Both Germ Line and Somatic Genetics of the p53 Pathway Affect Ovarian Cancer Incidence and Survival

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Abstract

Purpose: Although p53 is one of the most studied genes/proteins in ovarian carcinomas, the predictive value of p53 alterations is still ambiguous.

Experimental Design: We performed analyses of the *TP53* mutational status and its protein expression using immunohistochemistry. Moreover, the single nucleotide polymorphism SNP309 in the P2 promoter of the *MDM2* gene was investigated. We correlated the results with age of onset and outcome from 107 patients with ovarian carcinoma.

Results: In our study, we identified a large group of patients with p53 overexpression despite having a wild-type gene (49% of all patients with wild-type TP53). This was associated with a significantly shortened overall survival time ($P = 0.019$). Patients with p53 alterations (especially those with overexpression of wild-type TP53) were also more refractory to chemotherapy compared with patients with normal p53 ($P = 0.027$). The *G*-allele of SNP309 is associated with an earlier age of onset in patients with estrogen receptor – overexpressing FIGO stage III disease ($P = 0.048$). In contrast, in patients with FIGO stage III disease, a weakened p53 pathway (either the *G*-allele of SNP309 or a *TP53* mutation) was correlated with increased overall survival compared with patients whose tumors were wild-type for both *TP53* and SNP309 ($P = 0.0035$).

Conclusion: Our study provides evidence that both germ line and somatic alterations of the p53 pathway influence the incidence and survival of ovarian carcinoma, and it underscores the importance of assessing the functionality of p53 in order to predict the sensitivity of platinum-based chemotherapies and patient outcome.

Ovarian cancer is the leading cause of death among patients with gynecological cancer in the Western world (1). Most women with ovarian cancer present with advanced stages of disease at the time of diagnosis (2). The 5-year overall survival rates are only 15% to 25% for advanced stages (International Federation of Gynecology and Obstetrics, FIGO stages III and IV) of ovarian cancer (1, 3). Despite many efforts to establish molecular alterations as prognostic markers, residual disease and ascites are still the only significant variables in multivariate analyses in patients with advanced disease. The standard treatment for patients with advanced ovarian carcinoma is tumor debulking, followed by chemotherapy with carboplatin and taxol. Unfortunately, the disease will recur in nearly all patients due to acquired chemoresistance. Therefore, factors modulating chemoresistance are of outmost importance in the treatment of patients with ovarian carcinomas. An important factor for a cellular response to platinum-based chemotherapeutic agents is p53 whose functionality is attenuated by both germ line and somatic genetic events of TP53 itself or of regulators located upstream and downstream in the p53 tumor suppressor pathway. Therefore, resistance to these drugs may be due to the lack of functional p53.

In ovarian carcinomas, mutation frequencies, determined by direct sequencing, range from 40% to 80% (4 – 7). There seems to be a trend in which overexpression of TP53 (8 – 12) rather than mutation of *TP53* (4, 13 – 15), is correlated with shortened overall survival. In other studies, no significant association between p53 overexpression and a poorer outcome for patients with ovarian cancer was found (15 – 19). A problem related to studies separately analyzing *TP53* mutations and p53 protein is that immunohistochemistry will miss cases with truncating mutations, such as nonsense mutations and deletions/insertions, and on the other hand, overexpression of TP53 is not necessarily associated with mutations. Therefore, the status of p53 alterations (gene mutations and/or overexpression) will be more informative than the mutational and protein expression status alone. Although a few studies have evaluated both the *TP53* gene and protein expression status (i.e., refs. 5, 6), in

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Received 5/15/07; revised 8/1/07; accepted 10/8/07.

Grant support: The work in the authors' laboratories is supported by the Wilhelm-Roux-Programm of the University of Halle (grant 12/40 and 14/09). A. Böhnke is supported by a graduate student fellowship from the Wilhelm-Sander-Foundation (grant 2005.102.1).

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Note: F. Bartel and J. Jung contributed equally to the results of this study.

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doi:10.1158/1078-0432.CCR-07-1192

none of them have the authors correlated the combined status of p53 alterations with survival or response to chemotherapy.

The MDM2 oncogene is the key negative regulator of the p53 protein (20). It has been shown that the *G*-allele of a single nucleotide polymorphism (SNP309) in the p53-sensitive P2 promoter of the *MDM2* gene is associated with the attenuation of the p53 tumor suppressor pathway (21). Numerous reports provide evidence that the *G*-allele of SNP309 is correlated with an earlier age of onset and an increased risk of tumorigenesis (22–25). MDM2 expression is also regulated by estrogen receptor (ER) signaling with transcription of the MDM2-P2 transcript induced in ERα-positive cell lines (26). Interestingly, the ERα-binding site is located within the region that contains SNP309. In a previous report, we showed that SNP309 alters the effect of hormones, such as estrogen, on tumorigenesis, and contributes to the gender differences observed in many cancers (27).

The aim of this study was therefore to investigate the status of SNP309, *TP53* sequence alterations, and p53 protein expression in a group of 107 ovarian cancer patients with complete clinical data collected at the Institute of Pathology, University of Halle (Germany) and to test whether both germ line and somatic p53 alterations were associated with tumor characteristics that could be used as reproducible markers for clinical outcome.

Materials and Methods

Patient population and clinical data. Paraffin-embedded tissue samples from 107 invasive ovarian carcinomas which were diagnosed at the Institute of Pathology, Martin-Luther-University Halle-Wittenberg between 1997 and 2005 were selected based on the availability of tissue. The study was approved by the local ethical committee. All histologic slides were re-evaluated by two pathologists (E. Gradhand and S. Hauptmann) using a multihead microscope. Tumor patients and tissue samples were, in part, described elsewhere (28). Histology was classified according to the WHO, and grading was assessed according to Silverberg (29). Data retrieved from clinical files included the patient's age, amount of residual tumor, FIGO stage, adjuvant chemotherapy, and follow-up (Table 1).

Immunohistochemistry. The analysis of the p53 protein expression was carried out using the DO-7 mouse monoclonal antibody (DAKO), and analysis of the ER used the anti-ER antibody SP1 (Labvision, Germany). The immunoreactivity for p53 and ER was scored as the percentage of stained cells by counting 150 tumor cells on average. Tumors with >10% stained cells were considered to be either p53 or ER-positive.

***Single-strand conformation polymorphism analysis for* TP53.** DNA from paraffin-embedded tissue sections were isolated according to standard procedures (30). One section of each tumor sample was stained with H&E in order to confirm that the majority of the tissue was comprised of tumor cells.

We used PCR to amplify exons 3 to 9 of the *TP53* gene. The primers for each exon were located in intronic sequences; therefore, flanking splice sites could be analyzed. The sequences of the primers and the condition for the PCR amplification have been published previously (6). Ten to 20 μL of each PCR product was precipitated overnight at -20°C and the conformational changes of the PCR products were subsequently analyzed by single-strand conformational polymorphism (SSCP) on a denaturing polyacrylamide gel. DNA from healthy volunteers served as wild-type controls.

***Sequence analysis for* TP53.** All samples identified as having conformational changes, and in addition, all samples from exons 5 to 8, were analyzed by direct sequencing in both sense and antisense directions using the BigDye Terminator Cycle Sequencing 3.1 Kit (Applied Biosystems). The sequencing reactions were carried out according to the manufacturer's instructions.

Determination of SNP309 status. The SNP309 status of 103 ovarian carcinoma samples was determined by PCR and subsequent direct sequencing of the P2-promoter region of the *MDM2* gene as described elsewhere (21).

Statistical evaluation. All statistics, including Cox's proportional regression hazard model and the Kaplan-Meier survival estimates, were carried out using the SPSS 12.0G software (SPSS Science). $P < 0.05$ was considered to be significant.

Results

TP53 *mutations and p53 protein expression.* In 107 patient samples, we detected a total of 111 sequence alterations in exons and introns of the *TP53* gene (Table 2; Fig. 1), of which 44 were mutations in exons 4 to 8, including two mutations in introns. Twenty-two of the sequence alterations were a 16-bp insertion in intron 3, and 45 sequence alterations were found in codon 72, which are known as polymorphisms.

Of the mutations that affected the amino acid sequence of p53, 93% (39 of 42) were found in exons 5 to 8 (Table 2). Sixty-three percent (27 of 42) of the mutations were missense mutations, 9.3% (4 of 42) were nonsense mutations in which a single nucleotide exchange resulted in a premature stop codon. Furthermore, we detected small frameshifting mutations of which seven were deletions and three were insertions of 1 or 2 bp, respectively. In addition, there was one large deletion of 32 bp in exon 8 (Table 2). The most frequently affected were

Table 1. Summary of clinicopathologic data of patients with ovarian cancer

Characteristics	Patients (107)
	No. (%)
Tumor cell type	
Serous	62 (57.9)
Endometrioid	14 (13.1)
Mixed	11 (10.3)
Clear cell	9 (8.4)
TCC	1 (0.9)
UC	8 (7.5)
MC	2 (1.9)
FIGO tumor stage	
I	30 (28.0)
II	9 (8.4)
III	63 (58.8)
IV	5 (4.7)
Patient age (y)	
Mean	63.5
Median	64.0
SD	11.5
Type of therapy	
Cisplatin + taxol	62 (57.9)
Platinum-based chemotherapy without taxol	21 (29.7)
Other	2 (1.9)
None (FIGO Ia)	5 (4.7)
Refused/dead	13 (12.1)
Missing	4 (3.7)
Residual tumor	
None	39 (41.0)
<1 cm	22 (23.1)
>1 cm	34 (35.8)

Abbreviations: TCC, transitional cell carcinoma of the ovary; UC, undifferentiated carcinoma of the ovary; MC, mucinous carcinoma of the ovary.

Table 2. *TP53* mutations in ovarian cancer

Case no.	Histology	Exon	Codon	Conserved region	Mutation type	Change*	Wild-type	Mutated	Wild-type AA	Mutated AA
9	Mixed	4	91	NC	nonsense	G>A	TGG	TAG	Trp	STOP
37	ser	4	91	NC	nonsense	G>A	TGG	TAG	Trp	STOP
64	ser	4	98	NC	Missense	C>T	CCT	CCT	Pro	Leu
84	ser	5	138	Co	Deletion	-GC (*)				
115	mixed	5	141	Co	Missense	G>A	TGC	TAC	Cys	Tyr
116	ser	5	141	Co	Missense	G>A	TGC	TAC	Cys	Tyr
8	endo	5	149	NC	Insertion	+T	TCC	TTC	Ser	Phe
109	ser	5	150	NC	Missense	C>T	ACA	ATA	Thr	Ile
99	ser	5	152	NC	Deletion	C	CCG		STOP	
68	ser	5	153	NC	Insertion	+T				
34	un	5	175	Co	Missense	G>A	CGC	CAC	Arg	His
39	ser	5	175	Co	Missense	G>A	CGC	CAC	Arg	His
98	ser	5	175	Co	Missense	G>A	CGC	CAC	Arg	His
67	ser	5	178	Co	Missense	C>T	CAC	TAC	His	Tyr
111	ser	5	179	Co	Missense	A>G	CAT	CGT	His	Arg
31	un	Int5								
3	ser	6	195	NC	Missense	T>C	ATC	ACC	Ile	Thr
24	TCC	6	195	NC	Missense	T>C	ATC	ACC	Ile	Thr
20	EC	6	200	NC	Deletion	T				
61	ser	6	201	NC	nonsense	T>A	TTG	TAG	Leu	STOP
91	ser	6	206	NC	Deletion	TT (*)				
60	un	6	216	NC	Missense	G>A	GTG	ATG	Val	Met
32	ser	6	220	NC	Missense	A>G	TAT	TGT	Tyr	Cys
15	ser	7	225	NC	Missense	G>T	GTT	TTT	Val	Phe
106	endo	7	229	NC	Insertion	+T (*)	GAC	TGA	Asp	STOP
79	ser	7	239	Co	Missense	A>G	AAC	AGC	Asn	Ser
45	ser	7	245	Co	Missense	G>A	GGC	AGC	Gly	Ser
47	un	7	245	Co	Missense	G>T	GGC	TGC	Gly	Cys
59	ser	7	245	Co	Missense	G>A	GGC	GAC	Gly	Asp
4	ser	7	248	Co	Missense	G>A	CGG	CAG	Arg	Glu
69	ser	7	248	Co	Missense	G>A	CGG	CAG	Arg	Gln
40	clear	7	257	Co	Missense	T>A	CTG	CAG	Leu	Gln
33	clear	7	259	NC	Missense	G>T	GAC	TAC	Asp	Tyr
118	ser	Int7	Splice							
81	ser	8	267	NC	Deletion	-G				
76	mixed	8	272	Co	Missense	G>A	GTG	ATG	Val	Met
42	mixed	8	273	Co	Missense	G>A	CGT	CAT	Arg	His
41	ser	8	274	Co	Missense	G>T	GTT	TTT	Val	Phe
27	ser	8	275	Co	Missense	G>T	TGT	TTT	Cys	Phe
80	mixed	8	280	Co	Missense	G>C	AGA	ACA	Arg	Thr
78	clear	8	285	Co	Deletion	-32 (*)				
7	mixed	8	291	NC	Deletion	-G				
43	ser	8	294	NC	nonsense	G>T	GAG	TAG	Glu	STOP
86	ser	8	306	NC	Deletion	-C	CGA			

Abbreviations: Ser, serous ovarian carcinoma; Endo, endometroid ovarian carcinoma; TCC, transitional cell carcinoma of the ovary; UC, undifferentiated carcinoma of the ovary; MC, mucinous carcinoma of the ovary; NC, non-conserved region of the *TP53* gene; Co, highly conserved region of the *TP53* gene.
*Mutations which have not been described as of October 2006 according to Petitjean et al. (31).

codons 175 and 245, with three cases each. To our knowledge, 12 of the 42 exonic mutations have been described here for the first time to occur in ovarian cancer, including 4 that have not been published thus far (31).

We found that 51.4% (55 of 107) cases showed overexpression of p53. Twenty cases (18.7%) showed immunostaining of 10% or less of the cells and 32 cases (30.2%) were negative. Benign ovarian control tissue was negative for TP53.

Relationship of p53 protein expression and* TP53 *gene status. In our study, overexpression of p53, as detected by immunohistochemistry, was not correlated with the *TP53* mutational status (P = 0.59; χ^2 test). Fifty-four percent (24 of 44) of cases with a mutated *TP53* gene also showed overexpression of p53 protein; however, the percentage of cases with a wild-type *TP53* gene that overexpress p53 was 49% (31 of 63).

We divided the cases according to their combined p53 mutational/protein expression status into four groups. Cases with a wild-type *TP53* gene and undetectable p53 protein were designated as "p53 normal" and considered to have functional p53 (32 of 107 cases, 29.9%); other groups included cases with an alteration of p53, i.e. (*a*) cases that overexpressed wild-type p53 (31 of 107, 29%), (*b*) cases with *TP53* mutations and overexpression of the protein (24 of 107, 22.4%), and (*c*) cases with *TP53* mutations but undetectable p53 protein (20 of 107, 18.7%).

TP53 mutations and p53 overexpression were more frequent in advanced stage ovarian carcinomas; however, only the latter

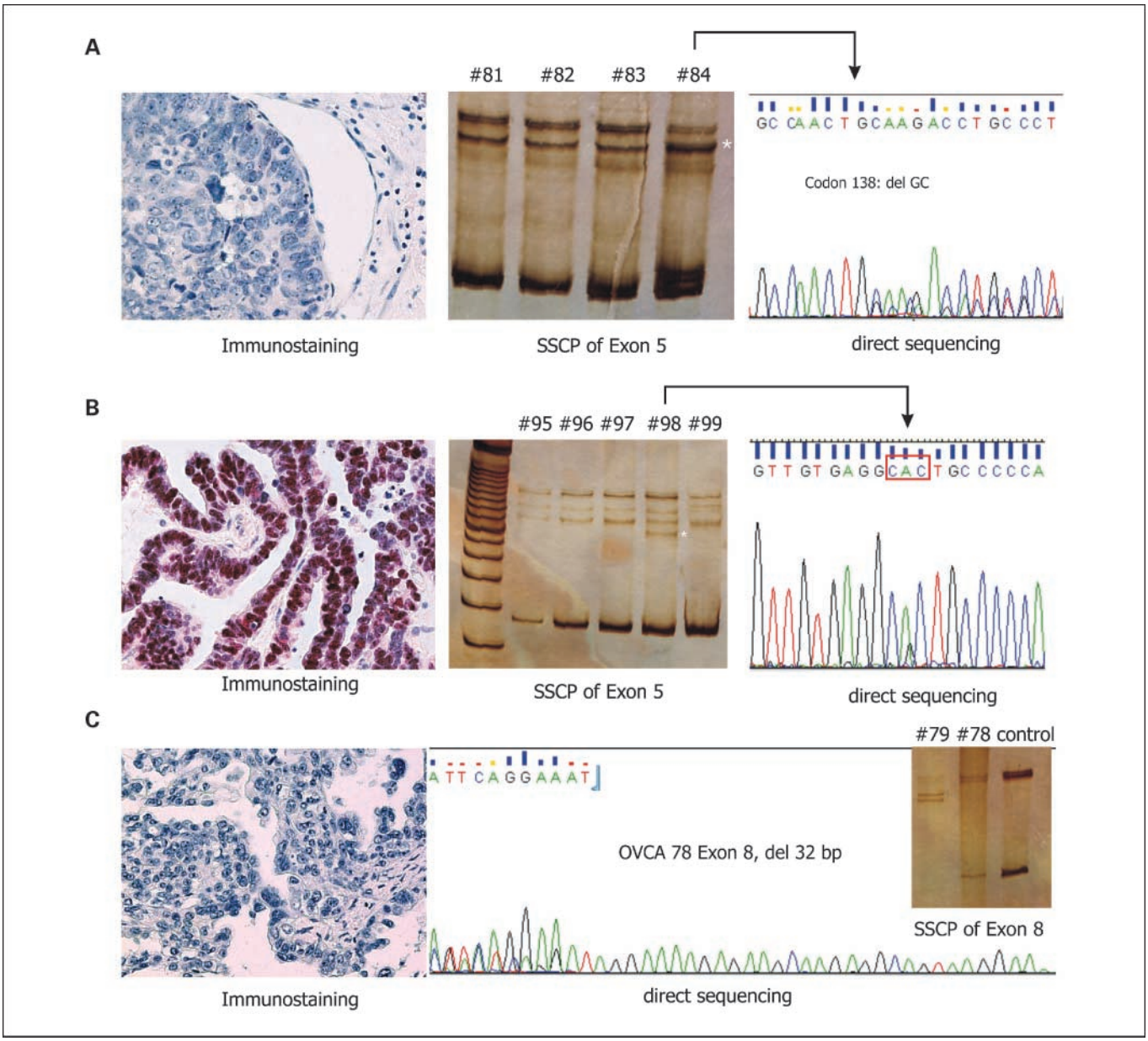

Fig. 1. SSCP, DNA sequencing, and immunohistochemical detection of p53 expression in ovarian cancer. *A,* the SSCP analysis of exon 5 from case no. 84 (serous ovarian carcinoma) clearly shows the appearance of an additional band (*). The subsequent DNA sequencing confirmed a 2-bp deletion (GC) in codon 138 that results in a premature stop codon. P53 was not detectable by immunohistochemistry in case no. 84. *B,* SSCP and DNA sequencing of exon 5 from case no. 98 (serous ovarian carcinoma) revealed a missense mutation in codon 175 (CGC to CAC) resulting in an Arg to His substitution. P53 immunostaining showed overexpression of the TP53 protein. *C,* SSCP, DNA sequencing, and immunohistochemical detection of TP53 expression in case no. 78 (clear cell ovarian carcinoma) which is characterized by a 32-bp deletion in exon 8 of the *TP53* gene and the absence of p53 immunostaining.

correlation was significant (P = 0.096 and P = 0.01, respectively; Table 3).

P53 status and response to chemotherapy. We found that patients whose tumors have p53 alterations are significantly more resistant (78%) than patients whose tumors harbor normal p53 (52%; P = 0.027). This was also true for protein expression status when comparing tumors either with overexpression of p53 (83%; P = 0.02) or no p53 (58%). Patients with *TP53* mutations were also more resistant to chemotherapy (79%) compared with patients with wild-type *TP53* (64%). However, this association was not significant (P = 0.148). Patients with altered TP53 (mutation and/or overexpression)

Table 3. Association of FIGO stage and p53 status in ovarian cancer

FIGO stage	*TP53* mutations		p53 immunostaining	
	Wild-type	Mutated	Negative	Positive
I	22	8 (26%)	22	8 (27%)
II	7	2 (22%)	5	4 (44%)
III	32	31 (49.2%)	23	40 (63%)
IV	2	3 (60%)	2	3 (66%)
P	0.096		0.01	
Summary	63	44 (41.1%)	53	55 (51.4%)

had a shorter time before relapsing than patients with normal p53 (28 versus 51 months; $P = 0.075$).

Status of the SNP309 in the MDM2 P2 promoter. SNP309 was analyzed in 103 of 107 patients with ovarian carcinoma. We found SNP309 with a relatively high frequency in the heterozygous T/G state (52.4%) and at a lower percentage in the homozygous G/G state (7.8%) compared with healthy volunteers (T/G, 40%; G/G, 12%; ref. 21), although the difference was not significant ($P = 0.53$). We have previously shown that the *G*-allele of SNP309 predominantly acts in women with an active estrogen signaling pathway (27). Therefore, we analyzed whether the association of SNP309 and the age of onset were affected by the expression of ER in patients with ovarian carcinoma. We found that 43% (46 of 107) of the tumors were ER-negative, 17% (18 of 107) showed low ER expression, and 40% (43 of 107) showed high ER expression (data not shown). In patients with FIGO stage III disease, the occurrence of SNP309 was associated with an onset almost 6 years earlier for patients with detectable expression of the ER (T/T, 70.6 years; T/G + G/G, 64.4 years), although the difference did not reach statistical significance ($P = 0.101$) and was 8.5 years earlier in patients with strongly elevated ER expression ($P = 0.048$). In ER-negative patients, there was no difference regarding the age of onset ($P = 0.44$). These results support the hypothesis that the *G*-allele of SNP309 requires an intact estrogen signaling pathway to accelerate tumorigenesis (27).

P53, SNP309 status, and overall survival. When combining the p53 mutational and protein expression status, patients with

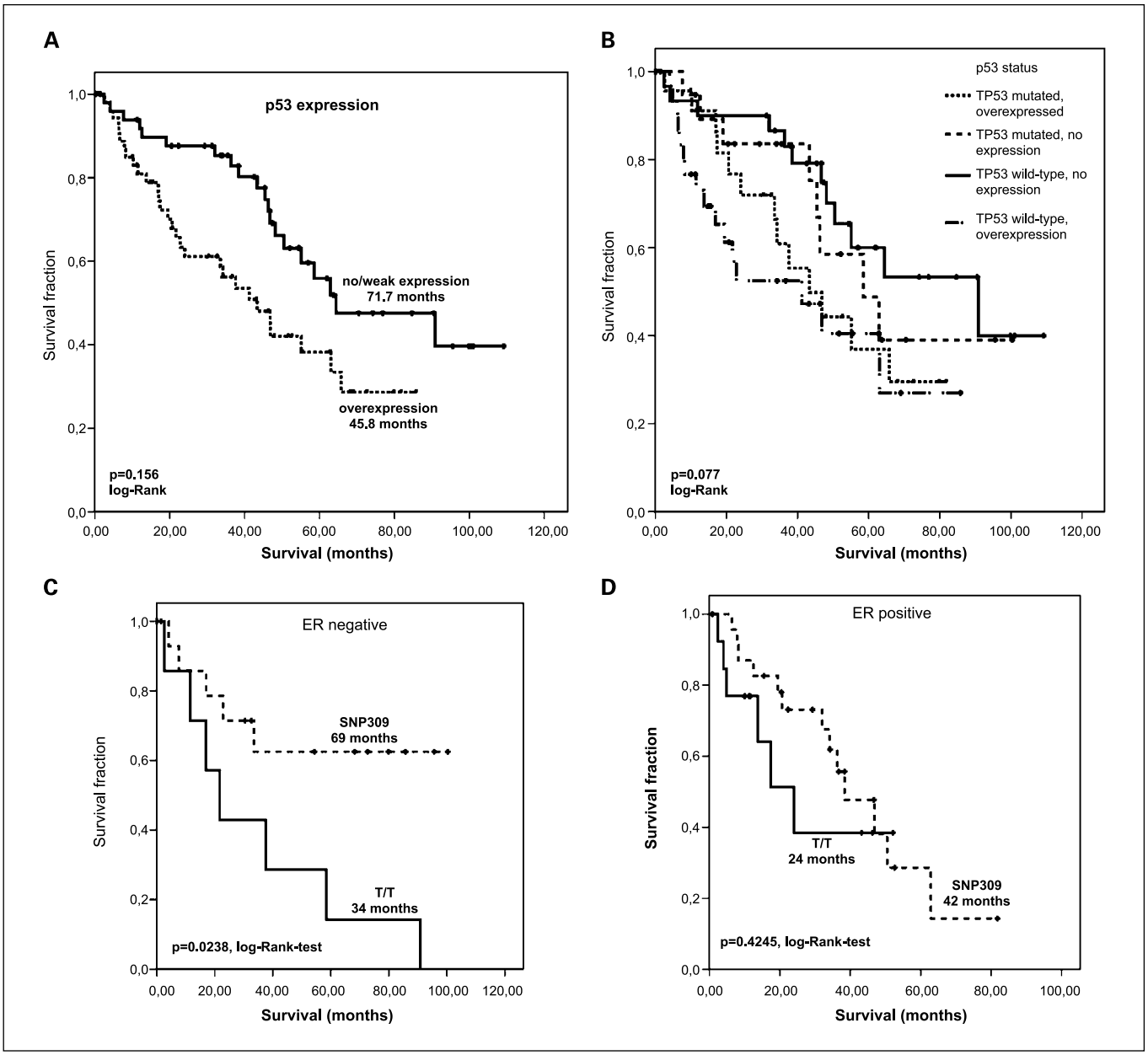

Fig. 2. *A,* overall survival of ovarian cancer patients with different p53 expression status. *B,* overall survival of ovarian cancer patients with combined p53 protein and mutational status (refer to text for further details). *C* and *D,* overall survival of ovarian cancer patients with different SNP309 genotypes and ER expression levels.

Table 4. Prognostic significance of molecular and clinical factors identified by multivariate Cox regression analysis

Variables	Univariate analysis		Multivariate analysis*	
	Unadjusted RR (95% CI)	*P*	Adjusted RR (95% CI)	*P*
p53 immunostaining				
Negative	1.00		1.00	
Positive	2.021 (1.13-3.616)	0.018	1.331 (0.703-2.522)	0.38
TP53 mutations				
Wild-type	1.00		1.00	
Mutated	1.051 (0.596-1.851)	0.864	0.648 (0.333-1.26)	0.201
p53 immunohistochemistry + mutation				
Normal	1.00		1.00	
Wild-type, overexpression	2.548 (1.188-5.465)	0.016	1.598 (0.662-3.856)	0.297
Mutated, no expression	1.922 (0.87-4.246)		0.884 (0.336-2.325)	
Mutated, overexpression	1.284 (0.524-3.145)		0.82 (0.299-2.246)	
p53 altered				
Normal	1.00		1.00	
Altered	1.901 (0.985-3.667)	0.045	1.107 (0.498-2.461)	0.804
Arg72Pro SNP				
Arg/Arg	1.00		1.00	
Arg/Pro	0.747 (0.386-1.446)		1.693 (0.769-3.728)	
Pro/Pro	1.371 (0.588-3.200)	0.412	6.452 (1.072-6.452)	0.035
Histology				
Serous	1.00		1.00	
Nonserous	1.938 (1.011-3.717)		1.449 (0.672-3.124)	
Undifferentiated	3.679 (1.284-10.54)	0.032	1.590 (0.419-6.033)	0.496
Ascites				
No	1.00		1.00	
Yes	3.015 (1.27-7.161)	0.01	1.681 (0.695-1.681)	0.249
Relapse				
No	1.00		1.00	
Yes	22.34 (3.072-162.54)	0.002	11.769 (1.374-100.77)	0.024
Residual disease				
None	1.00		1.00	
Any	6.556 (2.976-14.444)	<0.001	5.255 (1.648-29.588)	0.008
FIGO stage				
I	1.00		1.00	
II	1.462 (0.449-4.764)		1.403 (0.378-5.210)	
III	3.175 (1.505-6.697)		1.542 (0.523-4.541)	
IV	9.922 (2.932-33.577)	<0.001	6.983 (1.648-29.588)	0.008

Abbreviations: RR, relative risk; 95% CI, 95% confidence interval.
*Adjusted to FIGO stage and residual disease.

an up-regulation of the wild-type *TP53* gene had the shortest overall survival time (42.8 months), compared with patients with an overexpression of a mutated *TP53* gene (48.2 months) and with patients with no detectable p53 expression (*TP53* wild-type, 74.2 months; *TP53* mutated, 64.4 months); however, the difference in survival time between all groups was only marginally significant ($P = 0.077$, log-rank test; Fig. 2B). The difference in survival time reached statistical significance, however, when comparing p53 expression in patients with a wild-type *TP53* gene ($P = 0.019$, log-rank test). To summarize, patients with normal p53 (wild-type *TP53* gene and no detectable protein expression) had a longer survival time compared with patients whose tumors showed an overexpression of wild-type or mutated *TP53*.

Patients with p53 overexpression, compared with patients with no or weak expression, showed a significantly decreased overall survival time (45.8 versus 71.7 months; $P = 0.016$; log-rank test; Fig. 2A), but there was no difference in the overall survival for patients with wild-type or mutated *TP53* genes ($P = 0.86$; log-rank test). It is noteworthy that patients with FIGO III carcinomas and a wild-type *TP53* gene had a shorter overall survival than patients with a mutated *TP53* gene; this difference nearly reached statistical significance (41 versus 59 months; $P = 0.058$). We made a similar observation when we analyzed the effect of SNP309 on overall survival. The average survival time for women with the T/T genotype was 59 months; for women with the *G*-allele, this was 62 months ($P = 0.994$). In patients with FIGO stage III disease, the *G*-allele was significantly associated with prolonged overall survival (57.5 months) compared with patients with a T/T genotype (35.3 months; $P = 0.045$; log-rank test). When patients with FIGO stage III disease were further divided into groups according to their ER expression status (Fig. 2C and D), we found that ER-negative patients with a T/G or G/G genotype had a significantly increased survival time ($P = 0.024$, log-rank test) compared with patients with a T/T genotype of SNP309 (69 versus 34 months, respectively). In contrast, the *G*-allele did not influence the overall survival for FIGO stage III patients with ER-positive tumors (low expression, $P = 0.424$; strong expression, $P = 0.828$). These observations resulted in a model in which a weakened p53 pathway,

either through a mutant *TP53* or the *G*-allele of SNP309, was associated with a better outcome for patients with ovarian cancer.

In a univariate Cox's regression model (Table 4), we could show that overexpression of wild-type p53 ($P = 0.016$) and positive p53 immunostaining ($P = 0.018$) were prognostic factors, whereas the occurrence of *TP53* mutations alone did not increase the risk of tumor-related death ($P = 0.864$). Other factors with prognostic effects were an undifferentiated histology ($P = 0.032$), the occurrence of ascites ($P = 0.01$), residual disease ($P < 0.001$), FIGO stage ($P < 0.001$), and the progression of disease (relative risk, 22.3; $P = 0.002$). In a multivariate Cox regression, however, only FIGO stage ($P = 0.008$) and residual disease ($P = 0.008$) were independent prognostic factors for overall survival. Interestingly, the Pro/Pro alleles of the Arg72Pro SNP were correlated with a 6.4-fold increased risk of tumor-related death ($P = 0.032$) in a multivariate Cox regression that was adjusted for FIGO stage and residual disease.

Discussion

In our study, we analyzed the *TP53* gene, its protein expression, and the status of SNP309 within the p53-sensitive MDM2-P2 promoter in a cohort of 107 ovarian carcinomas. In 39% of the ovarian carcinomas (42 of 107), we found *TP53* mutations that resulted in a change of the amino acid sequence. This percentage is near the range of 40% to 80% which was reported in the literature for ovarian cancer (reviewed in ref. 7). The TP53 immunopositivity of 51% in our study is near the mean value of ~49% within a range of 29% to 62% published for FIGO I to IV paraffin-embedded samples (8–12, 32–34).

Our results clearly show that immunohistochemistry is not a surrogate marker of the *TP53* gene status. We found that 49.2% of the cases with a wild-type *TP53* gene were also positive for the p53 immunostaining, a percentage that is higher than those previously reported by others (5, 6). Havrilesky et al. (5) and Reles et al. (6) found 28% and 38%, respectively, of tumors with a wild-type sequence and overexpression of the p53 protein. Wild-type p53 is usually very unstable and is maintained at low levels because it is a key regulator of cell growth (35). In this regard, it is surprising that 90% of the wild-type p53-overexpressing tumors were high-grade ovarian carcinomas. The reasons for this abnormal stability are thus far unknown. It is conceivable that there is either a change in the functionality of proteins that interact and control the activity and the levels of p53, such as MDM2 (36) and MDMX (37), or a constitutive phosphorylation of TP53 that prevents an interaction with these negative regulators. It has also been suggested that a yet unknown mediator between ubiquitinylated p53 and the proteasome might be down-regulated in these tumors (38). Wang et al. (39) and Kraus et al. (40) have reported that the stabilization of wild-type p53 correlates with the expression of MDM2 splice variants. To summarize, the reason for the accumulation of wild-type p53 and its biological significance are currently unknown. Further studies are necessary to clarify the cause of the abnormal stability of wild-type p53, because in our study, 82% of the tumors that recur belong to this group whereas only 52% of the tumors with normal p53 (wild-type gene, no expression) had a relapse.

P53 plays a key role in platinum-induced apoptosis. Therefore, one can assume that alterations in p53 might confer a platinum-resistant phenotype. The ability of a tumor cell to respond to a given drug, such as cisplatin, depends on the type of mutations and probably on the status of the Arg72Pro SNP. Vikhanskaya et al. (41) have analyzed several *TP53* hotspot mutants in conjunction with the Arg72Pro SNP and found that cells homozygous for the Arg allele are—albeit slightly—more resistant than cells homozygous for the Pro allele. In our study, there was no significant difference in the time to progression in patients with tumors homozygous for the Arg allele ($P = 0.87$; log-rank test); in contrast, in patients with Arg72Pro heterozygous tumors, the mean time to progression was 61 months for wild-type *TP53* compared with 19 months when *TP53* was mutated and/or overexpressed ($P = 0.02$). On the other hand, patients with FIGO stage III disease whose tumors exhibited *TP53* mutations had a longer overall survival than patients with wild-type TP53 (data not shown). This is at least partly consistent with data from Havrilesky et al. (5), who showed that patients with mutant *TP53* had a reduced short-term risk of disease progression. Many chemotherapeutics cause DNA damage and normal p53 may contribute to enhanced DNA repair rather than undergoing apoptosis; this might provide a favorable prognosis for patients with tumors exhibiting specific *TP53* mutations. Other authors also described a better response from tumors with mutated *TP53* to a cisplatin-containing treatment (42, 43). In contrast, Reles et al. (6) reported that *TP53* mutations correlated with early relapse in both early and advanced stage ovarian carcinomas.

There seems to be a trend in which overexpression of p53 (8, 9), rather than mutation of *TP53* (4, 15), is correlated with shortened overall survival. Our results, showing that p53 immunostaining in >10% of the tumor cells is correlated with a decreased overall survival ($P = 0.0065$) and that the occurrence of *TP53* mutations is not ($P = 0.86$), are therefore consistent with the findings of other authors. We further showed that patients with altered p53 generally have a worse prognosis ($P = 0.047$) and a shortened overall survival ($P = 0.05$) compared with patients with normal p53. Of patients with altered p53, those with an overexpression of wild-type p53 surprisingly have the shortest average overall survival time. Because only a few studies have evaluated both the *TP53* gene and protein expression status (i.e., refs. 5, 6, 44), the predictive value of overexpression of wild-type p53 has thus far been underestimated. Because p53 is stabilized and functionally inactivated by as yet unknown mechanisms, its effect on chemosensitivity and tumor progression may be more important than the loss of p53.

In addition to p53 status, we also analyzed SNP309, a single nucleotide polymorphism that resides within the p53-sensitive P2 promoter of the *MDM2* gene. We have previously shown that the *G*-allele of SNP309 is only associated with an earlier age of tumor onset in females, but not in males, i.e., in diffuse large B-cell lymphoma and soft tissue sarcomas (27). Data from patients with invasive ductal carcinomas of the breast provided further evidence that the *G*-allele requires an intact estrogen-signaling pathway in order to accelerate tumorigenesis (27). Indeed, in our present study, the *G*-allele of SNP309 only associates with an earlier age of onset in high ER-positive (8 years earlier, $P = 0.048$) but not ER-negative (1 year earlier, $P = 0.77$) ovarian carcinomas. If one ignores the level of ER expression in ovarian carcinomas, no differences in the age of onset were observed between the different genotypes of SNP309, as has been published recently (45). In another study, Galic and coworkers analyzed the SNP309 status in 150 patients with ovarian cancer (46). In their study, there was also no association of the *G*-allele with the age of onset. The

distribution of the respective genotypes was different from our study. They found that 12% were G/G, 40% were T/G, and 47% were T/T, compared with 7% G/G, 50% T/G, and 43% T/T, respectively, in our study. Furthermore, patients with or without ER expression were not analyzed separately. Our results provide further evidence for the association of the estrogen pathway and the *G*-allele of SNP309, as has been previously shown for breast cancer (27).

The best outcome was determined for patients with normal TP53 status as opposed to patients with overexpression of p53 regardless of the existence of mutations. Importantly, we identified a large group of patients whose tumors overexpressed p53 despite a wild-type gene status. These patients were characterized by the highest percentage of early relapse and the shortest overall survival. Further studies are mandatory to evaluate the mechanism of wild-type p53 overexpression to circumvent chemoresistance in this subset of ovarian cancer. In addition, our study underscores the importance of assessing the functionality of p53 rather than separately looking at *TP53* mutations and overexpression in order to predict sensitivity to platinum-based chemotherapies and patient outcome.

Acknowledgments

We thank Martin Köbel from the Vancouver General Hospital, and Gareth L. Bond from the Institute of Advanced Studies, Princeton, NJ, for helpful discussions and revision of the manuscript. Furthermore, we appreciate the contributions of other members of our laboratories: Birgit Wypior, Ilona Wiederhold, Ute Rolle, Jana Beer, Tina Groβe, and Kathrin Spröte.

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Clin Cancer Res 2008;14:89-96.