p53 is not related to Ki-67 immunostaining in the epithelial and mesenchymal components of female genital tract carcinosarcomas

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Abstract. Carcinosarcomas (CSs) are composed of two separate histological components and are rare neoplasms of the female genital tract. Therefore, CS pathogenesis has not yet been fully elucidated. In the present study, immunohistochemical techniques were used to determine the role of p53 and Ki-67 overexpression in female genital tract CSs. The study group was comprised of 36 patients with CSs originating from the uterus (n=31), cervix (n=3) and ovary (n=2), as well as 3 metastatic tissues. p53 was overexpressed in the epithelial component of 23 out of 36 (64%) tumors, and in the mesenchymal component of 20 out of 36 (56%) tumors. In both CS components, there was a significant correlation between p53 overexpression and patient age and ovarian metastases. Ki-67 overexpression was detected in the epithelial component in 15 out of 36 (42%) cases, and in the mesenchymal component in 13 out of 36 (36%) neoplasms. There was a significant correlation of p53 overexpression between the carcinomatous and sarcomatous components (R=0.884, P<0.001). A significant correlation was also found in Ki-67 immunoreactivity between the two CS components (R=0.676, P<0.001). However, p53 overexpression was not correlated with Ki-67 immunostaining in both tumor components. In conclusion, based on immunohistochemical results, p53 was overexpressed in more than half of the female genital tract CSs included in the present study, either at the epithelial or mesenchymal component. The correlation between p53 or Ki-67 overexpression in both tumor components supports the combination theory of histogenesis in the majority of these tumors.

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Introduction

Carcinosarcomas (CSs), formerly known as malignant mixed Müllerian tumors (1), are relatively rare neoplasms of the female genital tract (2-4). These tumors are generally characterized by aggressive clinical course and, consequently, demonstrate an unfavorable outcome (3,5). The incidence of CSs within the female genital tract is low (1-2%), since they are generally detected at advanced clinical stages in postmenopausal women. They are distinctly biphasic as they are composed of malignant epithelial and mesenchymal components (6,7). Based on the presence or absence of heterologous mesenchymal components, CSs are divided into two subtypes: homologous and heterologous, such as rhabdomyosarcomas or chondrosarcomas (7,8).

Due to diversity of phenotypic manifestation, the pathogenesis of CSs still remains to be fully elucidated. To date, three theories, collision, combination and composition theories, have been proposed to explain this peculiar histogenesis (2,8,9). The first (collision or 'multiclonal') theory suggests that CSs are due to two intertwining malignant processes producing one final tumor. The combination or 'monoclonal' hypothesis assumes that CSs are monoclonal in origin, i.e. developing from a single multipotential stem cell differentiating into epithelial and mesenchymal pathways. Finally, the composition theory underlines that CS stromal components are considered to be not truly neoplastic but to represent a reactive process in response to malignant epithelial component differentiation. In 2002, McCluggage (6) reported

that these neoplasms are metaplastic carcinomas, where the sarcomatous component is a manifestation of increased aggressiveness. Recently, this theory has been questioned due to the fact that anatomopathological data confirmed that both CS components are truly malignant. However, some authors reported that a small proportion of female genital tract CSs represent collision neoplasms, composed of independently developed carcinomas and sarcomas, although this phenomenon is rare (10,11).

TP53 pathway alterations have been reported to be implicated in the development and progression of various neoplasms originating from the female genital tract organs (12-19). Several studies concerning the role of *TP53* pathway alterations in female genital CSs have been conducted (9,10,13,15,1,20-22), but the presented data are contradictory. Particularly, Blom *et al* (20) found that 61% of uterine tumors overexpressed p53, and that 25% were positive for mdm-2 immunostaining. Notably, all p53-positive cases showed a concordant immunostaining within the carcinomatous and sarcomatous areas. According to another study, 70 and 75% of uterine CSs showed positive staining for p53 and Ki-67, respectively (21). Wada *et al* (10) suggested that although uterine CSs are mostly combination tumors, some of them might develop as collision neoplasms. Moreover, the evaluation of clonality might help predict the prognosis of individual cases and improve subsequent clinical management.

The aim of the present study was to investigate the immunohistochemical markers, p53 and Ki-67, in 36 CSs derived from female genital tract organs in order to determine CS histogenesis. Clinical and pathological variables of the tumors were related to immunohistochemical data. Finally, based on our data, the role of combination theory in the development of CS was assessed.

Materials and methods

Patients and tissue samples. The study group was comprised of 36 patients with CSs originating from the uterus (n=31), cervix (n=3) and ovary (n=2). These patients were surgically treated during a 12-year period (2001-2012) in four centers: 2nd Department of Gynecology, Lublin Medical University, Lublin, Poland; Department of Gynecology, Otto von Guericke University, Magdeburg, Germany; Department of Gynecology and Gynecologic Oncology, Medical University of Białystok, Białystok, Poland; and Oncology Hospital, Brzozów, Poland. The patients were not administered any additional treatment prior to surgery. Initially, 42 CSs were collected. Six cases were excluded from the analysis due to insufficient material. From the remaining 36 CSs, the mean patient age was 65.2 years (range, 36-89). The clinical and pathological characteristics of the included patients are listed in Table I. The study was approved by the Ethics Committee of the Lublin Medical University.

The surgical specimens were immediately fixed in 10% buffered formalin and representative tissue samples were taken. Subsequently, the samples were routinely processed, embedded in paraffin blocks, stained with hematoxylin and eosin (H&E) and observed under a light microscope. Two experienced pathologists (Dr Danuta Skomra until 2011, and J.S. thereafter) reviewed and graded the tumors based on the WHO Staging System (7,23). Clinical staging was performed according to the modified FIGO classification (1,24,25). Regarding ovarian tumors, staging was performed according to FIGO classification from 1990 (26). Three metastases of primary uterine tumors, originating from the ovary, lymph nodes and omentum, were also examined.

Table I. Clinicopathological characteristics of 36 patients with carcinosarcomas (CSs).

Characteristic	No. of patients (n=36), n (%)
Patient age (years)	
<50	3 (8)
50-60	11 (31)
>60	22 (61)
Clinical stage	
I	12 (33)
II	7 (19)
III	13 (36)
IV	4 (12)
Histological type	
Homologous	29 (81)
Heterologous	7 (19)
Primary tumor localization	
Uterine corpus	31 (87)
Uterine cervix	3 (8)
Ovary	2 (5)
Myometrial invasion	
<50%	7 (19)
>50%	29 (81)
Lymphovascular space invasion	
Positive	24 (67)
Negative	12 (33)
Presence of tumor in the oviduct	
Yes	11 (31)
No	25 (69)
Ovarian metastases[a]	
Present	11 (32)
Absent	23 (68)
Presence of tumor in the uterine cervix[b]	
Yes	13 (39)
No	20 (61)
Lymph node metastases (n=20)	
Present	9 (45)
Absent	11 (55)
Omental metastases (n=8)	
Present	3 (38)
Absent	5 (62)

[a]CSs of the uterine corpus and cervix were evaluated; [b]CSs of the uterine corpus and ovary were included.

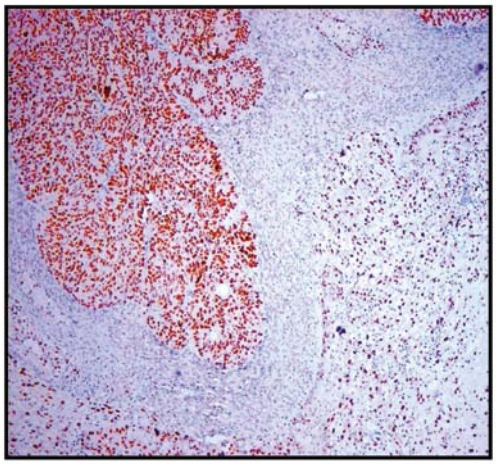

Figure 1. Immunohistochemical localization of p53 in epithelial and mesenchymal components of uterine carcinosarcoma. Magnification, x50.

Immunohistochemical analysis. Formalin-fixed, paraffin-embedded tissue samples were used for immunohistochemical analysis. Tissue sections (4-μm) were gently cut and mounted on adhesive slides (Poly-Prep™; Sigma, St. Louis, MO, USA). Antigen retrieval technique with microwave pretreatment was carried out by applying Dako buffer (pH 9.0) for 20 min at 700 W, and then cooled to room temperature. Endogenous peroxidase activity was blocked by 3% hydrogen peroxidase for 5 min. After washing with TBS buffer, the slides were incubated with primary antibodies against p53 (clone DO-7; dilution, 1:25; DakoCytomation, Copenhagen, Denmark) and Ki-67 (clone MIB-1; dilution, 1:100; DakoCytomation) for 30 min. Dako REAL™ EnVision™ Detection System Peroxidase/DAB+ (DakoCytomation) was then applied, and visualization was performed using 0.1% 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution for 5 min. The sections were finally counterstained with Mayer's hematoxylin, dehydrated and coverslipped after being embedded in mounting medium.

Known positive controls (primary human endometrioid-type endometrial adenocarcinomas overexpressing p53 and Ki-67) were included in each experiment (17,27,28). Primary antibody was replaced by normal rabbit antibody, diluted 1:100, as a negative control (DakoCytomation).

The representative areas (each of ~500 tumor cells) were counted. The analysis was performed by 3 independent researchers (Dr Danuta Skomra, J.S. and A.S.) reaching a full agreement in 85% of the sections counted. When consensus was not reached, immunostaining was evaluated cooperatively region by region. Regarding nuclear p53 reactivity, a semi-quantitative scoring system proposed by Alkushi *et al* (29) was applied: 0, p53 expressed in <10% tumor cells; 1, p53 expressed in 10-50% of tumor cells; and 2, p53 expressed in >50% of tumor cells. Nuclear p53 expression was defined as score 1, while overexpression of p53 was defined as score 2. Nuclear Ki-67 immunoreactivity was assessed semi-quantitatively using a two-point score (21): 0, ≤30% of positively stained tumor cells; and 1, >30% positively stained tumor cells. Positive nuclear Ki-67 immunoreactivity (overexpression) was defined as score 1.

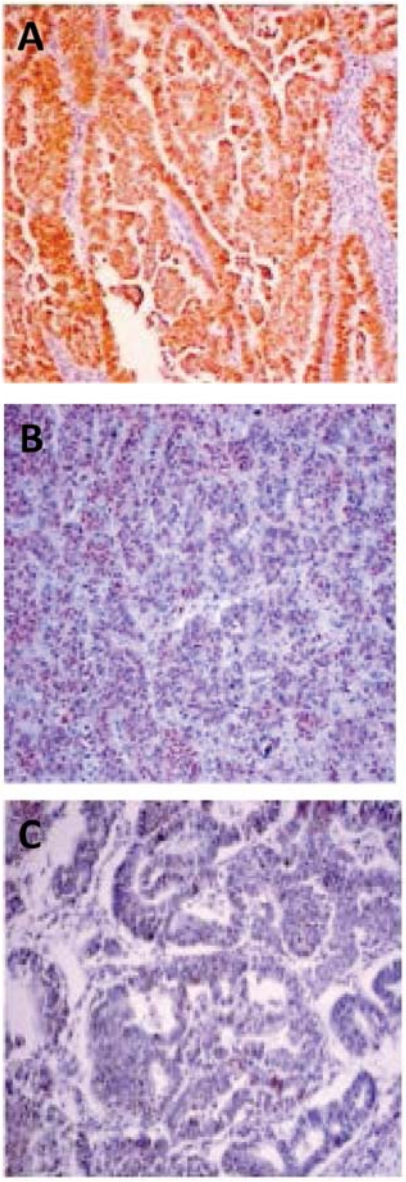

Figure 2. (A) Overexpression, (B) expression and (C) lack of staining for p53 in the epithelial component of carcinosarcoma. Magnification, x200.

Statistical analysis. Statistical analysis was carried out using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA). χ^2 or Fisher's exact test was applied when appropriate. Spearman's rank correlation coefficient was used to determine correlations between the expression of proteins and patient age. $P<0.05$ was considered to indicate a statistically significant difference.

Results

p53 immunostaining. Thirty-six primary human CSs were investigated for p53 immunoreactivity in the epithelial and stromal components applying immunohistochemical analysis (Fig. 1). p53 was overexpressed in 23 of 36 (64%) tumors at the epithelial component and in 20 of 36 (56%) tumors at the mesenchymal component (Figs. 2 and 3). Significant difference of p53 immunoreactivity between the two components was established in 3 cases (Table II). p53 protein was not

Table II. Differences of p53 and Ki-67 immunoreactivity in epithelial and mesenchymal components of the carcinosarcomas.

Patient no.	Primary tumor localization	p53		Ki-67	
		Epithelial component	Mesenchymal component	Epithelial component	Mesenchymal component
1	Uterine corpus	2	2	**86**	**20**
4	Uterine corpus	2	2	**60**	**0**
14	Ovary	**2**	**1**	20	5
18	Uterine corpus	**2**	**0**	20	10
20	Uterine corpus	2	2	**30**	**20**
33	Uterine corpus	2	2	**15**	**60**
34	Uterine corpus	**2**	**1**	**30**	**20**
36	Uterine corpus	1	1	**15**	**80**

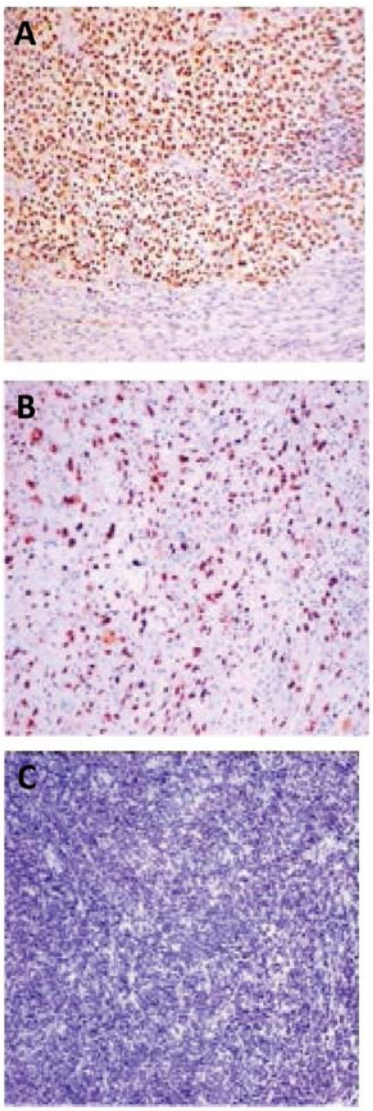

Figure 3. (A) Overexpression, (B) expression and (C) lack of staining for p53 in the mesenchymal component of carcinosarcoma. Magnification, x200.

overexpressed neither by the epithelial nor mesenchymal component in 5 (14%) cases.

A significant correlation between p53 overexpression and patient age was found in both epithelial and mesenchymal components (P=0.009 and P=0.034, respectively). Moreover, p53 immunostaining was related to cases harboring ovarian metastases (P=0.025 and P=0.032 for the epithelial and mesenchymal components, respectively). There was no significant correlation between p53 overexpression and other clinical and pathological characteristics of cancer, including clinical stage, depth of myometrial infiltration or lymphovascular space invasion. There was no correlation between primary tumor localization (uterine corpus, cervix or ovary) and p53 immunoreactivity.

p53 immunostaining was also evaluated in 3 primary and paired metastatic tumors (Table III). Simultaneous p53 overexpression was found in 2 tumor-metastasis pairs, while in the remaining case a marked p53 expression in the primary tumor was accompanied by only weak immunostaining in lymph node metastasis.

Ki-67 immunoreactivity. Ki-67 overexpression was observed in 15 of 36 (42%) tumors in the epithelial component, while 13 of 36 (36%) tumors displayed a Ki-67 index of ≥30% in the mesenchymal component (Fig. 4). Only one primary tumor-metastasis pair showed a significant difference in Ki-67 immunoreactivity (Table II). When clinicopathological characteristics of Ki-67-immunoreactivity were related to known clinical and pathological variables of CSs, no correlation was found between Ki-67 overexpression in the epithelial or mesenchymal components. Moreover, there was no correlation between primary tumor localization and Ki-67 immunoreactivity in both tumor components.

Correlation between p53 and Ki-67 expression. There was a significant correlation between p53 overexpression in the epithelial and mesenchymal components (R=0.884, P<0.001; Table IV). A significant correlation was also found between the Ki-67 immunoreactivity of the two CS components (R=0.676, P<0.001; Table IV). However, p53 overexpression was not correlated to Ki-67 immunostaining in both components. Moreover, neither p53 nor Ki-67 reactivity correlated with patient age in 36 cases of CSs.

Table III. p53 and Ki-67 immunostaining in primary uterine carcinosarcomas and the corresponding metastases.

Patient no.	Type of tissue and metastasis	p53[a]		Ki-67[a]	
		Epithelial component	Mesenchymal component	Epithelial component	Mesenchymal component
1	Primary tumor	2	2	86	20
	Ovarian metastasis	2		90	
5	Primary tumor	2	2	20	20
	Omental metastasis	2		20	
11	Primary tumor	1	1	30	30
	Lymph node metastasis	0		27	

[a]Immunohistochemical scoring is described in the Materials and methods section.

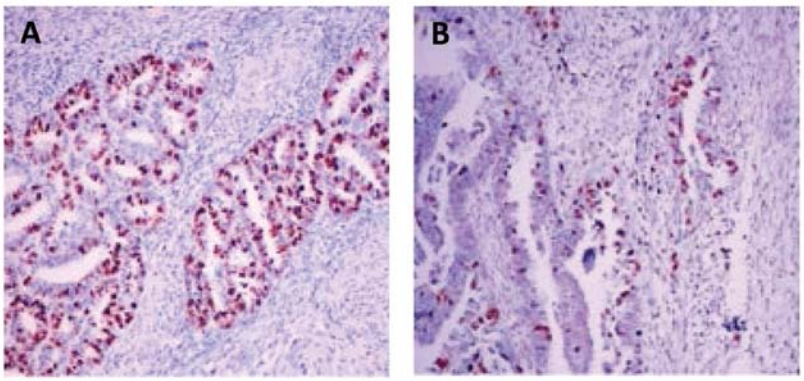

Figure 4. Ki-67 immunostaining in the epithelial component of carcinosarcoma; (A) ≥30 and (B) <30% of positively stained tumor cells. Magnification, x200.

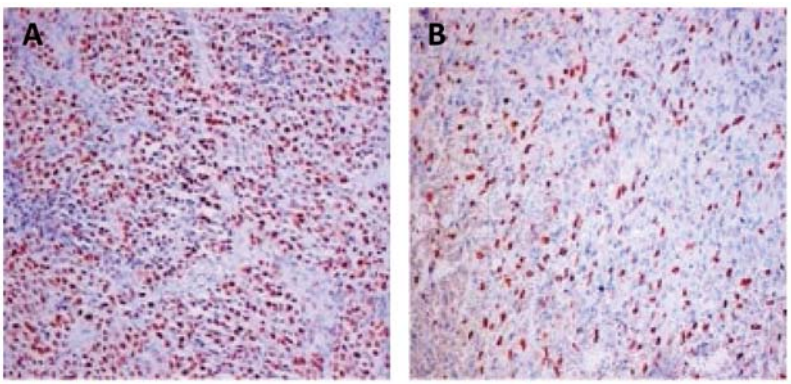

Figure 5. Ki-67 immunostaining in the mesenchymal component of carcinosarcoma; (A) ≥30 and (B) <30% of positively stained tumor cells. Magnification, x200.

Discussion

Carcinosarcomas are rare female genital tract tumors composed of two distinct carcinomatous and sarcomatous components (2,3). These highly aggressive neoplasms are characterized by a median overall survival of only 21 months, and for patients with advanced or recurrent disease this survival time could be shorter (3,5). The survival of women with uterine CSs was found to be substantially shorter compared with high-risk grade 3 endometrioid and non-endometrioid endometrial carcinomas (18). Clinical stage, low myometrial infiltration and late onset of menopause appear to be independent, prognostic indicators of overall survival (30,31). CSs are characterized by more aggressive tumor biology and reveal a wider pattern of spread compared with high-risk endometrioid-type endometrial carcinomas (32).

The aim of the present study was to independently investigate the expression of p53 in two coexisting components

Table IV. Correlation between patient age, p53 and Ki-67 immunostaining in epithelial and mesenchymal components of carcinosarcoma.

	Patient age	p53 epithelial component	p53 mesenchymal component	Ki-67 epithelial component	Ki-67 mesenchymal component
Patient age		R=0.283 P=0.095	R=0.280 P=0.098	R=-0.064 P=0.711	R=-0.028 P=0.873
p53 epithelial component	R=0.283 P=0.095		**R=0.884** **P<0.001**	R=0.209 P=0.222	R=-0.068 P=0.693
p53 mesenchymal component	R=0.280 P=0.098	**R=0.884** **P<0.001**		R=0.185 P=0.280	R=0.008 P=0.965
Ki-67 epithelial component	R=-0.064 P=0.711	R=0.209 P=0.222	R=0.185 P=0.280		**R=0.676** **P<0.001**
Ki-67 mesenchymal component	R=-0.028 P=0.873	R=-0.068 P=0.693	R=0.008 P=0.965	**R=0.676** **P<0.001**	

of female genital tract CSs. p53 was overexpressed in more than half of the CSs investigated. A highly significant correlation of p53 overexpression between CS components was established, thus supporting the combination theory of histogenesis in the majority of the included patients. The results of this study are in accordance with previously published studies, where p53 overexpression was observed in 58-78% of female genital tract CSs (14,20,21,33,34). However, some data demonstrated a significantly lower rate of p53-positive CSs. Particularly, Mayall *et al* (35) found p53-positivity only in 5 of 17 (30%) uterine CSs, a finding leaning towards the monoclonality of the neoplasm. According to another study, p53 overexpression was detected only in 30% of uterine CSs, while no p53 overexpression was detected in uterine adenosarcomas (36). Taken together, the differences in the frequency of p53 overexpression in female genital tract CSs could be associated with the application of different antibodies, detection systems and scoring counting. This variability could be also related to the relatively small numbers of observations involved.

p53 overexpression has been associated with *TP53* alterations in various human malignancies, particularly at 'hot-spot' regions of the gene (10,13,16,37,38). Notably, both point mutations and allelic lost at *TP53* occur in female genital CSs and have been used for clonal tumor analysis (10,39). As high as 32% (8/25) of uterine CSs revealed *TP53* point mutations, confirming the identical alterations in both tumor components (10). Based on combined application of molecular and immunohistochemical markers, Wada *et al* (10) suggested that most cases of CSs represent combination tumors. A high incidence of p53 expression concordance between two CS components was reported by Szukala *et al* (40). The same exon 8 *TP53* point mutation (codon 282, CGG→TGG) was detected in both components of a uterine CS (16). *TP53* alterations and protein overexpression are considered to be early events during CS tumorigenesis (10,14,20,40,41). Alterations in the *TP53* gene have been reported not only in primary tumors (14,15), but also in cell lines derived from female genital CSs (42).

The metastatic process involves several mechanisms including decreased adhesion between cells, basement membrane degradation, and invasion into the bloodstream and to locoregional lymph nodes (43). The presence of metastases (local or distant) at diagnosis is one of the most unfavorable prognostic indicators for women with CSs (43,44). In our laboratory, *TP53* alterations have been studied not only in primary human endometrial carcinomas, but also in corresponding metastases (45,46). According to Swisher *et al* (36), p53 overexpression was detected in 2 of 4 cases with primary tumors and in corresponding metastases, while immunoreactivity for Ki-67 was comparable. Recently, de Jong *et al* (18) demonstrated that there was similar p53 expression in 18 primary tumors and paired metastatic tissues. In the present study, 2 out of 3 cases displayed similar p53 immunoreactivity in primary tumors and corresponding metastases. Studies evaluating the molecular mechanisms, particularly the underlying mechanisms of the p53 pathway, involved in the formation of metastases in CS patients should be conducted.

The established correlation between p53 and Ki-67 overexpression in both tumor components strongly supports the combination theory in most cases of female genital CSs. Monoclonal origin of CSs stemming from the cervix, uterus, ovary and oviduct has been suggested by Fujii *et al* (39). Several other studies have reported similar p53 immunoreactivity in both tumor components (10,13,35,40,41,47-50). Moreover, in a model of uterine CS histogenesis proposed by Taylor *et al* (41), more than 71% of uterine CSs shared similar genetic alterations, while molecular defects acquired at a later stage were proved to be discordant between the two components. However, further studies are needeed for the investigation of the genetic mechanisms that are involved in the development of CSs into collision tumors. Different patterns of chromosome X inactivation in tumor cells genotyped from epithelial and mesenchymal lesions support the collision histogenesis (11). In the present study, 3 out of 36 (8%) cases showed a distinct p53 reactivity in both components, suggesting the 'biclonal' (collision) histogenesis. Future verification of genetic altera-

tions in the *TP53* gene in different p53-stained components of CS is needed.

In conclusion, based on immunohistochemical data, p53 is overexpressed in more than half of the female genital tract CSs included in the present study, either in the epithelial or mesenchymal component. The correlation between p53 or Ki-67 overexpression in both tumor components supports the combination theory of histogenesis in the majority of these tumors.

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