SHORT COMMUNICATION

Mutation analysis of the c-mos proto-oncogene and the endothelin-B receptor gene in medullary thyroid carcinoma and phaeochromocytoma

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Summary The characteristic tumours of MEN 2 are medullary thyroid carcinoma (MTC) and phaeochromocytoma. Somatic RET mutations have been found in only 23–40% of sporadic MTC and 10% of sporadic phaeochromocytomas. Thus, we sought other genes which may play a role in the pathogenesis of these tumours. We carried out direct sequence analysis of human c-mos and human ENRB in a series of sporadic MTC and phaeochromocytomas to determine if somatic mutations in these two genes could account for some of the sporadic MEN 2-related tumours in which no RET mutations are detected. No somatic mutations were found.

Keywords: MEN 2; RET; c-mos; endothelin-B receptor; medullary thyroid carcinoma; phaeochromocytoma

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominantly inherited cancer syndrome associated with germ line mutations in the RET proto-oncogene, which codes for a receptor tyrosine kinase expressed in tissues and tumours of neural crest origin (Bohlin et al., 1995; Carlson et al., 1994; Donis-Keller et al., 1993; Eng et al., 1994, 1995b; Hofstra et al., 1994; Mulligan et al., 1993, 1994, 1995). MEN 2 is characterised by the presence of MTC and phaeochromocytoma. Somatic RET mutations have been detected in 23–40% of sporadic MTC in series comprising ten or more tumours (Eng et al., 1994, 1995b; Hofstra et al., 1994; Kominoh et al., 1995; Zedenius et al., 1994) and 10–20% of sporadic phaeochromocytomas (Beldjord et al., 1995; Eng et al., 1994, 1995a; Lindor et al., 1995).

The human c-mos proto-oncogene encodes a serine–threonine protein kinase expressed at high levels in germ cells (reviewed in Yew et al., 1993). It is required for meiosis and plays a role in the initiation of oogenesis (Colledge et al., 1994; Sagata et al., 1988). Transgenic mice ectopically overexpressing mos develop medullary thyroid carcinomas (MTC) and phaeochromocytomas (Schulz et al., 1992). The endothelin-B receptor gene (ENRB) codes for a G-protein-coupled receptor, which is expressed in several tissues, including the kidneys, adrenal medulla and phaeochromocytomas (reviewed in Davenport, 1996; Davenport et al., 1994). Recently, germ line mutations in ENRB have been found in patients with Hirschprung disease (HSCR) (Puffenberger et al., 1995), a common congenital condition characterised by the lack of enteric ganglia leading to intestinal obstruction (Okamoto and Ueda, 1967). Up to 40% of patients with HSCR have mutations in the RET proto-oncogene (Attié et al., 1995).

To determine if mutations in the c-mos or ENRB genes play a role in the pathogenesis of the sporadic tumours in which no RET mutations have been detected, the coding sequence of c-mos and ENRB was examined in DNA from ten sporadic MTC and ten sporadic phaeochromocytomas which do not have known somatic RET mutations.

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Received 1 December 1995; accepted without revision 15 February 1996

Materials and methods

Tumours

The ten MTCs and nine of ten phaeochromocytomas studied here have been described previously (Eng et al., 1994, 1995a,b). The remaining phaeochromocytoma was a unilateral tumour occurring in a patient without first- or second-degree relatives with these syndromes, known phaeochromocytoma or MTC, or other stigmata of MEN 2/von Hippel–Lindau disease. Genomic DNA was extracted as described by Mathew et al. (1987).

Polymerase chain reaction (PCR) and DNA sequence analysis

PCR was performed, using 50–100 ng of template genomic DNA and red hot Thermus aquaticus DNA polymerase, according to the manufacturer’s recommendations (Advanced Biotechnologies, Surrey, UK) in the presence of 1.5 mM magnesium chloride.

A total of 30 to 40 cycles of amplification were carried out at 95°C for 1 min, 59°C (c-mos) or 60°C (ENRB) for 1 min and 72°C for 1 min, followed by a final 10 min cycle at 72°C. For c-mos, primer pairs used were Hu Mus 1F (5'-CTTCTATTCATCCACGCGG-3') and Hu Mos 1R (5'-AAATTCGCCCTTGACACGAGG-3'), Hu Mos 2F (5'-GGTGTGCTTGTCTGAGAGG-3') and Hu Mos 2R (5'-CGCCGAGATGACTTCTTGTG-3'), Hu Mos 3F (5'-CACTGAGACACATGAGTGAG-3') and Hu Mos 3R (5'-GTGCTGAAACACAGCGAGA-3') or Hu Mos 4R (5'-GGACAGCGGCAGTGATGGCGCC-3') and Hu Mos 4R (5'-CTAGCTGAGCGAGGATCTGAA-3') and Hu Mos 5R (5'-CTGACCAAGTTTCTGACGACG-3'). For ENRB, the primer pairs used were ETB-0F (5'-CACACCCCTTCCAGAAGCC-3') and ETB-1R (5'-CGCGGTTATCTGACAGCATT-3') [exon 1], ETB-2F (5'-CGCTGAGCGAGGAGCTGG-3') and ETB-3R (5'-AAGGAGTGGGGAACGAGG-3') [exons 2 and 3], ETB-4F (5'-GCTATGATTACAAATAGCCATG-3') and ETB-4R (5'-GCTGCGATACACAAGAGTTG-3') [exon 5], ETB-5F (5'-CAGGATCTGAGCTTCTCC-3') and ETB-5R (5'-CTGAGGATGTTTCTGTTG-3') [exon 6], and ETB-6F (5'-ATACAAAGAATGCGAAGGCCCTG-3') and ETB-6R (5'-TTTTTTGTGGTTTGTGGTGGTA-3') [exon 7]. Primers for exon 4 have been previously described (Puffenberger et al., 1995).

PCR products were gel and column purified (Eng et al., 1996).
1994). Twenty aliquots of 100 ng of PCR product served as the template for cycle sequencing (Cyclist kit or the Cyclist EXO-Pfu DNA sequence kit, Strategene). Sequence variants were confirmed by digestion with an appropriate restriction enzyme.

Results

No somatic mutations were detected in the coding sequence of c-mos or the endothelin-B receptor gene among ten sporadic MTC and ten sporadic phaeochromocytomas. In addition, all PCR products were the expected sizes, suggesting no deletions or splicing variants which could have been missed by sequence analysis.

A novel sequence polymorphism was detected within the kinase domain of c-mos: a G (A1) to T (A2) conversion, changing an alanine to a serine at codon 105. This causes loss of a Bpl restriction site. The A1 and A2 alleles occurred at frequencies of 0.85 and 0.15, respectively, among 122 Caucasian chromosomes.

Discussion

Although the tumours that developed in transgenic c-mos mice resembled those of MEN 2, suggesting that the human homologue may be involved in MEN 2-related tumours, no specific c-mos mutations or rearrangements could be found in human MTC or phaeochromocytomas. However, gene amplification in the tumours could not be excluded with our analyses, and sufficient DNA was not available for gene dosage studies. The lack of c-mos mutations in human tumours might be because in transgenic mice, the mos constructs were transfected from the Moloney murine sarcoma virus LTR, a relatively strong promoter which may also cause ectopic patterns of expression; or because of species-specific differences. Finally, it is possible that c-mos may play a role in rare MTC and phaeochromocytomas but the numbers examined precluded detection. In light of our data, it is interesting to note that in vitro, many deletion or missense mutations of conserved residues, except for one RT residue deletion, in Xenopus mos result in the elimination of biological and kinase activity (Fukasawa et al., 1995).

The finding of germine mutations of ENR2 in HSCR and its expression pattern suggested that this gene would be a good candidate for involvement in the pathogenesis of MTC and phaeochromocytoma. However, no mutations were detected. Thus, mutations of neither human c-mos nor ENR2 appears to play a major role in the tumorigenesis of MTC and phaeochromocytoma.

A novel sequence polymorphism in the kinase domain of c-mos was detected. The polymorphisms could serve as a useful marker in the region of human c-mos on chromosome subband 8q12, and may in addition help in the understanding of computer-modelled structure-function relationships of kinase domains.

Acknowledgements

We thank Dr Darrin P Smith for critical review of this manuscript. CDW is grateful to Dr Jan Vig for his continued support and encouragement. This work was supported by core and programme grants from the Cancer Research Campaign (CRC), the CRC Dana-Farber Cancer Institute Fellowship (CE), the Susan and Larry Marx Fellowship in Cancer Genetics (CE), the Dana-Farber Cancer Institute (CE), and the National Science Foundation Graduate Research Fellowship (KAF), the Overseas Research Student Awards Scheme (KAF), an MRC-Canada Operating Grant (LMM) and the Kingston Hospital Foundation (LMM). BAJP is a Gibb Fellow of the CRC.

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