**SHORT COMMUNICATION**

**p53 mutation is associated with high S-phase fraction in primary fallopian tube adenocarcinoma**

IB Runnebaum, T Köhler, E Stickeler, HE Rosenthal DG Kieback and R Kreienberg

**Department of Obstetrics and Gynaecology, Molecular Biology Laboratory, University of Ulm, 89075 Ulm, Germany.**

**Summary** Fallopian tube carcinoma (FTC) is a rare but lethal gynaecological malignancy. Four out of seven FTCs were identified with three point missense mutations, one single base deletion and one silent point mutation in the p53 gene. Genital-type HPV sequences were not detected. The S-phase fraction of tumours with mutant and wild-type p53 was 25.74% (median) and 12.55% (median) respectively.

**Keywords:** fallopian tube cancer; p53 mutation; cell cycle; human papilloma virus (HPV)

Primary fallopian tube carcinoma (FTC) is an aggressive, malignant tumour of the female genital tract with an unfavourable prognosis and an approximately 10% 5 year survival in the later stages (Eddy et al., 1984; Rose et al., 1990; Rosen et al., 1993). FTCs are rare, constituting about 1% of all female genital tract cancers. The vast majority represent adenocarcinomas and show histopathological similarities to epithelial ovarian cancer. In non-familial ovarian cancer, alteration of the p53 tumour-suppressor gene by somatic mutation is the most common single-gene alteration identified so far (Marks et al., 1991; Okamoto et al., 1991; Tsa et al., 1991; Kupryjanczyk et al., 1993; Milner et al., 1993; Runnebaum et al., 1994a; Runnebaum et al., 1995a). Wild-type p53 is a potent suppressor of tumorigenesis in different tumour types (Eliyahu et al., 1989; Baker et al., 1990; Cheng et al., 1992; Runnebaum et al., 1994c; Runnebaum and Kreienberg 1995). p53 acts as a transcription factor (Kern et al., 1991; Unger et al., 1992) regulating cellular functions such as DNA damage response (Kastan et al., 1991, 1992), induction of apoptosis by transactivating Bax and transrepressing Bel-2 (Miyashita et al., 1994; Miyashita and Reed, 1995) or inducing G1 cell cycle arrest by transactivating p21(WAF1/CIP1) (Lowe and Ruley 1993; Yonish-Rouach et al., 1993; Runnebaum et al., 1995b). A p53 mutation in FTC has first been identified in the cell line FT-MZ-1 established in our laboratory (Runnebaum et al., 1994b). We tested primary FTCs for p53 gene mutation and aberrant protein accumulation, integration of human papillomavirus (HPV) sequences and the association of cell cycle parameters with p53 alterations.

**Material and methods**

Pretreatment tumour samples from seven German Caucasian patients diagnosed with primary fallopian tube adenocarcinoma between 1986 and 1993 were analysed. Abdominal hysterectomy with bilateral salpingo-oophorectomy, resection of the omentum and retroperitoneal lymphadenectomy were performed. The tumour specimens contained more than 90% tumour cells as examined on haematoxylin and cosin stained sections taken from the same tumour block preceding and subsequent to the segment analysed. Tissue sections were analysed by two pathologists.

Mutation screening of the entire coding region comprising exon 2 to 11 was carried out using genomic tumour DNA extracted from the paraffin-embedded formalin-fixed sections as described previously (Runnebaum et al., 1991, 1994b). Polymerase chain reaction (PCR) fragments were sequenced on an automated sequencer (ALF express, Pharmacia Biotech, Uppsala, Sweden). Sense and antisense strands were analysed three times each. Ovarian cancer cell lines with characterised p53 mutations served as positive controls. The p53 protein was detected by immunohistochemistry with the anti-human-p53 mouse monoclonal antibody DO-1 (Dianova, Hamburg, Germany). Cell line FT-MZ-1, with a point missense mutation in codon 175 of the p53 gene, served as a control for positive staining (Runnebaum et al., 1994b). The evaluation was performed by two investigators unaware of the results of the molecular analysis. An immunoreactive score (IRS, see Table II) was assessed for recording staining intensity and proportion of stained cells (Remele and Stegner 1987; Runnebaum et al., 1996).

Genital-type HPV sequences were screened for using an L1 gene consensus primer PCR and dot blot analysis as well as an E6 gene multiplex PCR (Runnebaum et al., 1995c). Genomic DNA from the cervical cancer cell lines HeLa (10 to 50 integrated copies of HPV 18 per genome), SiHa (one to two integrated copies of HPV 16) and CaSkI (500 to 600 integrated copies of HPV 16) cells served as positive controls.

Flow cytometric analysis (Becton-Dickinson) was carried out using sections of 30 μm. The samples were coded and the experiments were carried out in a blinded fashion to eliminate observer bias.

**Results**

Seven patients with fallopian tube adenocarcinoma were included in the study. Four patients had stage IIA disease (Table I), according to the staging classification of the International Federation of Gynaecologists and Obstetricians (FIGO) with extension to the uterus or the ovaries (Nordin, 1994). Three other patients had a FIGO stage IIIC (Table I).
of the disease with intraabdominal spread. Tumour no. 777 had metastasised to paraaortic lymph nodes.

Five mutations were identified by nucleotide sequencing (Table II). Two mutations were identified in tumour no. 777, one silent point mutation in exon 8 not leading to an amino acid change and one single base deletion in exon 6 shifting the reading frame (Figure 1). The presence of mutant (mt) and wild-type (wt) sequence in nos. 339 and 777 indicated heterozygosity at the p53 locus.

Immunohistochemistry showed accumulation of p53 protein with a marked stain in three tumours. In tumours 906, with a homozygous point missense mutation in codon 285, and 6097, with the His175 mutant, the strong stain of more than 80% of the tumour cells resulted in an IRS of 12 (Figure 2). Tumour no. 777 showed an intermediate staining intensity in more than 80% of the cells with an IRS of 8 (Figure 2). Two tumours stained weakly with few cells (IRS 2), i.e. tumour no. 339 with an identified p53 point missense mutation and tumour no. 1417 with wild-type p53. Two tumours showed no detectable p53 expression (data not shown).

Integrated HPV sequences were found in none of the FTCs as tested by LI consensus primer PCR and dot blot. No HPV16-E6 or HPV18-E6 oncogene was detectable by multiplex PCR (data not shown).

The results of the FACScan analysis of the cell cycle distribution are summarised in Table III. The percentage of cells in S-phase varied between 7.06% and 33.90% in the different tumour samples. The S-phase fraction of tumours with mutant p53 was 25.74% (median) and of tumours with wild-type p53, 12.55% (median). The G1/G0 fractions varied between 58.84% and 82.60%. The median G1/G0 fraction of mutant p53 samples was 62.82% (median) and of wild-type p53 samples, 82.48% (median). All FTC samples were diploid.

### Discussion

The majority of FTCs are adenocarcinomas, stages II or III (Eddy et al., 1984; Rosen et al., 1993; Lacy et al., 1995). The tumours in this study were papillary adenocarcinomas representative for FTC. The tumours were metastatic at the time of diagnosis with FIGO stages IIA and IIC (Nordin, 1994). In four out of seven FTCs, mutations in the p53 tumour-suppressor gene were identified. The four point mutations were transition mutations, three of which changed the primary amino acid sequence. p53 transition mutations have frequently been found in other carcinoma types such as ovarian cancer, breast cancer and colon cancer and are considered to occur spontaneously. The point mutation in tumour no. 777 occurred at the third base position of codon 267 as a silent mutation. In the same tumour, an additional p53 mutation was found. The single base deletion in codon 194 leads to a frame shift. Small deletions could be caused by DNA replication errors and appear to occur relatively frequently in ovarian cancer (Okamoto et al., 1991; Milner et al., 1993; Runnebaum et al., 1994a). The loss of heterozygosity (LOH) rate at the p53 gene locus on chromosome arm 17p is not known for FTC. In various tumours the rate of LOH at this locus exceeds the rate of p53 mutations. Two out of four FTCs with mutant p53, however, remained heterozygous at the p53 locus.

The identified mutations were located in the 'core region', which comprises codons 102 to 292 of the p53 gene (Cho et al., 1994). The core region conveys the sequence-specific DNA-binding activity, a key function for the biological effect of p53. Missense mutations in this region are commonly
Table III  p53 status and cell cycle parameters

| Tumour no. | p53 status | G0/G1 (%) | S (%) | G2/M (%) |
|------------|------------|-----------|-------|----------|
| 339        | Mutant     | 71.33     | 24.44 | 4.23     |
| 777        | Mutant     | 64.48     | 23.12 | 12.40    |
| 906        | Mutant     | 61.16     | 33.90 | 4.94     |
| 6097       | Mutant     | 58.84     | 27.03 | 14.13    |
| 459        | Wild-type  | 82.60     | 12.55 | 4.85     |
| 1417       | Wild-type  | 65.49     | 16.37 | 18.14    |
| 1773       | Wild-type  | 82.48     | 7.06  | 10.46    |

predict clinical outcome (Lacy et al., 1995); the staining intensity but not the fraction of stained cells was recorded. Superior to the assessment of staining intensity or percentage of stained cells alone could be the IRS as demonstrated in a study on the prognostic value of steroid receptor expression in ovarian cancer (Kieback et al., 1993). Not all point missense mutations lead to accumulation of denatured p53 protein which was observed in one out of four tumours with p53 missense mutations in our study. An immunohistochemical study without molecular analysis may therefore mistakenly rate a significant number of tumours as containing wild-type p53. It appears difficult to value p53 immunohistochemical data with regard to clinical outcome.

Binding of p53 to the HPV E6 oncogene product mediated by the E6-associated protein has been shown to be a mechanism of p53 inactivation in squamous cell carcinoma of the cervix. Genital-type HPV sequences were not detected in the seven FTC samples analysed. HPV-related p53 inactivation (Scheffner et al., 1990) may not play a role in the development of FTC.

FTCs with mutant p53 presented with a higher S-phase, even in the p53 heterozygous tumours. Wild-type p53 protein, increased by DNA damage, increased DNA damage, transcriptionally induces p21WAF1/CIP1 (El-Deiry et al., 1993; Harper et al., 1993). p21WAF1/CIP1 complexes with and inhibits factors essential for cell cycle progression at the G1 checkpoint and DNA replication, cyclin-dependent kinases and the proliferating cell nuclear antigen (PCNA) (Chen et al., 1995; Luo et al., 1995). In the p53 heterozygous tumour no. 339, wild-type p53 may be inactivated in the presence of a dominant negative mutant protein possibly sequestering the wild-type protein by oligomerisation (Cloré et al., 1994).

In a small number of fallopian tube cancers, we have demonstrated that the p53 tumour-suppressor gene can be mutated with accumulation of aberrant p53 protein. FTCs with a p53 mutation may present with a higher S and a lower G1/G0 fraction. Larger studies will define the role of p53 mutation and cell cycle parameters as indicators of clinical outcome. Because of the rarity of FTC, such studies could best be performed in multicentre cooperative study groups.

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reflected by a nuclear accumulation of p53 protein stabilised in a denatured state (Cho et al., 1994). Studies have been conducted based on immunohistochemical screening to establish accumulation of p53 protein as a marker of prognostic significance in diverse tumour types. Accumulation of p53 as studied in 43 FTC cases has not been found to
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