Germline and Somatic Mutations in an Oncogene: RET Mutations in Inherited Medullary Thyroid Carcinoma


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Abstract

Inherited cancer syndromes predispose an individual to the development of specific tumors. Somatic and germline mutations in the same tumor suppressor gene, as described in Knudson’s two-mutation model, are well recognized. Inherited mutations in the RET proto-oncogene, which encodes a receptor tyrosine kinase, predispose individuals to the multiple endocrine neoplasia type 2 (MEN 2) cancer syndromes. The major component tumor of these syndromes is medullary thyroid carcinoma (MTC). To date, somatic mutations in RET have not been identified in tumors from individuals with MEN 2, although they have been well documented in sporadic MEN 2-related tumors. We have identified, among 16 MEN 2 cases with well-defined RET germline mutations, a somatic missense mutation at codon 918 of RET in 3 of 15 MTCs and in a sample with hyperplastic C-cells (the presumed precursor to hereditary MTC). We suggest that the presence of a somatic mutation, in addition to the preexisting germline mutation in hereditary MTCs, may contribute to tumorigenesis in vivo.

Introduction

MTC is a malignancy of the thyroid C-cells and is the characteristic component tumor of the inherited cancer syndromes MEN types 2A and 2B and FMTC. Germline mutations in the RET proto-oncogene, which encodes a receptor tyrosine kinase, cause MEN 2A, MEN 2B, and FMTC (1-9). The majority of germline mutations that have been found in MEN 2A and FMTC families lie within exons 10 and 11 of RET at codons 609, 611, 618, 620, and 634 and result in the substitution of a cysteine by an alternative amino acid (1, 2). A single mutation at codon 918 causing the substitution of a methionine (ATG) with a threonine (ACG) is associated with MEN 2B and sporadic MTC (3-5, 10-12). Somatic mutations in RET codons 768 and 883 have also been described in sporadic MTC at very low frequencies (7, 8, 13). Studies using transient transfection and other methods have shown that RET codon 634 missense mutations result in increased receptor dimerization independent of ligand binding, and the codon 918 mutation leads to altered substrate specificity (6, 9, 14). Both mutations increase receptor tyrosine kinase activity (6, 9).

Inherited cancer syndromes are generally caused by germline mutations in tumor suppressor genes (15). Activating mutations in the RET proto-oncogene in the germline of MEN 2 and FMTC individuals represent an exception to this rule. According to Knudson’s two-mutation model originally invoked for tumor suppressor genes (16), a primary mutation in a tumor suppressor gene occurs in the germline, with a secondary mutation occurring in somatic tissue within the same gene on the other allele leading to tumorigenesis (17, 18). It has been thought that this double mutation is not necessary in the case of oncogenes since a single activating mutation in RET has been shown to transform cells (6, 9). However, although the RET germline mutation is obviously present from birth in an affected individual, the factors that determine how CCH, the presumed precursor to hereditary MTC (19, 20), or MTC develop in C-cells are yet to be elucidated. We hypothesized that the presence of a somatic mutation, in addition to the preexisting germline mutation in hereditary MTCs, may contribute to tumorigenesis in vivo.

Materials and Methods

Patients. Thyroid tissue, from 16 members of MEN 2A or FMTC families, in which germline RET mutations had been identified previously (21-23), was studied. Patient 3, aged 11 years, was identified as having a high risk of inheriting the MEN 2A phenotype by linkage analysis, prior to the advent of direct mutation detection, and thyroidectomy was performed (24). CCH was diagnosed in the thyroid specimen from this patient. All other patients were adult and had MTCs that varied in extent from 8 mm (patient 1) to widespread metastatic disease in the remaining patients, with the exception of patients 6 and 16.

Mutation Detection. DNA was extracted from 5 paraffin-embedded and 11 frozen thyroid tissue specimens by methods described previously (25, 26). Sequencing of PCR products was performed using the Cyclist DNA Sequencing Kit (Stratagene, La Jolla, CA). Exon 16 was amplified using PCR and the primers CRT5G and CRT5H (3, 27). PCR products from this primary amplification were used as templates in a secondary PCR, which used a modified forward primer, to generate fragments specifically designed to be cleaved by the restriction endonuclease Rsal (New England Biolabs, Inc., Beverly, MA) only in the presence of the codon 918ATG → ACG mutation (28). Ten MTCs, whose codon 918 mutation status has been reported previously as negative (patients 6-8 and 10-16; Table 1; Ref. 12), were also re-analyzed with this sensitive primer-mediated technique. Samples were screened for mutations at codons 768 and 883 (7, 8, 13) by PCR amplification with CRT4E and CRT4F (exon 13), and CRT17B and CRT17G or CRT17S and CRT17A (exon 15), respectively (27), and digestion with the restriction endonuclease Alul (New England Biolabs, Inc.). The presence of each of the mutations causes loss of an Alul site. All results were independently replicated in separate laboratories.
Table 1  Germline and somatic RET mutations in MTCs of MEN 2A and FMTC origin

<table>
<thead>
<tr>
<th>Patient</th>
<th>Phenotype</th>
<th>Germline mutation</th>
<th>Presence of somatic codon 918 mutation in hereditary MTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*(a)</td>
<td>FMTC</td>
<td>618TGC-&gt;CGC</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>FMTC</td>
<td>620TGC-&gt;CGC</td>
<td>Positive</td>
</tr>
<tr>
<td>3*(a)</td>
<td>MEN 2A</td>
<td>634TGC-&gt;TCC</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>MEN 2A</td>
<td>634TGC-&gt;CGC</td>
<td>Positive</td>
</tr>
<tr>
<td>5*(a)</td>
<td>FMTC</td>
<td>618TGC-&gt;CGC</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>FMTC</td>
<td>634TGC-&gt;TCC</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>MEN 2A</td>
<td>618TGC-&gt;GGC</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>MEN 2A</td>
<td>620TGC-&gt;CGC</td>
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<tr>
<td>9</td>
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<td>MEN 2A</td>
<td>634TGC-&gt;CGC</td>
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<td>15</td>
<td>MEN 2A</td>
<td>634TGC-&gt;CGC</td>
<td>Negative</td>
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<tr>
<td>16</td>
<td>MEN 2A</td>
<td>634TGC-&gt;TAC</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Individuals from the same FMTC family.  
* CCH only.

Results

Fifteen MEN 2 MTCs and a single thyroid sample containing hyperplastic C-cells were analyzed for the presence of the somatic codon 918 mutation (ATG→ACG) in exon 16 of RET. A codon 918 mutation was present in 3 of 15 MTCs and in the sample with CCH (Fig. 1; Table 1). Screening of germline DNA samples for the codon 918 mutation, both by sequence analysis and primer-mediated mutation detection, was negative in all patients studied (data not shown). We also examined our tumors for the presence of two other somatic mutations, at codons 768 (exon 13) and 883 (exon 15), reported previously to be associated with sporadic MTC and/or FMTC (7, 8, 13). Neither mutation was detected in our tumor samples (data not shown).

Discussion

This is the first report of both germline and somatic mutations in a single dominantly acting oncogene occurring in the same tumor. Furthermore, the presence of a codon 918 somatic mutation in hyperplastic C-cells suggests that in this sample, the second genetic event occurred early in tumorigenesis.

By analogy to other receptor tyrosine kinases, wild-type RET should require ligand binding for dimerization and subsequent activation, leading to autophosphorylation and phosphorylation of specific downstream targets (29). Transient transfection experiments have shown that MEN2A mutations at cysteine codon 634 increase
autophosphorylation and cause covalent dimerization of the RET receptor in the absence of ligand (6-9). The ability of the codon 918 mutation to cause changes in RET receptor autophosphorylation and substrate specificity in the absence of covalent dimerization of the RET receptor has also been demonstrated, although the level of phosphorylation of the mutant codon 918 monomer is less than that of the mutant MEN 2A dimer (9, 14). A level of receptor activation surpassing that achieved in the presence of either mutation alone may occur as a consequence of the combination of a dimer-inducing MEN2A mutation and a syntenic codon 918 mutation. Alternatively, if mutations are located on different alleles, then every RET molecule is mutant. MEN 2A mutant receptors would be constitutively dimerized, and the monomeric codon 918 mutant receptors would exhibit altered substrate specificity. This combination may also enhance the potential for neoplastic transformation in MEN 2 individuals.

Germline mutations in the RET proto-oncogene represent the predisposing event in the development of MTC in MEN 2 and FMTC. With the number of target cells increased, further somatic mutations in RET, in combination with loss of heterozygosity at other chromosomal loci (30), may contribute to tumorigenesis. Although Knudson’s two-mutation hypothesis has been validated in several inherited cancer syndromes with germline mutations in tumor suppressor genes, this is the first example of a two-mutation mechanism occurring in a familial cancer syndrome caused by a dominantly acting oncogene.

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References

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