Immunochemistry of p53 protein in ovarian carcinoma: correlation with histopathological data and clinical outcome

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Abstract Objective: The objective of this study was to analyze the incidence of immunohistochemically detectable p53 protein accumulation in epithelial ovarian carcinomas and to correlate these data with the clinical outcome so as to clarify further the role of p53 mutations in prognosis with these patients. Methods: Tumor tissues from 179 patients with epithelial ovarian carcinoma were used for immunohistochemical analysis with monoclonal antibody DO1 and BP 53-12-1 on formalin-fixed, paraffin-embedded tissue. Results: A total of 78 cases (44%) showed positive nuclear p53 staining. The p53-positive cases were found in all histological types of epithelial ovarian tumors. p53 staining was found in tumors of all stages with a higher percentage of positive cases in stage IV ovarian carcinomas (not significant). Poorly differentiated carcinomas showed a significantly higher percentage of p53 protein expression than did highly differentiated tumors (P = 0.0002). Clinical follow-up of up to 14 years (median 25 months) showed a slightly but not significantly shortened disease-free and overall survival time for patients with p53-positive epithelial ovarian carcinomas. Conclusions: We conclude from our data that p53 expression in ovarian carcinoma is associated with poor differentiation but not with the disease being in an advanced stage. There was a tendency for shortened disease-free and overall survival for patients with p53-positive tumors.

Key words p53 • Tumor-suppressor gene • Ovarian carcinoma • Immunohistochemistry

Introduction

The p53 tumor-suppressor gene is located at chromosome 17p13.1 and encodes a 53-kDa nuclear phosphoprotein that is thought to play a central role in the regulation of cell growth and proliferation (Miller et al. 1986; Levine et al. 1991; Casey et al. 1991; Finlay et al. 1989). Mutations of the p53 gene have been found in a wide variety of human cancers and to date they represent the most common genetic event to be described in human malignancies (Nigro et al. 1989; Hollstein et al. 1991; Vogelstein and Kinzler 1992).

Wild-type p53 is expressed at low levels in nontransformed cells and is supposed to restrain cellular proliferation by acting as a transcription factor, which promotes transcription from a defined DNA recognition site (El Deiry et al. 1992; Zambetti et al. 1992; Funk et al. 1992), possibly increasing the production of growth-suppressing proteins (Hinds and Weinberg 1994). However, by different and as yet unidentified mechanisms, an abundance of normal p53 protein can also inhibit the expression of genes that are important for cell growth, including proto-oncogenes encoding nuclear transcription factors (Hinds and Weinberg 1994; Harris and Hollstein 1993).

DNA damage due to exogenous stimuli, like X-rays or DNA-affecting drugs, causes a rapid increase in the level of wild-type p53 protein, which arrests the cell in the G1 phase of the cell cycle thus preventing it from further replicating damaged DNA sequences and accumulating mutations (Hinds and Weinberg 1994; Kastan et al. 1991; Kuérbitz et al. 1992). In contrast, cells with a mutant p53 gene are only partially blocked and continue to divide (Vogelstein and Kinzler 1992). In addition, overexpression of wild-type p53 can cause apoptotic cell death (Younish-Rouach et al. 1991).

Of all the p53 mutations, 98% occur within a sequence encompassing exons 5 through 8 where the codons for most
of the evolutionarily conserved amino acids are concentrated (Nigro et al. 1989; Hollstein et al. 1991; Soussi et al. 1990); 80% of the p53 mutations are missense mutations, causing the expression and nuclear accumulation (Harris and Hollstein 1993; Bartek et al. 1991) of a structurally altered, dysfunctional protein with increased stability (Finlay et al. 1988; Hinds et al. 1989; Kraiss et al. 1991), reduced sequence-specific DNA binding and reduced transcription factor activity (Vogelstein and Kinzler 1992). In addition, elevated levels of mutated p53 protein may inactivate intrinsic wild-type p53 by complex formation thus accounting for the loss of a potential negative growth-regulatory function mediated by the wild-type p53 (Finlay et al. 1989; Vogelstein and Kinzler 1992). Moreover, a missense mutation of one p53 allele is often accompanied by a deletion of the other allele resulting in the absence of the wild-type p53 protein (Vogelstein and Kinzler 1992). Accumulated p53 protein, with its increased half-life of hours instead of minutes, is detectable by immunohistochemistry using monoclonal antibodies (Levine et al. 1991; Bartek et al. 1991; Finlay et al. 1988; Banks et al. 1986; Vojtesek et al. 1992).

p53 mutations have been found in 36%-52% of ovarian carcinomas in exons 5 through 9 (Mazars et al. 1991; Naito et al. 1992; Kihana et al. 1992; Milner et al. 1993) and 79% in exons 2 through 11 (Kupryjanczyk et al. 1993). The frequency of immunohistochemical detection of the p53 protein was found to correlate well with the frequency of mutations detected by polymerase chain reaction/single-strand polymorphism (PCR/SSCP) analysis and DNA sequencing in breast and ovarian cancers (Kihana et al. 1992; Kupryjanczyk et al. 1993; Thor et al. 1992).

The objective of this study was to analyze the incidence of p53 protein accumulation in epithelial ovarian carcinomas of different histology, stage and grade and to correlate these data with the clinical outcome so as to clarify further the role of p53 mutations for prognosis in these patients.

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**Materials and methods**

**Tumor tissue**

The immunohistochemical analysis was performed using archival, formalin-fixed, paraffin-embedded tissue from 179 patients with epithelial ovarian carcinoma who were treated at the Department of Gynecology and Obstetrics, Virchow-Klinikum of the Humboldt-University in Berlin between 1981 and 1995.

The tumor histologies of the ovarian carcinomas included 98 serous-papillary, 34 endometrioid, 12 mucinous, 9 clear-cell, 3 Brenner, 13 undifferentiated, 2 unclassified and 8 mixed epithelial carcinomas. Histological differentiation was graded from I to III (according to Day et al. 1975) classifying 43 tumors (25%) as well differentiated, 68 tumors (38%) as moderately differentiated and 66 tumors (37%) as poorly differentiated.

**Clinical data and statistics**

Patient age ranged from 25 to 80 years with a median of 62 years at the time of surgery. At the time of diagnosis, 58 tumors (33%) were in stage I, 16 tumors (9%) in stage II, 87 tumors (50%) in stage III and 14 tumors (8%) in stage IV according to the International Federation of Gynecologists and Obstetricians (FIGO).

Laparotomy with bilateral oophorectomy, hysterectomy if not carried out previously and omentectomy was the standard surgical procedure. Pelvic lymphadenectomy was performed in 85 cases (48%) and additional aortic lymphadenectomy in 37 cases (21%). Complete surgical resection of the tumor was achieved in 102 cases (57%), whereas a residual tumor mass of less than 2 cm was left in 43 cases (24%) and of more than 2 cm in 30 cases (17%). In four cases the size of the residual tumor was not available from the patient records.

Most of the patients were seen at the Rudolf Virchow University Hospital outpatient clinic for follow-up. Clinical follow-up data were available from patients’ medical records and also from physicians in private practice. Follow-up ranged from 2 months to 14 years. The median follow-up was 25 months in the total cohort as well as for the survivors. Survival rates were calculated according to life table analysis (Lee 1980). Correlation coefficients for p53 overexpression were calculated by the U-test of Wilcoxon, Mann and Whitney.