669 [PubMed: 14089625]
18. Pearse AG. Common cytochemical properties of cells producing polypeptide hormones, with particular reference to calcitonin and the thyroid C cells. *The Veterinary record*. 1966; 79(21):587–590. [PubMed: 4291207]
19. Pearse AG. 5-hydroxytryptophan uptake by dog thyroid 'C' cells, and its possible significance in polypeptide hormone production. *Nature*. 1966; 211(5049):598–600. [PubMed: 5298184]
20. Copp DH, Cockcroft DW, Kueh Y. Calcitonin from ultimobranchial glands of dogfish and chickens. *Science*. 1967; 158(3803):924–925. [PubMed: 6054163]
21. Moseley JM, Matthews EW, Breed RH, Galante L, Tse A, MacIntyre I. The ultimobranchial origin of calcitonin. *Lancet*. 1968; 1(7534):108–110. [PubMed: 4169600]
22. Pearse AG, Carvalheira AF. Cytochemical evidence for an ultimobranchial origin of rodent thyroid C cells. *Nature*. 1967; 214(5091):929–930. [PubMed: 5299162]
23. Stoeckel ME, Porte A. Ultrastructural study of ultimobranchial body of chicken. I. Normal aspect and embryonal development. *Zeitschrift fur Zellforschung und mikroskopische Anatomie*. 1969; 94(4):495–512. [PubMed: 5809185]
24. Tauber SD. The ultimobranchial origin of thyrocalcitonin. *Proceedings of the National Academy of Sciences of the United States of America*. 1967; 58(4):1684–1687. [PubMed: 5237896]
25. Kracht J, Hachmeister J, Kruse H. Thyrocalcitonin and the C cells of the thyroid gland. *Munchener medizinische Wochenschrift*. 1968; 110(4):203–208. [PubMed: 5695030]
26. Kracht J, Hachmeister U, Breustedt HJ, Lenke M. Immunohistological studies on thyrocalcitonin in C-cells. *Endokrinologie*. 1968; 52(5):395–401. [PubMed: 4883514]
27. Pearse AG. The cytochemistry of the thyroid C cells and their relationship to calcitonin. *Proceedings of the Royal Society of London Series B, Containing papers of a Biological character Royal Society*. 1966; 164(996):478–487.
28. Fagman H, Nilsson M. Morphogenesis of the thyroid gland. *Molecular and cellular endocrinology*. 2010; 323(1):35–54. DOI: 10.1016/j.mce.2009.12.008 [PubMed: 20026174]
29. Nilsson M, Fagman H. Mechanisms of thyroid development and dysgenesis: an analysis based on developmental stages and concurrent embryonic anatomy. *Current topics in developmental biology*. 2013; 106:123–170. DOI: 10.1016/B978-0-12-416021-7.00004-3 [PubMed: 24290349]
30. Le Douarin N, Le Lievre C. Demonstration of neural origin of calcitonin cells of ultimobranchial body of chick embryo. *Comptes rendus hebdomadaires des seances de l'Academie des sciences Serie D: Sciences naturelles*. 1970; 270(23):2857–2860.
31. Polak JM, Pearse AG, Le Lievre C, Fontaine J, Le Douarin NM. Immunocytochemical confirmation of the neural crest origin of avian calcitonin-producing cells. *Histochemistry*. 1974; 40(3):209–214. [PubMed: 4604566]
32. Kent ML, Harper C, Wolf JC. Documented and potential research impacts of subclinical diseases in zebrafish. *ILAR journal/National Research Council, Institute of Laboratory Animal Resources*. 2012; 53(2):126–134. DOI: 10.1093/ilar.53.2.126
33. Spitsbergen JM, Buhler DR, Peterson TS. Neoplasia and neoplasm-associated lesions in laboratory colonies of zebrafish emphasizing key influences of diet and aquaculture system design. *ILAR journal/National Research Council, Institute of Laboratory Animal Resources*. 2012; 53(2):114–125. DOI: 10.1093/ilar.53.2.114
34. Godwin MC. Complex IV in the dog with special emphasis on the relation of the ultimobranchial bodies to inter-follicular cells in the post-natal thyroid gland. *The American Journal of Anatomy*. 1937; 60:299–339.

35. Pearse AG, Polak JM. Cytochemical evidence for the neural crest origin of mammalian ultimobranchial C cells. *Histochemie Histochemistry Histochimie*. 1971; 27(2):96–102. [PubMed: 5092696]
36. Jordan RK, Scothorne RJ. An electron microscopic study of the ultimobranchial body of the sheep embryo. *Journal of anatomy*. 1972; 111(Pt 3):501–503.
37. Fagman H, Andersson L, Nilsson M. The developing mouse thyroid: embryonic vessel contacts and parenchymal growth pattern during specification, budding, migration, and lobulation. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2006; 235(2):444–455. DOI: 10.1002/dvdy.20653 [PubMed: 16331648]
38. Westerlund J, Andersson L, Carlsson T, Fagman H, Nilsson M. Misguided migration of C cell precursors to extra-thyroidal locations related to defective pharyngeal pouch development in *Shh* deficient mice. *Cell Dev Biol*. 2013; 2:129.
39. Kusakabe T, Hoshi N, Kimura S. Origin of the ultimobranchial body cyst: *T/ebp/Nkx2.1* expression is required for development and fusion of the ultimobranchial body to the thyroid. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2006; 235(5):1300–1309. DOI: 10.1002/dvdy.20655 [PubMed: 16342117]
40. Liao J, Kochilas L, Nowotschin S, Arnold JS, Aggarwal VS, Epstein JA, Brown MC, Adams J, Morrow BE. Full spectrum of malformations in velo-cardio-facial syndrome/DiGeorge syndrome mouse models by altering *Tbx1* dosage. *Human molecular genetics*. 2004; 13(15):1577–1585. DOI: 10.1093/hmg/ddh176 [PubMed: 15190012]
41. Manley NR, Capecchi MR. Hox group 3 paralogs regulate the development and migration of the thymus, thyroid, and parathyroid glands. *Developmental biology*. 1998; 195(1):1–15. DOI: 10.1006/dbio.1997.8827 [PubMed: 9520319]
42. Xu PX, Zheng W, Laclef C, Maire P, Maas RL, Peters H, Xu X. *Eya1* is required for the morphogenesis of mammalian thymus, parathyroid and thyroid. *Development*. 2002; 129(13):3033–3044. [PubMed: 12070080]
43. Kameda Y, Nishimaki T, Miura M, Jiang SX, Guillemot F. *Mash1* regulates the development of C cells in mouse thyroid glands. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2007; 236(1):262–270. DOI: 10.1002/dvdy.21018 [PubMed: 17103415]
44. Lanigan TM, DeRaad SK, Russo AF. Requirement of the MASH-1 transcription factor for neuroendocrine differentiation of thyroid C cells. *Journal of neurobiology*. 1998; 34(2):126–134. [PubMed: 9468384]
45. Lindahl M, Timmusk T, Rossi J, Saarma M, Airaksinen MS. Expression and alternative splicing of mouse *Gfra4* suggest roles in endocrine cell development. *Molecular and cellular neurosciences*. 2000; 15(6):522–533. DOI: 10.1006/mcne.2000.0845 [PubMed: 10860579]
46. Kameda Y, Nishimaki T, Chisaka O, Iseki S, Sucov HM. Expression of the epithelial marker E-cadherin by thyroid C cells and their precursors during murine development. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*. 2007; 55(10):1075–1088. DOI: 10.1369/jhc.7A7179.2007 [PubMed: 17595340]
47. May CL, Kaestner KH. Gut endocrine cell development. *Molecular and cellular endocrinology*. 2010; 323(1):70–75. DOI: 10.1016/j.mce.2009.12.009 [PubMed: 20025933]
48. Ahlgren SC, Bronner-Fraser M. Inhibition of sonic hedgehog signaling in vivo results in craniofacial neural crest cell death. *Current biology : CB*. 1999; 9(22):1304–1314. [PubMed: 10574760]
49. Brito JM, Teillet MA, Le Douarin NM. An early role for sonic hedgehog from foregut endoderm in jaw development: ensuring neural crest cell survival. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103(31):11607–11612. DOI: 10.1073/pnas.0604751103 [PubMed: 16868080]
50. Testaz S, Jarov A, Williams KP, Ling LE, Koteliensky VE, Fournier-Thibault C, Duband JL. Sonic hedgehog restricts adhesion and migration of neural crest cells independently of the Patched-Smoothed-Gli signaling pathway. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98(22):12521–12526. DOI: 10.1073/pnas.221108698 [PubMed: 11592978]

51. Pueblitz S, Weinberg AG, Albores-Saavedra J. Thyroid C cells in the DiGeorge anomaly: a quantitative study. *Pediatric pathology/affiliated with the International Paediatric Pathology Association*. 1993; 13(4):463–473.
52. Abu-Khudir R, Paquette J, Lefort A, Libert F, Chanoine JP, Vassart G, Deladoey J. Transcriptome, methylome and genomic variations analysis of ectopic thyroid glands. *PloS one*. 2010; 5(10):e13420.doi: 10.1371/journal.pone.0013420 [PubMed: 20976176]
53. Vandernoot I, Sartelet H, Abu-Khudir R, Chanoine JP, Deladoey J. Evidence for calcitonin-producing cells in human lingual thyroids. *The Journal of clinical endocrinology and metabolism*. 2012; 97(3):951–956. DOI: 10.1210/jc.2011-2772 [PubMed: 22238389]
54. Fernández-Santos, JM., Jesús Morillo-Bernal, J., Rocío García-Marín, R., José Carmelo Utrilla, JC., Inés Martín-Lacave, I. Paracrine Regulation of Thyroid-Hormone Synthesis by C Cells. In: Agrawal, NK., editor. *Thyroid Hormone*. InTech; Rijeka, Croatia: 2012.
55. Copp DH. Endocrine regulation of calcium metabolism. *Annual review of physiology*. 1970; 32:61–86. DOI: 10.1146/annurev.ph.32.030170.000425
56. Brown EM. Role of the calcium-sensing receptor in extracellular calcium homeostasis. *Best practice & research Clinical endocrinology & metabolism*. 2013; 27(3):333–343. DOI: 10.1016/j.beem.2013.02.006 [PubMed: 23856263]
57. Davey RA, Findlay DM. Calcitonin: physiology or fantasy? *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2013; 28(5):973–979. DOI: 10.1002/jbmr.1869
58. Okabe M, Graham A. The origin of the parathyroid gland. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(51):17716–17719. DOI: 10.1073/pnas.0406116101 [PubMed: 15591343]
59. Cooper CW, Schwesinger WH, Mahgoub AM, Ontjes DA. Thyrocalcitonin: stimulation of secretion by pentagastrin. *Science*. 1971; 172(3989):1238–1240. [PubMed: 5576157]
60. Hennessy JF, Gray TK, Cooper CW, Ontjes DA. Stimulation of thyrocalcitonin secretion by pentagastrin and calcium in 2 patients with medullary carcinoma of the thyroid. *The Journal of clinical endocrinology and metabolism*. 1973; 36(1):200–203. DOI: 10.1210/jcem-36-1-200 [PubMed: 4681885]
61. Reubi JC, Waser B. Unexpected high incidence of cholecystokinin-B/gastrin receptors in human medullary thyroid carcinomas. *International journal of cancer Journal international du cancer*. 1996; 67(5):644–647. DOI: 10.1002/(SICI)1097-0215(19960904)67:5<644::AID-IJC9>3.0.CO;2-U [PubMed: 8782652]
62. Desai AJ, Roberts DJ, Richards GO, Skerry TM. Role of receptor activity modifying protein 1 in function of the calcium sensing receptor in the human TT thyroid carcinoma cell line. *PloS one*. 2014; 9(1):e85237.doi: 10.1371/journal.pone.0085237 [PubMed: 24454825]
63. Garrett JE, Tamir H, Kifor O, Simin RT, Rogers KV, Mithal A, Gagel RF, Brown EM. Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. *Endocrinology*. 1995; 136(11):5202–5211. DOI: 10.1210/endo.136.11.7588259 [PubMed: 7588259]
64. Fisher DA. The unique endocrine milieu of the fetus. *The Journal of clinical investigation*. 1986; 78(3):603–611. DOI: 10.1172/JCI112616 [PubMed: 3018041]
65. Wolfe HJ, Voelkel EF, Tashjian AH Jr. Distribution of calcitonin-containing cells in the normal adult human thyroid gland: a correlation of morphology with peptide content. *The Journal of clinical endocrinology and metabolism*. 1974; 38(4):688–694. DOI: 10.1210/jcem-38-4-688 [PubMed: 4132222]
66. Bjerre Knudsen L, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ, Gotfredsen C, Egerod FL, Hegelund AC, Jacobsen H, Jacobsen SD, Moses AC, Molck AM, Nielsen HS, Nowak J, Solberg H, Thi TD, Zdravkovic M, Moerch U. Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. *Endocrinology*. 2010; 151(4):1473–1486. DOI: 10.1210/en.2009-1272 [PubMed: 20203154]
67. O'Toole K, Fenoglio-Preiser C, Pushparaj N. Endocrine changes associated with the human aging process: III. Effect of age on the number of calcitonin immunoreactive cells in the thyroid gland. *Human pathology*. 1985; 16(10):991–1000. [PubMed: 3899904]

68. Lafont AG, Wang YF, Chen GD, Liao BK, Tseng YC, Huang CJ, Hwang PP. Involvement of calcitonin and its receptor in the control of calcium-regulating genes and calcium homeostasis in zebrafish (*Danio rerio*). *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2011; 26(5):1072–1083. DOI: 10.1002/jbmr.301
69. Thomas PM, Nasonkin I, Zhang H, Gagel RF, Cote GJ. Structure of the mouse calcitonin/calcitonin gene-related peptide alpha and beta genes. *DNA sequence : the journal of DNA sequencing and mapping*. 2001; 12(2):131–135. [PubMed: 11761712]
70. Nguyen TT, Heath H 3rd, Bryant SC, O’Fallon WM, Melton LJ 3rd. Fractures after thyroidectomy in men: a population-based cohort study. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 1997; 12(7):1092–1099. DOI: 10.1359/jbmr.1997.12.7.1092
71. Daripa M, Paula FJ, Rufino AC, Foss MC. Impact of congenital calcitonin deficiency due to dysgenetic hypothyroidism on bone mineral density. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas/Sociedade Brasileira de Biofisica [et al]*. 2004; 37(1):61–68.
72. Axelrad AA, Leblond CP. Induction of thyroid tumors in rats by a low iodine diet. *Cancer*. 1955; 8(2):339–367. [PubMed: 14352174]
73. Doniach I. The effect of radioactive iodine alone and in combination with methylthiouracil upon tumour production in the rat’s thyroid gland. *British journal of cancer*. 1953; 7(2):181–202. [PubMed: 13081899]
74. Jubb KV, Mc EK. The relationship of ultimobranchial remnants and derivatives to tumors of the thyroid gland in cattle. *The Cornell veterinarian*. 1959; 49(1):41–43. passim. [PubMed: 13619293]
75. Hazard JB, Hawk WA, Crile G Jr. Medullary (solid) carcinoma of the thyroid; a clinicopathologic entity. *The Journal of clinical endocrinology and metabolism*. 1959; 19(1):152–161. DOI: 10.1210/jcem-19-1-152 [PubMed: 13620740]
76. Williams ED. Histogenesis of medullary carcinoma of the thyroid. *Journal of clinical pathology*. 1966; 19(2):114–118. [PubMed: 5948665]
77. Melvin KE, Tashjian AH Jr. The syndrome of excessive thyrocalcitonin produced by medullary carcinoma of the thyroid. *Proceedings of the National Academy of Sciences of the United States of America*. 1968; 59(4):1216–1222. [PubMed: 5240025]
78. Woodhouse NJ, Gudmundsson TV, Galante L, Nadarajah A, Buckle RM, Joplin GF, MacIntyre I. Biochemical studies on four patients with medullary carcinoma of the thyroid. *The Journal of endocrinology*. 1969; 45(1 Suppl):xvi.
79. Melvin KE, Miller HH, Tashjian AH Jr. Early diagnosis of medullary carcinoma of the thyroid gland by means of calcitonin assay. *The New England journal of medicine*. 1971; 285(20):1115–1120. DOI: 10.1056/NEJM197111112852004 [PubMed: 5095737]
80. Lindsay S, Nichols CW. Medullary thyroid carcinoma and parathyroid hyperplasia in rats. *Archives of pathology*. 1969; 88(4):402–406. [PubMed: 5822058]
81. Martin-Lacave I, Bernab R, Sampedro C, Conde E, Fernandez-Santos JM, San Martin MV, Beato A, Galera-Davidson H. Correlation between gender and spontaneous C-cell tumors in the thyroid gland of the Wistar rat. *Cell and tissue research*. 1999; 297(3):451–457. [PubMed: 10460491]
82. DeLellis RA, Nunnemacher G, Bitman WR, Gagel RF, Tashjian AH Jr, Blount M, Wolfe HJ. C-cell hyperplasia and medullary thyroid carcinoma in the rat. An immunohistochemical and ultrastructural analysis. *Laboratory investigation; a journal of technical methods and pathology*. 1979; 40(2):140–154. [PubMed: 431034]
83. Boorman GA, van Noord MJ, Hollander CF. Naturally occurring medullary thyroid carcinoma in the rat. *Archives of pathology*. 1972; 94(1):35–41. [PubMed: 5033297]
84. Lausson S, Volle GE, Bourges M, Pidoux E, Borrel C, Milhaud G, Moukhtar MS, Jullienne A, Treilhou-Lahille F. Calcitonin secretion, C cell differentiation and proliferation during the spontaneous development of murine medullary thyroid carcinoma. *Virchows Archiv : an international journal of pathology*. 1995; 426(6):611–617. [PubMed: 7655743]
85. Fritz A, Walch A, Piotrowska K, Rosemann M, Schaffer E, Weber K, Timper A, Wildner G, Graw J, Hofler H, Atkinson MJ. Recessive transmission of a multiple endocrine neoplasia syndrome in the rat. *Cancer research*. 2002; 62(11):3048–3051. [PubMed: 12036912]

86. Ward JM, Goodman DG, Squire RA, Chu KC, Linhart MS. Neoplastic and nonneoplastic lesions in aging (C57BL/6N × C3H/HeN)F1 (B6C3F1) mice. *Journal of the National Cancer Institute*. 1979; 63(3):849–854. [PubMed: 288939]
87. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer Journal international du cancer*. 2015; 136(5):E359–386. DOI: 10.1002/ijc.29210 [PubMed: 25220842]
88. Pasini B, Hofstra RM, Yin L, Bocciardi R, Santamaria G, Grootsholten PM, Ceccherini I, Patrone G, Priolo M, Buys CH, et al. The physical map of the human RET proto-oncogene. *Oncogene*. 1995; 11(9):1737–1743. [PubMed: 7478601]
89. Liu X, Vega QC, Decker RA, Pandey A, Worby CA, Dixon JE. Oncogenic RET receptors display different autophosphorylation sites and substrate binding specificities. *The Journal of biological chemistry*. 1996; 271(10):5309–5312. [PubMed: 8621380]
90. 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. *The Journal of biological chemistry*. 2004; 279(14):14213–14224. DOI: 10.1074/jbc.M312600200 [PubMed: 14711813]
91. Knowles PP, Murray-Rust J, Kjaer S, Scott RP, Hanrahan S, Santoro M, Ibanez CF, McDonald NQ. Structure and chemical inhibition of the RET tyrosine kinase domain. *The Journal of biological chemistry*. 2006; 281(44):33577–33587. DOI: 10.1074/jbc.M605604200 [PubMed: 16928683]
92. Asai N, Murakami H, Iwashita T, Takahashi M. A mutation at tyrosine 1062 in MEN2A-Ret and MEN2B-Ret impairs their transforming activity and association with shc adaptor proteins. *The Journal of biological chemistry*. 1996; 271(30):17644–17649. [PubMed: 8663426]
93. Borrello MG, Alberti L, Arighi E, Bongarzone I, Battistini C, Bardelli A, Pasini B, Piutti C, Rizzetti MG, Mondellini P, Radice MT, Pierotti MA. The full oncogenic activity of Ret/ptc2 depends on tyrosine 539, a docking site for phospholipase Cgamma. *Molecular and cellular biology*. 1996; 16(5):2151–2163. [PubMed: 8628282]
94. Encinas M, Crowder RJ, Milbrandt J, Johnson EM Jr. Tyrosine 981, a novel ret autophosphorylation site, binds c-Src to mediate neuronal survival. *The Journal of biological chemistry*. 2004; 279(18):18262–18269. DOI: 10.1074/jbc.M400505200 [PubMed: 14766744]
95. Mulligan LM. RET revisited: expanding the oncogenic portfolio. *Nature reviews Cancer*. 2014; 14(3):173–186. DOI: 10.1038/nrc3680 [PubMed: 24561444]
96. Plaza-Menacho I, Mologni L, McDonald NQ. Mechanisms of RET signaling in cancer: current and future implications for targeted therapy. *Cellular signalling*. 2014; 26(8):1743–1752. DOI: 10.1016/j.cellsig.2014.03.032 [PubMed: 24705026]
97. Lindfors PH, Lindahl M, Rossi J, Saarma M, Airaksinen MS. Ablation of persephin receptor glial cell line-derived neurotrophic factor family receptor alpha4 impairs thyroid calcitonin production in young mice. *Endocrinology*. 2006; 147(5):2237–2244. DOI: 10.1210/en.2005-1620 [PubMed: 16497798]
98. Kramer ER, Aron L, Ramakers GM, Seitz S, Zhuang X, Beyer K, Smidt MP, Klein R. Absence of Ret signaling in mice causes progressive and late degeneration of the nigrostriatal system. *PLoS biology*. 2007; 5(3):e39.doi: 10.1371/journal.pbio.0050039 [PubMed: 17298183]
99. Luesma MJ, Cantarero I, Alvarez-Dotu JM, Santander S, Junquera C. New insights into c-Ret signalling pathway in the enteric nervous system and its relationship with ALS. *BioMed research international*. 2014; 2014:328348.doi: 10.1155/2014/328348 [PubMed: 24868525]
100. Schuchardt A, D'Agati V, Larsson-Blomberg L, Costantini F, Pachnis V. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature*. 1994; 367(6461):380–383. DOI: 10.1038/367380a0 [PubMed: 8114940]
101. Carniti C, Belluco S, Riccardi E, Cranston AN, Mondellini P, Ponder BA, Scanziani E, Pierotti MA, Bongarzone I. The Ret(C620R) mutation affects renal and enteric development in a mouse model of Hirschsprung's disease. *The American journal of pathology*. 2006; 168(4):1262–1275. DOI: 10.2353/ajpath.2006.050607 [PubMed: 16565500]
102. Yin L, Puliti A, Bonora E, Evangelisti C, Conti V, Tong WM, Medard JJ, Lavoue MF, Forey N, Wang LC, Manie S, Morel G, Raccurt M, Wang ZQ, Romeo G. C620R mutation of the murine

ret proto-oncogene: loss of function effect in homozygotes and possible gain of function effect in heterozygotes. *International journal of cancer Journal international du cancer*. 2007; 121(2):292–300. DOI: 10.1002/ijc.22378 [PubMed: 17372903]

103. Meng X, Lindahl M, Hyvonen ME, Parvinen M, de Rooij DG, Hess MW, Raatikainen-Ahokas A, Sainio K, Rauvala H, Lakso M, Pichel JG, Westphal H, Saarma M, Sariola H. Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. *Science*. 2000; 287(5457):1489–1493. [PubMed: 10688798]
104. Hofmann MC. Gdnf signaling pathways within the mammalian spermatogonial stem cell niche. *Molecular and cellular endocrinology*. 2008; 288(1–2):95–103. DOI: 10.1016/j.mce.2008.04.012 [PubMed: 18485583]
105. Hofmann MC, Braydich-Stolle L, Dym M. Isolation of male germ-line stem cells; influence of GDNF. *Developmental biology*. 2005; 279(1):114–124. DOI: 10.1016/j.ydbio.2004.12.006 [PubMed: 15708562]
106. Braydich-Stolle L, Kostereva N, Dym M, Hofmann MC. Role of Src family kinases and N-Myc in spermatogonial stem cell proliferation. *Developmental biology*. 2007; 304(1):34–45. DOI: 10.1016/j.ydbio.2006.12.013 [PubMed: 17222400]
107. Essegir S, Todd SK, Hunt T, Poulson R, Plaza-Menacho I, Reis-Filho JS, Isacke CM. A role for glial cell derived neurotrophic factor induced expression by inflammatory cytokines and RET/GFR alpha 1 receptor up-regulation in breast cancer. *Cancer research*. 2007; 67(24):11732–11741. DOI: 10.1158/0008-5472.CAN-07-2343 [PubMed: 18089803]
108. Gainor JF, Shaw AT. The new kid on the block: RET in lung cancer. *Cancer discovery*. 2013; 3(6):604–606. DOI: 10.1158/2159-8290.CD-13-0174 [PubMed: 23749525]
109. Ito Y, Okada Y, Sato M, Sawai H, Funahashi H, Murase T, Hayakawa T, Manabe T. Expression of glial cell line-derived neurotrophic factor family members and their receptors in pancreatic cancers. *Surgery*. 2005; 138(4):788–794. DOI: 10.1016/j.surg.2005.07.007 [PubMed: 16269310]
110. Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, Sakamoto H, Tsuta K, Furuta K, Shimada Y, Iwakawa R, Ogiwara H, Oike T, Enari M, Schetter AJ, Okayama H, Haugen A, Skaug V, Chiku S, Yamanaka I, Arai Y, Watanabe S, Sekine I, Ogawa S, Harris CC, Tsuda H, Yoshida T, Yokota J, Shibata T. KIF5B-RET fusions in lung adenocarcinoma. *Nature medicine*. 2012; 18(3):375–377. DOI: 10.1038/nm.2644
111. Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, Curran JA, Balasubramanian S, Bloom T, Brennan KW, Donahue A, Downing SR, Frampton GM, Garcia L, Juhn F, Mitchell KC, White E, White J, Zwirko Z, Peretz T, Nechushtan H, Soussan-Gutman L, Kim J, Sasaki H, Kim HR, Park SI, Ercan D, Sheehan CE, Ross JS, Cronin MT, Janne PA, Stephens PJ. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nature medicine*. 2012; 18(3):382–384. DOI: 10.1038/nm.2673
112. Morandi A, Martin LA, Gao Q, Pancholi S, Mackay A, Robertson D, Zvelebil M, Dowsett M, Plaza-Menacho I, Isacke CM. GDNF-RET signaling in ER-positive breast cancers is a key determinant of response and resistance to aromatase inhibitors. *Cancer research*. 2013; 73(12):3783–3795. DOI: 10.1158/0008-5472.CAN-12-4265 [PubMed: 23650283]
113. Plaza-Menacho I, Morandi A, Robertson D, Pancholi S, Drury S, Dowsett M, Martin LA, Isacke CM. Targeting the receptor tyrosine kinase RET sensitizes breast cancer cells to tamoxifen treatment and reveals a role for RET in endocrine resistance. *Oncogene*. 2010; 29(33):4648–4657. DOI: 10.1038/onc.2010.209 [PubMed: 20531297]
114. Sawai H, Okada Y, Kazanjian K, Kim J, Hasan S, Hines OJ, Reber HA, Hoon DS, Eibl G. The G691S RET polymorphism increases glial cell line-derived neurotrophic factor-induced pancreatic cancer cell invasion by amplifying mitogen-activated protein kinase signaling. *Cancer research*. 2005; 65(24):11536–11544. DOI: 10.1158/0008-5472.CAN-05-2843 [PubMed: 16357163]
115. Figlioli G, Landi S, Romei C, Elisei R, Gemignani F. Medullary thyroid carcinoma (MTC) and RET proto-oncogene: mutation spectrum in the familial cases and a meta-analysis of studies on the sporadic form. *Mutation research*. 2013; 752(1):36–44. DOI: 10.1016/j.mrrev.2012.09.002 [PubMed: 23059849]

116. 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(5196):381–383. [PubMed: 7824936]
117. Arighi E, Borrello MG, Sariola H. RET tyrosine kinase signaling in development and cancer. *Cytokine & growth factor reviews*. 2005; 16(4–5):441–467. DOI: 10.1016/j.cytogfr.2005.05.010 [PubMed: 15982921]
118. Santoro M, Carlomagno F. Central role of RET in thyroid cancer. *Cold Spring Harbor perspectives in biology*. 2013; 5(12):a009233.doi: 10.1101/cshperspect.a009233 [PubMed: 24296167]
119. Asai N, Iwashita T, Matsuyama M, Takahashi M. Mechanism of activation of the ret proto-oncogene by multiple endocrine neoplasia 2A mutations. *Molecular and cellular biology*. 1995; 15(3):1613–1619. [PubMed: 7532281]
120. Borrello MG, Smith DP, Pasini B, Bongarzone I, Greco A, Lorenzo MJ, Arighi E, Miranda C, Eng C, Alberti L, et al. RET activation by germline MEN2A and MEN2B mutations. *Oncogene*. 1995; 11(11):2419–2427. [PubMed: 8570194]
121. Salvatore D, Melillo RM, Monaco C, Visconti R, Fenzi G, Vecchio G, Fusco A, Santoro M. Increased in vivo phosphorylation of ret tyrosine 1062 is a potential pathogenetic mechanism of multiple endocrine neoplasia type 2B. *Cancer research*. 2001; 61(4):1426–1431. [PubMed: 11245446]
122. Songyang Z, Carraway KL 3rd, Eck MJ, Harrison SC, Feldman RA, Mohammadi M, Schlessinger J, Hubbard SR, Smith DP, Eng C, et al. Catalytic specificity of protein-tyrosine kinases is critical for selective signalling. *Nature*. 1995; 373(6514):536–539. DOI: 10.1038/373536a0 [PubMed: 7845468]
123. Malumbres M, Pellicer A. RAS pathways to cell cycle control and cell transformation. *Frontiers in bioscience : a journal and virtual library*. 1998; 3:d887–912. [PubMed: 9696882]
124. Wennerberg K, Rossman KL, Der CJ. The Ras superfamily at a glance. *Journal of cell science*. 2005; 118(Pt 5):843–846. DOI: 10.1242/jcs.01660 [PubMed: 15731001]
125. Buss JE, Soliski PA, Schaeffer JP, MacDonald MJ, Der CJ. Activation of the cellular proto-oncogene product p21Ras by addition of a myristylation signal. *Science*. 1989; 243(4898):1600–1603. [PubMed: 2648572]
126. Carboni JM, Yan N, Cox AD, Bustelo X, Graham SM, Lynch MJ, Weinmann R, Seizinger BR, Der CJ, Barbacid M, et al. Farnesyltransferase inhibitors are inhibitors of Ras but not R-Ras2/TC21, transformation. *Oncogene*. 1995; 10(10):1905–1913. [PubMed: 7761092]
127. Cox AD, Hisaka MM, Buss JE, Der CJ. Specific isoprenoid modification is required for function of normal, but not oncogenic, Ras protein. *Molecular and cellular biology*. 1992; 12(6):2606–2615. [PubMed: 1375323]
128. Cherfils J, Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiological reviews*. 2013; 93(1):269–309. DOI: 10.1152/physrev.00003.2012 [PubMed: 23303910]
129. Avruch J, Khokhlatchev A, Kyriakis JM, Luo Z, Tzivion G, Vavvas D, Zhang XF. Ras activation of the Raf kinase: tyrosine kinase recruitment of the MAP kinase cascade. *Recent progress in hormone research*. 2001; 56:127–155. [PubMed: 11237210]
130. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A, Stivala F, Libra M, Basecke J, Evangelisti C, Martelli AM, Franklin RA. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochimica et biophysica acta*. 2007; 1773(8):1263–1284. DOI: 10.1016/j.bbamcr.2006.10.001 [PubMed: 17126425]
131. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nature reviews Molecular cell biology*. 2009; 10(5):307–318. DOI: 10.1038/nrm2672 [PubMed: 19339977]
132. Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, Waterfield MD, Downward J. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature*. 1994; 370(6490):527–532. DOI: 10.1038/370527a0 [PubMed: 8052307]

133. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*. 2005; 307(5712):1098–1101. DOI: 10.1126/science.1106148 [PubMed: 15718470]
134. Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proceedings of the National Academy of Sciences of the United States of America*. 1987; 84(14):5034–5037. [PubMed: 3037531]
135. Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. *Nature reviews Molecular cell biology*. 2010; 11(5):329–341. DOI: 10.1038/nrm2882 [PubMed: 20379207]
136. Castellano E, Downward J. RAS Interaction with PI3K: More Than Just Another Effector Pathway. *Genes & cancer*. 2011; 2(3):261–274. DOI: 10.1177/1947601911408079 [PubMed: 21779497]
137. Stephen AG, Esposito D, Bagni RK, McCormick F. Dragging ras back in the ring. *Cancer Cell*. 2014; 25(3):272–281. DOI: 10.1016/j.ccr.2014.02.017 [PubMed: 24651010]
138. Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. *Cancer research*. 2012; 72(10):2457–2467. DOI: 10.1158/0008-5472.CAN-11-2612 [PubMed: 22589270]
139. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: Mission possible? *Nature reviews Drug discovery*. 2014; 13(11):828–851. DOI: 10.1038/nrd4389 [PubMed: 25323927]
140. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nature reviews Cancer*. 2011; 11(11):761–774. DOI: 10.1038/nrc3106 [PubMed: 21993244]
141. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. *Nature reviews Cancer*. 2003; 3(6):459–465. DOI: 10.1038/nrc1097 [PubMed: 12778136]
142. Esteban LM, Vicario-Abejon C, Fernandez-Salguero P, Fernandez-Medarde A, Swaminathan N, Yienger K, Lopez E, Malumbres M, McKay R, Ward JM, Pellicer A, Santos E. Targeted genomic disruption of H-ras and N-ras, individually or in combination, reveals the dispensability of both loci for mouse growth and development. *Molecular and cellular biology*. 2001; 21(5):1444–1452. DOI: 10.1128/MCB.21.5.1444-1452.2001 [PubMed: 11238881]
143. Johnson L, Greenbaum D, Cichowski K, Mercer K, Murphy E, Schmitt E, Bronson RT, Umanoff H, Edelmann W, Kucherlapati R, Jacks T. K-ras is an essential gene in the mouse with partial functional overlap with N-ras. *Genes & development*. 1997; 11(19):2468–2481. [PubMed: 9334313]
144. Koera K, Nakamura K, Nakao K, Miyoshi J, Toyoshima K, Hatta T, Otani H, Aiba A, Katsuki M. K-ras is essential for the development of the mouse embryo. *Oncogene*. 1997; 15(10):1151–1159. DOI: 10.1038/sj.onc.1201284 [PubMed: 9294608]
145. Umanoff H, Edelmann W, Pellicer A, Kucherlapati R. The murine N-ras gene is not essential for growth and development. *Proceedings of the National Academy of Sciences of the United States of America*. 1995; 92(5):1709–1713. [PubMed: 7878045]
146. Shirasawa S, Furuse M, Yokoyama N, Sasazuki T. Altered growth of human colon cancer cell lines disrupted at activated Ki-ras. *Science*. 1993; 260(5104):85–88. [PubMed: 8465203]
147. Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes & development*. 2000; 14(19):2501–2514. [PubMed: 11018017]
148. Cirstea IC, Kutsche K, Dvorsky R, Gremer L, Carta C, Horn D, Roberts AE, Lepri F, Merbitz-Zahradnik T, Konig R, Kratz CP, Pantaleoni F, Dentici ML, Joshi VA, Kucherlapati RS, Mazzanti L, Mundlos S, Patton MA, Silengo MC, Rossi C, Zampino G, Digilio C, Stuppia L, Seemanova E, Pennacchio LA, Gelb BD, Dallapiccola B, Wittinghofer A, Ahmadian MR, Tartaglia M, Zenker M. A restricted spectrum of NRAS mutations causes Noonan syndrome. *Nature genetics*. 2010; 42(1):27–29. DOI: 10.1038/ng.497 [PubMed: 19966803]
149. Schubbert S, Zenker M, Rowe SL, Boll S, Klein C, Bollag G, van der Burgt I, Musante L, Kalscheuer V, Wehner LE, Nguyen H, West B, Zhang KY, Sistermans E, Rauch A, Niemeyer

- CM, Shannon K, Kratz CP. Germline KRAS mutations cause Noonan syndrome. *Nature genetics*. 2006; 38(3):331–336. DOI: 10.1038/ng1748 [PubMed: 16474405]
150. Aoki Y, Niihori T, Kawame H, Kurosawa K, Ohashi H, Tanaka Y, Filocamo M, Kato K, Suzuki Y, Kure S, Matsubara Y. Germline mutations in HRAS proto-oncogene cause Costello syndrome. *Nature genetics*. 2005; 37(10):1038–1040. DOI: 10.1038/ng1641 [PubMed: 16170316]
 151. Gripp KW. Tumor predisposition in Costello syndrome. *American journal of medical genetics Part C, Seminars in medical genetics*. 2005; 137C(1):72–77. DOI: 10.1002/ajmg.c.30065
 152. Rauen KA. The RASopathies. *Annual review of genomics and human genetics*. 2013; 14:355–369. DOI: 10.1146/annurev-genom-091212-153523
 153. Bezniakow N, Gos M, Obersztyn E. The RASopathies as an example of RAS/MAPK pathway disturbances - clinical presentation and molecular pathogenesis of selected syndromes. *Developmental period medicine*. 2014; 18(3):285–296. [PubMed: 25182392]
 154. Milburn MV, Tong L, deVos AM, Brunger A, Yamaizumi Z, Nishimura S, Kim SH. Molecular switch for signal transduction: structural differences between active and inactive forms of protooncogenic ras proteins. *Science*. 1990; 247(4945):939–945. [PubMed: 2406906]
 155. Okazaki M, Miya A, Tanaka N, Miki T, Yamamoto M, Motomura K, Miyauchi A, Mori T, Takai S. Allele loss on chromosome 10 and point mutation of ras oncogenes are infrequent in tumors of MEN 2A. *Henry Ford Hospital medical journal*. 1989; 37(3–4):112–115. [PubMed: 2576939]
 156. Bockhorn M, Frilling A, Kalinin V, Schroder S, Broelsch CE. Absence of H- and K-ras oncogene mutations in sporadic medullary thyroid carcinoma. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association*. 2000; 108(1):49–53.
 157. Horie H, Yokogoshi Y, Tsuyuguchi M, Saito S. Point mutations of ras and Gs alpha subunit genes in thyroid tumors. *Japanese journal of cancer research : Gann*. 1995; 86(8):737–742. [PubMed: 7559096]
 158. Moley JF, Brother MB, Wells SA, Spengler BA, Biedler JL, Brodeur GM. Low frequency of ras gene mutations in neuroblastomas, pheochromocytomas, and medullary thyroid cancers. *Cancer research*. 1991; 51(6):1596–1599. [PubMed: 1998949]
 159. Carson-Walter EB, Smith DP, Ponder BA, Baylin SB, Nelkin BD. Post-transcriptional silencing of RET occurs, but is not required, during raf-1 mediated differentiation of medullary thyroid carcinoma cells. *Oncogene*. 1998; 17(3):367–376. DOI: 10.1038/sj.onc.1201938 [PubMed: 9690518]
 160. Nakagawa T, Mabry M, de Bustros A, Ihle JN, Nelkin BD, Baylin SB. Introduction of v-Ha-ras oncogene induces differentiation of cultured human medullary thyroid carcinoma cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1987; 84(16):5923–5927. [PubMed: 3112776]
 161. Johnston D, Hatzis D, Sunday ME. Expression of v-Ha-ras driven by the calcitonin/calcitonin gene-related peptide promoter: a novel transgenic murine model for medullary thyroid carcinoma. *Oncogene*. 1998; 16(2):167–177. DOI: 10.1038/sj.onc.1201478 [PubMed: 9464534]
 162. Schulz N, Propst F, Rosenberg MP, Linnoila RI, Paules RS, Kovatch R, Ogiso Y, Vande Woude G. Pheochromocytomas and C-cell thyroid neoplasms in transgenic c-mos mice: a model for the human multiple endocrine neoplasia type 2 syndrome. *Cancer research*. 1992; 52(2):450–455. [PubMed: 1345812]
 163. Moura MM, Cavaco BM, Pinto AE, Leite V. High prevalence of RAS mutations in RET-negative sporadic medullary thyroid carcinomas. *The Journal of clinical endocrinology and metabolism*. 2011; 96(5):E863–868. DOI: 10.1210/jc.2010-1921 [PubMed: 21325462]
 164. Agrawal N, Jiao Y, Sausen M, Leary R, Bettegowda C, Roberts NJ, Bhan S, Ho AS, Khan Z, Bishop J, Westra WH, Wood LD, Hruban RH, Tufano RP, Robinson B, Dralle H, Toledo SP, Toledo RA, Morris LG, Ghossein RA, Fagin JA, Chan TA, Velculescu VE, Vogelstein B, Kinzler KW, Papadopoulos N, Nelkin BD, Ball DW. Exomic sequencing of medullary thyroid cancer reveals dominant and mutually exclusive oncogenic mutations in RET and RAS. *The Journal of clinical endocrinology and metabolism*. 2013; 98(2):E364–369. DOI: 10.1210/jc.2012-2703 [PubMed: 23264394]

165. Boichard A, Croux L, Al Ghuzlan A, Broutin S, Dupuy C, Leboulleux S, Schlumberger M, Bidart JM, Lacroix L. Somatic RAS mutations occur in a large proportion of sporadic RET-negative medullary thyroid carcinomas and extend to a previously unidentified exon. *The Journal of clinical endocrinology and metabolism*. 2012; 97(10):E2031–2035. DOI: 10.1210/jc.2012-2092 [PubMed: 22865907]
166. Ciampi R, Mian C, Fugazzola L, Cosci B, Romei C, Barollo S, Cirello V, Bottici V, Marconcini G, Rosa PM, Borrello MG, Basolo F, Ugolini C, Materazzi G, Pinchera A, Elisei R. Evidence of a low prevalence of RAS mutations in a large medullary thyroid cancer series. *Thyroid : official journal of the American Thyroid Association*. 2013; 23(1):50–57. DOI: 10.1089/thy.2012.0207 [PubMed: 23240926]
167. Schulten HJ, Al-Maghrabi J, Al-Ghamdi K, Salama S, Al-Muhayawi S, Chaudhary A, Hamour O, Abuzenadah A, Gari M, Al-Qahtani M. Mutational screening of RET, HRAS, KRAS, NRAS, BRAF, AKT1, and CTNNB1 in medullary thyroid carcinoma. *Anticancer research*. 2011; 31(12):4179–4183. [PubMed: 22199277]
168. Flicker K, Ulz P, Hoger H, Zeithofer P, Haas OA, Behmel A, Buchinger W, Scheuba C, Niederle B, Pfragner R, Speicher MR. High-resolution analysis of alterations in medullary thyroid carcinoma genomes. *International journal of cancer Journal international du cancer*. 2012; 131(2):E66–73. DOI: 10.1002/ijc.26494 [PubMed: 22038905]
169. Ye L, Santarpia L, Cote GJ, El-Naggar AK, Gagel RF. High resolution array-comparative genomic hybridization profiling reveals deoxyribonucleic acid copy number alterations associated with medullary thyroid carcinoma. *The Journal of clinical endocrinology and metabolism*. 2008; 93(11):4367–4372. DOI: 10.1210/jc.2008-0912 [PubMed: 18765511]
170. Barbieri RB, Bufalo NE, Secolin R, Assumpcao LV, Maciel RM, Cerutti JM, Ward LS. Polymorphisms of cell cycle control genes influence the development of sporadic medullary thyroid carcinoma. *European journal of endocrinology/European Federation of Endocrine Societies*. 2014; 171(6):761–767. DOI: 10.1530/EJE-14-0461
171. Knudsen ES, Wang JY. Targeting the RB-pathway in cancer therapy. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2010; 16(4):1094–1099. DOI: 10.1158/1078-0432.CCR-09-0787 [PubMed: 20145169]
172. Zhu L, Lu Z, Zhao H. Antitumor mechanisms when pRb and p53 are genetically inactivated. *Oncogene*. 2014; doi: 10.1038/onc.2014.399
173. Chinnam M, Goodrich DW. RB1, development, and cancer. *Current topics in developmental biology*. 2011; 94:129–169. DOI: 10.1016/B978-0-12-380916-2.00005-X [PubMed: 21295686]
174. Di Fiore R, D'Anneo A, Tesoriere G, Vento R. RB1 in cancer: different mechanisms of RB1 inactivation and alterations of pRb pathway in tumorigenesis. *Journal of cellular physiology*. 2013; 228(8):1676–1687. DOI: 10.1002/jcp.24329 [PubMed: 23359405]
175. Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America*. 1971; 68(4):820–823. [PubMed: 5279523]
176. Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. Effects of an Rb mutation in the mouse. *Nature*. 1992; 359(6393):295–300. DOI: 10.1038/359295a0 [PubMed: 1406933]
177. Williams BO, Remington L, Albert DM, Mukai S, Bronson RT, Jacks T. Cooperative tumorigenic effects of germline mutations in Rb and p53. *Nature genetics*. 1994; 7(4):480–484. DOI: 10.1038/ng0894-480 [PubMed: 7951317]
178. Harrison DJ, Hooper ML, Armstrong JF, Clarke AR. Effects of heterozygosity for the Rb-1t19neo allele in the mouse. *Oncogene*. 1995; 10(8):1615–1620. [PubMed: 7731716]
179. Kanagal-Shamanna R, Portier BP, Singh RR, Routhort MJ, Aldape KD, Handal BA, Rahimi H, Reddy NG, Barkoh BA, Mishra BM, Paladugu AV, Manekia JH, Kalhor N, Chowdhuri SR, Staerkel GA, Medeiros LJ, Luthra R, Patel KP. Next-generation sequencing-based multi-gene mutation profiling of solid tumors using fine needle aspiration samples: promises and challenges for routine clinical diagnostics. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc*. 2014; 27(2):314–327. DOI: 10.1038/modpathol.2013.122
180. Simbolo M, Mian C, Barollo S, Fassan M, Mafficini A, Neves D, Scardoni M, Pennelli G, Rugge M, Pelizzo MR, Cavedon E, Fugazzola L, Scarpa A. High-throughput mutation profiling

improves diagnostic stratification of sporadic medullary thyroid carcinomas. *Virchows Archiv : an international journal of pathology*. 2014; 465(1):73–78. DOI: 10.1007/s00428-014-1589-3 [PubMed: 24828033]

181. Holm R, Nesland JM. Retinoblastoma and p53 tumour suppressor gene protein expression in carcinomas of the thyroid gland. *The Journal of pathology*. 1994; 172(3):267–272. DOI: 10.1002/path.1711720307 [PubMed: 8195930]
182. Mathew CG, Smith BA, Thorpe K, Wong Z, Royle NJ, Jeffreys AJ, Ponder BA. Deletion of genes on chromosome 1 in endocrine neoplasia. *Nature*. 1987; 328(6130):524–526. DOI: 10.1038/328524a0 [PubMed: 3614355]
183. van Veelen W, Klompmaker R, Gloerich M, van Gasteren CJ, Kalkhoven E, Berger R, Lips CJ, Medema RH, Hoppener JW, Acton DS. P18 is a tumor suppressor gene involved in human medullary thyroid carcinoma and pheochromocytoma development. *International journal of cancer Journal international du cancer*. 2009; 124(2):339–345. DOI: 10.1002/ijc.23977 [PubMed: 18942719]
184. Ito Y, Yoshida H, Nakamura Y, Tomoda C, Uruno T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Kuma K, Kakudo K, Miyauchi A. Expression of Jun activation domain-binding protein 1 and p27 (Kip1) in thyroid medullary carcinoma. *Pathology*. 2005; 37(3):216–219. [PubMed: 16175894]
185. Muszynski M, Birnbaum RS, Roos BA. Glucocorticoids stimulate the production of preprocalcitonin-derived secretory peptides by a rat medullary thyroid carcinoma cell line. *The Journal of biological chemistry*. 1983; 258(19):11678–11683. [PubMed: 6311819]
186. Zeytinoglu FN, Brazeau P, Mougins C. Regulation of neurotensin secretion in a mammalian C cell line: effect of dexamethasone. *Regulatory peptides*. 1983; 6(2):147–154. [PubMed: 6878753]
187. Zeytinoglu FN, DeLellis RA, Gagel RF, Wolfe HJ, Tashjian AH Jr. Establishment of a calcitonin-producing rat medullary thyroid carcinoma cell line. I. Morphological studies of the tumor and cells in culture. *Endocrinology*. 1980; 107(2):509–515. DOI: 10.1210/endo-107-2-509 [PubMed: 6248326]
188. Pellegata NS, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, Hofler H, Fend F, Graw J, Atkinson MJ. Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103(42):15558–15563. DOI: 10.1073/pnas.0603877103 [PubMed: 17030811]
189. De Miguel M, Fernandez-Santos JM, Trigo-Sanchez I, Matera I, Ceccherini I, Martin I, Romeo G, Galera-Davidson H. The Ret proto-oncogene in the WAG/Rij rat strain: an animal model for inherited C-cell carcinoma? *Laboratory animals*. 2003; 37(3):215–221. DOI: 10.1258/002367703766453065 [PubMed: 12869284]
190. Acton DS, Velthuyzen D, Lips CJ, Hoppener JW. Multiple endocrine neoplasia type 2B mutation in human RET oncogene induces medullary thyroid carcinoma in transgenic mice. *Oncogene*. 2000; 19(27):3121–3125. DOI: 10.1038/sj.onc.1203648 [PubMed: 10871866]
191. Michiels FM, Chappuis S, Caillou B, Pasini A, Talbot M, Monier R, Lenoir GM, Feunteun J, Billaud M. Development of medullary thyroid carcinoma in transgenic mice expressing the RET protooncogene altered by a multiple endocrine neoplasia type 2A mutation. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94(7):3330–3335. [PubMed: 9096393]
192. Reynolds L, Jones K, Winton DJ, Cranston A, Houghton C, Howard L, Ponder BA, Smith DP. C-cell and thyroid epithelial tumours and altered follicular development in transgenic mice expressing the long isoform of MEN 2A RET. *Oncogene*. 2001; 20(30):3986–3994. DOI: 10.1038/sj.onc.1204434 [PubMed: 11494127]
193. Kawai K, Iwashita T, Murakami H, Hiraiwa N, Yoshiki A, Kusakabe M, Ono K, Iida K, Nakayama A, Takahashi M. Tissue-specific carcinogenesis in transgenic mice expressing the RET proto-oncogene with a multiple endocrine neoplasia type 2A mutation. *Cancer research*. 2000; 60(18):5254–5260. [PubMed: 11016655]
194. Smith-Hicks CL, Sizer KC, Powers JF, Tischler AS, Costantini F. C-cell hyperplasia, pheochromocytoma and sympathoadrenal malformation in a mouse model of multiple endocrine neoplasia type 2B. *The EMBO journal*. 2000; 19(4):612–622. DOI: 10.1093/emboj/19.4.612 [PubMed: 10675330]

195. Cranston AN, Ponder BA. Modulation of medullary thyroid carcinoma penetrance suggests the presence of modifier genes in a RET transgenic mouse model. *Cancer research*. 2003; 63(16): 4777–4780. [PubMed: 12941793]
196. van Veelen W, van Gasteren CJ, Acton DS, Franklin DS, Berger R, Lips CJ, Hoppener JW. Synergistic effect of oncogenic RET and loss of p18 on medullary thyroid carcinoma development. *Cancer research*. 2008; 68(5):1329–1337. DOI: 10.1158/0008-5472.CAN-07-5754 [PubMed: 18316595]
197. Chen X, Mitsutake N, LaPerle K, Akeno N, Zanzonico P, Longo VA, Mitsutake S, Kimura ET, Geiger H, Santos E, Wendel HG, Franco A, Knauf JA, Fagin JA. Endogenous expression of Hras(G12V) induces developmental defects and neoplasms with copy number imbalances of the oncogene. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106(19):7979–7984. DOI: 10.1073/pnas.0900343106 [PubMed: 19416908]
198. Schuhmacher AJ, Guerra C, Sauzeau V, Canamero M, Bustelo XR, Barbacid M. A mouse model for Costello syndrome reveals an Ang II-mediated hypertensive condition. *The Journal of clinical investigation*. 2008; 118(6):2169–2179. DOI: 10.1172/JCI34385 [PubMed: 18483625]
199. Baetscher M, Schmidt E, Shimizu A, Leder P, Fishman MC. SV40 T antigen transforms calcitonin cells of the thyroid but not CGRP-containing neurons in transgenic mice. *Oncogene*. 1991; 6(7):1133–1138. [PubMed: 1650439]
200. Harvey M, Vogel H, Lee EY, Bradley A, Donehower LA. Mice deficient in both p53 and Rb develop tumors primarily of endocrine origin. *Cancer research*. 1995; 55(5):1146–1151. [PubMed: 7867001]
201. Okazaki K, Sagata N. MAP kinase activation is essential for oncogenic transformation of NIH3T3 cells by Mos. *Oncogene*. 1995; 10(6):1149–1157. [PubMed: 7700641]
202. DeCaprio JA, Ludlow JW, Figge J, Shew JY, Huang CM, Lee WH, Marsilio E, Paucha E, Livingston DM. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. *Cell*. 1988; 54(2):275–283. [PubMed: 2839300]
203. Kedzia C, Lacroix L, Ameur N, Ragot T, Kelly PA, Caillou B, Binart N. Medullary thyroid carcinoma arises in the absence of prolactin signaling. *Cancer research*. 2005; 65(18):8497–8503. DOI: 10.1158/0008-5472.CAN-04-3937 [PubMed: 16166330]
204. Schuff KG, Hentges ST, Kelly MA, Binart N, Kelly PA, Iuvone PM, Asa SL, Low MJ. Lack of prolactin receptor signaling in mice results in lactotroph proliferation and prolactinomas by dopamine-dependent and -independent mechanisms. *The Journal of clinical investigation*. 2002; 110(7):973–981. DOI: 10.1172/JCI15912 [PubMed: 12370275]
205. Pozo K, Castro-Rivera E, Tan C, Plattner F, Schwach G, Siegl V, Meyer D, Guo A, Gundara J, Mettlach G, Richer E, Guevara J, Ning L, Gupta A, Hao G, Tsai L, Sun X, Antich P, Sidhu S, Robinson B, Chen H, Nwariaku F, Pfragner R, Richardson J, Bibb J. The role of Cdk5 in neuroendocrine thyroid cancer. *Cancer Cell*. 2013; 24(4):499–511. [PubMed: 24135281]
206. Ziebold U, Lee EY, Bronson RT, Lees JA. 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*. 2003; 23(18):6542–6552. [PubMed: 12944480]
207. Takahashi C, Contreras B, Iwanaga T, Takegami Y, Bakker A, Bronson RT, Noda M, Loda M, Hunt JL, Ewen ME. Nras loss induces metastatic conversion of Rb1-deficient neuroendocrine thyroid tumor. *Nature genetics*. 2006; 38(1):118–123. DOI: 10.1038/ng1703 [PubMed: 16369533]
208. Hu MI, Cote GJ. Medullary thyroid carcinoma: who's on first? *Thyroid : official journal of the American Thyroid Association*. 2012; 22(5):451–453. DOI: 10.1089/thy.2012.2205.ed [PubMed: 22545752]
209. Drost M, Putzer BM. Mechanisms of Disease: cancer targeting and the impact of oncogenic RET for medullary thyroid carcinoma therapy. *Nature clinical practice Oncology*. 2006; 3(10): 564–574. DOI: 10.1038/ncponc0610
210. 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. *Endocrine-related cancer*. 2011; 18(1):1–11. DOI: 10.1677/ERC-09-0292 [PubMed: 20943719]

211. Elisei R, Schlumberger MJ, Muller SP, Schoffski P, Brose MS, Shah MH, Licitra L, Jarzab B, Medvedev V, Kreissl MC, Niederle B, Cohen EE, Wirth LJ, Ali H, Hessel C, Yaron Y, Ball D, Nelkin B, Sherman SI. Cabozantinib in progressive medullary thyroid cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013; 31(29):3639–3646. DOI: 10.1200/JCO.2012.48.4659 [PubMed: 24002501]
212. 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. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012; 30(2):134–141. DOI: 10.1200/JCO.2011.35.5040 [PubMed: 22025146]
213. Cai J, Li L, Ye L, Jiang X, Shen L, Gao Z, Fang W, Huang F, Su T, Zhou Y, Wang W, Ning G. Exome sequencing reveals mutant genes with low penetrance involved in MEN2A-associated tumorigenesis. *Endocrine-related cancer*. 2015; 22(1):23–33. DOI: 10.1530/ERC-14-0225 [PubMed: 25404689]
214. Wu X, Schmidt JA, Avarbock MR, Tobias JW, Carlson CA, Kolon TF, Ginsberg JP, Brinster RL. Prepubertal human spermatogonia and mouse gonocytes share conserved gene expression of germline stem cell regulatory molecules. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106(51):21672–21677. DOI: 10.1073/pnas.0912432106 [PubMed: 20018717]
215. Iwashita T, Kruger GM, Pardal R, Kiel MJ, Morrison SJ. Hirschsprung disease is linked to defects in neural crest stem cell function. *Science*. 2003; 301(5635):972–976. DOI: 10.1126/science.1085649 [PubMed: 12920301]
216. Choi SK, Yoon SR, Calabrese P, Arnheim N. Positive selection for new disease mutations in the human germline: evidence from the heritable cancer syndrome multiple endocrine neoplasia type 2B. *PLoS genetics*. 2012; 8(2):e1002420.doi: 10.1371/journal.pgen.1002420 [PubMed: 22359510]
217. Thomas D, Friedman S, Lin RY. Thyroid stem cells: lessons from normal development and thyroid cancer. *Endocrine-related cancer*. 2008; 15(1):51–58. DOI: 10.1677/ERC-07-0210 [PubMed: 18310275]
218. Zhang P, Zuo H, Ozaki T, Nakagomi N, Kakudo K. Cancer stem cell hypothesis in thyroid cancer. *Pathology international*. 2006; 56(9):485–489. DOI: 10.1111/j.1440-1827.2006.01995.x [PubMed: 16930327]
219. Hardin H, Montemayor-Garcia C, Lloyd RV. Thyroid cancer stem-like cells and epithelial-mesenchymal transition in thyroid cancers. *Human pathology*. 2013; 44(9):1707–1713. DOI: 10.1016/j.humpath.2013.01.009 [PubMed: 23528862]
220. Ozaki T, Matsubara T, Seo D, Okamoto M, Nagashima K, Sasaki Y, Hayase S, Murata T, Liao XH, Hanson J, Rodriguez-Canales J, Thorgeirsson SS, Kakudo K, Refetoff S, Kimura S. Thyroid regeneration: characterization of clear cells after partial thyroidectomy. *Endocrinology*. 2012; 153(5):2514–2525. DOI: 10.1210/en.2011-1365 [PubMed: 22454152]
221. Zhu W, Hai T, Ye L, Cote GJ. Medullary thyroid carcinoma cell lines contain a self-renewing CD133+ population that is dependent on ret proto-oncogene activity. *The Journal of clinical endocrinology and metabolism*. 2010; 95(1):439–444. DOI: 10.1210/jc.2009-1485 [PubMed: 19897677]
222. Kucerova L, Feketeova L, Kozovska Z, Poturnajova M, Matuskova M, Nencka R, Babal P. In vivo 5FU-exposed human medullary thyroid carcinoma cells contain a chemoresistant CD133+ tumor-initiating cell subset. *Thyroid : official journal of the American Thyroid Association*. 2014; 24(3):520–532. DOI: 10.1089/thy.2013.0277 [PubMed: 24073856]
223. Grubbs EG, Ng PK, Bui J, Busaidy NL, Chen K, Lee JE, Lu X, Lu H, Meric-Bernstam F, Mills GB, Palmer G, Perrier ND, Scott KL, Shaw KR, Waguespack SG, Williams MD, Yelensky R, Cote GJ. RET Fusion as a Novel Driver of Medullary Thyroid Carcinoma. *The Journal of clinical endocrinology and metabolism*. 2015; 100(3):788–793. DOI: 10.1210/jc.2014-4153 [PubMed: 25546157]
224. Berindan-Neagoe I, del Monroig PC, Pasculli B, Calin GA. MicroRNAome genome: a treasure for cancer diagnosis and therapy. *CA: a cancer journal for clinicians*. 2014; 64(5):311–336. DOI: 10.3322/caac.21244 [PubMed: 25104502]

225. Yang G, Lu X, Yuan L. LncRNA: a link between RNA and cancer. *Biochimica et biophysica acta*. 2014; 1839(11):1097–1109. DOI: 10.1016/j.bbagr.2014.08.012 [PubMed: 25159663]
226. Davoli T, Xu AW, Mengwasser KE, Sack LM, Yoon JC, Park PJ, Elledge SJ. Cumulative haploinsufficiency and triplosensitivity drive aneuploidy patterns and shape the cancer genome. *Cell*. 2013; 155(4):948–962. DOI: 10.1016/j.cell.2013.10.011 [PubMed: 24183448]
227. Manikandan M, Raksha G, Munirajan AK. Haploinsufficiency of Tumor Suppressor Genes is Driven by the Cumulative Effect of microRNAs, microRNA Binding Site Polymorphisms and microRNA Polymorphisms: An In silico Approach. *Cancer informatics*. 2012; 11:157–171. DOI: 10.4137/CIN.S10176 [PubMed: 23032637]
228. Park MS, Rosai J, Nguyen HT, Capodieci P, Cordon-Cardo C, Koff A. p27 and Rb are on overlapping pathways suppressing tumorigenesis in mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96(11):6382–6387. [PubMed: 10339596]
229. Zhou Z, Flesken-Nikitin A, Levine CG, Shmidt EN, Eng JP, Nikitina EY, Spencer DM, Nikitin AY. Suppression of melanotroph carcinogenesis leads to accelerated progression of pituitary anterior lobe tumors and medullary thyroid carcinomas in Rb+/- mice. *Cancer research*. 2005; 65(3):787–796. [PubMed: 15705875]
230. Pasquali D, Circelli L, Faggiano A, Pancione M, Renzullo A, Elisei R, Romei C, Accardo G, Coppola VR, De Palma M, Ferolla P, Grimaldi F, Colao A, Colantuoni V. CDKN1B V109G polymorphism a new prognostic factor in sporadic medullary thyroid carcinoma. *European journal of endocrinology/European Federation of Endocrine Societies*. 2011; 164(3):397–404. DOI: 10.1530/EJE-10-0929
231. Sekiya T, Bronstein MD, Benfini K, Longuini VC, Jallad RS, Machado MC, Goncalves TD, Osaki LH, Higashi L, Viana J Jr, Kater C, Lee M, Molatore S, Francisco G, Chammass R, Naslavsky MS, Schlesinger D, Gama P, Duarte YA, Lebrao ML, Zatz M, Meirelles O, Liberman B, Fragoso MC, Toledo SP, Pellegata NS, Toledo RA. p27 variant and corticotropinoma susceptibility: a genetic and in vitro study. *Endocrine-related cancer*. 2014; 21(3):395–404. DOI: 10.1530/ERC-13-0486 [PubMed: 24532476]
232. Hoff AO, Catala-Lehnen P, Thomas PM, Priemel M, Rueger JM, Nasonkin I, Bradley A, Hughes MR, Ordonez N, Cote GJ, Amling M, Gagel RF. Increased bone mass is an unexpected phenotype associated with deletion of the calcitonin gene. *The Journal of clinical investigation*. 2002; 110(12):1849–1857. DOI: 10.1172/JCI14218 [PubMed: 12488435]
233. Andersson L, Westerlund J, Liang S, Carlsson T, Amendola E, Fagman H, Nilsson M. Role of EphA4 receptor signaling in thyroid development: regulation of folliculogenesis and propagation of the C-cell lineage. *Endocrinology*. 2011; 152(3):1154–1164. DOI: 10.1210/en.2010-0232 [PubMed: 21266507]
234. Lupulescu A. Effect of calcitonin on rabbit thyroid glands. *Endocrinology*. 1972; 90(4):1046–1054. DOI: 10.1210/endo-90-4-1046 [PubMed: 5011046]
235. Toki H, Inoue M, Minowa O, Motegi H, Saiki Y, Wakana S, Masuya H, Gondo Y, Shiroishi T, Yao R, Noda T. Novel retinoblastoma mutation abrogating the interaction to E2F2/3, but not E2F1, led to selective suppression of thyroid tumors. *Cancer science*. 2014; 105(10):1360–1368. DOI: 10.1111/cas.12495 [PubMed: 25088905]
236. Coxon AB, Ward JM, Geradts J, Otterson GA, Zajac-Kaye M, Kaye FJ. RET cooperates with RB/p53 inactivation in a somatic multi-step model for murine thyroid cancer. *Oncogene*. 1998; 17(12):1625–1628. DOI: 10.1038/sj.onc.1202381 [PubMed: 9794240]
237. Matoso A, Zhou Z, Hayama R, Flesken-Nikitin A, Nikitin AY. Cell lineage-specific interactions between Men1 and Rb in neuroendocrine neoplasia. *Carcinogenesis*. 2008; 29(3):620–628. DOI: 10.1093/carcin/bgm207 [PubMed: 17893233]
238. Nikitin AY, Liu CY, Flesken-Nikitin A, Chen CF, Chen PL, Lee WH. Cell lineage-specific effects associated with multiple deficiencies of tumor susceptibility genes in Msh2(-/-)Rb(+/-) mice. *Cancer research*. 2002; 62(18):5134–5138. [PubMed: 12234974]
239. Franklin DS, Godfrey VL, O'Brien DA, Deng C, Xiong Y. Functional collaboration between different cyclin-dependent kinase inhibitors suppresses tumor growth with distinct tissue specificity. *Molecular and cellular biology*. 2000; 20(16):6147–6158. [PubMed: 10913196]

240. Bai F, Pei XH, Nishikawa T, Smith MD, Xiong Y. p18Ink4c, but not p27Kip1, collaborates with Men1 to suppress neuroendocrine organ tumors. *Molecular and cellular biology*. 2007; 27(4): 1495–1504. DOI: 10.1128/MCB.01764-06 [PubMed: 17145768]
241. Damo LA, Snyder PW, Franklin DS. Tumorigenesis in p27/p53- and p18/p53-double null mice: functional collaboration between the pRb and p53 pathways. *Molecular carcinogenesis*. 2005; 42(2):109–120. DOI: 10.1002/mc.20068 [PubMed: 15584024]
242. Yamasaki L, Bronson R, Williams BO, Dyson NJ, Harlow E, Jacks T. Loss of E2F-1 reduces tumorigenesis and extends the lifespan of Rb1(+/-)mice. *Nature genetics*. 1998; 18(4):360–364. DOI: 10.1038/ng0498-360 [PubMed: 9537419]

Fig. 1. RET receptor activation

Shown is a schematic figure outlining the basic structural organization of the trans-membrane receptor tyrosine kinase RET and the pathway to its activation. One of four GDNF family ligands is recruited to the receptor upon binding to its cognate co-receptor. Solid arrows indicate the primary ligand/co-receptor interactions. Dotted arrows show secondary binding interactions. Generation of the heterotrimeric complex allows for receptor dimerization causing kinase activation and auto-phosphorylation (P). The predominant receptor complex observed in MTC cells is comprised of PSPN and GFRA4.

Fig. 2. Mechanism of oncogenic RET activation and the signal transduction network
 Shown is a schematic figure outlining the normal ligand-dependent activation of RET compared with ligand-independent mechanisms resulting from receptor mutation. The asterisks show the general location of two common RET activating mutations. In all cases RET activation is associated with phosphorylation (P) of tyrosines (Y) at seven possible positions located within the intracellular domain. Note that Y1096 only exists on the long isoform of RET. Tyrosine phosphorylation results in the recruitment of adaptor proteins (green boxes) that lead to the activation of multiple signaling pathways including: JAK/STAT, PKC, PI3K/AKT and RAS/MAPK.

Fig. 3. RAS signaling pathway

The figure shows the activation of RAS initiated through interaction with phosphorylated RET. As shown in Figure 2, signal transduction is primarily mediated by tyrosines 1062 and 1096 through adaptor proteins GRB2, SHC1, and GAB1. The guanine nucleotide exchange factor SOS is responsible for initiating the formation of active GTP-bound RAS. Active RAS is capable of both activation of MAPK signaling through RAF or PI3K activation leading to AKT signaling. Major components of both pathways are shown with their specific outcome of regulating cellular functions indicated.

Fig. 4. RB1-mediated pathway of cell cycle regulation

The figure shows a schematic representation of major regulators RB1-mediated cell cycle regulation. RB1 is responsible of inactivation of E2F transcription factors involved the expression of proteins required for G1 to S transition. Phosphorylation (P) of RB1 results in release from E2F and activation of transcription. RB1 phosphorylation is positively regulated through induction of Cyclins and CKDs, such as RET-mediated increase of Cyclin D, and negatively regulated through activation of CDK inhibitory proteins, the CDKN2 and CDKN1 families.

Table 1**Mouse Models of MTC**

Genetic Target	C Cell Phenotype	Reference
RET		
Human RET C634R	MTC seen in 3 of 4 founder lines	[191]
Tg-RET C634R short iso-form	CCH or MTC in 93% of mice	
Rat CT/CGRP promoter	MTC seen by 8, 13, 14 mo in each founder	
Human RET C634R	Two founder lines only 1 with MTC	[193]
Tg-RET C634R long iso-form	Line 121 (22 cop-ies)	
MoMuLV LTR promoter	MTC in 100% of mice by 9 mo	
Human RET M918T	MTC seen in 3 founders, highest in line 42	[190]
Tg-RET M918T short iso-form	CCH in 77% of line 42 mice by 8 mo	
Human CT/CGRP promoter	MTC in 13% of line 42 mice by 11 mo	
MEN2B M919T knockin		[194]
ret ^{MEN2B/+}	Nodular CCH in 14% at 8–12 mo	
ret ^{MEN2B/MEN2B}	Nodular CCH in 60% at 6–10 mo	
MEN2A C620R knockin	No thyroid pheno-type out to 24 mo	[101]
ret ^{C620R/+}	Homozygous is a neonatal lethal	
MEN2A C620R knockin		
ret ^{C620R/+}	CCH in 48.4% compared to 22.8% in control mice at 20–30 mo	[102]
Human RET C634R	MTC in 0 FVB/n mice at 43 wk	[195,192]
Tg-RET C634R long iso-form	MTC in 14% of BALB/c mice at 43 wk	
Human CT/CGRP promoter	MTC in 64% of C57BL/6J 9 mice at 43 wk	
CT-2A-3 (1 copy)	MTC in 98% of CBA/ca mice at 43 wk	
Tg-RET M918T; p18+/-	MTC in 0% of mice by 12 mo	[196]
No Tg-RET; p18+/-	MTC in 0% of mice by 12 mo	
Tg-RET M918T; p18+/-	MTC in 26% of mice by 12 mo	
No Tg-RET; p18-/-	MTC in 11% of mice by 12 mo	
Tg-RET M918T; p18-/-	MTC in 43% of mice by 12 mo	
No Tg-RET; p18+/-; p27+/-	MTC in 0% of mice by 12 mo	
Tg-RET M918T; p18+/-; p27+/-	MTC in 27% of mice by 12 mo	
No Tg-RET; p18-/-; p27+/-	MTC in 88% of mice by 9 mo	
Tg-RET M918T; p18+/-; p27-/-	MTC in 100% of mice by 9 mo	
RAS		
Tg-v-Ha-Ras	MTC seen in 5 founder lines	[161]
Rat CT/CGRP promoter	Expanded lines with 1 and 12 copies	
v-Ha-ras-p21	MTC in 85% of mice at 6–12 mo	
OTHER GENES		
Tg-SV40 T-Antigen		[199]

Genetic Target	C Cell Phenotype	Reference
Rat CT/CGRP promoter	MTC in 5 founder lines	
SV40 T antigen	MTC in 100% of mice with death by 3 mo	
Tg-mos	MTC in 3 of 4 founder lines	[162]
Moloney virus LTR	MTC in 4–63% of mice by 8 mo	
Mouse mos		
Rb1+/-1t19neo	CCH in 43% of mice	[178]
Rb1+/-	MTC in 96% of mice by 77 wk	[229]
Rb1 D326V/+ (M1326)	No thyroid tu-mors	[235]
Rb1 intron 10 3' ss/+ (M1033)	MTC in 90% of mice by 70 wk	
Rb1 intron 21 5' ss/+ (M1032)	MTC in 90% of mice by 70 wk	
Rb1+/-	MTC in 70% of mice over 6 mo	[177]
Rb1+/-; p53+/-	MTC in 83% of mice over 6 mo	
Rb1+/-; p53-/-	MTC in 60% of mice by 6 mo	
Rb1+/-	MTC in 0% of mice by 18 mo	[200]
Rb1+/-; p53+/-	MTC in 37% of mice by 16 mo	
Rb1+/-; p53+/-	CCH or MTC in 40% of mice	[236]
Rb1+/-	MTC in 60% of mice by 12 mo	[228]
p27-/-	MTC in 30% of mice by 15 mo	
Rb1+/-; p27+/-	MTC in 80% of mice by 15 mo	
Rb1+/-; p27-/-	MTC in 77% of mice by 7 mo	
Rb1+/-; Men1+/-	No effect on RB1 phenotype	[237]
Rb1+/-; Msh2-/-	No effect on RB1 phenotype	[238]
p18-/-	CCH 12%, Nodu-lar CCH 2% in mice over 12 mo	[239]
p27-/-	CCH 4% of mice by 12 mo	
p18-/-; p27+/-	CCH in 46%, Nodular CCH 8% in mice by 9 mo	
p18-/-; p27-/-	CCH in 81%, Nodular CCH 6% in mice by 3.5 mo	
p18-/-; men1+/-	Nodular CCH in 80% of mice by 22 mo	[240]
p27-/-; men1+/-	Nodular CCH in 6% of mice by 12 mo	
p18-/-	Nodular CCH in 2.4% of mice at 14 mo	[241]
p18-/-; p53-/-	Nodular CCH in 5% of mice at 8 mo	
Tg-TetOp-p25-GFP; Tg-NSE5021	MTC in 100% of mice by 16 wk	[205]
Rb1+/-	MTC in 53% of mice by 500 days	[242]
Rb1+/-; E2f1+/-	MTC in 6% of mice at 575 days	
Rb+/-; E2f1-/-	MTC in 0% of mice at 700 days	
Rb1+/-	MTC in 40% of mice at 210–270 d	[206]
Rb1+/-; E2f3+/-	MTC in 88% of mice at 210–270 d	
Rb1+/-; E2f3-/-	MTC in 100% of mice at 150–270 d	
Rb1+/-	Nodular CCH or MTC in 32% of mice	[207]
Rb1+/-; Nras+/-	MTC in 50% of mice, sig-nificantly larger tumors	
Rb1+/-; Nras-/-	MTC in 100% of mice, significantly larger tumors	
PRLR-/-	MTC in 29% of mice at 12 mo	[203]