American Journal of Pathology, Vol. 147, No. 6, December 1995

Commentary

RET Proto-Oncogene Mutations and Rearrangements in Endocrine Diseases

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Introduction

The *RET* proto-oncogene encodes a member of the receptor tyrosine kinase family of transmembrane receptors and has been mapped to the centromeric region of chromosome 10q11.2.[1–4] It is expressed in various normal tissues including thyroid, adrenal, nerve tissue, developing kidney and in some neuroendocrine tumors including medullary thyroid carcinoma (MTC) and pheochromocytomas, as well as in parathyroid hyperplasia and neoplasias. Recent studies have shown that in patients with familial forms of MTC *RET* proto-oncogene is usually mutated in specific exons associated with certain diseases such as multiple endocrine neoplasia (MEN) syndromes. In this issue of the Journal Padberg et al[5] searched for *RET* proto-oncogene point mutations in sporadic hyperplastic and neoplastic lesions of the parathyroid glands. Hyperplastic parathyroid glands from six patients with MEN 2A, which is associated with MTCs, pheochromocytomas, and parathyroid disease, all had germ line mutations in exon 11 with a Cys 634 → Arg substitution. The solitary patient with MEN 2B, which is associated with MTCs, pheochromocytomas, a marfanoid habitus, mucosal neuromas, intestinal ganglioneuromas, skeletal abnormalities, and only rarely parathyroid disease, had a mutation in codon 918 of exon 16. None of the 32 patients with hyperparathyroidism secondary to sporadic hyperplasia or neoplasias had any mutations in exons 10, 11, and 16 detected in the analysis by Padberg et al[5] of paraffin-embedded tissue sections. These findings add to the rapidly accumulating new data from analyses of the *RET* proto-oncogene in various disorders and raises questions about the role of *RET* proto-oncogene in sporadic and familial neuroendocrine and related diseases.

The transmembrane tyrosine kinase receptor encoded by *RET* proto-oncogene has a calcium-binding cysteine-rich extracellular domain along with a cadherin-like ligand binding site, a tyrosine kinase-containing intracellular domain, and a short transmembrane domain.[2,3] The *RET* gene consists of at least 20 exons spanning more than 60 kb of genomic DNA and encodes 5 major mRNA species.[4–7] The proto-oncogene products are expressed as polypeptides of 1072 and 1114 amino acid residues that differ in C-terminal amino acid composition. The *RET* ligand is still unknown.

Both familial and sporadic pheochromocytomas and MTCs express normal-sized transcripts of *RET* proto-oncogene.[8] *RET* proto-oncogene is also frequently expressed in neuroblastoma cell lines[9,10] as well as in human neuroblastomas.[9] The detection of *RET* proto-oncogene in a variety of normal and neoplastic neural and neuroendocrine tissues including adrenal glands, normal thyroid, sympathetic ganglia, and parathyroid parenchymal cell precursors derived from the posterior branchial arches, which are *RET*-positive,[11,12] helps to explain the diverse expression of *RET* in neuroendocrine cells and tissues involved with multiple endocrine neoplasia. Interestingly, Pachinis et al[12] found *RET* expression during mouse embryogenesis not only in cell lineages from the peripheral and central nervous system, but also in the nephric or Wolffian duct, the ureteric bud epithelium and the growing tips of the renal collecting ducts, suggesting that the c-*RET* gene may encode the receptor for a factor involved in the prolif-

Accepted for publication September 13, 1995.

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Table 1. *Summary of ret Proto-Oncogene Mutations in Various Diseases*

Disease	Tissue/Tumor	ret proto-oncogene mutation	Reference
MEN 2A	Thyroid C cell Pheochromocytoma Parathyroid	Exons 10 and 11 Codons 609, 611, 618, 620, 630, and 634	14, 19, 20 23, 24, 27, 28
MEN 2B	Thyroid C cell Pheochromocytoma Parathyroid	Exon 16 Codon 918	16, 17, 21, 22
FMTC	Thyroid C cell	Exons 10 and 11 Exon 13 Codon 768	15, 19, 24
Sporadic MTC*	Thyroid	Exon 16 Codon 918	29, 9
Sporadic† Pheochromocytoma	Pheochromocytoma	Exons 11 and 16	30
Hirschsprung's	Ganglion cells	Exons 2, 3, 5, and 6	32, 33, 34

*Sporadic MTC: somatic mutations in about 20 to 40% of patients.[9,29]
†Sporadic pheochromocytomas: somatic mutations in about 10% of patients.[30]

eration, migration, and differentiation of neuronal cell lineage as well as renal organogenesis. Recent studies by Schuchardt et al[13] in which mice homozygous for a targeted mutation in c-*RET* developed to term but died soon after birth with renal agenesis or severe dysgenesis and absence of enteric neurons throughout the digestive tract support the concept that *RET* is an essential component of pathways needed for enteric neurogenesis and renal organogenesis.[13]

Analysis of *RET* proto-oncogene expression and mutations have had the greatest clinical impact in the diagnosis of MEN syndromes.[9] These diseases, which are inherited as autosomal dominant disorders, are associated with abnormalities of the *RET* proto-oncogene and include MEN 2A, MEN 2B, and familial MTCs (FMTCs). Patients with FMTC develop C-cell tumors in a familial pattern, but do not develop the other abnormalities affecting MEN 2A and 2B patients such as parathyroid and adrenal medullary abnormalities.

Several studies have characterized germ line mutations in MEN 2A, MEN 2B, and FMTC.[14–29] These studies have shown that various mutations are commonly associated with specific disease subtypes (Table 1). Thus, 95% of patients with MEN 2A have germ line mutation in exons 10 and 11, whereas >90% of MEN 2B patients have germline mutations in exon 16 codon 918.[9] In contrast FMTC is associated with mutations in exon 10 and 11 as well as exon 13 codon 768 mutations.

In an analysis of 118 families with MTC, Mulligan et al[27] found that one of five cysteines in the extracellular domain in 97% of MEN 2A patients and 86% of FMTC patients was usually involved with *RET* proto-oncogene mutations. Most of the mutations in 84% of these MEN 2A patients affected codon 634. MEN 2A patients with a Cys 634 → Arg substitution had a greater risk of developing parathyroid disease than those with other codon 634 mutations. Komminoth et al[24,25] reported similar findings that strongly link specific disease phenotype with the nature and position of the *RET* mutation.

Some patients with sporadic MTC have tumor-specific mutations in codon 918, and a few have codon 768 mutations.[18,29] It is possible that exon 10 or 11 mutations may not lead to MTC development in most cases when they occur somatically, but may have a developmental role in priming the C cells before transformation occurs.[9]

The utility of DNA analysis for the unambiguous identification of MEN 2A gene carriers, unlike the less exact biochemical tests, was recently documented by Lips et al[28] in a study of 300 subjects from four large families. Patients with MEN 2B usually have mutations in exon 16 codon 918.[16,21] The observation of mutations affecting exon 16 codon 918 in 23% of patients with sporadic MTC from a study of 71 sporadic MTC suggests that codon 918 of the *RET* gene may play a role in the development of sporadic C-cell tumors as it does with MTC in most MEN 2B patients.

Pheochromocytomas, like neuroblastomas, usually arise in the adrenal medulla and both tumors commonly express *RET* proto-oncogene.[23] Komminoth et al[23] detected mutations in exons 10 and 11 in

pheochromocytomas from patients with MEN 2A using paraffin embedded pheochromocytoma specimens with nonradioactive single-strand conformation polymorphism analysis and direct sequencing. With these same techniques, analysis of seven patients with sporadic pheochromocytomas did not have mutations in exons 10,11 or 16. However, Lindor et al[30] using fresh frozen tissues recently detected mutations in exons 10, 11, and 16 in 3 of 30 patients (10%) with sporadic pheochromocytomas. The mutations were somatic in origin, since they were not found in the peripheral blood from positive cases and suggested that the *RET* proto-oncogene could contribute to the development of pheochromocytomas in some sporadic tumors.[30] In contrast to sporadic MTC and FMTC, mutations in codon 768 have not been reported in sporadic pheochromocytomas.[9]

The absence of *RET* proto-oncogene mutations in sporadic parathyroid tumors, as reported by Padberg et al,[5] is not too surprising, although based on other neuroendocrine tumors involved in MEN such as MTC and pheochromocytoma, a small percentage of parathyroid hyperplasias or neoplasms with *RET* mutations may have been expected. It is possible that *RET* mutations in parathyroid lesions may be located in exons other than 10, 11, and 16; that the sample size of Padberg et al[5] was too small to detect rare mutations; or that there is a slight decrease in the sensitivity of the assay systems when the starting DNA is from paraffin-embedded tissue.

Hirschsprung's disease is associated with decreased or absent enteric ganglion cells. Most patients develop obstructive constipation during the neonatal period or megacolon as infants or adults. The association of Hirschsprung's disease with other neurological abnormalities and with Down's syndrome has suggested that this disease may be one manifestation of more extensive abnormalities in neural crest development.[31] This concept was supported by recent observations that *RET* mutations are involved in Hirschsprung's disease.[32–34] Various missense and nonsense mutations in exons 2, 3, 5, and 6 have been found. The mutations included amino acid substitutions, deletions, and premature stop codons, which led to loss of *RET* function, and which support the observation that *RET* proto-oncogene plays a critical role in the embryogenesis of the enteric nervous system in addition to its role in tumorigenesis and in renal development.

Interestingly, patients with MEN 2A and MEN 2B do not usually have a higher incidence of Hirschsprung's disease; only six families with MEN 2A or FMTC and Hirschsprung's disease have been reported.[9] This may be partially explained by the different portions of the *RET* gene affected in these diseases (Table 1) and results from various studies indicating that MEN 2A and MEN 2B represent proliferative conditions while patients with Hirschsprung's disease have a loss of function secondary to the *RET* proto-oncogene mutations.

The *RET* proto-oncogene is not only involved in the development of neuroendocrine tumors. Several investigators have linked papillary thyroid carcinomas with chromosomal rearrangements of the *RET* gene resulting in activation of the *RET* proto-oncogene tyrosine kinase by fusion with other genes.[35–41] Both *RET* and *trk* proto-oncogenes encode receptor tyrosine kinases and are both rearranged in a small group of papillary carcinomas where they form chimeric oncogenes by fusion of the tyrosine kinase domains with the 5′ end sequences of different activating genes.[39–41] This fusion protein has constitutive tyrosine kinase activity, which is probably associated with its transforming ability in experiments with NIH 3T3 cells.[39] In one study, activation of *RET* proto-oncogene was observed in 18 of 52 cases of papillary thyroid carcinomas examined by transfection assay in NIH 3T3 cells.[39,41] All of the *RET* breakpoints characterized to date have been clustered in the 1843-bp intron 11 of the *RET* proto-oncogene between the transmembrane and the first tyrosine kinase domain encoding exons, so the *RET* exon 12 is always joined to coding sequences of the various activating genes.[39,41] In a recent report Ito et al[42] observed that *in vitro* irradiation of an undifferentiated human thyroid carcinoma cell line was able to cause *RET* activation by a rearrangement similar to that producing one of the *RET* rearrangements, *RET*/PTC1, suggesting that radiation-induced thyroid carcinomas may develop by this molecular mechanism.

Although the specific mutations in MEN 2A, MEN 2B, FMTC, and sporadic MTC have been extensively characterized, it is not known how these alterations contribute to neoplastic development. The mutated *RET* allele could act as a dominant transforming gene, or the *RET* proto-oncogene mutations could inactivate a possible tumor suppressor fraction of *RET*, which could lead to transformation directly or indirectly by inhibition of the function of the wild-type protein in a dominant negative role.[43] To test these various possibilities Santoro et al[17] used engineered eukaryotic expression vectors encoding proto-*RET*, three different MEN 2A alleles, and the MEN 2B allele. There were differences in the effects of mutations in MEN 2A and MEN 2B. Mutations in MEN 2A led to *RET* dimerization at steady state while the MEN 2B mutation altered *RET* catalytic properties. After

transformation with NIH 3T3 fibroblasts, the *RET* mutants, but not the proto-*RET*, had a high transforming efficiency, and the MEN 2A and MEN 2B transfectants showed high clonogenic ability in soft agar while the proto-*RET* transfectants did not. Interestingly, although MEN 2B is clinically more aggressive than MEN 2A, the *RET* MEN 2B protein did not display increased kinase activity or transforming ability as compared with *RET* MEN 2A. Moreover, other studies showed that MEN 2B mutations altered the substrate specificity of *RET* while MEN 2A mutations did not. These experiments indicated that mutations in MEN 2A and MEN 2B convert *RET* into a dominant transforming gene and that the underlying mechanism is a dominant oncogenic conversion rather than a loss of suppressor function.

Although the precise roles of the *RET* gene in normal targeted neuroendocrine cell development and in the function of thyroid C cells are not known, recent studies by Carson et al[44] with the MTC cell line TT have provided new insight into the role of *RET* gene expression. These investigators showed that when the TT cell line had the raf-1 signal transduction pathway activated, the TT cells were induced within 48 hours to resemble mature MTC cells. During this time expression of both the mutant and wild-type *RET* gene alleles were silenced at the protein and mRNA levels. These investigators proposed that *RET* proto-oncogene mutations causing increased function may lead to abnormal proliferation rather than differentiation, which could produce C-cell hyperplasia and tumor development. The silencing of *RET* proto-oncogene by raf with inducible differentiation suggests that the mechanism may be similar to the down-regulation of tyrosine kinases in other systems.[45]

The molecular mechanisms involved in the development of sporadic parathyroid neoplasms remains uncertain.[5] Various candidate oncogenes, including PRAD1/cyclin D1, which has been found to be abnormally expressed in some parathyroid adenomas;[46,47] and the retinoblastoma suppressor gene, which has been associated with allelic loss on chromosome 13q14 and inactivation from mutations in parathyroid carcinomas, have been reported.[48] The relationship of PRAD1 and *RET* proto-oncogene in the development of parathyroid hyperplasias and adenomas in MEN 2A remains to be explored.

In summary, the analysis of disorders with *RET* proto-oncogene mutations and rearrangements has provided a great deal of insight into diseases involving the neuronal and neuroendocrine systems such as MEN syndrome types 2A and 2B and Hirschsprung's disease, in addition to developmental anomalies of the nephrogenic system and the pathogenesis of papillary thyroid carcinomas. Although many major questions about the function of the ligand, the exact functions of the *RET* protein, and the precise role of *RET* proto-oncogene in tumor development and progression remain to be elucidated, the studies to date have provided the foundation to address many unanswered questions about the role of *RET* proto-oncogene in endocrine diseases.

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