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Short Communication

Polymorphisms in *P21*$^{CIP1/WAF1}$ are not Correlated with *TP53* Status in Sporadic Ovarian Tumours

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In breast cancers and sarcomas, a variant polymorphism in the cell cycle inhibitor P21$^{CIP1/WAF1}$ is under-represented in those individuals whose tumours contain mutated *TP53*. The aim of this study was to determine whether this variant polymorphism was also under-represented in those with ovarian carcinoma and *TP53* mutations. We studied lymphocyte DNA from 104 women with ovarian carcinoma, 15 with borderline tumours and 16 with benign tumours, using a previously-reported PCR-RFLP technique. 96 of the ovarian carcinoma cases had been previously examined for mutations in *TP53* and/or for overexpression of the TP53 protein. The variant allele was seen in 11 out of 104 women (10.6%) with ovarian carcinoma. There was no significant difference in the distribution of the variant allele in the women whose tumours had (7/47) or did not have (4/49) TP53 mutations ($P = 0.523$). It does not appear that the presence of this variant allele of *P21*$^{CIP1/WAF1}$ has any aetiological role in ovarian carcinomas. Studies in other tumours support this finding.

Key words: ovarian carcinoma, *P21*$^{CIP1/WAF1}$, cell cycle inhibitors, TP53, polymorphisms

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INTRODUCTION

MUTATIONS IN *TP53* are seen at a high frequency in human cancers. In ovarian carcinoma, we and others [1–3], have found that approximately 50% of cancers contain aberrant TP53, measured either by mutation analysis, immunohistochemistry or both. The frequency is much lower in non-malignant ovarian tumours [2]. As aberration in TP53 function is so central to tumour progression, it is of interest to determine whether TP53 may be inactivated in tumours by mechanisms other than mutation in *TP53* itself.

The cell cycle inhibitor gene, *P21*$^{CIP1/WAF1}$ encodes a 21 kDa protein which can bind to and inhibit cyclin–Cdk complexes. In addition, there is a TP53 binding site 2.4 kb (kilobases) upstream of the *P21*$^{CIP1/WAF1}$ gene [4]. The gene has tumour suppressor properties [4] and alterations in this downstream TP53 effector could, therefore, have a role in cancer. However, mutations in the coding region have been very rarely seen in human cancers, and specifically not in ovarian cancer [5–8]. Two polymorphisms, one at codon 31 (resulting in a change from serine to arginine in a conserved region of the protein) and another, 20 nucleotides downstream of the stop codon in the 3′ UTR have been reported [5, 7–9]. These point mutations do not appear to be more frequent in cancer patients than in controls [5, 7, 8]. However, it has been suggested that the variant polymorphisms are under-represented in breast cancer and sarcoma patients whose tumours possess somatic *TP53* mutations compared to those without mutations [8]. This result is intriguing, as it could imply that one or both polymorphisms influence P21$^{CIP1/WAF1}$ function, in such a way as to obviate the requirement for *TP53* mutations to deregulate cell cycle function.

We have, therefore, sought to determine whether this polymorphism is differentially distributed in a large series of ovarian tumours where the presence or absence of *TP53* mutations had already been determined by direct sequencing, immunohistochemistry or both [1, 2].

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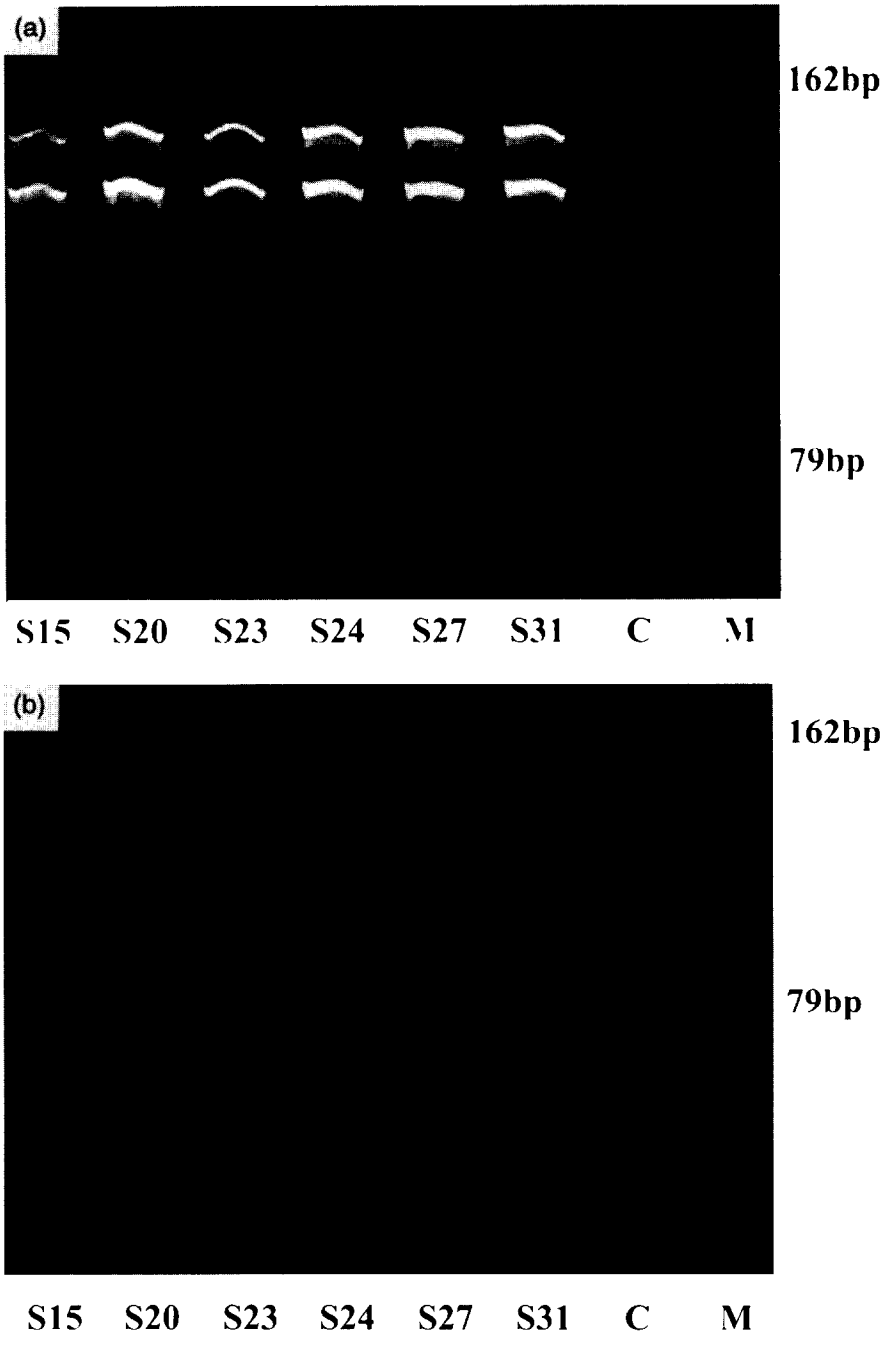

Figure 1. (a) Codon 31 polymorphism detection on 10% polyacrylamide gel. Heterozygote individuals S15, S20 and S23 are identified as having *Bsm*AI digestion products of 75 bp and 67 bp in addition to the wild-type 142 bp and 131 bp fragments. C, PCR contamination control, i.e. no DNA template (DNA bands seen are probably primer multimers and were a consistent feature); M, molecular markers. (b), 3′ UTR polymorphism detection on 10% polyacrylamide gel. Heterozygote patients S15, S20 and S23 are identified as having an allele that can not be digested with *Pst*I (123 bp) in addition to the digested allele (66 bp and 57 bp). C, PCR contamination control, i.e. no DNA template; M, molecular markers.

MATERIALS AND METHODS

135 ovarian tumours (104 carcinomas, and 31 non-malignant tumours (15 benign and 16 borderline)), which have been extensively characterised by molecular and histochemical methods [1, 2, 10–12], were studied. For $P21^{CIP1/WAF1}$ polymorphism analysis, we used lymphocyte DNA from the affected women. The PCR primers used were as described by Mousses and associates [8]. The PCR products were digested with the appropriate restriction endonucleases (*Bsm*AI for the codon 31 polymorphism and *Pst*I for the 3′ UTR polymorphism). The digested and undigested products were run side by side on 2% agarose and/or 10% polyacrylamide gels. The gels were stained with ethidium bromide and photographed under UV light. For *TP53* mutation analysis, tumour DNA was studied. PCR conditions, primer sequences and gel conditions were as described previously [2]. For those tumours where immunohistochemistry was carried out ($n = 75$), we employed a standard avidin–biotin complex technique, with diaminobenzidine, as previously described [1]. Fisher's exact test was used to assess

Table 1. Frequency of variant alleles in women with ovarian carcinoma

	TP53 mutation absent in tumour	*TP53* mutation present in tumour	Total
$P21^{CIP1/WAF1}$ variant allele absent (wt/wt)	45	40*	85
$P21^{CIP1/WAF1}$ variant allele present (wt/v)	4	7	11
Total	49	47	96

Fisher's exact test: $P = 0.523$ (two-sided).

*In two cases, the immunohistochemical analysis of *TP53* showed that > 50% of the cells were positive, but no mutation was found in exons 5–8 of *TP53*. These tumours have been included in this total. 7 cases showed mutations in exons 5–8 of *TP53*, but the immunohistochemistry was negative. They have also been included in this total. None of these tumours had the variant $P21^{CIP1/WAF1}$ allele. Exclusion of these cases does not significantly affect the results. Parts of the *TP53* data have been published previously [1, 2, 12].

the significance of the differences in the frequency of the variant alleles in a 2 × 2 table.

RESULTS

The frequency of the variant polymorphism in this series of women with ovarian tumours was 15/135 (11.1%), in accordance with previously published data [5–8]. The frequency of the $P21^{CIP1/WAF1}$ codon 31 variant polymorphism (Figure 1a) did not differ between those with carcinomas, borderline or benign tumours (11/104 (10.6%), 2/15 (13.3%), 2/16 (12.5%), respectively). The variant 3′ UTR polymorphism (Figure 1b) always appeared in concert with codon 31 polymorphism. Ninety-six of the carcinomas had been previously screened for *TP53* mutations [1, 2, 12]. There was no significant difference in the frequency of the variant polymorphism at codon 31 in those women whose tumours contained *TP53* mutation compared to those without *TP53* mutation (Table 1).

DISCUSSION

The products of the genes *TP53* and $P21^{CIP1/WAF1}$ bind at specific sequences resulting in $P21^{CIP1/WAF1}$ activation. $P21^{CIP1/WAF1}$ then binds the cyclin–Cdk complex, inhibiting its action and thus negatively regulating the cell cycle. Germ-line and somatic mutations in $P21^{CIP1/WAF1}$ are extremely rare. The loss of topological control of $P21^{CIP1/WAF1}$ observed in colonic neoplasia [13] does not appear to be mediated via mutation [5], and, therefore, subtle variations in activity might be an alternative mechanism. Polymorphisms in $P21^{CIP1/WAF1}$ have been reported [5, 7–9], and one group has shown a highly significant deficit of cases of breast cancer and sarcoma patients with somatic *TP53* mutations possessing a variant $P21^{CIP1/WAF1}$ codon 31 or 3′ UTR polymorphism [8]. Our study has shown that this relationship does not hold for ovarian carcinoma. Additionally, expression of $P21^{CIP1/WAF1}$ in ovarian carcinomas does not correlate with the presence or absence of *TP53* mutations, whether at the mRNA or protein level ([14] and Milner B.J., University of Aberdeen, U.K.). In a study of 158 brain tumours [15], there was no association between somatic *TP53* mutations and the Arg31Ser polymorphism in $P21^{CIP1/WAF1}$, which had a frequency of 8.5%. In addition, no mutations were found in exon 2 of $P21^{CIP1/WAF1}$.

The lack of association between the codon 31 $P21^{CIP1/WAF1}$ polymorphism and the likelihood of a *TP53* mutation is supported by data from assays of tumour suppressor activity. Chedid and colleagues demonstrated that the Ser13 allele and the Arg31 allele had almost equal abilities to inhibit colony formation [9], thus both forms of $P21^{CIP1/WAF1}$ appear to have equal tumour suppressing ability.

We found that 11 out of 104 women with ovarian carcinoma possessed the variant form of $P21^{CIP1/WAF1}$. There were no homozygotes. A previous report [7] showed that the frequency of this polymorphism in Italians did not differ between those with cancer (16/183, 8.7%) and those without cancer (9/102, 8.8%). Similarly, Mousses and associates found that 30 out of 186 (16.1%) cases and 11 out of 92 (12.0%) normal controls carried the variant [8]. This difference was not significant ($P = 0.472$, two-sided). Therefore, codon 31 3′ UTR $P21^{CIP1/WAF1}$ polymorphisms are not differentially represented in women with ovarian carcinomas, and among women with ovarian carcinomas, the presence of the variant form of $P21^{CIP1/WAF1}$ in the heterozygous state does not have any association with the occurrence of *TP53* mutations in the tumours themselves. These results concur with those recently published by Wan and associates [16].

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