Polymerase chain reaction allelotyping of human ovarian cancer

R.J. Osborne & V. Leech

Cancer Research Campaign Department of Clinical Oncology, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK.

Summary  We have used a set of microsatellite polymorphisms (MSPs) to examine the location and frequency of allele loss throughout the genome in a panel of 25 human epithelial ovarian tumours. When more than one MSP was employed per arm, mean informativity was 85.2% (range 64–100%). The average fractional allelic loss was 0.28 (range 0–0.65). A high frequency of allele loss was seen at 5q (40%), 9q (48%), 11p (43%), 14q (46%), 15q (40%), 17p (61%), 17q (64%), 19p (45%) and Xp (40%), confirming previous findings at some sites, but also suggesting the existence of new tumour-suppressor genes in regions (9q, 14q, 15q) which have not previously been studied in ovarian cancer. For 9q and 14q, partial loss of the arm was more common than loss of heterozygosity for all loci. There was a significant relationship between allele loss affecting the short arm of chromosome 17 and allele loss affecting 17q (P<0.001). No other relationship was detected between allele losses at different sites. Polymerase chain reaction allelotyping is suitable for the examination of very small tumour samples and tumours in which classical karyotyping is problematic.

In the presence of a mutated tumour-suppressor gene, loss of the normal homologue unmasks the defective gene and allows unopposed dysfunction. A variety of mechanisms, including whole homologue loss, mitotic recombination and deletion, may result in loss of the normal gene. These varied phenomena may be manifested by loss of heterozygosity (LOH) at one allele of a heterozygous locus. The term 'deletion' is often used where LOH is observed, regardless of the underlying mechanism. In ovarian cancer, several chromosome regions (3p, 6p, 6q, 11p, 11q, 13q, 17p, 17q, Xp) have been reported to be frequently affected by allele loss (Eihen & Dubeau, 1990; Okamoto et al., 1991; Zheng et al., 1991; Eccles et al., 1992; Gallion et al., 1992; Jones & Nakamura, 1992; Saito et al., 1992; Viau et al., 1992; Yang-Feng et al., 1992; Jacobs et al., 1993; Foulkes et al., 1993a, b). In most sites, the genes involved are not yet characterised, though the high rate of deletion implies the presence of tumour-suppressor genes of considerable importance.

Studies of tumour progression in colonic neoplasia (Vogelstein et al., 1988) suggest that the accumulation of genetic lesions may occur in a relatively consistent and ordered manner, with correlations between particular lesions and phenotypic and clinical parameters. In ovarian cancer, individual studies which have defined frequently deleted regions have also included assessments of clinical or pathological relationships (Zheng et al., 1991; Gallion et al., 1992; Viau et al., 1992; Foulkes et al., 1993a). However, because of the wide range of lesions which occur, this approach has not provided a clear insight into the disease process. Previous studies of limited numbers of regions have also failed to assess the total number of genetic lesions, another important factor in tumour phenotype (Vogelstein et al., 1988).

Ideally, analysis of all relevant loci is required for a valid assessment of the relationship between genotype and phenotype. For tumour-suppressor genes (known and putative) this can be achieved in two ways: by direct visualisation of chromosomes and by allele loss studies which involve every arm of every chromosome ("allelotyping"). Although conventional karyotyping has provided pointers to regions where deletions are frequent (Whang-Peng et al., 1984; Pejovic et al., 1989), it has not been applied to sufficient tumours for conclusions to be drawn about tumor progression or other clinical features. Allelotyping using restriction fragment length polymorphisms (RFLPs) (Sato et al., 1991; Cliby et al., 1993) is limited by the low informativity of many loci, the limited number of RFLPs available and the requirement for relatively large amounts of tumour DNA. The recent development of large numbers of highly informative, well-distributed microsatellite polymorphisms (MSPs) (Todd, 1992) may allow a more comprehensive allelotype to be rapidly performed, using very small samples if necessary. We have used MSPs spanning every arm of every chromosome (excluding the short arms of the acrocentric chromosomes) to examine 25 paired ovarian tumour–blood lymphocyte DNA samples. We report on the feasibility of this approach, and the abnormalities detected.

Materials and methods

Tumours

Twenty-five malignant epithelial ovarian tumours were studied. Samples comprised either surgically resected solid masses or ascites cells. Tumour masses were frozen at −70°C before use. Ascites cells and lymphocytes were processed...
| Chromosome arm | D number | Location | Reference, sequence | Reference, locus | Sequence |
|---------------|----------|----------|---------------------|----------------|----------|
| 1p            | AMY2B    | 1p21     | G 1990-7-97         | PNAS 1983-80-932 | AAA CTC CTC GCA GTC GTC AAC TAC TAT GTA TTA CTC CTA TAT AGG TGG |
| 1q            | APOA2    | 1q21-q23 | AJHG 1989-44-388    | AJHG 1989-44-388 | CTCGATACCTTTTGGGACGGGTGGGCTGAGACTATAG |
| 1q            | D15103   | 1q32-q44 | NAR 1990-18-2199    | S 1992-258-67   | AGGAAACTATCAGATAC TTTGAGAAGACTGCTT |
| 2p            | TPO      | 2p23-pter | HMG 1992-1-137     | HMG 1992-1-137  | CAC TAG CACCA GAA CCG TC CCT TAT GCT CTT TGT GTG CTC |
| 2p            | CD8A     | 2p12     | NAR 1991-19-1718    | I 1989-30-393   | AGCTGCTCATTGACCTGACGAGGCTGACCTGAGATT |
| 2q            | D2572    | 2q       | NAR 1990-18-2200    | S 1992-258-67   | AAC TAT AAT TCG ATC ATT GCA TGG TCT TAT AAT TTA GCA TTA TG |
| 3q            | ACPP     | 3q21-pter | NAR 1991-19-4792    | CCG 1989-52-68  | GGGCCAACTCCACGATCTTCT GCA CGCTTACTTCCCTT |
| 3q            | D3S196   | 3q       | NAR 1990-18-4635    | S 1992-258-67   | ACT CTT TOT TGA ATT CCC AT TTT CCA CTC GGG AAC ATG CT |
| 4p            | D4S174   | 4p11-p15 | NAR 1990-18-4636    | G 1992-14-209   | AGAGGACGCTGACGT GACTTTCAGAATGAGAAGCCTGT |
| 4p            | GABARB1  | 4p12-p13 | AJHG 1991-49-621    | AJHG 1991-49-621 | GCTCCTAAATACACTCTCTGTTCTAATAGAGAAGCTAGG |
| 4q            | D4S175   | 4p25-34  | G 1992-14-209       | G 1992-14-209   | ATC TCT TTT CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC 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Table II – cont.

| Chromosome arm | D number | Location | Reference, sequence | Reference, locus | Sequences |
|----------------|----------|----------|---------------------|-----------------|-----------|
| 13q            | FLT-1    | 13q12    | NAR 1991-19-2803    | O 1990-5-519    | TTTGGCGAAGCTGGGTTAAGACAAAACATGTTTT |
| 13q            | D13S115  | 13q      | G 1992-13-622       | S1992-258-67    | TCTACTATTCTGCTGGAAAGGAGATTCAGACAA |
| 14q            | D14S34   | 14q      | NAR1990-18-4638     | MFD 42          | GCCCTACCAAGAAGTACCACCTCTCTTATGAA |
| 14q            | D14S50   | 14q      | G 1992-13-35        | S 1992-258-67   | TCTACTATCAAGAAGTACCACCTCTCTTATGAA |
| 14q            | D14S49   | 14q      | G 1992-13-35        | S 1992-258-67   | TCTACTATCAAGAAGTACCACCTCTCTTATGAA |
| 14q            | D14S51   | 14q      | G 1992-13-35        | S 1992-258-67   | TCTACTATCAAGAAGTACCACCTCTCTTATGAA |
| 14q            | D14S48   | 14q      | G 1992-13-35        | S 1992-258-67   | TCTACTATCAAGAAGTACCACCTCTCTTATGAA |
| 15q            | FES      | 15q26.1  | NAR 1991-19-4018    | CCG 1993-63-33  | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 16p            | D16S292  | 16p13    | G 1992-13-402       | G 1992-13-402   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 16q            | D16S265  | 16q21    | NAR 1990-18-4034    | S 1992-258-67   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 17p            | D17S520  | 17p12    | MFD 144             | CCG 1991-58-728 | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 17p            | TP5      | 17p13.1  | GCC 1992-5-89       | N 1986-320-84   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 17q            | D17S250  | 17q11.2–12q | NAR 1990-18-4640 | NAR 1990-18-4640 | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 17q            | D17S588  | 17q12–21q | CR 1993-53-1218     | CR 1993-53-1218 | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 18p            | D18S40   | 18p11.21–pter | G 1993-15-48 | G 1993-15-48   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 18q            | D18S53   | 18q21.2–21.3 | NAR 1990-18-6465 | G 1993-15-48   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 19p            | D19S177  | 19p13.3  | CCG 1991-58-1190    | S 1992-258-67   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 19q            | D19S49   | 19q12    | NAR 1990-18-1927    | S 1992-258-67   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 20p            | D20S27   | 20p12    | NAR 1990-18-2202    | S 1992-258-67   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 20q            | D20S54   | 20q      | G 1992-12-183       | G 1992-12-183   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
|                | D20S46   | 20q      | G 1992-12-183       | G 1992-12-183   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 21q            | D21S120  | 21q11.2  | NAR 1990-18-4969    | NAR 1990-18-4969 | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 21q            | D21S171  | 21q22.3–qter | HG 1991-87-401 | HG 1991-87-401  | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 21q            | D21S167  | 21q22.3  | NAR 1990-18-4967    | NAR 1990-18-4967 | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 22q            | D22S156  | 22q      | NAR 1990-18-4639    | S 1992-258-67   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| 22q            | TOP1P2   | 22q11.2–13.1 | HMG 1992-1-6 | HG 1989-84-6   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| Xp             | DXS538   | Xp11.21–21.1 | NAR 1991-19-1161 | AJHG 1990-46-776 | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |
| Xq             | DXS454   | Xq       | NAR 1990-18-4037    | S 1992-258-67   | TCTGCAAGAAGTCTGCTGCTGCTGCTGCTGCTG |

MSPs are listed by their official locus name ('D number'), chromosomal location and oligonucleotide primer sequences. References relate to the published details of the sequence and location of each MSP. Journal abbreviations are listed below.

Journal abbreviations: AJHG, American Journal of Human Genetics; C, Cell; CCG, Cytogenetics and Cell Genetics; GCC, Cancer Genetics and Cytogenetics; CR, Cancer Research; G, Genomics; GCC, Genes, Chromosomes and Cancer; HG, Human Genetics; HMG, Human Molecular Genetics; I, Immunogenetics; MFD, Marshfield Markers Release 10–7/1/93 (J. Weber, personal communication); N, Nature; NAR, Nucleic Acids Research; O, Oncogene; PNAS, Proceedings of the National Academy of Sciences of the USA; S, Science.

Fresh. Histological type, grade, clinical stage and origin of the tumours are detailed in Table I.

DNA preparation

Ten micron haematoxylin and eosin-stained frozen sections were examined to identify regions of tumour which were free from significant contamination with normal tissue. Two to ten further 10 μm sections were cut, and where necessary normal tissue was scraped away. No tumour sample contained more than 40% normal tissue. The sections were digested with proteinase K at 55°C in polymerase chain reaction (PCR) buffer for 1 h then boiled for 10 min. The resulting solution was used directly in the PCR reaction without further purification. Cytospin examination of ascites cells was performed, and only samples comprising greater than 60% tumour were used. Cells from 1 ml of fluid were added to a buffer containing detergents which lysed cytoplasmic membranes (Higuchi, 1989). The nuclei were pelleted and washed, then the nuclear membranes were digested in 1 ml of PCR buffer. Normal DNA was derived from the lymphocytes in 1 ml of whole blood, treated in the same way as ascites. An aliquot of the resulting solution was used directly in the PCR reaction without further purification.

Oligonucleotides

Oligonucleotides were obtained from the HGMp Resource Centre (Harrow, UK), or were synthesised locally. They were selected on the basis of their high informativeness, accurate
Table III Results for all loci

| ARM LOCUS | LOCATION |
|-----------|----------|
| 1p AMY2B | 1p21     |
| 1q * APOA2 | 1q21-q23 |
| 1q D15S03 | 1q32-q44 |
| 2p TPO | 2p23-pter |
| 2p CDA8 | 2p12     |
| 2q D25S72 | 2q       |
| 3p # TH4B | 3p24     |
| 3p # D3S15S2 | 3p21 |
| 3p # D3S30 | 3p13-p14 |
| 3q * ADH | 3q21-qter |
| 3q D3S156 | 3q       |
| 4p D4S174 | 4p11-p15 |
| 4p * GABBR1 | 4p12-q13 |
| 4q D4S175 | 4q25-q34 |
| 4q D4S171 | 4q35-qter |
| 5p * DSS268 | 5p     |
| 5q D5S117 | 5p15.1-15.3 |
| 5q D5S346 | 5q21-q22 |
| 6p F13A1 | 6p24-25 |
| 6p D6S109 | 6p23.3-p24 |
| 6p * FTH1 | 6p12-p21.3 |
| 6q D6S67 | 6q23.1   |
| 7p EGR | 7p11.2-p12 |
| 7q D7S23 | 7q31     |
| 7q CPTF | 7q31     |
| 8p LPL | 8p22     |
| 8p D8S135 | 8p       |
| 8p ANK1 | 8p11.2   |
| 8q D8S161 | 8q22-qter |
| 9p D9S54 | 9p22-pter |
| 9q D9S15 | 9q13-q21.1 |
| 9q GGN | 9q33     |
| 9q ASS | 9q34.1   |
| 9q D9S64 | 9q34-qter |
| 10p D10S89 | 10p11.2-p2 |
| 10p D10S111 | 10p11.2-pter |
| 10p D10S179 | 10p     |
| 10q D10S173 | 10q     |
| 11p D11S419 | 11p13-p15.1 |
| 11q D11S534 | 11q13   |
| 11q D11S536 | 11q     |
| 12p* RFBWP | 12p12-pter |
| 12q D12S80 | 12q     |
| 13q PLT1 | 13q12    |
| 13q D13S115 | 13q    |
| 14q D14S34 | 14q     |
| 14q D14S50 | 14q     |
| 14q D14S49 | 14q     |
| 14q D14S51 | 14q     |
| 14q D14S48 | 14q     |
| 15q FEBS | 15q26.1  |

localisation and even distribution. The loci and chromosomal regions examined and their corresponding oligonucleotides are detailed in Table II.

PCR
A 50–200 ng aliquot of genomic DNA (1 μl of solution described above) was amplified in a reaction volume of 12.5 μl as previously described (Jacobs et al., 1993). For all reactions except those indicated with an asterisk in Table II, PCR consisted of 1 min at 95°C, 2 min at 55°C and 2 min at 72°C for 30 cycles followed by a final extension for 10 min at 72°C. After chloroform extraction, PCR products were processed and analysed as previously described (Jacobs et al., 1993). For chromosome 3p an RFLP-PCR technique (Ganly et al., 1992) was used to examine five polymorphisms at the
Allelotyping results for all tumours at all loci are presented. The symbols used are explained in the key below. The percentage loss of heterozygosity (LOH) at each locus and for each chromosomal arm has been calculated and is listed, with the percentage informativeness of each locus, in the three columns at the right of the table.

Key: O, retained heterozygosity; ●, loss of heterozygosity; Blank, non-informative or failed. *Refer to original paper for PCR conditions; #RFLP-PCR (Osborne et al., 1992).

### Table III – cont.

| Loci | 16p | 16q | 17p | 17q | 18p | 18q | 19p | 19q | 20q | 20q* | 21q | 21q* | 21q* | 22q | 22q | Xp | Xq |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| D16S292 | 16p13 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D16S265 | 16q21 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D17S520 | 17p12 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| TP53 | 17p13.1 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D17S250 | 17q11.2-q12 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D17S588 | 17q12-q21 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D18S40 | 18p11.21-pter | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D18S535 | 18q21.2-q21.3 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D19S177 | 19p13.3 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D19S49 | 19q12 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D20S27 | 20q12 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D20S54 | 20q | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D20S46 | 20q | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D21S120 | 21q11.2 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D21S171 | 21q22.3-qter | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D21S167 | 21q22.3 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| D22S1568 | 22q | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| T0P2 | 22q11.2-q13.1 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| DXS538 | Xp11.21-p21.1 | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| DXS454 | Xq | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |

**Results**

PCR allelotyping of the tumour panel was relatively rapid and easy. Examination of a single locus, involving 50 samples plus controls, was completed within one working day (excluding autoradiography). Results for all tumours at all loci are shown in Table III. Table IV comprises a summary of these results analysed by chromosomal arm. Figure 1 summarises the percentage allele loss at each chromosomal arm. Informativeness for individual MSPs ranged between 20% and 88% (mean 59.1%). However, when more than one MSP was employed per arm, informativeness increased to a mean of 85.2% (range 64–100%).

Interpretation of results was facilitated by the use of tumours in which contamination with normal tissue had been minimised. The additional 'shadow' bands of smaller products routinely seen with this technique (Litt, 1991) did not hamper interpretation of results. Representative findings for one tumour–normal pair are shown in Figure 2. All results were scrutinised for evidence of microsatellite mutation (Thibodeau et al., 1993). Only two examples were identified, each affecting a single locus (Figure 3).

Only two tumours (tumours 23 and 39) showed no evidence of deletion at any locus, whereas tumour 8 had allele loss affecting 65% of informative chromosomal arms. The mean allele loss per tumour was 28% (s.d. 22.8%). Because the material studied was derived predominantly from poorly differentiated serous stage III tumours, it was not possible to explore the relationship between frequency of allele loss and parameters such as tumour histology, grade or stage.

Frequency of loss of heterozygosity for individual chromosomal arms varied between 0% (16p) and 64% (17q). Forty per cent or more of informative tumours showed loss of heterozygosity at chromosomal arms 5q (40%), 9q (48%), 11p (43%), 14q (46%), 15q (40%), 17p (61%), 17q (64%), 19p (45%) or Xp (40%). There was a significant relationship between allele loss affecting the short arm of chromosome 17 and allele loss affecting 17q (P < 0.001). Non-disjunction is a possible explanation for this association. No other relationship was detected between allele losses at different sites in this cohort of tumours. Although allele loss usually affected all loci examined for a particular chromosomal arm, there were notable exceptions. For 9q and 14q partial loss of the arm was more common than loss of heterozygosity for all loci. This observation may explain the discrepancy between these results and those obtained in an earlier allelotyping study (Sato et al., 1991) in which fewer loci were studied.

### Discussion

This paper describes the use of a set of microsatellite polymorphisms which permits a comprehensive evaluation of the numerous deletions which may occur throughout the genome of tumours. The MSPs selected are easy to use, particularly since the vast majority share common PCR conditions. The use of silver staining or automated sequencing techniques (Cawkwell et al., 1993) to detect products are possible refinements which will further increase the utility of the method.

This approach depends upon the assumption that chromosome deletions are sufficiently large to allow their detection using probes which examine only a small number of loci per arm. Mapping studies employing large numbers of probes for a particular chromosome reveal that the majority of deletions are extensive, usually involving an entire arm (Jacobs et al., 1993; Foulkes et al., 1993a). Small interstitial or terminal deletions are relatively uncommon. In the present
Table IV  Summary of results for individual chromosomal arms

| Chromosome | TUMOURS | % LOH | % INF |
|------------|---------|-------|-------|
| 3          |         |       |       |
| 4          |         |       |       |
| 5          |         |       |       |
| 6          |         |       |       |
| 7          |         |       |       |
| 8          |         |       |       |
| 9          |         |       |       |
| 10         |         |       |       |
| 11         |         |       |       |
| 12         |         |       |       |
| 13         |         |       |       |
| 14         |         |       |       |
| 15         |         |       |       |
| 16         |         |       |       |
| 17         |         |       |       |
| 18         |         |       |       |
| 19         |         |       |       |
| 20         |         |       |       |
| 21         |         |       |       |
| 22         |         |       |       |
| X          |         |       |       |
| Xq         |         |       |       |
| % loss     | 6 65 41 53 3 5 42 54 41 8 0 0 3 44 50 15 3 22 4 31 61 50 4 50 41 | 21 56 |

Allelotyping results for all loci studied on a chromosomal arm are amalgamated to indicate the frequency with which individual arms are affected. When loss of heterozygosity is found at one locus on an arm, with retention of heterozygosity at another locus on that arm, the overall result is scored as loss of heterozygosity. The symbols used are explained in the key to Table III. The percentage of chromosomal arms affected by allele loss in individual tumours is listed at the bottom of the table.
Figure 1 Percentage loss of heterozygosity on individual chromosomal arms.

Figure 2 Autoradiographs of microsatellite polymorphism PCR products separated by polyacrylamide gel electrophoresis, showing examples of allele loss found in tumour no. 52. Left lane, normal lymphocyte DNA; right lane, tumour DNA; R, retention of heterozygosity; L, loss of heterozygosity.

study examination of only two loci on both 17p and 17q detected rates of allele loss for both chromosome arms which were almost identical to those expected from previous studies (Okamoto et al., 1991; Eccles et al., 1992; Gallion et al., 1992; Jacobs et al., 1993). These observations support the validity of using a small number of MSPs per chromosomal arm. Optimum density of MSPs should take into account the relative sizes of the chromosomes, but compromises are forced by the limited number of accurately localised highly informative probes for some arms. In this study efforts were
made to achieve even coverage of the genome with the materials available. With the rapid expansion in numbers of MSPs, even greater probe density is now feasible.

In the tumours studied, a considerable level of genetic damage was evident, particularly affecting 5q, 9q, 11p, 14q, 15q, 17p, 17q, 19p and Xp. The high rate of allele loss for 17p and 17q is in keeping with results from previous studies (Okamoto et al., 1991; Eccles et al., 1992; Gallion et al., 1992; Jacobs et al., 1993). Similar frequencies of allele loss to those observed here have been reported for 11p (Zheng et al., 1991; Gallion et al., 1992; Viel et al., 1992) and Xp (Yang-Feng et al., 1992) in ovarian cancer, for 5q in colon cancer (Solomon et al., 1987) and for 9q in urothelial cancer (Tsai et al., 1990). Rearrangement of the short arm of chromosome 19 has been consistently observed in ovarian cancer (Pezovis et al., 1989). Until recently, the long arms of chromosomes 14 and 15 have only been the subject of a limited examination (Sato et al., 1991) in ovarian cancer, which did not detect frequent allele loss. However, a more extensive RFLP-allelotyping study (Cliby et al., 1993) has cast more light on all the areas mentioned above, with 14q and 15q allele loss being found in 47% and 36% of tumours respectively. Overall, considerable similarities are evident when the results from the present study and the allelotyping study based on RFLP analysis are compared (Figure 4). The discrepancies observed may result from the relatively small numbers of tumours studied or the inclusion of low-grade tumours in the RFLP study, or may be due to differences in the distribution of the probes employed. This last possibility is unlikely, since the regions of the chromosomes examined in the instances where greatest differences were evident were common to both studies.

Although this study was not performed with the intention of achieving genotypic–phenotypic correlations, the genetic abnormalities revealed are likely to prove clinically relevant. Firstly, the high frequency of allele loss affecting the long arms of chromosomes 9 and 14 is a new finding in ovarian cancer, and strongly suggests that these are the sites of as yet uncharacterised tumour-suppressor genes. This supposition is supported by the recent observation of frequent 9q deletion in urothelial malignancy (Tsai et al., 1990) and lymphoma (Offit et al., 1993). The high incidence of partial loss of 9q in the tumours in this study permits initial localisation of a smallest region of the overlap of the deletions (R.J. Osborne et al., in preparation).

Secondly, a surprisingly high overall prevalence of LOH was observed, with 29/41 arms showing deletion in more than 20% of informative tumours (mean percentage LOH = 28%). Similar results (mean percentage LOH = 35%) have been reported recently (Cliby et al., 1993) in ovarian cancer. The high rate of allele loss in this disease contrasts with that reported in endometrial cancer (<10%) (Fujino et al., 1993), suggesting that tumours derived from different tissues, which presumably have different pathogenesis, differ in the extent of genetic damage which accumulates during tumour progression.

Finally, this study reveals that microsatellite mutations (Aaltonen et al., 1993) are very rare in ovarian cancer. Only two mutations were observed in 25 tumours examined with 68 MSPs (total 1,700 experiments). This finding distinguishes ovarian cancer from colon cancer in terms of the genetic

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**Figure 3** Microsatellite mutations observed in two tumours, one at the D10S173 locus and one at the D16S265 locus. N, normal lymphocyte DNA; T, tumour DNA; ai, allele 1; aii, allele 2; M, mutant alleles.

**Figure 4** Comparison of allele loss frequencies observed on individual chromosomal arms in the present study (■) and in a previous RFLP-based allelotyping study (□) (Cliby et al., 1993).
lesions involved in tumorigenesis, since 28% of sporadic colon tumours showed microsatellite instability in a recent study (Thibodeau et al., 1993). The possibility that genetic dysfunction leading to microsatellite mutation is involved in some forms of hereditary ovarian cancer has not yet been explored, since the tumours studied here were all derived from sporadic cases.

The use of PCR allelotyping to detect the multiple deletions which represent dysfunction of tumour-suppressor genes is applicable to all tumour types (assuming tissue free from excessive normal cell contamination can be obtained). Analysis of a representative panel of tumours with well-characterised clinical or pathological features will permit correlations between genetic and phenotypic parameters which are more wide-ranging and complete than those based on examination of a very limited number of genetic lesions in tumours, as was previously done. Detailed studies of tumour progression, using very small amounts of microdissected tissue or archival (formalin fixed, wax embedded) material (Greer et al., 1991), are possible with this technique. Examination of epithelium from benign cysts and borderline tumours which sometimes occur synchronously with frankly malignant ovarian neoplasms will greatly clarify understanding of the pathogenesis of familial colorectal cancer. Concurrent examination of malignant epithelium and underlying stroma will be similarly important.

Although PCR allelotyping is capable to revealing losses of genetic material in tumours, it is unsuitable for detection of gene amplifications and thus the technique may not provide a full picture of the genetic disturbances in a particular tumour. It is also unable to detect point mutations or rearrangements, and small deletions may also be missed. The newly developed technique of comparative genomic hybridisation (CGH) (Kallioniemi et al., 1992) is capable of detecting both amplification and deletion of genetic material affecting any part of any chromosome. Although this approach therefore offers some advantages over PCR allelotyping, the two methods are probably complementary. PCR allelotyping provides information about microsatellite instability and, if necessary, can be applied to map sites of interest identified by CGH, using increased numbers of MSPs.

In conclusion, we have compiled and validated a set of MSPs for detecting deletions on all chromosomes in a simple and rapid fashion. Use of this approach will not only increase understanding of the relationship between genetic lesions and clinical behaviour for particular tumour types, but will also reveal similarities and differences between neoplasms derived from histologically distinct tissues.

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References

AALTONEN, L.A., PELTOMAKI, P., LEACH, F.S., SISTONEN, P., PYLKANEN, L., MECKLIN, J.K., JARVINEN, H., POWELL, S.M., JEN, J., HAMILTON, S.R., PETERSEN, G.M., KINZLER, K.W., VOGELSTEIN, B. & DE LA CHAPELLE, A. (1993). Clues to the pathogenesis of familial colorectal cancer. Science, 260, 812–816.

CAWKWELL, L., BELL, S.M., LEWIS, F.A., DIXON, M.F., TAYLOR, G.R. & QUIRKE, P. (1993). Rapid detection of allele loss in colorectal tumours using microsatellites and fluorescent DNA technology. Br. J. Cancer, 67, 1262–1267.

CLIBY, W., RITLAND, S., HARTMANN, L., GODDSON, M., HALLING, K.C., KEENLEY, G., PODRATZ, K.C. & JENKINS, R.B. (1993). Human epithelial ovarian cancer allelotype. Cancer Res., 53, 2393–2398.

ECHEL, D.M., RUSSELL, S.E.H., HAITES, N.E. & THE ABE OVARIAN CANCER GENETICS GROUP (1992). Early loss of heterozygosity on 17q in ovarian cancer. Oncogene, 7, 2069–2072.

EIHLEN, T. & DEBEAU, L. (1990). Loss of heterozygosity on chromosomal segments 3p, 6p and 11p in human ovarian carcinomas. Oncogene, 5, 219–223.

FOULKES, W.D., RAGOUSSIS, J., STAMP, G.W.H., ALLAN, G.J. & TROWSDALE, J. (1993a). Frequent loss of heterozygosity on chromosome 6 in ovarian carcinoma. Br. J. Cancer, 67, 551–559.

FOULKES, W.D., CAMPBELL, J.G., STAMP, G.W.H. & TROWSDALE, J. (1993b). Loss of heterozygosity and amplification on chromosome 11q in human ovarian cancer. Br. J. Cancer, 67, 268–273.

FUJINO, T., RISINGER, J.J., NICHI, H., COLLINS, N.K., LIU, F.S., CHOI, C., TAKASHASHI, H., SASAKI, H., KOHLER, M., BERCHUCK, A., BARRETT, J.C. & BOYD, J. (1993). Allelotype of epithelial ovarian carcinoma. Proc. Am. Assoc. Cancer Res., 34, 540.

GALLION, H.H., POWELL, D.E., MORROW, J.K., PIETTERI, M., CASE, E., TURKER, M.S., DEPRIEST, P.D., HUNTER, J.E. & VAN NAGELL, J.R. (1992). Molecular genetic changes in human epithelial ovarian malignancies. Gynecol. Oncol., 47, 137–142.

GALNY, P.S., GRAY, N., RUDO, N. & RABBITS, P.H. (1992). PCR-based RFLP analysis allows genotyping of the short arm of chromosome 3 in small biopsies from patients with lung cancer. Genomics, 12, 221–228.

GREEN, C.E., PETERSON, S.L., KVIAT, N.B. & MANOS, M.M. (1991). PCR amplification from paraffin-embedded tissues. Am. J. Clin. Pathol., 95, 117–124.

HIGUCHI, R. (1989). Simple and rapid preparation of samples for PCR. In PCR Technology, Erlich, H.A. (ed.) pp. 31–38. Stockton Press: New York.

JACOBS, I.J., SMITH, S.A., WISEMAN, R.W., FUTREAL, P.A., HARRINGTON, T., OSBORNE, R.J., LEECH, V., MOLYNEUX, A., BERCHUCK, A., PONDER, B.A.J. & BAST, R.C. (1993). A deletion unit on chromosome 17q in epithelial ovarian tumours distal to the familial breast/ovarian cancer locus. Cancer Res., 53, 1218–1221.

JONES, M.H. & NAKAMURA, Y. (1992). Deletion mapping of chromosome 3p in female genital tract malignancies using microsatellite polymorphisms. Oncogene, 7, 1631–1634.

KALLIONIEMI, A., KALLIONIEMI, O.-P., SUDAR, D., RUTOVITZ, D., GRAY, J.W., WALDMAN, F. & PINKEL, D. (1992). Comparative genomic hybridisation for molecular cytogenic analysis of solid tumours. Science, 258, 818–821.

LITT, M. (1991). PCR of TG microsatellites. In PCR: A Practical Approach, McPherson, M.J., QUIRKE, P. & TAYLOR, G.R. (eds) pp. 55–99. Oxford University Press, New York.

OFFIT, K., PAKSA, N.Z., JHANWAR, S.C., FILIPPA, D., WACHTEL, M. & CHAGANTI, R.S.K. (1993). Clusters of chromosome 9 aberrations are associated with clinicopathologic subsets of non-Hodgkin's lymphoma. Genes Chrom. Cancer, 1, 1–7.

OKAMOTO, A., SAMESHIMA, Y., YOKOYAMA, S., TERASHIMA, Y., SUGIMURA, T., TERADA, M. & YOKOTA, J. (1991). Frequent allele losses and mutations of the p53 gene in human ovarian cancer. Cancer Res., 51, 5171–5176.

OSBORNE, R., LEECH, V., GANLY, P., MOLYNEUX, A. & RABBITS, P. (1992). Chromosome 3p deletion in epithelial ovarian tumours. Proc. Am. Assoc. Cancer Res., 33, 384.

PEJOVIC, T., HEIM, S., MANDAL, N., ELMFORS, B., FLODERUS, U.-M., FURGVIK, S., HELM, G., WILLEN, H. & MITELMAN, F. (1989). Consistent occurrence of a 19p+ marker chromosome and loss of 11p material in ovarian serous papillary cystadenocarcinomas. Genes Chrom. Cancer, 1, 167–171.

SAITO, S., SAITO, H., KIO, S., SAGAE, S., KUDO, R., SAITO, J., NODA, K. & NAKAMURA, Y. (1992). Fine-scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian carcinomas. Cancer Res., 52, 5812–5820.

SAITO, T., SAITO, H., MORITA, R., KIO, S., LEE, J.H. & NAKAMURA, Y. (1991). Allelotype of human ovarian cancer. Cancer Res., 51, 5118–5122.

SOLOMON, E., VOSS, R., HALL, V., BODMER, W.F., JASS, J.R., REYES, A.J., LUCIBELLO, F.C., PATEL, I. & RIDER, S.H. (1987). Chromosome 5 allele loss in human colorectal carcinomas. Nature, 328, 616–619.
THIBODEAU, S.N., BREN, G. & SCHAID, D. (1993). Microsatellite instability in cancer of the proximal colon. *Science, 260*, 816–819.

TODD, J.A. (1992). La carte des microsatellites est arrivée! *Hum. Mol. Genet.*, 1, 663–666.

TSAI, Y.C., NICHOLS, P.W., HITI, A.L., WILLIAMS, Z., SKINNER, D.G. & JONES, P.A. (1990). Allelic losses of chromosomes 9, 11 and 17 in human bladder cancer. *Cancer Res.*, 50, 44–47.

VIEL, A., GIANNINI, F., TUMIOTTO, L., SOPRACORDEVOLE, F., VISSENTIN, M.C. & BOIOCCHI, M. (1992). Chromosomal localisation of two putative 11p oncosuppressor genes involved in human ovarian tumours. *Br. J. Cancer, 66*, 1030–1036.

VOGELSTEIN, B., FEARON, E.R., HAMILTON, S.R., KERN, S.E., PREISINGER, A.C., LEPPERT, M., NAKAMURA, Y., WHITE, R., SMITS, A.M.M. & BOS, J.J. (1988). Genetic alterations during colorectal tumor development. *N. Engl. J. Med.*, 319, 525–532.

WHANG-PENG, J., KNUTSEN, T., DOUGLASS, E.C., CHU, E., OZOLS, R.F., HOGAN, W.M. & YOUNG, R.C. (1984). Cytogenetic studies in ovarian cancer. *Cancer Genet. Cytogenet.*, 11, 91–106.

YANG-FENG, T.L., LI, S., HAN, H. & SCHWARTZ, P.E. (1992). Frequent loss of heterozygosity on chromosomes Xp and 13q in human ovarian cancer. *Int. J. Cancer, 52*, 575–580.

ZHENG, J., ROBINSON, W.R., EHLEN, T., YU, M.C. & DUBEAU, L. (1991). Distinction of low grade from high grade human ovarian carcinomas on the basis of losses of heterozygosity on chromosomes 3, 6 and 11 and HER-2/neu amplification. *Cancer Res.*, 51, 4045–4051.