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*P*53 mutations in tissue from Danish ovarian cancer patients From the Danish "MALOVA" ovarian cancer study

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Abstract

Objectives. The *p*53 gene, a tumor suppressor gene located on the short arm of chromosome 17 (17p13), has been found mutated in 30–80% of epithelial ovarian cancers (OC), with the most frequently detected mutations in the conserved regions of the gene. A small number of studies investigated the survival of patients with *p*53 mutations in OC, but their conclusions are not in agreement.

Methods. We analyzed the frequency of *p*53 mutations in 124 Danish women with OC, using Single-Stranded Conformation Polymorphism analysis in addition with DNA sequencing and evaluated if mutations correlated with clinicopathological parameters and with patient survival.

Results. Thirty-five (28%) ovarian tumors were found to contain one or more *p*53 variations, two of which were considered polymorphisms. Twenty-seven (82%) mutations were single nucleotide substitutions of which 23 (85%) were missense mutations and therefore led to amino acid substitutions. Significantly shorter survival was found for stage III/IV patients with a *p*53 missense mutation compared to stage III/IV OC patients with wild type *p*53 ($P = 0.0018$). Multivariate Cox regression analysis restricted to 107 OC patients with a *p*53 missense mutation or *p*53 wild type in the tumor tissue and with information on radicality of primary surgery showed that missense *p*53 mutation (HR = 2.5, 95% CI: 1.21–4.98), radicality after primary surgery (HR = 1.7, 95% CI: 1.04–2.88), tetranectin (mg/l: HR = 0.78, 95% CI: 0.67–0.91) and stage (I vs. III: HR = 0.30, 95% CI: 0.10–0.92, II vs. III: HR = 0.24, 95% CI: 0.05–1.05, IV vs. III: HR = 2.70, 95% CI: 1.22–5.98) were independent prognostic factors.

Conclusion. Missense mutations in the conserved regions of *p*53 may be of prognostic value in Danish OC patients.

Keywords: *p*53; Tumor suppressor gene; Mutations; Ovarian cancer; CA125; Tetranectin; Prognosis

Introduction

Ovarian cancer (OC) is the leading cause of death among gynaecologic cancer patients and is the fifth most frequent female cancer type and the fourth most frequent cause of death from cancer among women in Denmark [1]. If diagnosed and treated at early stage of disease, approximately 90% of OC patients survive 5 years or longer. However, if diagnosed at an advanced stage of disease (FIGO stage III and IV) (The International Federation of Gynecology and Obstetrics staging), the survival rate diminishes to less than 20%. Unfortunately, 70% of cases are diagnosed at the late stage of disease [2,3]. The high frequency and poor prognosis of OC emphasize the need for both additional and better prognostic factors.

Abbreviations: OC, ovarian cancer; PCR, polymerase chain reaction; SSCP, Single-Stranded Conformation Polymorphism; CA125, cancerantigen 125; ELISA, enzyme-linked immunosorbent assay; CV, coefficient of variation; CI, confidence interval; RH, relative hazard; MALOVA, MALignant OVArian cancer study; FIGO, The International Federation of Gynaecology and Obstetrics; WHO, World Health Organisation; IARC, International Agency for Research on Cancer.

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The molecular genetic events underlying ovarian neoplasms are complex and poorly understood. The transformation of a normal cell into a malignant cell is thought to be a multistep process that progresses through an accumulation of genetic alterations in two categories of normal cellular genes: proto-oncogenes and tumor suppressor genes [4–6].

The *p*53 tumor suppressor gene, on chromosome 17p13.1, encodes a 393 amino acid nuclear phosphoprotein (the *p*53 protein), thought to have a major function as a negative regulator of the cell cycle [7]. The *p*53 tumor suppressor gene has been found mutated in 30–80% of epithelial OCs depending on the methods used [5,8–16]. Generally, most of these missense mutations are found corresponding to the sequence-specific DNA-binding domain of the *p*53 protein, resulting in loss of function [17–19]. Only few studies investigated the prognostic role of *p*53 mutations in OC patients, and the results of the five most recently published studies are not consistent [11–13,15,20]. The prognostic role of *p*53 mutations is therefore still a subject of debate.

Serum levels of CA125, a biochemical marker for OC, are elevated in more than 80% of epithelial OCs. In studies using univariate life tables analysis, the level of CA125 has been reported to be a valuable prognostic marker of primary OC [21,22]. However, the prognostic value of CA125 determination disappears when FIGO stages are included in multivariate Cox analyses [22,23]. In contrast, tetranectin (TN) has been shown of prognostic value in OC patients when adjusted for FIGO stage [24–26]. Low serum or plasma TN levels may be due to absorption of TN from the blood to the tumor site for the purpose of proteolysis. This hypothesis is supported by the immunohistochemical findings of high extracellular TN concentrations in the malignant tumors in combination with low plasma TN values [27].

The aim of this study was to determine the frequency of *p*53 mutations in tissue from 124 Danish OC patients, to evaluate whether mutations correlated with clinicopathological parameters, to investigate the correlation of *p*53 mutations with CA125 and TN and finally to evaluate the role of *p*53 mutation as a prognostic factor in Danish OC patients.

Materials and methods

Study population

The material consists of the first 124 epithelial OC patients included in the MALOVA study (described below) where blood samples and corresponding tissue samples were available (30 stage I, 11 stage II, 67 stage III and 16 stage IV). The median age of the patients at diagnosis was 60 years (range 35–79). Clinical characteristics of the study population are shown in Table 1. The MALOVA study ("MALignant OVArian cancer study") is a multidisciplinary Danish study on OC, covering epidemiology (lifestyle factors), biochemistry and molecular biology with the purpose of identifying risk factors and prognostic factors for OC. The MALOVA study is described in detail elsewhere [28–31]. Briefly, preoperative blood samples as well as tumor tissue samples were obtained from most of the patients with a primary epithelial ovarian tumor. Histopathological classifications of the ovarian tumors were based on the typing criteria of the WHO. Pathology reports and tissue specimens were collected from the participating hospitals. One pathologist, specialized in ovarian tumors, reviewed the tissue specimen without knowledge of the original diagnosis. Subsequently, the reviewed diagnosis was compared to the original diagnosis. In terms of invasiveness, agreement between the original hospital diagnosis and the review diagnosis was present in 98% of the cases. Through the reviewing procedure, it was possible to define the histological type of tumor more precisely in 14% of the cases. FIGO stages were obtained from clinical records and were reviewed by two gynecologists, both specialized in OC. Furthermore, in the clinical

Table 1
Clinical characteristics of the study population (N = 124)

Characteristic	No. of patients (%)	*P*53 variations, no. of patients (%)			*P* value (missense or WT)
		Missense	WT	Other	
Patient age at diagnosis (media N = 60 years)					
35–50	28 (22)	7 (5)	20 (16)	1 (1)	
51–65	48 (39)	5 (4)	38 (31)	5 (4)	
66–79	48 (39)	11 (9)	31 (25)	6 (5)	
FIGO stage					
I	30 (24)	6 (5)	23 (18)	1 (1)	0.97
II	11 (9)	2 (2)	7 (5)	2 (2)	
III	67 (54)	12 (10)	49 (39)	6 (5)	
IV	16 (13)	3 (2)	10 (9)	3 (2)	
Histological type					
Undiff. carc. and pap. adenocarc. NOS	11 (9)	3 (2)	6 (5)	2 (2)	0.32[a]
Serous adenocarcinomas	81 (65)	12 (10)	60 (48)	9 (7)	
Mucinous adenocarcinomas	9 (7)	2 (2)	7 (5)	–	
Endometrioid adenocarcinomas	17 (14)	6 (5)	10 (8)	1 (1)	
Clear-cell neoplasms	6 (5)	–	6 (5)	–	
Radicality of primary surgery					
Radical (no macroscopic residual tumor)	44 (35)	9 (7)	34 (27)	1 (1)	0.92[b]
Non-radical (visible macroscopic residual tumor)	75 (60)	14 (11)	50 (40)	11 (9)	
No information	5 (5)	–	5 (5)	–	

[a] Serous adenocarcinoma, mucinous adenocarcinoma, endometroid adenocarcinoma and other histological types.
[b] Radical or non-radical.