CLINICAL STUDY

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 (GTA GCT to GTT 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.

European Journal of Endocrinology 142 573–575

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 at 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

Figure 1 Ethidium bromide-stained polyacrylamide gel of AluI restriction digestion of *RET* exon 15 amplicons. Lane 1, negative control; lane 2, tumour DNA from patient; lane 3, corresponding germline DNA for tumour in lane 2. For the mutant allele, restriction digestion produces bands of 175 and 118 bp. For the normal allele, restriction digestion produces bands of 135, 118 and 40 bp. M denotes the lane containing the 100 bp ladder marker (Gibco/BRL, Gaithersburg, MD, USA).

in Fig. 1, the restriction pattern suggested the presence of the A883F mutation.

Sequence analysis not only confirmed the expected mutation but also documented a second sequence alteration involving codon 882 (Fig. 2). This last event is a silent mutation, GTA→GTT (Val→Val) which, by itself, also destroys the same restriction point for AluI. Since the restriction pattern corresponding to peripheral blood DNA was normal, as shown in lane 3 of Fig. 1, the second variant could not be interpreted as a polymorphism.

Discussion

No occult or *de novo* germline mutations in *RET* were found in this patient with an apparently sporadic MTC.

Figure 2 DNA sequence analysis of exon 15 PCR amplification products from tumour DNA. Heterozygosity at codon 882 (GTA→GTT) and at codon 883 (GCT→TTT) can be observed.

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 can 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.

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Received 14 December 1999

Accepted 8 March 2000