Loss of heterozygosity on chromosome 22 in ovarian carcinoma is distal to and is not accompanied by mutations in *NF2* at 22q12

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Summary Frequent loss of heterozygosity (LOH) has been reported on 22q in ovarian carcinoma, implying the presence of a tumour-suppressor gene. The neurofibromatosis type 2 gene (*NF2*) at 22q12 is a plausible candidate. Analysis of 9 of the 17 exons of *NF2* by single-strand conformational polymorphism (SSCP) in 67 ovarian carcinomas did not detect any somatic mutations, suggesting that *NF2* is not involved in the pathogenesis of ovarian carcinoma. LOH data support this conclusion and that the putative tumour-suppressor gene lies distal to *NF2*, beyond D22S283.

Cytogenetic and allele loss studies point to the involvement of a chromosome 22 tumour-suppressor gene in a variety of malignancies. Loss of 22q markers has been observed in 11–38% of breast cancers (Sato *et al.*, 1990; Chen *et al.*, 1992; Lindblom *et al.*, 1993), 21–50% of colon carcinomas (Miyaki *et al.*, 1990; Okamoto *et al.*, 1988) and 33% of hepatocellular carcinomas (Takahashi *et al.*, 1993). In ovarian carcinoma, loss of 22q markers has been reported at frequencies of up to 71% (Cliby *et al.*, 1993; Dodson *et al.*, 1993), although others have reported lower frequencies in the range of 25% (Sato *et al.*, 1991; Yang-Feng *et al.*, 1993; Osborne & Leech, 1994). These studies have not defined a minimum region of LOH. However, with the exception of hepatocellular carcinomas, the 22q losses are consistent with deletion of the region containing *NF2* on 22q12 (Rouleau *et al.*, 1993; Trofatter *et al.*, 1993) and this therefore may be the target for the LOH.

Neurofibromatosis type 2 (*NF2*) is an autosomal dominant syndrome which predisposes to schwannomas, meningiomas, ependymomas and juvenile cataracts. LOH for DNA markers on chromosome 22 has been found in both hereditary and sporadic tumours associated with the *NF2* phenotype (Sanson *et al.*, 1993), suggesting that *NF2* is acting as a tumour-suppressor gene. *NF2* is expressed in a variety of non-central nervous system tissues and therefore could be implicated in tumours arising from these tissues. Although ovarian carcinoma is not part of the *NF2* syndrome, somatic mutations in the tumour-suppressor gene *NF1*, for example, have been reported in tumour types not usually associated with the hereditary syndrome (Li *et al.*, 1992). The strong genetic evidence for the presence of a tumour-suppressor gene on 22q prompted us to undertake a mutation analysis of *NF2* in ovarian carcinomas and, if appropriate, a deletion analysis to determine the smallest region of loss.

Materials and methods

Tumour specimens and DNA extraction

Tumour and blood samples were obtained from 67 patients undergoing primary surgery for ovarian carcinoma. Thirty-eight of the samples have been described previously and have been the subject of extensive molecular studies (Foulkes *et al.*, 1993a–c, 1994; Allan *et al.*, 1994). The remaining 29 samples were collected from hospitals in and around Southampton. The 67 tumours studied included 46 serous tumours, 11 mucinous, two mixed Müllerian, seven endomet-

rioid and one granulosa cell tumour. DNA was extracted from the tumours and blood as described by Foulkes *et al.* (1993a).

Polymerase chain reactions (PCRs)

PCRs were performed on 10–200 ng of genomic DNA in a reaction volume of 20 µl with the inclusion of 1 µCi of [α -³²P]dCTP (Foulkes *et al.*, 1993c) using the published PCR cycle conditions.

SSCP analysis

NF2 exons were amplified with primers within surrounding intronic sequence. Details of these primers, including the regions amplified by each set and the PCR conditions used, have been reported by Twist *et al.* (1994). Samples were prepared for single-strand conformational polymorphism analysis as described previously (Campbell *et al.*, 1994) and electrophoresis carried out at room temperature in 6% non-denaturing acrylamide gels in the presence of 5% and 10% glycerol.

Microsatellite analysis

The chromosome 22q primers used were D22S430 (Sainz *et al.*, 1993), D22S282, D22S283 and D22S274 (Weissenbach *et al.*, 1992). PCR products were resolved on 8% non-denaturing polyacrylamide gels.

Results

Nine of the 17 coding exons of *NF2* (exons 2, 5, 7–12 and 15) were examined for the presence of somatic mutations. The SSCP analysis was biased toward more 5' exons since the N-terminal region of the merlin protein is more frequently associated with mutations (Deprez *et al.*, 1994), however no mutations were detected in any of the 67 ovarian carcinomas. The sensitivity of SSCP analysis has been shown to be related to the size of the PCR product (Sheffield *et al.*, 1993). In our study, this was 188 bp, and we therefore estimate the SSCP analysis sensitivity to be 70–75%.

In view of the absence of detectable *NF2* mutations we undertook a preliminary allelotyping analysis of 37 ovarian carcinomas (from the collection described by Foulkes *et al.*, 1993a–c) in order to identify tumours with partial loss of 22q, since previous studies have not enabled an assignment of the gene to any particular region on chromosome 22. All four 22q markers used map distal to *NF2*, with the most proximal marker, D22S430, located in a region approximately 300 kb distal to *NF2*. The relative genetic and

Figure 1 Allelic deletion pattern for ovarian tumours 28, 72 and 91 showing partial loss of chromosome 22q. Unshaded boxes, no LOH; black filled boxes, LOH; grey filled boxes, constitutional homozygosity. For each informative locus, the autoradiograph of the normal (N) and tumour (T) DNA PCR is shown to the right. A genetic linkage map of the four markers used is shown to the right of a karyogram of chromosome 22 and is based on published maps (Matise *et al.*, 1994; Buetow *et al.*, 1994). The inter-locus distances are in centimorgans. The position of the *NF2* gene is indicated just proximal to D22S430.

cytogenetic positions of these markers are shown in Figure 1. Twenty-three of 32 informative tumours (72%) showed loss of one or more 22q markers. In three of these tumours partial loss of 22q was observed (Figure 1). In tumour 28, complete LOH was observed with D22S274 and D22S282, while only partial LOH was seen with D22S283. The finding was consistently found on repeated testing and therefore might be accounted for by clonal variation. In tumour 72, heterozygosity is clearly maintained with D22S283 and completely lost with the more distal markers. In tumour 91, there was maintenance of heterozygosity with the most proximal marker D22S430 but clear loss with the distal markers D22S283 and D22S274.

Discussion

LOH data suggest that a tumour-suppressor gene relevant in ovarian, breast and colon carcinomas resides on chromosome 22, but it is unclear at this stage if the same gene is involved in these and other malignancies. *NF2*, located at 22q12, is a plausible candidate for this gene, but to date somatic mutation analysis has been restricted largely to tumours of the central nervous system (CNS). However, in two reports, mutations were detected in the coding region of *NF2* in a total of 1 of 69 breast carcinomas, 6 of 17 melanomas and 2 of 64 colon carcinomas (Arakawa *et al.*, 1994; Biachi *et al.*, 1994). The significance of the low frequencies of *NF2* mutation in the breast and colon carcinomas is unclear. However, in this study of 67 ovarian carcinomas, the absence of *NF2* mutations supports the conclusion that *NF2* is not involved in the pathogenesis of ovarian carcinoma. Although we have not examined all the *NF2* coding exons, studies in *NF2*-related tumours (Rouleau *et al.*, 1993; Deprez *et al.*, 1994; Rubio *et al.*, 1994; Rutledge *et al.*, 1994; Twist *et al.*, 1994)

and non-CNS tumours (Arakawa *et al.*, 1994; Biachi *et al.*, 1994) have shown that mutations are distributed throughout the gene with a bias towards more 5' exons. The distribution of *NF2* mutations is unlikely to differ significantly in ovarian carcinomas, and given our large sample size we consider it improbable that *NF2* is the 22q ovarian carcinoma tumour-suppressor gene.

In our preliminary allelotype study we demonstrated 72% LOH of 22q markers, consistent with the frequencies reported by Cliby *et al.* (1993) and Dodson *et al.* (1993). Three tumours were identified which had retained heterozygosity for markers proximal to *NF2* but showed LOH for more distal markers. Although some somatic deletions might be the result of generalised genomic instability unrelated to tumour pathogenesis, the finding of three tumours with consistent losses distal to *NF2*, coupled with the absence of mutations in the *NF2* gene, support the conclusion that the 22q ovarian carcinoma tumour-suppressor gene lies distal to *NF2*, beyond D22S283. Detailed deletion mapping of 22q in an expanded set of ovarian carcinomas is currently under way to refine the location of this gene.

Abbreviations: LOH, loss of heterozygosity; *NF2*, neurofibromatosis type 2; PCR, polymerase chain reaction; CNS, central nervous system.

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