Association of p21, p21 p27 and p21 p53 Status to Histological Subtypes and Prognosis in Low-stage Epithelial Ovarian Cancer

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Abstract. *Aim: The objective of this study was to evaluate the prognostic value of p21 alone and in combination with p53 or p27 for different histological subtypes of epithelial ovarian cancer and disease-free survival. Patients and Methods: The specimens were obtained at primary surgery from a series of 129 ovarian carcinomas in FIGO stages I-II. The technique of tissue microarray and immunohistochemistry was used for detection of positivity of the markers. Results: Positive staining for p21, p27 and p53 was detected in 36%, 58% and 25% of cases, respectively. The p21 status, p27 status and concomitant p21 p27 and p21 p53 status in four subgroups were related to histological subtypes (p=0.016, p=0.036, p=0.004 and p=0.001). Mucinous tumors mostly stained negatively for p27 and concomitantly negatively for p21 and p53. Clear cell tumors generally stained positively for p21 and p27 but negatively for p53. Serous tumors usually stained concomitantly negatively for p21 and positively for p53. In a multivariate Cox regression analysis, FIGO stage, p21 p53 and p53 status were independent prognostic factors for disease-free survival. Conclusion: A subgroup, constituting 25/129 (19%) of the patients with predominantly serous tumors with concomitant p21 negativity and p53 positivity had a poor survival. Another subgroup of 11/129 (9%) patients with non-serous tumors with concomitant p21 and p27 positivity had excellent survival.*

Epithelial ovarian cancer (EOC) is the most lethal malignancy of all gynecological cancers. Various clinical and pathological features of ovarian cancer are used as predictors of clinical outcome (1). The five histopathological subtypes of EOC represent five different diseases with different biological and genetic background (2, 3). Proliferation and cell cycle control are central processes in the biology of cancer (4). The cell cycle is regulated by two major families of cyclin kinase inhibitors (CKIs). One of these is the Cip/kip family including p21 and p27, which inhibit cyclin E/CDK (5). Down-regulation or inactivation of p21 and p27, which normally cause G_1 arrest by binding to cyclin CDKs, result in dysregulation of normal cell cycle control and increased cell proliferation, and contribute to malignancy (6).

Frequently mutated in a wide range of human cancer types, wild type p53 is a negative regulator of cell cycle control, which inhibits cell cycle progression in part by activating p21 expression, and also controls the exit of cells from the cell cycle into apoptosis (7). The p21 gene is the primary mediator of p53-induced cell cycle arrest, and cells lacking functional p53 express only low levels of p21. It was demonstrated in a study on ovarian A2780 cells that p21 not only inhibits CDK activity directly, but also increases the level of p27 as inhibitor through stabilizing the p27 protein (8). In contrast to p53 gene, mutation of the p21 and p27 genes occurs infrequently in human cancer, and epigenetic silencing seems to be another means of negative regulation of these tumor suppressors (9, 10). Findings, regarding the predictive and prognostic relevance of expression of p21 in EOC are inconsistent. Some studies have reported that immunostaining (IHC) of p21 alone was an independent prognostic factor for survival (6-7, 9) for EOC or endometrial cancer (11). The p21 p53 status was an independent prognostic factor for survival in a Cox multivariate analysis in at least three studies (6, 12, 13). Concomitant loss of p21 (negativity) and overexpression of p53 (positivity) were related to worse prognosis than the other combinations of staining (13). In two studies, the combination of low p21 and p27 expression was associated with worse overall survival (14, 15). In the present retrospective clinical study on 129 patients with EOC in FIGO stages I and II the aim was to evaluate the prognostic value of p21 separately and in combination with p53 and p27, respectively according to histological subtype and disease-free survival (DFS).

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Key Words: Ovarian cancer, histological subtypes, prognosis, cyclin kinase inhibitors, p21, p21 p53, p21p27.

Materials and Methods

Patients and treatment. This study assessed 129 patients with tumors available for analysis of p21 (because of two missing cases for staining), p27 and p53 out of a total series of 131 patients with FIGO stages I or II EOC, who all underwent primary surgery and postoperative adjuvant chemotherapy. A total of 140 consecutive patients with FIGO stage I or II EOC, who underwent primary surgery and adjuvant chemotherapy in the Örebro-Uppsala Medical Region during the 5-year period from January 1, 2000 to December 31, 2004, were entered into this study. All samples were collected with the patient's informed consent in compliance with the Helsinki Declaration (16) and used in accordance with the Swedish Biobank Legislation and Ethical Review Act (approval by Uppsala Ethical Review Board, decision ref.UPS-03-477). Thus, after the patient's informed consent, there were 131 patients remaining in the study.

The primary surgery was performed at nine different surgical gynecological departments and the staging procedure was done at the time of primary surgery. Modified surgical staging according to the (EORTC) surgical staging categories in early ovarian cancer (17) was undertaken in 38 (29%) out of the 129 cases, and in the remaining 91 (71%) patients surgical staging was regarded as inadequate according to the same guidelines. Patients' characteristics, *e.g.* age, performance status (WHO), type of chemotherapy, FIGO stage, histological subtype and FIGO grade are shown in Table I. All patients underwent adjuvant platinum-containing chemotherapy 4-6 weeks after primary surgery. No patients were lost from follow-up and the mean follow-up time was 67 months (range 9-110 months). Survival was taken from the date of confirmed histological diagnosis after primary surgery to the date of recurrence or last visit.

Table I. *Patient characteristics (N=129).*

Age (median)	59.0 (range 25-84)
WHO performance status	(%)
0	35 (27.0)
1	66 (51.1)
2	21 (16.3)
3	6 (4.6)
Type of chemotherapy	
Taxane/carboplatin	104 (80.6)
Carboplatin (single drug)	25 (19.4)
FIGO-stage	
IA	38 (29.4)
IB	6 (4.6)
IC	65 (50.4)
II	20 (15.6)
Histopathology	
Serous	50 (38.7)
Mucinous	19 (14.7)
Endometrioid	42 (32.6)
Clear cell	16 (12.4)
Anaplastic	2 (1.6)
FIGO-grade	
Grade 1	30 (23.2)
Grade 2	46 (35.7)
Grade 3	53 (41.1)

Sampling and tissue microarray construction of ovarian cancer tissue. The specimens were obtained from paraffin blocks containing the embedded tissue removed from the tumor at primary surgery. After staining with hemotoxylin and eosin, they were classified and graded by a single pathologist. The tissue microarrays were constructed as described previously (18). In brief, tumor tissues were embedded in paraffin and 5 μm sections stained with hematoxylin-eosin were obtained to select representative areas for biopsies. Core tissue biopsy specimens (diameter 0.6 mm) were taken from these areas of individual donor paraffin blocks and precisely arrayed into a new recipient paraffin block with a custom-built instrument. Tissue core specimens from 131 ovarian carcinomas were arranged in three recipient paraffin blocks. Two core biopsies were obtained from each specimen. The presence of tumor tissue on the arrayed samples was verified by hematoxylin-eosin-stained section by a pathologist.

Immunohistochemistry and interpretation. Five μm thick sections were cut from each multi tissue block, were put on coated slides and dried overnight at 37˚C. The sections were pre-treated by heat-induced epitope retrieval in target retrieval solution (Dako, Glostrup, Denmark), pH=6 or EDTA buffer pH=9, for 7+7 minutes in a microwave oven (99˚C). Blocking with peroxidase was performed for 5 minutes. The slides were counterstained with hematoxylin for 2 minutes. The following monoclonal primary antibodies were used: DO-7, directed against p53 protein (dilution 1:1000; Dako), NCL-p27 (dilution 1:40; Vision Biosystems Novocastra, Newcastle, UK) and p21 protein (dilution 1:50; Dako). The immunostainings were performed in an Autostainer automated machine (Dako) using the REAL Envision detection system (Dako). The work of tissue-microarray construction was undertaken at the Department of Pathology, the University Hospital MAS in Malmö in South-Sweden, but the immunohistochemical analyses and interpretation were performed at the department of Pathology, Halmstad Medical Central Hospital.

The IHC stains were interpreted by the authors (IS and TS). At the time of evaluation, no information was available on the specific diagnosis and prognosis for the individual cases. A semi-quantitative analysis (19) was used and the stainings were graded as negative, +, ++, and +++ for p21, p53, and p27. All markers were then dichotomized into negative and positive cases (3). The staining for p53 was considered to be positive when strong and widespread granular staining of the nuclei of the tumor cells was found. The staining for p27 was considered to be positive when strong and widespread granular staining of the nuclei and cytoplasma of the tumor cells was found. The staining for p21 was considered to be positive when there was a strong and widespread granular staining of the nuclei of the tumor cells.

Statistical analysis. The Pearson's Chi-square test was used for testing proportional differences in univariate analyses. The survival curves were generated by using the Kaplan Meier technique and differences between these curves were tested by the log-rank test. All tests were two-sided and the level of statistical significance was accepted at $p \leq 0.05$. The Statistica 11.0 (StatSoft™) statistical package for personal computers was used for the analyses. For multivariate analyses the Cox regression model was used with disease-free survival as the endpoint.

Table II. *Status of p21 and p27 proteins according to histological subtypes (N=129).*

Expression (N%)	p21 +	p21 -	p27 +	p27 -	p21 + p27 +	p21 + p27 -	p21 - p27 +	p21 - p27-
	46 (36)	83 (64)	74 (57)	55 (43)	11 (9)	7 (5)	63 (49)	48 (37)
Histology								
Serous	14 (31)	36 (44)	30 (40)	20 (37)	0 (0)	4 (56)	30 (48)	16 (33)
Mucinous	4 (9)	15 (18)	5 (7)	14 (25)	1 (10)	1 (14)	4 (6)	13 (27)
Endometrioid	17 (36)	25 (30)	26 (35)	16 (29)	5 (45)	1 (14)	21 (33)	15 (32)
Clear cell	11 (24)	5 (6)	11 (15)	5 (9)	5 (45)	1 (14)	6 (10)	4 (8)
Anaplastic	0 (0)	2 (2)	2 (3)	0 (0)	0 (0)	0 (0)	2 (3)	0 (0)
p-value (chi-2)	0.016		0.036		0.004(chi-2)			

Results

Patients (tumor recurrences and survival rate). Primary cure was achieved in all 129 patients (100%). The total number of recurrences in the complete series was 34 out of 129 (26%), and 21 of the patients (62%) died due to their disease. Five patients (15%) with recurrent disease died due to incurrent disease and 8 (23%) patients were still alive at the time of the last follow-up. Recurrent disease was statistically significantly associated with FIGO substages (p=0.0003), FIGO-grade (p=0.019), and residual disease (p=0.0012). Five out of the six patients (83%) with FIGO stage II disease with residual tumor after primary surgery had recurrent disease. However, histopathology (p=0.482), cystic rupture at surgery (p=0.331), ascites at primary surgery (p=0.885), and type of surgical staging (p=0.078) were not related to recurrent disease. In the complete series, the 5-year DFS rate was 68%, the disease-specific survival rate 76%, and the overall survival rate 71%.

Results from immunohistochemistry. p21 staining was confined to the nucleus, and p21 positivity was observed in 46 (36%) out of 129 tumors. The p21 status was significantly (p=0.016) related to histological subtype (Table II). Thus, positive staining for p21 was seen more frequently in clear cell tumors but was infrequent in mucinous carcinomas. The p21 status alone was not associated with serous/non-serous tumors, tumor grade, FIGO stage or survival.

p27 staining was confined predominantly to the nucleus. p27 positivity was observed in 74 (57%) out of 129 tumors. The p27 status was statistically significantly (p=0.036) associated with histological subtype (Table II). Thus, positive p27 staining was seen more frequently in clear cell tumors but was infrequent in mucinous tumors. However, the p27 status was not associated (p=0.430) with serous/non-serous tumors. Tumor grade was statistically significantly (p=0.006) associated with p27 status. Thus positive staining for p27 was most frequently seen in poorly differentiated (G3) tumours. On the contrary, well differentiated (G1) tumors usually stained negatively for p27. The p27 status alone was not related to FIGO stage (p=0.959) or DFS (p=0.311).

p53 staining was confined to the nucleus, and p53 positivity was observed in 32 (25%) out of 129 tumors. The p53 status was not related to histological subtype (p=0.127). The most striking findings was that all 16 clear cell tumors stained negatively for p53. The p53 status was not related to serous/non-serous tumors (p=0.382), tumor grade (p=0.464) or FIGO stage (p=0.220). However, the p53 status was significantly (p=0.0007) associated with DFS in univariate analysis. Survival analysis demonstrated significant (p=0.003) differences for patients according to the p53 status. Patients with p53 negative tumors had a 5-year survival (DFS) of 82% compared with 62% survival for those with p53 positive tumors.

Relationship between p21and p27, and p21 and p53 and their association to clinicopathological data and prognosis. There were no differences in mean age between the groups of patients with p21-positive and p21-negative tumors, p27-positive and p27-negative tumors, or p53-positive and p53-negative tumors. There was a borderline significant difference (p=0.060) between staining of p21 and p53 in carcinomas, but no significance (p=0.331) between staining of p21 and p27 was found. Furthermore, a statistical trend (p=0.055) was detected for the associations of staining of p53 with that of p27 in tumors. In a previous study (20) including the total series of patients (N=131), the distribution of four subgroups was analyzed after the p53 p27 status of tumors according to the same variables as shown in the tables. The complete series of 129 patients was split into four subgroups according to p21 and p27 status (Table II) and to p21 and p53 status (Table III) of the tumors and their distribution was analyzed according to clinicopathological features.

The p21 p27 status (Table II) in four subgroups was related to the histological subtypes (p=0.004) and was also related (p=0.011) to tumor grade (not shown in the Tables). Thus, the frequency of positive staining for p27 in

Table III. *Status of the p21 and p53 proteins according to clinicopathological features (N=129).*

Expression (N %)	p21 + p53+	p21 + p53-	p21 -p53 +	p21 -p53 -
	7 (6)	39 (30)	25 (19)	58 (45)
Histopathology				
Serous	2 (29)	12 (31)	13 (52)	23 (40)
Mucinous	3 (44)	1 (3)	3 (12)	12 (21)
Endometrioid	2 (29)	15 (38)	9 (36)	16 (28)
Clear cell	0 (0)	11(28)	0 (0)	5 (9)
Anaplastic	0 (0)	0 (0)	0 (0)	2 (2)
p-value (chi-2)				0.007
Tumor grade				
G1	2 (29)	6 (15)	5 (20)	17 (29)
G2	2 (29)	14 (36)	7 (28)	23 (40)
G3	3 (44)	19 (49)	13 (52)	18 (31)
p-value (chi-2)				0.466
FIGO-stage				
IA-IB	3 (43)	12 (31)	6 (24)	23 (40)
IC	4 (57)	21 (54)	11(44)	29 (50)
II	0 (00)	6 (15)	8 (32)	6 (10)
p-value (chi-2)				0.211
Disease-free Survival	5(71)	33 (85)	11(44)	47 (81)
Dead of disease or alive with recurrent disease	2(29)	6 (15)	14 (56)	11(19)
p-value (chi-2)				0.001

carcinomas without regard to the p21 status was higher in G3 tumors and concomitant negativity for both p21 and p27 was detected in 36 (75%) out of the 48 G1 and G2 tumors.

No patient in the subgroup of patients (N=11) with tumors concomitantly positive for p21 and p27 (all of non-serous histology) had recurrent disease (p=0.042) in spite of the fact that 7 (67%) out of the 11 tumors were G3 tumors. In a further analysis (Table IV) this subgroup (p21 + p27 +) was compared to other three subgroups combined in one group (p21 + p27-, p21 -p27 +, p21 -p27 -) for the same variables as before. In survival analysis (Figure 1) there was significantly better (p=0.043) DFS for this subgroup of patients. The subgroup (N=11) with non-serous tumors with concomitant positivity for p21 and p27 had a DFS of 100% at 60 and 100 months after diagnosis. Patients in the other subgroups combined in one had a survival rate of 85% at 60 months.

The p21 p53 status was significantly (p=0.007) related to histological subtype. Eleven (69%) out of the 16 clear cell tumors in the study had concomitant positivity for p21 and negativity for p53. In the third subgroup of patients with concomitant negativity for p21 and positivity for p53 of (Table III), serous tumors were seen more frequently (52%) compared to the other three subgroups, and also more frequently (52%) compared to other histological subtypes in this subgroup. In addition, more (p=0.001) recurrences were detected among patients from this subgroup. In a new analysis (Table IV) this subgroup (p21 - p53 +) was compared to other three subgroups combined (p21 + p53 +, p21 + p53 -, p21 - p53 -) after clinicopathological data and survival. The difference was significant (p=0.036) for FIGO stages. Survival analysis (Figure 2) showed significantly worse (p=0.0002) DFS for the subgroup of patients with concomitant p21 negative and p53 positive tumors compared to other subgroups combined. The difference was statistically highly significant and patients from this subgroup had a poor survival rate of only 21% at 100 months compared to the 62% survival rate for to other subgroups combined.

Multivariate analysis. In a multivariate Cox regression analyses with DFS as the end point (Table VA), FIGO stage with hazard ratio (HR) of 2.6 and p21 p53 status with HR of 2.5 were significant and independent prognostic factors. An HR of 2.5 for concomitant p21 negativity and p53 positivity of tumors *versus* other combinations of p21 p53 status of tumors meant a 2.5- fold increased risk for recurrence or death due to disease for a patient who belonged to the first subgroup. In a separately Cox multivariate regression analysis (Table VB), both FIGO stage with an HR of 2.9 and p53 status with an HR of 2.3 were significant and independent prognostic factors. In a separate multivariate analysis (together with the same variables as before) the p21 p27 status (p21 + p27 + *versus* other combinations of p21 and p27) of tumors, with an HR of 1.5, was not a significantly independent prognostic factor.

Discussion

In recent years it has been accepted that the five different histological subtypes of EOC are different diseases with different distributions across early stages (FIGO stages I and II) and advanced stages (FIGO stages III and IV). Thus mucinous, endometrioid and clear cell carcinomas were relatively more common in stage I and II tumors compared to high grade serous ovarian carcinoma which, accounted for 36% of stage I-II tumors and 88% of stage III and IV tumors in two large independent patient series from Canada (21). Serous tumors accounted for 39% of tumors, all in FIGO stages I and II in the present study. These findings reflect important biological differences in the behavior of tumors which represent different diseases with different biological and genetic background (2, 3). Some molecular biological studies (22, 23) have revealed additional information about the association between genetic alterations and specific histological

Table IV. *Status of protein expression in tumors of the p21, p21-p53+/other in one group and p21+p27+/other in one group versus clinico-pathological features (N=129).*

	No (%)					
	46 (36)	83 (64)	25 (19)	104 (81)	11 (9)	118 (81)
Positivity	p21+	p21–	p21–p53+	p21+p53+ p21+p53– p21–53–	p21+p27 +	p21+p27– p21–p27+ p21–p27–
Histopathology						
Serous	14 (30)	38 (46)	13 (52)	39 (38)	0 (00)	52 (44)
Non-serous	32 (70)	45 (54)	12 (48)	65 (62)	11 (100)	66 (56)
	p=0.088 (chi-2)		p=0.184 (chi-2)		p=0.004 (chi-2)	
Tumor grade						
G1+G2	23 (50)	51 (61)	12 (48)	62 (60)	4 (36)	70 (59)
G3	23 (50)	32 (39)	13 (52)	42 (40)	7 (64)	48 (41)
	p=0.207 (chi-2)		p=0.291 (chi-2)		p=0.140 (chi-2)	
FIGO-stage						
IA-IB	15 (33)	29 (35)	6 (24)	38 (36)	2 (18)	42 (36)
IC	25 (54)	40 (48)	11 (44)	54 (52)	7 (64)	58 (49)
II	6 (13)	14 (17)	8 (32)	12 (12)	2 (18)	18 (15)
	p=0.760 (chi-2)		p=0.036 (chi-2)		p=0.504 (chi-2)	
Disease-free survival						
Survival	38 (83)	58 (70)	11 (44)	85 (82)	11 (100)	85 (72)
Dead of disease or alive with recurrent disease	8 (17)	25 (30)	14 (56)	19(18)	0 (00)	33 (28)
	p=0.112 (chi-2)		p=0.00010 (chi-2)		p=0.042 (chi-2)	

phenotypes. Furthermore, there is an increasing number of reports addressing the different immunohistochemical expressions of cell cycle regulatory proteins in ovarian carcinoma (24). Findings from one study (22) indicated that the several CKIs investigated were differently expressed among different histological subtypes of ovarian carcinomas.

In the present study mucinous tumors usually stained negatively for p27 and concomitant negatively for p21 and p53. Clear cell tumors usually stained positively for p21 and p27, and all stained negatively for p53. In a study (3) including 132 clear cell tumors, positivity for p53 was detected in fewer than in 10% of tumors. Our findings confirm the results of other studies (3, 14, 22, 25), which indicate that p21 and p27 are differently expressed among different histological subtypes of ovarian carcinoma. Furthermore, presence of the tumor suppressor p53 in combination with p21 and p27, respectively, was different in different histological subtypes. Serous tumors usually stained concomitantly negatively for p21 and positively for p53, but serous tumors were found to predominate (52%) in the subgroup of tumors with concomitant p21 negativity and p53 positivity, and mostly (60%) had concomitant p21 negativity and p27 positivity.

Some limitations of this work have to be noted. Firstly, the relative limited number of cases and secondly, the TMA technology used in this study where two 0.6 mm core biopsies were obtained from each specimen. As ovarian carcinomas can be very heterogeneous, such specimens may not be adequately representative for some cases. In addition, we used the method of semiquantitative analysis (19) for the interpretation. Thus, all markers were dichotomized into negative and positive groups (3). Some uncertainty in the interpretation of the staining pattern of p53 has been noted after findings from two studies (26-27) which showed that a complete lack of staining for p53 might be associated with missense mutation of TP53, leading to formation of nonimmuno-reactive protein. Thus, combining the two immunohistochemical patterns (0% and 60-100% of cells), TP53 mutation could be correctly identified in 94% of cases. Accordingly, a low-level pattern (10-50% of cells) should be indicative of wild TP53. However, these findings are contrary to results of the present study and findings in a meta-analysis

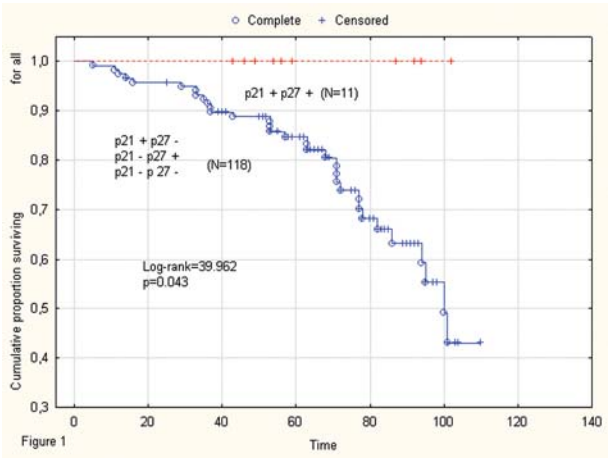

Figure 1. *Disease-free survival was better for the subgroup (N=11) of patients with tumors of concomitant p21 and p27 positivity compared to those combined in one group (N=118).*

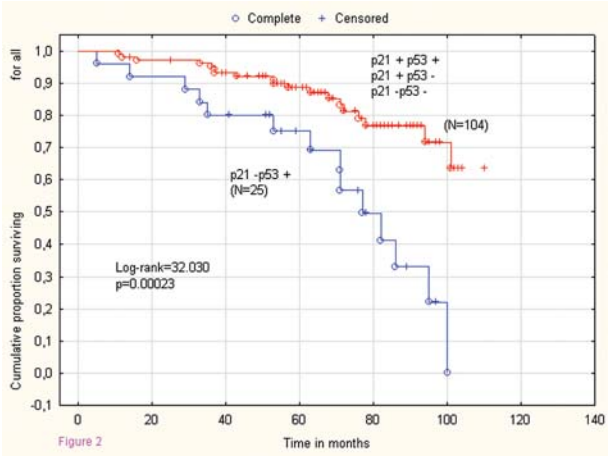

Figure 2. *Disease-free survival was worse for the subgroup (N=25) of patients with tumors of concomitant p21 negativity and p53 positivity compared to those combined in one group (N=104).*

including 62 studies reporting results of 75 analyses in 9448 patients for p53 (28).

In the present study, it was possible to indentify two prognostic subgroups of patients, one with favorable outcome and another with poor outcome. Thus, the subgroup of patients with only non-serous tumors with concomitant positivity for p21 and p27 had a DFS of 100% at 60 and 100 months. These findings could be supported only from studies separately analyzing the predictive and prognostic value of p21 and p27. In several studies, nuclear absence or low p27 expression significantly correlated with poor survival of patients in multivariate analyses (14, 29). There has been increasing evidence that loss of nuclear p27 expression and high expression of p27 in the cytoplasm are both poor prognostic factors in ovarian cancer (24). Some studies have reported that p21 positivity in ovarian tumors was an independent prognostic factor for survival (6-9, 11, 30). In a study on ovarian A2780 cells, it was demonstrated that the DNA-damaging agent DAB (cisplatin analogue IR, 2R-diamino- cyclohexane platinum) induced p21 and promoted the increase of p27 in CDK2 in complexes and contributed to the inhibition of CDK2 kinase activity (8). This study provided evidence that p27 up-regulation is linked directly to activation of the p21/p53 pathway by a DNA-damaging agent. Therefore, the p53/p21/p27 axis should become a new focus of attention in checkpoint response to DNA damage. Patients with tumors with concomitant p21 negativity and p53 positivity had a DFS of 75% at 60 months and 21% at 100 months in this study. Our findings confirm those of other authors (12, 31) in studies including patients with all disease stages and different histological subgroups. In multivariate analysis the p21 p53 status was an independent prognostic factor, which was stronger than that of the p53 status alone. The p21 p53 status was an independent prognostic factor for survival in a Cox multivariate analysis in at least three studies (6, 12-13).

Table V. *A. Multivariate Cox regression analysis with disease-free survival as endpoint (N=129).*

Variable	HR	95% C.I.	*p*-Value
Age	1.016	0.983-1.049	0.337
Stage (I *vs.* II)	2.587	1.213-5.518	0.014
Serous/non-serous	0.855	0.420-1.737	0.665
Grade#	1.735	0.834-3.606	0.140
Surgical staging*	0.590	0.234-1.489	0.264
p21p53**	2.448	1.184-5.059	0.016

#Grade (G3 *vs.* G2 + G1 tumors); *adequately performed *vs.* not performed; **p21 -p53 + *vs.* others; HR Hazard ratio; CI: Confidence Interval.

B. Multivariate Cox regression analysis with disease-free survival as endpoint (N=129).

Variable	HR	95% C.I.	*p*-Value
Age	1.023	0.991-1.056	0.152
Stage (I *vs.* II)	2.948	1.426-6.095	0.003
Serous/non-serous	0.885	0.443-1.69	0.729
Grade#	1.477	0.721-3.024	0.539
Surgical staging*	0.539	0.218-1.331	0.180
p53**	2.268	1.139-4.517	0.019

#Grade (G3 *vs.* G2 + G1 tumors); *adequately performed *vs.* not performed; **p53+ *vs.* p53-; HR Hazard ratio; CI: Confidence Interval.

Current treatment of recurrent disease, which is similar to treatment of primary disease, has proved ineffective and many studies have demonstrated the presence of cancer stem cells (CSCs) in ovarian carcinoma (32). From a study on CSCs in recurrent ovarian cancer samples, it was reported that different genetic profiles are displayed by primary and recurrent ovarian tumors (33). At the gene level, recurrent tumors appear to be associated with p53 p21 regulation where p53 regulation is enhanced and p21 regulation is no longer required in recurrent tumors. Thus, this altered p53 p21 regulation is the primary mechanisms through which tumors avoid apoptosis and stimulate cellular proliferation, which can result in recurrent disease. The poor prognosis for patients with tumors with concomitant p21 negativity and p53 positivity in the present study might be partly explained by findings from the study above. At present, adjuvant therapy is mainly dependent upon tumor stage and grade rather than histological subtype (34). Future studies undertaken for identification of therapeutic targets should have focus on these specific subtypes rather than studies of unselected series of patients. As high grade serous ovarian cancer accounts for the great majority of all cases of EOC, such studies might be conducted with a greater number of patients in all stages for this subtype. On the contrary, the non-serous (mucinous, endometrioid and clear cell) subtypes usually are detected in the early stages (I and II) and therefore represent uncommon diseases which require large scale research trials for searching for subtype specific biomarkers.

Conflicts of Interest

The Authors declare that they have no conflict of interests.

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