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Original Paper

TP53 Mutation in Ovarian Carcinoma

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This short report describes the detection of mutations of the TP53 tumour suppressor gene in sporadic ovarian carcinomas using archival paraffin-embedded tissues and automated fluorescent DNA sequencing. TP53 mutations were detected in eight tumours. Missense mutations predominated and all were transitions. Mutations were commonest in late-stage serous tumours. In three cases, where tissue was available, the mutations were homogeneous throughout several sections of the bilateral ovarian tumours and in omental metastases. These data confirm the findings of previous investigations describing TP53 mutation in ovarian carcinoma and demonstrate that archival paraffin-embedded tissues can be used for such analyses. © **1997 Elsevier Science Ltd.**

Key words: p53, mutations, ovarian carcinoma

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INTRODUCTION

THE TP53 gene is one of the most commonly altered tumour suppressor genes recognised to date [1, 2] and has a significant role in the biology of ovarian carcinoma. TP53 mutations occur in 30–50% of ovarian carcinomas [3–5] and are more common in stage III/IV [6] serous tumours [7].

We have previously studied p53 protein expression, TP53 mutation and deletion in a series of 44 paraffin-embedded archival ovarian carcinoma samples [8]. Mutation of the TP53 gene was found to be common and was associated with p53 protein expression, but mutation and protein expression were not synonymous and there was an excess of deletions over mutations. TP53 mutations were also associated with stage III/IV tumours, aneuploid DNA content and loss of heterozygosity (LOH) at the TP53 locus or *D17S513*, a closely related locus on 17p [9]. TP53 mutations were detected by non-isotopic single strand conformation polymorphism (SSCP) analysis of polymerase chain reaction (PCR) products amplified from tumour samples and normal tissues. We have now undertaken mutation analysis in selected cases showing aberrant bands on SSCP and/or p53 protein expression by direct automated fluorescent DNA sequencing.

MATERIALS AND METHODS

5 μm sections from tumour-rich and adjacent tumour-free areas were dewaxed and digested with proteinase K as previously described [7] to extract template DNA. The template DNA was PCR amplified for exons 5–9 of the TP53 gene [10] prior to cycle sequencing incorporating fluorescently labelled dideoxy chain terminators, and automated analysis using an ABIFS kit and an ABI 377 automated sequencer according to the manufacturer's instructions. All mutations were confirmed by sequencing in both directions. (Figure 1). Where mutations were detected, further sections from that tumour (where available) were examined for the presence of the same mutation.

RESULTS

Mutations in exons 5–9 of the TP53 gene were detected in eight tumours (Table 1 and Figure 1). Six of the mutations were missense, two were nonsense and all were transitions. With the exception of one stage II tumour, all the mutations were found in stage III or stage IV tumours. The serous histological subtype predominated, although numbers were small. In three of the eight tumours showing mutations, additional material was available from other sites of the tumour, from tumour in the contralateral ovary and/or from omental metastases. Direct sequencing showed the mutations to be homogeneous throughout all sites in the tumours concerned. The six tumours with missense mutations (Table 1) had all shown diffuse p53 expression when investigated previously by immunohistochemistry [8]. Mutations were found in two tumours which were immunonegative. Both these tumours exhibited the same stop codon in exon 8, with concomitant LOH involving the second TP53 allele.

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Table 1. p53 mutations in ovarian carcinoma

Case histology	Figo stage	Mutation	Exon	Codon	p53† protein expression	LOH
1 Mucinous	Stage III	CGA-TGA	8	306	0	LOH
2 Endometrioid	Stage III	CGA-TGA	8	306	0	LOH
3 Mucinous	Stage III	GGC-AGC	7	245	+++	LOH
4 Serous	Stage III	CGG-CAG	7	248	+++	HOM
5* Serous	Stage III	TGC-TAC	7	242	+++	LOH
6 Endometrioid	Stage II	CGG-CAG	7	248	+++	LOH
7* Serous	Stage III/IV	CGC-CAC	5	175	++	HET
8* Serous	Stage III	CGC-CAC	5	175	++	HOM

*In these three bilateral cases of serous cystadenocarcinoma, both ovarian tumours and omental metastases showed the same mutation throughout, with concordant diffuse p53 protein expression.
†p53 protein expression +++, >50% positive cells, ++, 10–50% positive cells, 0, no staining.

DISCUSSION

We have used automated DNA sequencing to characterise TP53 mutations in archival ovarian carcinomas previously screened for TP53 mutations by SSCP. Direct sequencing of the relevant exons of the TP53 gene to detect mutations is the most direct method of detecting inactivation in tumours, but conventional sequencing approaches are time-consuming. Some investigations have used immunohistochemical staining alone to infer mutation [11], but the relationship between p53 protein expression and TP53 mutation is increasingly recognised as complex [12].

SSCP has proven a relatively sensitive and specific screening technique to identify mutations, but provides no information about their nature or functional consequences. Although the SSCP and sequencing data correlated well, there were very occasionally SSCP positive tumours also showing p53 protein expression in which no mutation could be found, despite repeated sequencing. This discrepancy may have resulted from low amounts of the mutant allele falling below the detection threshold of sequencing in samples with a high ratio of stroma to epithelium.

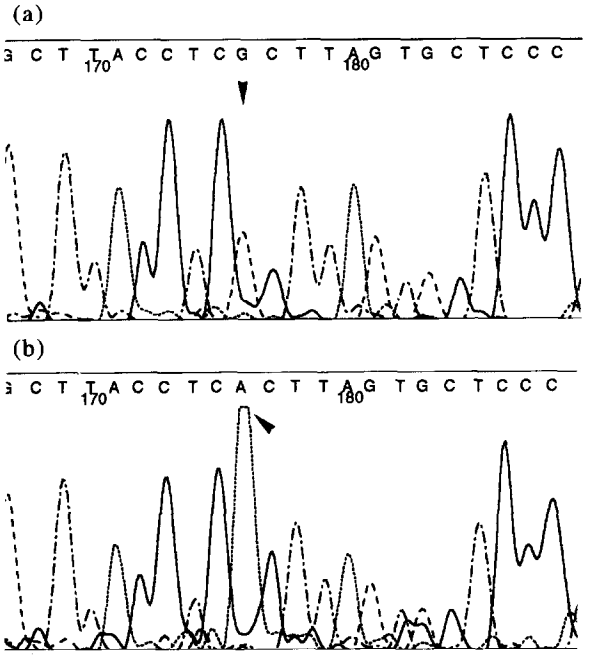

Figure 1. (a) Electrophoretogram showing wild type sequence from normal tissue. (b) Electrophoretogram showing mutation in Case 2. Reverse primer showing mutation at codon 306 GCT to ACT (i.e. CGA-TGA on sense strand, resulting in premature termination).

Detection of the same TP53 mutation in three bilaeral ovarian tumours as well as in omental deposits is in keeping with previous investigations [13, 14], which suggest that ovarian carcinoma usually has a unifocal origin and that TP53 mutation frequently occurs prior to metastasis. All the mutations detected were transitions, with four mutations occurring at two known CpG residues (GC:AT transitions at codons 175 and 248). These data are consistent with the mutational spectrum already described in ovarian cancer [3, 6, 15, 16].

In conclusion, most studies in ovarian carcinoma have used prospectively collected frozen tumour material for molecular analysis, but we have demonstrated that paraffin-embedded archival tissue provides a suitable material for automated DNA sequencing. This should facilitate retrospective investigation of specific subgroups of patients [17] that would only accrue slowly in prospective investigations and might also permit correlation of intratumour histological heterogeneity with molecular genetic abnormalities.

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