Somatic trinucleotide change encompassing codons 882 and 883 of the RET proto-oncogene in a patient with sporadic medullary thyroid carcinoma

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Abstract

Objective: Restriction analysis is a straightforward procedure for mutational analysis. It is commonly used for screening RET mutations. Incomplete digestion is a well-known cause of false results. Herein, we report another limitation of the method.

Design and Methods: Screening for somatic mutations in RET exons 16, 13 and 15 was performed in a patient with a sporadic medullary thyroid carcinoma. Genetic study was carried out by both restriction analysis and direct sequencing.

Results: A somatic trinucleotide change encompassing codons 882 and 883 of the RET proto-oncogene (GT\textsuperscript{A} GCT to GT\textsuperscript{T} TTT) was documented. Particular to this case is the silent mutation (GTA → GTT) at codon 882. Independently, both the novel silent mutation and the missense mutation at codon 883 may disrupt the same AluI restriction site. Based on the restriction pattern we were able to say that both mutations occurred in the same allele.

Conclusions: Restriction analysis is an easy approach for screening RET mutations; however, it is not enough to assign a final diagnosis.

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Introduction

RET proto-oncogene localizes to 10q11.2 (1) and encodes a tyrosine receptor. Activating mutations affecting different domains of RET proto-oncogene are associated with multiple endocrine neoplasia types 2A and 2B (MEN 2A and 2B) and with familial medullary thyroid carcinoma (FMTC) or sporadic medullary thyroid carcinoma (MTC). MEN 2 (OMIM no. 171400) and FMTC (OMIM no. 155240) are both autosomal dominant familial cancer syndromes. Although not frequently, mutations may occur within exon 15. Marsh et al. (2) described a somatic mutation at codon 883 in one of 32 sporadic tumors. Later, germline mutations were documented at codon 891 in FMTC patients (3) and at codon 883 in MEN 2B patients (4).

We here report the coexistence, in the same allele, of two somatic mutations affecting codons 882 and 883 of the RET proto-oncogene.

Materials and methods

The patient, a Caucasian male, was diagnosed and operated on at the age of 35 years. He is alive 3 years after surgery but has cervical and mediastinal lymph nodes as well as bone metastases.

PCR primers (5) were as follows: upstream (CRT 17B): GTC TCA CCA GGC CGC TAC and downstream (CRT 17G): ATG GTG CAC CTG GGA TCC CT. PCR conditions and analysis of PCR products were as follows: approximately 100 ng genomic DNA were amplified in a 40 μl reaction. Genomic DNA was denatured for 5 min at 95 °C prior to 35 cycles of 94 °C, 64 °C and 72 °C for 45 s each temperature, followed by a 7 min 72 °C polishing step. Amplified products were digested with AluI and separated electrophoretically on 10% polyacrylamide gels. The gels were stained with ethidium bromide and analysed under u.v. light. The purified PCR products were directly sequenced using Sequenase Version 2.0 kit (USB, Cleveland, OH, USA).

Results

After having excluded the presence of the most common germline mutations, the patient was screened for somatic mutations. Since the 883 somatic mutation destroys one restriction site for the AluI enzyme, exon 15 was initially screened by restriction analysis. As seen
The frequency of germline mutations, either inherited or de novo, in sporadic MTC patients depends on the series ranging from 1.5% to 6% (6, 7).

Somatic mutations in RET have been described in 23–69% of sporadic MTCs. The most common occurs within codon 918 (8, 9). Mutations in codons 768 (10) and 883 (2) have been reported less frequently. In a few cases, MEN 2A-like somatic mutations (11, 12), as well as deletions in exons 10 and 11, have been identified. Furthermore, sporadic MTCs may have different cell subpopulations, some of which carry somatic mutations while others do not or, alternatively, different subsets of cells may carry different somatic mutations (13).

In the present case, in addition to the previously described codon 883 mutation, we found a second sequence variant at codon 882 (GTA→GTT) present in a heterozygous state. The A→T substitution, at the third position, disrupts one restriction site for AluI independently of the 883 mutation that also destroys the same restriction site. Moreover, based on the heterozygous AluI restriction pattern it is possible to say that both mutations occurred in the same allele (in cis). As a silent mutation, it can hardly be responsible for the aggressive behaviour of disease noticed in our patient. Nevertheless, it is necessary to be cautious in the interpretation of apparently neutral variants as these kinds of sequence variants can lead to an aberrantly spliced product (14, 15).

Most of the time, restriction analysis is an easy and reliable detection strategy for RET mutations. The present case points out one limitation of this method. Given the possible coexistence of different mutations associated with the same restriction pattern, only sequencing analysis will further clarify and definitely establish the diagnosis of exon 15 somatic mutations.

References

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