Mutation analysis of the c-mos proto-oncogene in human ovarian teratomas

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Summary Female transgenic mice lacking a functional c-mos proto-oncogene develop ovarian teratomas, indicating that c-mos may behave as a tumour-suppressor gene for this type of tumour. We have analysed the entire coding region of the c-MOS gene in a series of human ovarian teratomas to determine whether there are any cancer-causing alterations. DNA from twenty teratomas was analysed by single-strand conformational analysis (SSCA) and heteroduplex analysis (HA) to screen for somatic and germine mutations. In nine of these tumours the entire gene was also sequenced. A previously reported polymorphism and a single new sequence variant were identified, neither of which we would predict to be disease-causing alterations. These results suggest that mutations in the coding region of the c-MOS gene do not play a significant role in the genesis of human ovarian teratomas.

Keywords: c-mos; teratoma; ovary; parthenogenesis

The mos gene was first identified as the oncogenic element of the murine Moloney sarcoma virus (Moloney, 1966). Overexpression of the gene causes transformation of NIH 3T3 cells and tumour growth in mice (Fefer et al, 1967; Blair et al, 1981). However, few studies have implicated c-mos in human oncogenesis (Parkar et al, 1988; Stenman et al, 1991). The human c-MOS gene, located on chromosome 8q11, consists of a single 1.2-kb exon encoding a serine–threonine kinase (Watson et al, 1982). The MOS protein is the active component of cytokinetic factor (CSF) that is responsible for arresting developing oocytes in metaphase II (Sagata et al, 1989; Lorca et al, 1991).

Two studies using knock-out mice provide evidence for a role for c-mos in the genesis of ovarian teratomas (Colledge et al, 1994; Hashimoto et al, 1994). In both studies, male mutants were phenotypically normal, but female mutant mice exhibited a significantly reduced fertility as well as an elevated risk of developing ovarian teratomas. The females’ reduced fertility was attributed to the fact that large numbers of their oocytes failed to arrest in metaphase II and became parthenogenetically activated. Genetic studies of mouse and human ovarian teratomas suggest that both arise from the development of parthenogenetically activated oocytes (Stevens and Varnum, 1974; Surti et al, 1990).

We have examined the c-MOS gene in a set of twenty human ovarian teratomas using single-strand conformational analysis (SSCA) and heteroduplex analysis (HA). The entire gene was also sequenced in nine of these tumours to ensure that mutations were not being missed. A previously reported polymorphism and a unique silent base change were found.

MATERIALS AND METHODS

Tumour material

DNA was prepared from 12 frozen and eight paraffin-embedded ovarian tumours classified as teratomas by histological examination. Frozen tumours were ground in liquid nitrogen and homogenized in 3 ml of TNE (0.5 m Tris pH 8.0, 0.1 m sodium chloride, 20 mm EDTA). Sodium dodecyl sulphate (SDS) was added to a final concentration of 1%, and the solution was treated with Proteinase K (2 mg ml⁻¹) at 55°C overnight. DNA was extracted with phenol-chloroform and ethanol precipitated. Paraffin-embedded tissue was scraped from slides into 200 μl of extraction buffer (1 × PCR buffer, 1.5 mm magnesium chloride, 0.45% Tween-20 and 0.45% NP-40) and treated with Proteinase K (200 mg ml⁻¹) for 3 h at 55°C.

SSCA/HA

The c-MOS gene was PCR amplified with Red Hot DNA Polymerase (Advanced Biotechnologies) in five overlapping fragments of approximately 250–300 bp, using the following primer pairs: Hu MOS 1F (5’-TCTTTCAATTCACTCCACGGC-3’) and Hu MOS 1R (5’-AGTGCTTTGGTACCCAGG-3’); Hu MOS 2F (5’-CTCCGCCGCTTGCCTGCAAG-3’) and Hu MOS 2R (5’-CCGCATAGATCTGTTG-3’); Hu MOS 3F (5’-CTCGGAGGATCATCCAT-3’) and Hu MOS 3R (5’-GGTGTCTGAAAGCCAGCA-3’); Hu MOS 4F (5’-CTGAGGCAGAGTCTGTTG-3’) and Hu MOS 4R (5’-GGAGGCAGGCTGTAAG-3’); and Hu MOS 5F (5’-CTTGAACAGTTCTAGTCAC-3’) and Hu MOS 5R (5’-CTTGACCAGTTCTAGTCAC-3’). SSCA/HA was performed as previously described (Gayther et al, 1995). PCR products were denatured at 99°C for 10 min in the presence of

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formamide, then left on ice for 10 min to allow some of the product to form homo- and heteroduplexes. SSCA and HA fragments were resolved on the same gel. Electrophoresis was performed using 0.8% MDE (JT Baker) polyacrylamide gels with and without 10% glycerol. Gels were silver-stained as described previously (Gayther et al, 1995).

Sequence analysis

Nine frozen teratomas with abundant DNA were selected for sequence analysis. DNA was PCR amplified with the primers described above. PCR products were purified from 2% low-melting-point agarose gels using the Wizard PCR system (Promega). Sequencing reactions were performed using the Exol(-) Pfu Cyclist DNA Sequencing kit (Stratagene) with [γ-32P]dATP as a label. The sequencing products were run for both 2 and 4 h at 65 W on 6% polyacrylamide gels (Sequagel-6, Flugown). All PCR products were sequenced in both the forward and reverse directions.

RESULTS

Six of the twenty samples revealed similar variant SSCA patterns with the Hu MOS 2 primers (Figure 1A). Sequence analysis showed this was due to a previously reported polymorphism (G to T transversion) at codon 105 (Eng et al, 1996). This polymorphism was present in our set of teratomas at the same frequency as it was reported in the overall population. A seventh sample showed variation in both SSCA and HA patterns with the Hu MOS 4 primers (Figure 1B and C). Sequencing revealed a silent C to A transversion at codon 260 in this tumour. The blood from this patient was not available to test whether this base change was somatic or germine. However, as it does not cause an amino acid change, we would predict that it does not affect the function of c-MOS. No further base substitutions were identified by sequencing the entire coding region in DNA from nine of the frozen teratomas.

DISCUSSION

Although c-mos has been shown to be oncogenic in some mammals and mammalian cell lines, a role for this gene in human tumour formation has remained elusive. Given the strong experimental evidence suggesting a role for c-mos as a tumour-suppressor gene in murine ovarian teratomas, the absence of mutations in this analysis is perhaps surprising. It is possible that MOS is involved in human teratomas not through coding mutations but by genetic alterations outside the c-MOS coding region that alter expression of the gene, or through mutations affecting other members of its signalling pathway. Loss of heterozygosity analysis of the c-MOS region would be complicated by the fact that the majority of ovarian teratomas arise from a failure of meiosis II and contain two almost identical sets of chromosomes (Surti et al, 1990).

The lack of mutations in human teratomas might also be explained by species-specific differences. The histological profile of ovarian teratomas appears to be similar between human and mouse tumours (Michael and Roth, 1993; Furuta et al, 1995). However, there is evidence that the mos protein plays different roles in different species. In Xenopus oocyte maturation, mos is required for the activation of maturation promoting factor, germinal vesicle breakdown and the extrusion of the first polar body, as well as being the active component of cytostatic factor (Sagata et al, 1988; Yew et al, 1993). In contrast, the phenotype of c-mos mutant mice suggests that murine mos is only needed to arrest developing oocytes in metaphase II (Colledge et al, 1994; Hashimoto et al, 1994). Perhaps the functions of human c-MOS do not render it as vulnerable a target for cancer induction as the murine version of the gene seems to be.

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