Loss of heterozygosity on chromosome 5q in ovarian cancer is frequently accompanied by TP53 mutation and identifies a tumour suppressor gene locus at 5q13.1–21

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Summary

Forty-nine ovarian tumours were examined for loss of heterozygosity (LOH) on chromosome 5 using eight microsatellite markers spanning both arms, including one at the APC locus. LOH on 5q was a frequent event, detectable in 23 of 49 (47%) tumours, whereas 5p LOH was detected in only 1 of 22 tumours (5%). Six tumours showed partial LOH on 5q, enabling the candidate region to be localised to a 22 cM region proximal to APC, flanked by DSS5424 and DSS644. An association was found between 5q LOH and TP53 mutation, with 18 of 23 (78%) tumours with LOH on 5q also harbouring a TP53 mutation. LOH on 5q was observed in 6 of 18 (33%) stage I tumours, suggesting that it may be an early event in the molecular pathogenesis of certain ovarian carcinomas.

Keywords: ovarian cancer; chromosome 5; loss of heterozygosity; TP53 mutation; tumour suppressor gene

Tumorigenesis results from the accumulation of multiple alterations in proto-oncogenes and tumour-suppressor genes (TSGs). Loss of heterozygosity (LOH) at specific chromosomal segments is often associated with the loss of function of TSGs and is frequently observed in a variety of human malignancies (reviewed by Weinberg, 1992). In ovarian cancer, multiple chromosomal deletions on chromosomes 3, 6, 11, 17, 18 and 22 among others have been reported (Okamoto et al., 1991; Sato et al., 1991; Yang-Feng et al., 1993; Cliby et al., 1993; Foulkes et al., 1993a,b; Tavassoli et al., 1993; Englefield et al., 1994). However, apart from TP53 and BRCA1, the TSGs which are the target of these allelic losses have not been cloned and in many cases even their approximate locations have yet to be defined. In some cases, LOH analysis has identified regions containing TSGs with proven involvement in other tumours, thus prompting investigations of the role of the TSG in ovarian cancer (Englefield et al., 1994; Foulkes et al., 1994). In particular, allelic deletions on chromosome 5 have been observed in ovarian carcinoma with the common region consistent with inactivation of the APC gene (Cliby et al., 1993; Allan et al., 1994). However, in an extensive mutation analysis, Allan et al. (1994) found no evidence of APC mutation, arguing against its involvement in ovarian tumorigenesis. They were able to confirm that chromosome 5 LOH was common in ovarian cancer but were unable to refine the location of the putative TSG beyond an exclusion of distal 5p. In an attempt to refine the location of the candidate region we have analysed for LOH using seven polymorphic microsatellite markers on chromosome 5q and one on 5p in a panel of 49 ovarian tumours. The same panel of tumours was also analysed for mutations in the TP53 gene.

Materials and methods

Tumour specimens and DNA extraction

Tumour and blood samples were obtained from 49 patients undergoing surgery for primary ovarian cancer. The tumours were collected from hospitals in and around Southampton except for those suffixed ‘m’, which were obtained from King’s College Hospital, London, and the Royal Sussex County Hospital, Brighton. Where possible tumours were staged according to FIGO staging (Shepherd, 1989). DNA was isolated from tumours and blood as described by Foulkes et al. (1993a).

Polymerase chain reaction

Microsatellite markers for chromosome 5 were amplified by the polymerase chain reaction (PCR) using the primers listed in Table I. PCR reactions were performed in 15 μl aliquots containing 10 pmol of each primer, 200 μM each of dATP, dTTP and dGTP, 50 mM dCTP, standard PCR reaction buffer containing 1.5 mM magnesium chloride, 0.5 u Tag DNA polymerase (Promega, USA), 50 ng of DNA and 0.05 mM [α-32P]dCTP. PCR conditions consisted of 30 cycles of 1 min at 94°C, 1 min at 53–58°C and 1 min at 72°C. The PCR products were analysed on standard 6% (29:1 acrylamide-bis-acrylamide) denaturing and/or non-denaturing polyacrylamide gels.

SSCP and sequencing analysis of TP53

PCR amplification of exons 5–8 of TP53 were performed using the primers and conditions described by Milner et al. (1993). SSCP analysis of the samples was performed as described by Campbell et al. (1994). Tumour samples showing abnormal band shifts were repeated together with matching normal DNA to ensure that it was not due to a germline polymorphism. DNA sequencing was performed on some of the tumours with band shifts using a dideoxy termination protocol (Foulkes et al., 1995).

Statistical analyses

Statistical analysis was performed using Spearman’s rank correlation (Gardner and Altman, 1989).

Results

LOH on chromosome 5

Forty-nine ovarian tumours were analysed for chromosome 5 LOH with up to eight microsatellite markers. One was located at Spter (DSS417) and the other seven spanned the 5q
arm, including one at the APC gene locus (DSS346). The LOH data together with the tumour histology and stage are presented in Table II. LOH of any marker on 5q was detected in 23 of 49 (47%) tumours. In contrast, LOH of the 5pter marker (DSS417) was detected in only 1 of 22 (5%) informative tumours and no tumour was identified with LOH on 5p only. In 13 tumours, partial LOH was detected. Seven of these tumours (12m, 22, 27, 32, 36, 49 and 71) retained heterozygosity at DSS417, three (11m, 13m and 86) retained heterozygosity at DSS118 and a further two (47 and 95) retained heterozygosity at DSS424 (Figure I), thereby excluding 5p and proximal 5q from the candidate region. The 5q distal boundary of the candidate region is indicated by tumours 71, 86 and 151, which show proximal 5q LOH but retain heterozygosity for the distal markers DSS644 (tumour 151) and DSS346 (tumours 71 and 86), as shown in Figure 1. The smallest common region of deletion defined by these tumours is flanked by the markers DSS424 and DSS444 representing a genetic distance of approximately 22 cM (Gyapay et al., 1994). This region at 5q13.1–21 is proximal to the APC locus.

Analysis of TP53 mutation

SSCP analysis of TP53 exons 5–8 detected abnormal band shifts in 22 of the 49 (45%) tumours examined (Table II and Table IV) in agreement with the frequency observed in a number of other studies (Foulkes et al., 1995; Kohler et al., 1993a,b). No band shifts were detected in the matching normal DNA from these samples, indicating that these were somatic alterations and not germline polymorphisms. Twelve of these tumours were sequenced, and in all cases a somatic mutation was detected. There was a striking concordance of TP53 mutation with chromosome 5q deletions (P<0.001; Table III). Eighteen of the 23 (78%) tumours with 5q LOH also harboured a mutation in TP53 compared with only 4 of 26 tumours heterozygous for 5q markers.

Correlation of 5q LOH and TP53 mutation with tumour stage and histological subtype

The LOH on chromosomes 5q and TP53 mutation was compared with tumour stage (Table IV). Six of 18 (33%) stage I tumours showed LOH at 5q, four of which also harboured TP53 mutations suggestive of the involvement of these loci in early stages of the development of some ovarian cancers. There was an increase in the incidence of both 5q and TP53 mutation with advancing stage, although this increase was not statistically significant. With respect to the main histological subtypes, 5q LOH is perhaps of less relevance in mucinous tumours since LOH was detected in only 20% (1/5) of the mucinous adenocarcinomas compared with 61% (16/26) of serous and undifferentiated adenocarcinomas and 55% (5/9) of endometrioid carcinomas. Among the other histological subtypes and borderline and benign tumours only one of the two mixed Müllèrian tumours showed 5q LOH.

Discussion

Deletions on chromosome 5 which include the APC gene have been observed in a variety of malignancies other than just colorectal cancer and include oesophageal, gastric, pancreatic and lung carcinomas (Boynton et al., 1992; D’Aminco et al., 1992; Hori et al., 1992a,b; Hosoe et al., 1994). In ovarian cancers, chromosome 5q LOH has been reported by some groups to be an infrequent event (Ehlen and Dubau, 1990; Sato et al., 1991; Yang-Feng et al., 1993) while others have shown frequent deletions (Cliby et al., 1993; Allain et al., 1994). These discrepancies are most likely

Table I  The sequence and location of chromosome 5 microsatellite markers

| Locus/ marker | Position | Primers* |
|---------------|----------|----------|
| DSS417        | 5pter    | TGGAAACTATGTATCTTGGAGG |
| AFM205        |          | GCCGGCTTTAGGTTG     |
| DSS118        | Scen-tq11.2 | CAATCTGTCAGCTTTCTCA |
| MFD63         |          | CAAACACAAAAACACAGGC |
| DSS424        | 5q13.1–14 | GGGTACAGGGGATCTATTAGG |
| DSS644        | 5q14–21  | TCTCATGTCAGGCCAGGATA |
| DSS346        | 5q21–22  | ACTAATCTGAGATCAATGTC |
| APC           |          | TGGATTGCTAAGACTGTTG |
| IL9           | Sq22.3–q31.3 | CTATGCTAGTTAGGCG |
| DSS399        | Sq22.3–q31.3 | GTGTTGTAAGAGCTGATA |
| DSS209        | Sq31.3–33.3 | GAGGTGTACAGCAGGTCG |
| MFD116        |          | GGCCTCACTTATAATCAA |

*Primer sequences are indicated in the 5' to 3' direction.
due to differences in the number, location and type of polymorphic markers used in each study as well as the small size of the tumour collections. The most comprehensive of these studies used five markers on each chromosomal arm and detected LOH in 50% of the 27 tumours examined (Allan et al., 1994). The LOH was consistent with the loss of APC, but no mutations were detected by SSCP in any of the exons containing published mutations suggesting that another gene was the target of the deletions.

In the present study we analysed for chromosome 5 LOH using eight microsatellite markers to verify the high frequency of LOH reported by some and refine the location of the putative 5q TSG. Consistent with the frequencies reported by Ciby et al. (1993) and Allan et al. (1994) we detected LOH on 5q in 23 of 49 ovarian tumours. Thirteen of these tumours exhibited LOH on only part of 5q including two with interstitial deletions permitting the refinement of the candidate TSG locus to the 22cM region, flanked by D5S424 and D5S644 at 5q 13.1–21. This region is proximal

| Table II | Tumour clinical, chromosome 5 LOH and TP53 mutation data |
|----------|---------------------------------------------------------|
| Tumour number | Type | Stage | S417 | S118 | S424 | S644 | S346 | IL9 | S399 | S209 | TP53 mutation | Codon, nucleotide and amino acid change |
| 11m Ac/UD Ia | Het | Het | LOH | LOH | LOH | LOH | exon 7 | NS |
| 12m Spac Ia | Het | NI | LOH | LOH | LOH | NI | exon 5 | NS |
| 13m Spac Ia | NI | Het | LOH | LOH | LOH | NI | exon 5 | NS |
| 17m Ac/UD III | NI | LOH | LOH | LOH | exon 5 | NS |
| 21m Spac na | NI | NI | LOH | LOH | NI | exon 7 | NS |
| 22 Spac III | Het | LOH | LOH | LOH | NI | NI | exon 6 | 220, TAG>TGT, Tyr>Cys |
| 26 Spac III | NI | LOH | LOH | LOH | NI | LOH | exon 7 | 242, TGC>TGG, Cys>Trp |
| 27 Ac/UD I |Het | LOH | NI | LOH | LOH | NI | n | |
| 36 EC I | NI | LOH | LOH | LOH | NI | n | |
| 43 Ac/UD II | NI | LOH | LOH | LOH | LOH | NI | exon 5 | 157, GTC>GAC, Val>Asp |
| 45 Spac III | NI | LOH | LOH | LOH | NI | LOH | n | |
| 47 Ac/UD II | Het | LOH | LOH | LOH | NI | LOH | exon 5 | 179, CGC>CAC, Arg>His |
| 50 MMT III | Het | LOH | LOH | LOH | NI | LOH | exon 5 | 276, GCC>GC, frame shift |
| 63 Ac/UD na | LOH | LOH | LOH | LOH | NI | LOH | exon 7 | 242, TGC>GGC, Cys>Gly |
| 71 Spac Ia | Het | LOH | LOH | Het | NI | Het | exon 5 | 151, CCC>CGC, Pro>Arg |
| 86 Spac IIIa | Het | LOH | LOH | Het | NI | Het | exon 5 | 151, CCC>CGC, Pro>Arg |
| 95 Ec I | Het | LOH | NI | n | |
| 121 Mac III | NI | LOH | n | |
| 131 Sac III | LOH | LOH | n | |
| 146 Ec Ic | LOH | LOH | n | |
| 151 Ec Ic | LOH | Het | NI | exon 7 | NS |
| 2m Bsa Ic | Het | NI | Het | NI | Het | n | |
| 4m Sa na | Het | NI | Het | NI | n | |
| 10m Ec Ia | Het | Het | Het | Het | n | |
| 14 Spac IIIb | Het | Het | Het | Het | n | |
| 15m Spac IIb | Het | Het | Het | Het | n | |
| 16m Ec III | Het | Het | Het | Het | n | |
| 18m Spac III | Het | Het | Het | n | |
| 19 Spac III | Het | Het | Het | Het | NI | NI | n | |
| 20 Bsa IIIa | Het | Het | Het | Het | Het | n | |
| 23 Spac I | Het | NI | Het | NI | Het | n | |
| 40 Mac II | Het | NI | Het | NI | Het | n | |
| 48 Spac III | NI | Het | Het | n | |
| 50 Mac I | Het | NI | Het | NI | Het | n | |
| 60 Gct Ia | Het | Het | n | |
| 75 MA na | Het | Het | n | |
| 80 Mac Ia | Het | NI | Het | n | |
| 97 Mmt III | Het | Het | n | |
| 119 Ec I | Het | NI | n | |
| 122 Ac/UD III | Het | NI | n | |
| 124 Gct I | Het | Het | n | |
| 128 Ec Ic | Het | Het | n | |
| 134 Spac III | Het | Het | n | |
| 135 Spac Ic | Het | Het | n | |
| 144 Mac Ic | Het | Het | n | |

| Table III | Comparison between LOH on chromosome 5q and TP53 mutation* |
|-----------|---------------------------------------------------------------|
| TP53 mutation | TP53 normal |
| 5q LOH | 18 | 5 |
| 5q Het | 4 | 22 |

*Correlation 0.631; P-value <0.001; 90% confidence interval (CI) (0.462–0.756). The correlations and their P-values were calculated by Spearman's rank correlation.
Table IV  Association between LOH on 5q and TP53 mutation with tumour stage

| Tumour stage | TP53 mutation | 5q LOH | TP53 mutations/5q LOH |
|--------------|---------------|-------|-----------------------|
| I            | 4/18 (22%)    | 6/18 (33%) | 3/6 (50%) |
| II           | 4/7 (57%)     | 5/7 (71%) | 4/5 (80%) |
| III          | 12/20 (60%)   | 10/20 (50%) | 9/10 (90%) |
| Unstaged     | 2/4 (50%)     | 2/4 (50%) | 2/2 (100%) |
| Totals       | 22/49 (45%)   | 23/49 (47%) | 18/23 (78%) |

aNumbers of tumours with TP53 mutation over the number of tumours of the stage indicated; figures in brackets are percentages. 
bNumbers of tumours with LOH anywhere on chromosome 5q divided by the total number of tumours of that stage with percentages in brackets. cNumber of tumours with TP53 mutation divided by the number of tumours with 5q LOH with percentages in brackets. dNumber of tumours of all stages with the indicated property.

to APC, thereby excluding it as the candidate TSG, consistent with the absence of APC mutations in ovarian cancer reported by Allan et al. (1994).

LOH on 5q occurred in six (33%) stage I tumours, suggesting that it may be an early event in the development of certain ovarian cancers. This finding is inconsistent with the study by Allan et al. (1994), who concluded 5q LOH was a late event in ovarian carcinogenesis. However, their conclusion was based on the absence of LOH in only three low-grade tumours, highlighting a difficulty encountered in studies of this type in ovarian cancer in which low-grade and early-stage tumours are relatively uncommon. Nevertheless, such studies are vital if the sequence of molecular genetic events in ovarian tumorigenesis is to be unravelled.

Comparison of the presence of LOH on chromosome 5 with mutation in TP53 revealed a significant association between the two genetic events (P<0.001). A similar observation has been reported in colorectal cancers (Smith et al., 1995), but this is more likely to reflect an association with APC inactivation than with another Sq TSG. Although the association between 5q LOH and TP53 mutation in ovarian cancer is striking, caution must be exercised in attributing this to a functional link between TP53 and the putative 5q TSG as this might simply reflect generalised chromosomal instability in tumours with advancing stage. Only when the 5q TSG is cloned and it can be examined for specific inactivating mutations will it be possible to determine the true relationship between the two events.

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