RET Proto-Oncogene and Thyroid Cancer

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Abstract

The *RET* proto-oncogene has not only conclusively been identified as responsible for the three subtypes of the inherited cancer syndrome multiple endocrine neoplasia type 2 (MEN-2) but also shown to be involved in the molecular evolution of sporadic medullary and papillary thyroid carcinoma as well as Hirschsprung's disease. A variety of recent studies have elucidated the pathophysiological mechanisms leading to neoplastic disease and we now understand that dominant activating germline mutations lead to MEN-2A, MEN-2B, and familial MTC; somatic mutations to sporadic medullary thyroid carcinoma; *RET* rearrangements to papillary thyroid carcinoma; and inactivating alterations to Hirschsprung's disease. The clinical significance, however, of *RET* alterations especially in sporadic thyroid tumors is still controversial and therapeutic concepts in MEN-2 gene carriers only start to emerge. This article is a short summary of the recent findings on the structure and physiology of the *RET* proto-oncogene and its role in familial and sporadic thyroid cancer.

Key Words: RET Thyroid; medullary thyroid carcinoma; papillary thyroid carcinoma.

Introduction

There have been many recent advances in our understanding of thyroid disease, including the molecular biology of thyroid neoplasms. Recent reports have described the involvement of specific genetic alterations in different types of thyroid neoplasms: *Ras* point mutations are frequently observed in tumors with follicular histology and a high prevalence of p53 point mutations have been found in anaplastic carcinomas. More recent studies revealed that the *RET* proto-oncogene is involved in the tumorigenesis of medullary thyroid carcinoma (MTC) and papillary thyroid carcinoma by activation of its tyrosine kinase either by point mutation or rearrangement.

The *RET* Proto-Oncogene

The *RET* proto-oncogene (REarranged during Transfection) is located on chromosome 10q11.2, has 21 exons, and encodes a transmembrane receptor with cytoplasmic tyrosine kinase activity [1]. *RET* transcripts and protein are expressed in cells and neoplasms of neuroendocrine differentiation, including parafollicular C cells and MTCs, adrenal medulla and pheochromocytomas as well as neuroblastomas, parathyroid parenchymal cell precursors and peripheral nerves, neurofibromas, and schwannomas. The *RET* protein is a functional receptor for the glial-cell-line-derived neurotrophic factor (GDNF), a distant member of the transforming growth factor (TGF)-β superfamily [2]. GDNF uses a multisubunit receptor system in which the GDNF receptor-α (GDNFR-α) and *RET* function as the ligand-binding and signaling components, respectively [3].

RET in Medullary Thyroid Carcinoma

Several groups have demonstrated that distinct germline mutations in the *RET*

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proto-oncogene are associated with the dominantly inherited cancer syndromes multiple endocrine neoplasia type 2A and 2B (MEN-2A and MEN-2B) and familial medullary thyroid carcinoma (FMTC) [4]. All three syndromes share heritable MTCs as part of the disease phenotype [5]. MEN-2A, which accounts for more than 90% of all cases of MEN-2, is characterized by the additional occurrence of pheochromocytoma and hyperparathyroidism resulting from parathyroid hyperplasia or adenoma. Activating mutations in the cysteine-rich extracellular region cause enhanced dimerization of the *RET* tyrosine kinase receptor and autophosphorylation, and are causative for MEN-2A and FMTC. Missense germline mutations in one of six codons for Cys in *RET* exons 10 (609, 611, 618, and 620) and 11 (630 and 634) have been identified in 97% of MEN-2A and 87% of FMTC families [4]. In FMTC families MTC is the only clinical feature and the course of the disease is more benign than that of MEN-2A or MEN-2B. In a few FMTC families, additional germline mutations have been identified at codons 768 and 804 in *RET* exons 13 and 14. The effects of these mutations are unclear. The former may alter kinase activity by changing the substrate specificity or the ATP-binding capacity of the receptor and the latter may activate the receptor by altering its interactions with normal cellular substrates or modifying the range of substrates the receptor can phosphorylate. In MEN-2B, which accounts for approx 5% of all patients with MEN-2, an activating mutation of the tyrosine kinase core domain has been identified in 94% of cases. This mutation at codon 918 in exon 16 replaces methionine with threonine and causes increased autophosphorylation and alteration of the substrate specificity of the tyrosine kinase. The MEN-2B syndrome is characterized by MTC and pheochromocytoma, rare involvement of parathyroids, myelinated corneal nerves, gastrointestinal ganglioneuromatosis, and a variety of skeletal abnormalities such as a marfanoid habitus, pes cavus, talipes equinovarus, slipped capital femoral epiphysis, kyphosis, scoliosis, and an increased joint laxity [5]. The thyroid tumors tend to occur at an earlier age and pursue a more aggressive course than in patients with MEN-2A and FMTC. The germline mutation in MEN-2B frequently represents new mutations (approx 50%) and it appears that the mutant allele is paternally derived, even though the father does not carry a germ line mutant allele.

Somatic mutations in *RET* have also been found in a proportion of patients with sporadic MTCs. By far the most common mutation involves codon 918 (Met → Thr). This type of missense mutation in exon 16 has been described in 23–85% of sporadic MTCs; in our own series of 16 examined tumors we found 44% MTCs harboring this type of *RET* mutation [6]. Other groups found additional mutations at codon 768 of exon 13, at codon 883 of exon 15, at codon 634 of exon 11, and in exon 10 in a small proportion of tumors [4]. Furthermore, microdeletions causing the loss of a Cys residue at codon 630 or 634 have been described in sporadic MTCs by several groups. The differences in mutational frequencies and codons involved that have been reported by various centers suggest that either regional and environmental or technical factors might be involved. Thus, in a recent study, Eng et al. [7] examined microdissected subpopulations from sporadic MTCs and multiple metastases from these tumors and found that approx 80% of sporadic MTCs had at least one subpopulation with the *RET* codon 918 mutation. The distribution of this mutation was nonhomogeneous, occurring only in subpopulations in most

tumors and among subsets of multiple metastases. These findings suggest either that the codon 918 mutation can arise as an event in progression within a metastatic clone, or that MTC can be of polyclonal origin. In the same study the authors also reported that one of two MTCs from MEN-2A patients carried a somatic mutation at codon 918 in addition to the *RET* mutation present in the germline.

Recently, two groups have reported that somatic mutations of the *RET* proto-oncogene in sporadic MTCs were significantly correlated with a poor outcome, whereas others have demonstrated that the presence or absence of the somatic mutation at the *RET* codon 918 was not correlated with clinical data, including most recent follow-up [8,9]. Thus, the clinical significance of *RET* mutations in sporadic MTCs remains to be elucidated on larger series of patients with long-time follow-up data.

We and others have demonstrated that the analysis of germline DNA for *RET* mutations may be helpful to determine the hereditary or sporadic nature of MTCs [10] and that DNA analysis can also be performed on DNA extracted from paraffin-embedded tissues [11]. The absence of a germline *RET* exon 10, 11, 13, 14, or 16 mutation appears to rule out MEN-2A, MEN-2B, or FMTC to a high probability, although a familial form of MTC other than classical MEN-2 cannot be excluded conclusively. Furthermore, the presence of a few MEN-2 families without detectable *RET* mutations indicate that this disease might also be caused by germline mutations in the gene encoding the GDNFR-α or other genes.

RET in Papillary Thyroid Carcinoma

The *RET* proto-oncogene has also been implicated in the causation of papillary thyroid carcinoma (PTC). It has been shown that *RET* is activated through somatic rearrangements in a subset of PTCs. The *RET*/PTC oncogenes, rearranged forms of the *RET* proto-oncogene, encode fusion proteins in which proto-*RET* tyrosine kinase and C-terminal domains are fused to different donor genes. The respective *RET*/PTC oncoproteins display constitutive tyrosine kinase activity and tyrosine phosphorylation. Three major forms of the *RET*/PTC oncogene have been identified: the *RET*/PTC-1 oncogene (where c-*RET* rearranges with the H4 gene D10S170 on chromosome 10q21), the *RET*/PTC-2 oncogene (where c-*RET* rearranges with the regulatory subunit R1 alpha of the protein kinase A on 17q23), and the *RET*/PTC-3 oncogene (where c-*RET* rearranges with the RFG2/Ele1 gene on 10q11.2). Thus, the two most common forms, *RET*/PTC-1 and *RET*/PTC-3, both result from a paracentric inversion of the long arm of chromosome 10 [12]. More recently, a novel type of Ele1/*RET* rearrangement designated *RET*/PTC-4 has been described in which the exon 5 of Ele1 is joined to exon 11 instead of exon 12 of *RET* and the cDNA sequence is 93 nucleotides larger than the regular one [13]. The *RET*/PTC-4 oncogene has been found in a post-Chernobyl PTC, indicating that targeted radiation effects could be responsible for the atypical *RET* rearrangement.

Wide differences (2.5–60%) in frequency of *RET* activation by *RET*/PTC in PTCs of different populations have been reported, and it is not clear whether these are owing to environmental factors, racial differences, or technical reasons. However, several studies have shown an association between ionizing radiation and development of PTC. In addition, in vitro irradiation of tumor cell lines induced rearrangements of *RET* similar to those observed in human PTCs. These two observations could be

related to the reported increased incidence of PTCs in children living in contaminated areas around Chernobyl. However, it has recently been demonstrated that age, *per se,* also plays a role in the development of *RET* positive PTCs [14]. Furthermore, it has been shown that *RET*/PTC activation is present in 11 of 26 (42%) occult PTCs and therefore concluded that *RET*/PTC rearrangement represents an early event in the process of thyroid cell transformation [15].

The clinical relevance of *RET*/PTC rearrangements in PTCs is controversial. Some have suggested that *RET*/PTC expression could serve as an indicator of aggressive behavior in PTCs, specifically for distant metastatic disease. In a recent study, Sugg et al. [16] analyzed 60 thyroid carcinomas by RT-PCR for *RET*/PTC expression to determine a possible correlation with clinical data or tumor morphology. They found *RET*/PTC oncogene rearrangements in 3 of 60 (5%) PTCs of young patients (<45 yr of age) with small thyroid carcinomas showing a predisposition for lymphatic involvement, suggesting a possible role in the development of this subgroup of tumors. Thus, more data are necessary to determine whether molecular analysis of *RET*/PTC rearrangements are required in patients with PTCs.

In addition to the tumor types mentioned, *RET* appears also to be causative for 10–40% of cases with Hirschsprung's disease [17] with or without MEN-2 [18], but seems not to be generally important in the formation of sporadic neuroendocrine tumors other than MTC and pheochromocytoma [6].

Summary and Conclusion

A variety of recent studies on the *RET* proto-oncogene have elucidated the pathophysiological mechanisms leading to neoplastic disease; we now understand that dominant activating germline mutations lead to MEN-2A, MEN-2B, and FMTC; somatic mutations to sporadic MTCs; *RET* rearrangements to PTCs; and inactivating alterations to Hirschsprung's disease. The clinical significance of *RET* alterations, especially in sporadic thyroid tumors, however, is still controversial and therapeutic concepts in MEN-2 gene carriers only start to emerge. Furthermore, a substantial proportion of sporadic MTCs and especially PTCs do not show *RET* mutations or activation and are likely caused by other genetic alterations. Several candidate genes, such as the GDNF and GDNFR-α [19], are currently under investigation and it is anticipated that the near future will bring new insights into the tumorigenesis of sporadic as well as hereditary thyroid carcinoma.

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