INVITED REVIEW

RET/PTC activation in papillary thyroid carcinoma: European Journal of Endocrinology Prize Lecture

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Abstract

Papillary thyroid carcinoma (PTC) is frequently associated with RET gene rearrangements that generate the so-called RET/PTC oncogenes. In this review, we examine the data about the mechanisms of thyroid cell transformation, activation of downstream signal transduction pathways and modulation of gene expression induced by RET/PTC. These findings have advanced our understanding of the processes underlying PTC formation and provide the basis for novel therapeutic approaches to this disease.

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RET/PTC rearrangements in papillary thyroid carcinoma

The rearranged during transfection (RET) proto-oncogene, located on chromosome 10q11.2, was isolated in 1985 and shown to be activated by a DNA rearrangement (rearranged during transfection) (1). As illustrated in Fig. 1, it encodes a single-pass transmembrane tyrosine kinase that functions as the receptor (receptor tyrosine kinase, RTK) for the growth factors of the glial cell line-derived neurotropic factor family (2). RET is essential for the development of the sympathetic, parasympathetic, and enteric nervous systems, the kidney and the testis (2). Germline point mutations in RET lead to multiple endocrine neoplasia type 2 syndromes thereby predisposing to thyroid C-cell-derived medullary thyroid carcinoma (3).

With an incidence ranging between 0.5 and 10 cases per 100 000 population, thyroid cancer is the most frequent endocrine malignancy (4). Papillary thyroid carcinoma (PTC), which accounts for approximately 80% of cases, is the most frequent of all thyroid malignancies (4). In PTC, genomic rearrangements juxtapose the RET kinase and COOH-terminus encoding domains (exons 11–21) to unrelated genes, thereby creating dominantly transforming oncogenes called RET/PTC (5, 6). Similar rearrangements of another neurotropic RTK, namely the neurotropic tyrosine receptor kinase type 1 (NTRK1), the receptor for nerve growth factor, have been described in a fraction of PTC patients (7). As illustrated in figure 1, many different genes have been found to be rearranged with RET in individual PTC patients. RET/PTC1 and 3 account for more than 90% of all rearrangements and are hence, by far, the most frequent variants (8–11). They result from the fusion of RET to the coiled-coil domain containing gene 6 (CCDC6, formerly called H4/D10S170) or to the nuclear receptor co-activator gene 4 (NcoA4, formerly called RET fused gene (RFG)/ELE1/androgen receptor activator 70(ARA70)) (8–11).

Prevalence of RET/PTC rearrangements

For a detailed discussion of the prevalence of RET/PTC rearrangements and their correlation with clinicopathological features of thyroid carcinomas, we refer the reader to three excellent reviews (8–10). The prevalence of RET/PTC rearrangements in thyroid cancer varies widely among studies. In an adult population from the United States, the prevalence of rearrangements was approximately 35% (9). Prevalences between 3 and 85% have been reported for other regions (8–10). This wide range of values probably reflects not only the geographic variation but also the different procedures used to identify RET/PTC rearrangements, i.e. various reverse transcriptase (RT)-PCR methods, Southern blot, and fluorescence in situ hybridization. An exhaustive study by Zhu and co-workers (12) demonstrated that the method used has a striking effect on the efficacy of RET/PTC detection, and hence on the prevalence reported. Tumor

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heterogeneity is another factor that can affect the evaluation of RET/PTC prevalence. In addition to PTC samples with ‘clonal’ RET/PTC rearrangements (those affecting the majority of tumor cells), there are samples with ‘non-clonal’ rearrangements (those affecting a small portion of tumor cells) (12, 13). Whereas RET/PTC is likely to be important for tumor formation in samples with clonal rearrangements, RET/PTC could not be a ‘driver’ mutation in PTC samples with non-clonal rearrangements (13). This distinction has important implications for the stratification of patients who could potentially benefit from novel therapeutic approaches based on the use of RET kinase inhibitors (see below). It is still controversial the presence of RET/PTC rearrangements in non-neoplastic cells in Hashimoto’s thyroiditis. It is possible that a heterogeneous presence of the rearrangement may account, at least in part, for such a controversy (8, 9, 13). Tumor heterogeneity and multiclonality is also indicated by the presence of multiple RET/PTC variants in individual PTC samples (14).

RET/PTC rearrangements have so far been identified only in thyroid lesions, and in particular in PTC (8–10). Most studies concur that RET/PTC rearrangements are rare or absent in benign follicular adenomas, and absent in follicular and medullary carcinomas (8–10). Various PTC histological variants have been identified, namely classic, follicular, diffuse-sclerosing, columnar-cell, Hurthle-cell, cribriform, solid and tall-cell variants (4). Classic (~45%) and follicular (~18%) variants are the most prevalent (4). RET/PTCs are more frequent in tumors that have a classic architecture (15) and in microcarcinomas (<1 cm) (16). They are rare in the follicular variant of PTC (15). RET/PTCs have been reported in the cribriform variant, which is typically associated with familial adenomatous polyposis (17), in the Hurthle-cell variant (18, 19), and in hyalinizing trabecular adenoma, a rare tumor that can be...
morphologically similar to PTC (20, 21). The solid variant, which is an aggressive PTC subtype, is closely associated with the RET/PTC3 oncogene, whereas the classic variant is associated with RET/PTC1 (22).

Clinical, epidemiologic, and pathologic evidence supports the possibility of a stepwise progression from well-differentiated carcinoma, including PTC, to poorly differentiated and anaplastic carcinoma (23). Accordingly, some anaplastic carcinomas are associated with genetic lesions (rat sarcoma viral oncogene homolog (RAS) and BRAF mutations) that overlap those present in PTC and in other well-differentiated carcinomas (23). Conflicting results have been reported about the presence of RET/PTC in poorly-differentiated and anaplastic carcinomas and, therefore about whether or not RET/PTC predisposes to thyroid tumor progression (23–28). Again, methodological differences and tumor heterogeneity may account for these controversies. A large multicenter study in which all centers use the same methodology should resolve this issue. It is also important to study the different RET/PTC variants individually, because, as mentioned above, they could confer different degrees of aggressiveness on PTC. It should be noted that intercross of RET/PTC transgenic mice with p53null mice induced the formation of anaplastic carcinomas, suggesting that at least in a specific genetic background RET/PTC is able to induce thyroid tumor progression (29).

Chromosomal basis for RET/PTC rearrangements

CCDC6 (the RET fusion partner in RET/PTC1) and NcoA4 (the RET fusion partner in RET/PTC3 and 4) map together with RET on chromosome 10, whereas the other RET fusion partners are located on different chromosomes. Therefore, RET/PTC recombination events are caused either by a paracentric inversion of chromosome 10 (in the case of RET/PTC1 and RET/PTC3) or balanced chromosomal translocations involving chromosome 10 and other chromosomes (in the case of the other RET/PTC subtypes). It is well known that ionizing radiations promote DNA double-strand breaks and that PTC is the most common form of solid neoplasm associated with radiation exposure. These findings prompted the notion that there is a direct link between radiations, RET/PTC rearrangements, and PTC formation. At least two observations support this concept. First, X-ray irradiation induces the formation of RET/PTC1 (30–32). Second, RET/PTC rearrangements are very frequent in PTC samples from patients who had received external radiations (33–35) and in post-Chernobyl PTCs (22, 36–39). The Chernobyl nuclear power plant accident in April 1986 resulted in the release of large amounts of iodine isotopes, mainly 131I, and, therefore, there was widespread radiation exposure to the thyroid (36). A high incidence of childhood PTC was reported in contaminated areas. Post-Chernobyl PTC featured a high prevalence (over 60%) of RET/PTC rearrangements (22, 36–39). RET/PTC3 was more prevalent among short latency solid PTCs, whereas RET/PTC1 was more frequently found after a long latency period (9, 22, 36–39). Rapid thyroid cell proliferation may account for the particularly high sensitivity to radiation-induced RET/PTC rearrangements among children (40). However, also in cases of PTC in non-exposed populations, the prevalence of RET/PTC was higher in children than in adults, which suggests that in general RET/PTC recombinations occur more frequently in young thyroid (41, 42).

RET/PTC rearrangements are not the only intrachromosomal aberration found in radiation-associated PTC. Also NTRK1 rearrangements, caused by paracentric inversions of chromosome 1q, have been reported in post-Chernobyl PTC (7, 38). Moreover, in 11% of the post-Chernobyl PTCs there was a paracentric inversion of chromosome 7q that resulted in the A-kinase anchor protein (AKAP)9–BRAF fusion oncogene (43). Differently, point mutations in BRAF, which are very frequent in sporadic PTC (see below) are rare in Chernobyl cancers affecting children (44). This suggests that young age and radiation exposure are specifically linked to gene rearrangements in PTC.

The reason for the high frequency of chromosomal rearrangements, and particularly paracentric inversions, in thyroid cancer after radiation exposure is not clear. Nikiforova and co-workers (45) shed new light on this issue when they demonstrated that, although the two loci participating in the formation of the RET/PTC1 fusion are about 30 megabases apart, they frequently juxtapose within the interfase nuclei of thyroid cells. This spatial contiguity can provide a structural basis for radiation-induced illegitimate recombination of the two genes. More recently, the same group showed that NcoA4 is also in close proximity to RET in thyroid cell chromatin (46). A similar mechanism was proposed for NTRK1 fusions in PTC (47). Taken together, these findings suggest that tissue-specific chromatin folding is the genomic basis underlying the high prevalence of gene rearrangements in PTC.

Molecular mechanisms of activation of RET/PTC oncoproteins

Adoptive expression of RET/PTC transforms murine fibroblasts (5, 6). Chronic RET/PTC expression causes thyrotropin-independent proliferation and impairs the expression of the thyroid differentiation markers of rat thyroid follicular PC Cl 3 cells (48–50). On the other hand, acute expression of RET/PTC oncogenes induces apoptosis of PC Cl 3 cells (49, 51), a phenomenon that is often seen when dominant oncogenes are acutely introduced in normal cells. RET/PTC expression in primary cultures of human thyrocytes results in changes
of nuclear morphology (irregular nuclear contours and euchromasia) resembling those characterizing human PTC (52). Finally, thyroid targeting of RET/PTC1 (53, 54) or RET/PTC3 (55) in transgenic mice leads to the development of tumors that histologically and cytologically resemble human PTC. Interestingly, mice carrying RET/PTC3 develop thyroid tumors, whose solid phenotype resembles those associated with activation of this RET/PTC variant in humans (22, 55). RET/PTC3 is more potently transforming than RET/PTC1 also when tested in cultured thyrocytes in vitro (56).

RET/PTC can exert oncogenic activity via various mechanisms (schematically illustrated in Fig. 2): altered expression, ligand-independent kinase activation, subcellular relocalization and functional alteration of the RET fusion partner. Whereas wild-type RET expression is tightly regulated (2), its fusion partners are ubiquitously expressed (57, 58). Therefore, RET/PTC rearrangements may lead to the unscheduled expression of RET in the thyroid cell (mechanism no. 1 in Fig. 2). However, also unarranged RET is expressed in thyroid follicular cells and in tumors that derive from them (59), and therefore the rearrangement might be not essential for RET expression in the thyroid. The mechanisms driving the ligand-independent kinase activity of RET/PTC oncoproteins have been partially elucidated. The rearrangement deletes the signal sequence, the extracellular ligand-binding domain and the intracellular juxtamembrane domains of the receptor (mechanism no. 2 in Fig. 2). As in the case of other receptors, the juxtamembrane domain negatively affects RET mitogenic signaling although the molecular mechanisms governing this process are not clear (60). On the other hand, all the translocated amino termini fused to RET are predicted to fold into coiled-coils (Figs 1 and 2 mechanism no. 3). Coiled-coils are protein–protein interaction domains able to mediate dimerization and activation of the kinase. Accordingly, RET/PTC proteins form dimers that are essential for oncogenic activation (58, 61). Whereas wild-type RET is a transmembrane protein, as a further consequence of the deletion (mechanism no. 4 in Fig. 2), RET/PTC oncoproteins are re-localized to the cytosolic compartment. This could prevent RET/PTC from interacting with negative regulators located at plasma membrane level, for instance, the tyrosine phosphatase protein tyrosine phosphatase receptor type-J (PTPRJ) (62) (Fig. 2). However, it should be noted that by interacting with proteins resident at the plasma membrane, e.g. Enigma (63) or FRS2 (64), RET/PTC proteins may be re-directed to the membrane compartment. Finally (mechanism no. 5 in Fig. 2), RET/PTC rearrangements, besides activating RET, can also alter the function of RET-fused genes. This could explain why the RET/PTC variants constituted by different fusion partners might have, at least in part, non-overlapping biological effects. Perhaps, the best example of a RET fusion partner, whose altered function may contribute to neoplastic transformation is the regulatory subunit type I-α of protein kinase A (PRKAR1A) that is involved in RET/PTC.
PTC2. PRKAR1A is, indeed, a *bona fide* tumor suppressor (65) and its ablation in the mouse induced the formation of thyroid tumors (66). The knowledge about the function of the other RET fusion partners is limited. Some of them are involved in transcriptional regulation, such as Ncoa4, tripartite motif-containing (TRIM)24 (formerly called HTIF1, human transcriptional intermediary factor 1), methyl-CpG binding domain protein 1 (MBD1) (formerly called PCM1, pericentriolar material 1), and RET finger protein (RFP) (formerly called TRIM27) (www.ncbi.nlm.nih.gov/entrez/), or in cell fate determination, such as TRIM33 (formerly called RFG7/Ectodermin/HTIFγ) (67). Ncoa4 functions as a co-activator of several nuclear receptors. It interacts with and promotes androgen receptor activity via the consensus FXXLF motif (located at amino acids 328–332) (68), and interacts with and promotes peroxisome-proliferator-activated receptor-γ via the LXXLL motif located at amino acids 92–96 (69). Therefore, its rearrangement may affect the function of these steroid hormone receptors. Surprisingly, we still know very little about CCDC6, the first isolated RET fusion partner (6, 57). According to the conserved domain database (www.ncbi.nlm.nih.gov/Structure/cdd/), this protein, like golgi autoantigen glogin subfamily A (GOLGA)5 (formerly called RFG5) and KTN1 (kinectin; the fusion partners in RET/PTC5 and 8 respectively) contains the signature sequence of the ATP-binding cassette family of ATPases, which are proteins involved in DNA maintenance and repair. It is still unknown whether such a function is altered consequent to the RET/PTC1 rearrangement. Of note, CCDC6 overexpression induces apoptosis, suggesting that abrogation of its function may promote PTC cell survival (57). Intriguingly, some of the RET fusion partners are found rearranged in tumors other than PTC; CCDC6 rearranges with the kinase domain of the platelet-derived growth factor receptor-β in myeloproliferative disorders (70), TRIM24 with BRAF in mouse hepatocellular carcinomas (71), and PCM1 with JAK2 in chronic myeloid leukemia (72).

**RET/PTC and the MAPK signaling cascade**

The most common genetic alteration found in PTC (up to 45% of cases) is the activating point mutation (most often V600E) in BRAF (73). Together with the other oncoproteins implicated in PTC (RET, NTRK1 and RAS), BRAF functions in the mitogen-activated protein kinase (MAPK) pathway. By promoting GTP loading on RAS, RET and NTRK1 stimulate RAF family kinases, which leads to the phosphorylation of MEK (MAP kinase or ERK) and ERK (extracellular signal-regulated kinases or MAPK) (74) (Fig. 3). One tyrosine residue (tyrosine 1062) in RET mediates the firing of this cascade. This tyrosine is the binding site for several proteins including Shc family adaptors, IRS1/2, FRS2, and DOK1/4/5 (2, 11, 75). Binding to Shc and FRS2 mediates recruitment of growth factor receptor-bound protein 2 (GRB2) that, in cooperation with the guanine nucleotide exchange factor SOS, activates RAS. In its GTP-bound form, RAS activates RAF kinases (such as BRAF), and its downstream signaling cascade (MEK and ERK). There are several negative regulators of this pathway but the possible role of these mediators in RET/PTC signaling has not been studied yet. These proteins include the sprouty family of proteins (SPRY), which can inhibit the pathway at various levels; RAF kinase inhibitor protein (RKIP); RAS and RAB Interactor 1 (RIN1); IMP (impedes mitogenic signal propagation); and the MKP (dual specificity phosphatase) family of MAPK phosphatases.

![Figure 3](https://www.eje-online.org)

**Figure 3** RET/PTC binds adaptor proteins such as growth factor receptor-bound protein 2 (GRB2) that, in cooperation with the guanine nucleotide exchange factor SOS, activates RAS. In its GTP-bound form, RAS activates RAF kinases (such as BRAF), and its downstream signaling cascade (MEK and ERK). There are several negative regulators of this pathway but the possible role of these mediators in RET/PTC signaling has not been studied yet. These proteins include the sprouty family of proteins (SPRY), which can inhibit the pathway at various levels; RAF kinase inhibitor protein (RKIP); RAS and RAB Interactor 1 (RIN1); IMP (impedes mitogenic signal propagation); and the MKP (dual specificity phosphatase) family of MAPK phosphatases.
corresponding to Y1062 in wild-type RET (77); and (iii) transcriptional responses to RET/PTC and BRAF partially overlap (see also below) (48, 76, 78). Such an assembly of oncogenic proteins in one signaling cascade has numerous implications: one is that other genes implicated in PTC formations may encode proteins functioning in the same cascade. Many proteins (schematically represented in Fig. 3) are potentially able to modulate the RET/PTC-BRAF-MAPK axis (79); it would be well worth investigating their activity in thyroid cell transformation.

Not all the biological activities of the RET/PTC and BRAF oncoproteins overlap. Indeed, activated BRAF but not RET/PTC induces genomic instability in cultured thyroid cells (80); BRAF (but not RET/PTC) transgenic mice develop PTCs that progress to undifferentiated carcinomas (81); and RET/PTC and BRAF regulate the expression of specific sets of genes as well as the expression of common genes (48, 78, 82, 83). Thus, although functioning on the same pathway, RET/PTC and BRAF are endowed with differential signaling abilities. This could explain the different clinicopathologic features of PTCs associated with the two oncogenes. In fact, whereas most RET/PTC-positive PTCs belong to the classic variant and are associated with a young age and an early stage at presentation, BRAF-positive PTCs belong to the classic and tall cell variants and are associated with old age and an advanced tumor stage (15, 73).

The pro-inflammatory transcriptional program regulated by RET/PTC

The transcriptional response to RET/PTC has been evaluated both in rat thyroid PC Cl 3 cells, either stably (48, 84) or conditionally (76, 85) expressing RET/PTC3, and in human thyrocytes expressing RET/PTC1 (86). Although some differences were noted (perhaps linked to the different model systems used), these studies reached two conclusions. First, as anticipated above, tyrosine 1062 and the downstream signaling cascade is particularly important for RET/PTC-mediated effects (48, 76, 84, 86). Second, RET/PTC induced the expression of a pro-inflammatory transcriptional program. Such a program included the up-regulation of various cytokines (OPN/SPP1, GM-CSF, M-CSF, G-CSF, IL1A, IL1B, IL6, and IL24) (84–87), chemokines (CCL2, CCL20, CXCL8, CXCL1, CXCL10, and CXCL12) (48, 84–86), chemokine receptors (CXCR4) (48, 86, 88), pro-inflammatory enzymes (cyclooxygenase-2, and microsomal prostaglandin E2 synthase) (84, 85, 89), and interferon-dependent genes (85). Many of these mediators were also upregulated in the thyroids of RET/PTC3 transgenics (90). Moreover, injection of RET/PTC-transformed PC Cl 3 cells induced angiogenesis and inflammatory responses in SCID mice (84). These findings suggest that induction of an inflammatory-type reaction might be part of the oncogenic effect of RET/PTC on thyroid cancer and may explain the chronic inflammatory reaction that is characteristic of this tumor type.

Concluding remarks

Since the RET/PTC oncogene was isolated almost three decades ago (5), much progress has been made in our understanding of the effect exerted by this oncogene. Its relationship with radiation exposure has been established, the chromosomal basis for its activation has been clarified, its transforming and signaling properties have been characterized, and finally we now know that RET/PTC triggers a pro-inflammatory response. Over the last few years, it has become clear that cancers characterized by activating mutations in dominant oncogenes can be treated by directly targeting the disease-causing oncoprotein. In this framework, RET and the downstream signaling cascade are promising therapeutic targets. Several inhibitors of RET, BRAF, and MEK kinases have been isolated (11, 91) and hopefully, their clinical use will lead to novel therapeutic options for patients afflicted by thyroid cancer.

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