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Expression of regulatory proteins and proliferative activity in relation to phenotypic characteristics of upper urothelial carcinoma

Ekspresija regulatornih proteina i proliferativna aktivnost u vezi sa fenotipskim karakteristikama karcinoma gornjeg urotelijuma

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Abstract

Background/Aim. Deregulation of the normal cell cycle is common in upper urothelial carcinoma (UUC). The aim of this study was to investigate the expression of regulatory proteins of the cell cycle (p53, p16, cyclin D1, HER-2) and proliferative Ki-67 activity in UUC, and to determine their interaction and influence on the phenotypic characteristics of UUC. **Methods.** In 44 patients with UUC, histopathological and immunohistochemical analyses (p53, p16, cyclin D1, HER-2, and Ki-67) of tumors were done. **Results.** Overexpression/altered expression of p53, p16, cyclin D1 or HER-2 was detected in 20%, 57%, 64%, and 57% of tumors, respectively. Eleven (25%) UUC had a high proliferative Ki-67 index. Forty patients (91%) had at least one marker altered, while four (9%) tumors had a wild-type status. Analysis of relationship between expressions of molecular markers showed that only high expression of p53 was significantly associated with altered p16 activity ($p < 0.05$). High Ki-67 index was associated with the high stage ($p < 0.005$), solid growth ($p < 0.01$), high grade ($p < 0.05$), and multifocality $p < 0.05$) of UUC, while high expression of p53 was associated with the solid growth ($p < 0.05$). In regression models that included all molecular markers and phenotypic characteristics, only Ki-67 correlated with the growth ($p < 0.0001$), stage ($p < 0.01$), grade ($p < 0.05$) and multifocality ($p < 0.05$) of UUC; Ki-67 and HER-2 expression correlated with the lymphovascular invasion ($p < 0.05$). **Conclusions.** This investigation showed that only negative regulatory proteins of the cell cycle, p53 and p16, were significantly associated in UUC, while proliferative marker Ki-67 was in relation to the key phenotypic characteristics of UUC in the best way.

Key words:
urinary bladder; carcinoma; cell cycle proteins; ki-67 antigen.

Apstrakt

Uvod/Cilj. Deregulacija normalnog ćelijskog ciklusa je česta kod karcinoma gornjeg urotelijuma (KGU). Cilj ovog rada bio je da se ispita ekspresija regulatornih proteina ćelijskog ciklusa (p53, p16, ciklin D1, HER-2) i proliferativna aktivnost Ki-67 kod KGU i da se utvrdi njihov međusobni uticaj i uticaj na fenotipske karakteristike KGU. **Metode.** Kod 44 bolesnika sa KGU urađene su patohistološka i imunohistohemijska analiza (p53, p16, cyclin D1, HER-2 i Ki-67) tumora. **Rezultati.** Prekomerna ekspresija/izmenjena ekspresija p53, p16, ciklina D1 i HER-2 otkrivena je kod 20%, 57%, 64% i 57% tumora, redom. Jedanaest (25%) KGU imalo je visoki proliferativni Ki-67 indeks. Četrdeset bolesnika (91%) imalo je alteraciju najmanje jednog markera, dok su četiri (9%) tumora imala *wild-type* status. Analiza povezanosti ekspresije molekularnih markera pokazala je da je samo visoka ekspresija p53 bila značajno udružena sa izmenjenom p16 aktivnošću ($p < 0,05$). Visoki Ki-67 indeks bio je udružen sa visokim stadijumom ($p < 0,005$), solidnim rastom ($p < 0,01$), visokim gradusom ($p < 0,05$) i multifokalnošću ($p < 0,05$) KGU, dok je visoka ekspresija p53 bila udružena sa solidnim rastom ($p < 0,05$). U regresionom modelu koji je uključivao sve molekularne markere i fenotipske karakteristike, samo je Ki-67 korelisao sa rastom ($p < 0,0001$), stadijumom ($p < 0,01$), gradusom ($p < 0,05$) i multifokalnošću ($p < 0,05$) KGU, a ekspresija Ki-67 i HER-2 korelisala je sa limfovaskularnom invazijom ($p < 0,05$). **Zaključak.** Ovo istraživanje pokazalo je da su samo negativni regulatorni proteini ćelijskog ciklusa, p53 i p16, bili značajno povezani kod KGU, dok je proliferativni Ki-67 marker bio povezan sa ključnim fenotipskim karakteristikama KGU na najbolji način.

Ključne reči:
mokraćna bešika; karcinomi; ćelija, ciklus, proteini; ki-67 antigen.

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Introduction

A normal cell has three different states: static, division, or apoptosis. Most urothelial cells are not dividing under physiological condition [1, 2]. Many proteins act during the process of DNA replication, but also in cell grown regulation. Defects in the regulation of the DNA replication can lead to cancer. Activated cell proliferation may produce the loss of cell cycle arrest due to encoding of cellular regulatory genes or deactivation of tumor suppressor genes [3–6].

Deregulation of the normal cell cycle is common in upper urothelial carcinoma (UUC). Mutations in the p53 gene were frequently found in invasive UUC as well as in high-grade superficial UUC, while these mutations were rare in well-differentiated superficial UUC. p53 mutations result in prolonged half-life and accumulation of the p53 protein to a level that makes it detectable immunocytochemically (ICH) in tumor cell nuclei. In contrast, the wild-type p53 protein has a short half-life, measured as only 6-30 minutes [7–11].

Also, a new family of negative cell cycle regulators, the cyclin-dependent kinase inhibitor gene INK4 and KIP, has been identified. $p16^{INK4A}$, as members of the INK4, and KIP family proteins ($p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$) are inhibitors of cyclin-dependent kinases (CDKs). $p16^{INK4A}$ increases the Rb protein level as a control of the G1-S cell cycle checkpoint, and inhibits the CDK4/6 kinase. The loss of p16 function leads to the loss of pRb function and inappropriate cell cycling [12, 13]. Some findings explain the importance of inactivation through the $p16^{INK4A}$ pathway during the oxidative stress that leads to the activation of transitional cell carcinoma (TCC) [14].

Cyclin D1 is an important positive regulator of the G1–S transition, and expressed in the early G1 in response to mitogenic signals. Its expression is maximal in the middle G1 phase. Cyclin D contributes to regulate G1 progression by forming a complex with different CDK catalytic component [15, 16]. Cyclin D1 gene (CCND1) is located at chromosome 11q13, and amplification of the chromosomal region is frequently detected in TCCs of the bladder. CCND1 overexpression occurs in TCCs of the bladder and may be associated with growth of low-grade papillary tumors [17].

The proto-oncogene HER-2 (located on 17q21) encodes tyrosine kinase growth factor receptor and regulates cell growth and differentiation. The amplification of HER-2 or overexpression of its product is associated with malignant cell transformation and a poor prognosis in prostate, bladder and breast tumors. In urothelial carcinomas, it has been associated with tumor grade, tumor stage, and a patient's outcome [18]. HER2 positive tumors were associated with greater metastatic potential [19].

Ki-67, a marker of cellular proliferation, is nuclear protein complex expressed in the G1, S, G2 and M phases of the cell cycle of proliferating cells. Ki-67 may be involved in ribosome biosynthesis during cell proliferation, suggesting that the Ki-67 antigen is not directly involved in the cell cycle regulation [20].

The aim of this study was to investigate the expression of regulatory proteins of the cell cycle (p53, p16, cyclin D1, HER-2) and proliferative Ki-67 activity in UUC, and to determine their interaction and influence to the phenotypic characteristics of UUC.

Methods

The investigation included 44 consecutive patients with UUC. The analysis was done on 37 pelvic and 7 ureteral tumors. The patients included in the study had undergone nephroureterectomy with removal of bladder cuff. Extended lymphadenectomy was not routinely performed. All the cases of UUC were diagnosed at the Institute of Pathology, School of Medicine, Niš. The histological sections were processed from tissue fixed in 10% formalin by standard techniques, and stained with haematoxylin and eosin (H&E). H&E-stained slides were used to assess histological grade (low and high grade) [21], pathologic stage (pT) [22], growth of tumor (papillary/solid), multifocality, and lymphovascular invasion (LVI). Authors compared low stage non-muscle invasive tumor (pTa-pT1) and high stage muscle invasive (pT2-pT4) tumor [23].

Immunohistochemistry and scoring

We performed p53, p16, cyclin D1, HER-2, and Ki-67 immunohistochemical staining using serial sections from the same paraffin-embedded blocks. Tumors were analyzed by using the mouse monoclonal antibody and a standard avidin-biotin immunoperoxidase complexes detection system, according to the manufacturer's protocol (Dako LSAB2R system-HRP). Staining and scoring protocols for p53, p16, cyclin D1, HER-2, and Ki-67 are given in Table 1.

Table 1

Immunostaining protocol

Antibody	Clone / Source	Dilution	Expression
p53	Pab 1801, IgG1 / Newcastle	1:50	nuclear
p16	Clone 6H12, IgG2b / Newcastle	1:40	nuclear
Cyclin D1	P2D11F11, IgG2a / Newcastle	Ready to use	nuclear
HER-2	Code A 0485 / Dako	1:300	cell membrane
Ki-67	MIB-1, Izotip IgG1, kappa / Dako	1:100	nuclear

Before quantifying the immunohistochemical results, the technique quality was assessed and those areas with greater positivity were selected, avoiding peripheral area measurement, necrosis or artifact.

Nuclear p53 immunoreactivity was considered altered when samples demonstrated at least 10% nuclear reactivity, as it has been shown that accumulation of p53 protein in 10% or more of the tumor cell nuclei strongly correlates with

mutations in the p53 gene [24]. The staining protocol for p16 nuclear protein includes heterogeneous, homogeneous and negative findings. Tumor was considered to have a normal heterogeneous p16 pattern if it had relatively weak nuclear staining with considerable differences in nuclear intensity, including many negative cells. Strong 16 staining considered if the majority of the malignant cells had intensive p16 nuclear expression and p16 negative tumor cells were rare. Tumor was termed p16 negative if no malignant cells had positive staining. Tumor without or with overexpression of p16 was categorized as altered [25]. The cutoff value for cyclin D1 in the tumor tissue was set at the level of expression in the normal tissue (5%). For testing HER-2 (C-erbB2) status we used the HercepTest scoring system devised by DAKO. HER-2 cell membrane specific immunoreactivity were scored by estimating the percentage of positive tumor cell as follows: score 0, no immunoreactive cells; score +1, positivity in < 5% cancer cells; score +2, positivity in 5–50% cancer cells; and score +3, positivity in > 50% of cancer cells. The specimens were considered positive for HER-2 expression when the score was +2 or +3. The nuclei in morphologically malignant cells were considered positive for Ki-67 antigen when they showed dark brown granular staining. Stromal or peritumoral lymphocytes were present in most cases and served as internal controls for Ki-67. The cutoff value for Ki-67 in the tumor tissue was 10% [25, 26].

Slides were reviewed independently by the three investigators. Interobserver discrepancies were resolved using a double headed microscope. Only nuclear expression was recorded for p53, p16, cyclin D1, and Ki-67. The number of distinctly positive tumor cell nuclei was counted under high power (× 400) using a 10 × 10 eyepiece grid. In total, 1,000 tumor cells were assessed. The number of positive nuclei was expressed as a percentage of all tumor cell nuclei counted.

Statistical analysis

For the purposes of statistical analysis, pathological tumor stage (low *vs* high), grade (low *vs* high), growth pattern (papillar *vs* solid), LVI (yes *vs* no), and multifocality (yes *vs* no) were evaluated as dichotomized variables. The Fisher's exact test and the χ^2 test were used to evaluate the association of p53, p16, cyclin D1, HER-2, Ki-67 with phenotypic characteristics of tumors (stage, grade, growth, lymphovascular invasion), and multifocality. To determinate influence of regulatory proteins (p53, p16, cyclin D1, HER-2, Ki-67) on conventional pathological parameters regression analysis was performed.

All analyses were performed with the SPSS statistical package (SPSS version 10.0 for Windows). The result was considered statistically significant if $p < 0.05$.

Results

Clinical and pathological features

The age of 44 patients with UUC ranged from 22 to 87 years, with a mean age of 63.5 years. There was a male predominance (29 patients) with relation M : F = 2 : 1.

The number of patients with low grade and high grade UUC was 27 (61%), and 17 (39%), respectively. The pathological stage was low in 15 (34%), and high stage in 29 (66%) patients. Tumors had papillary growth in 24 (55%), and solid in 20 (45%) specimens. Also, LVI and multifocality were detected in 12 (27%), and 9 (20%) UUC, respectively.

There was a significant association between pathological stage and grade, as well as pathological stage and LVI ($\chi^2 = 6.15$, $p < 0.05$, and $\chi^2 = 8.53$, $p < 0.005$, respectively). Growth pattern and multifocality of UUC were not in significant association with the pathological stage (Table 2).

Evaluation of immunohistochemical staining

The normal urothelium of the kidney pelvis and ureter showed heterogeneous expression of p16 (wild type) and the absence of p53 (wild type).

Table 2

Morphological finding and pathological stage

Morphological finding	Pathological stage		*p*
	low stage n (%)	high stage n (%)	
Grade			
low grade	13 (30)	14 (32)	< 0.05
high grade	2 (4)	15 (34)	
Growth			
papillary	11 (25)	13 (30)	N.S.
solid	4 (9)	16 (36)	
LVI			
yes	0 (0)	12 (27)	< 0.005
no	15 (34)	17 (39)	
Multifocality			
yes	3 (7)	6 (14)	N.S.
no	12 (27)	23 (52)	

LVI – lymphovascular invasion; N.S. – no significance

Tumor cells reacted positively for p53, p16, cyclin D1 (Figure 1, a–c), and Ki-67 were predominantly stained in the nucleus, while HER-2 had expression on the cell membrane (Figure 1d).

The mean p53 labeling index was 6.88 ± 15.67 (range, 0–89.2) and 18 specimens (41%) were positive for p53 ex-

pression. Expression of p53 was low in 35 (80%) and high in 9 (20%) specimens (Fig.1a). Immunohistochemical analysis of p16 showed that 25 (57%) tumors had altered p16 function, i.e. 16 (36%) UUC were p16 negative, while 9 (21%) tumors had strong, homogeneous nuclear staining. Cytoplasm p16 staining was common in the presence of nuclear staining, particularly in those tumors with the strong p16 expression (Figure 1b), but was not found in p16 negative tumors. A normal heterogeneous p16 staining pattern was detected in 19 (43%) UUC. The mean labeling index for cyclin D1 was 11.91 ± 11.63 (range, 0–41.23), and 30 specimens (68%) were positive for cyclin D1 expression. Expression of cyclin D1 was low in 16 (36%) and high in 28 (64%) specimens (Figure 1c). Investigation of the HER-2 status of 44 UUC showed that 25 (57%) tumors was HER-2 positive (≥ 2+ staining), while only 9 (20%) of them were classified as 3+ positive (Figure 1d). The mean Ki-67 labeling index was 10.49 ± 9.00 (range, 0.1–34.3). Expression of Ki-67 was low in 33 (75%) and high in 11 (25%) specimens (Figures 1, e and f).

Number of altered cell cycle regulatory proteins in UUC

Forty patients (91%) had at least one marker altered, while four (9%) tumors had wild-type status (p53, p16, cyclin D1, HER-2). The majority (64%) of UUC had alteration of one or two markers: 12 (27%) one and 16 (37%) two markers. Alterations in the three or four cell cycle regulatory proteins occurred in twelve (27%) patients with UUC, in 5 (11%) patients three and in 7 (16%) patients four markers (Table 3). An analysis of the relationship between expression of molecular markers showed that only high expression of p53 was significantly associated with altered p16 activity ($\chi^2 = 4.79$, $p < 0.05$) (data shown in Table 4 are only for markers which reached statistical significance).

Correlation of immunohistochemical staining with conventional pathological features

Tables 5 and 6 show the relationships between conventional pathological parameters and immunohistochemical

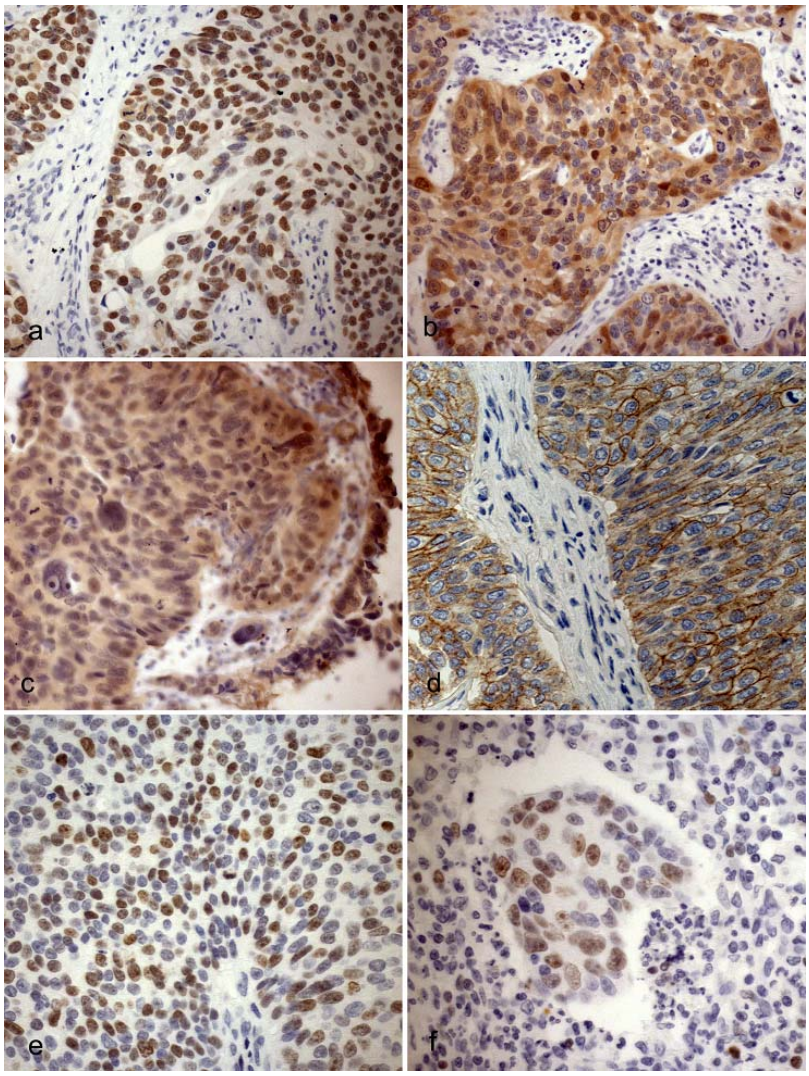

Fig. 1 – The representative positive staining of regulatory proteins in upper urothelial carcinoma (UUC), with strong nuclear p53 activity in solid growth of tumor (a), homogeneous p16 nuclear and cytoplasm expression (b), nuclear cyclin D1 staining in high grade tumor (c); HER-2 positive tumor cells classified as 3+ positive (d); high proliferative Ki-67 activity in solid growth (e), and LV invasion (f) (ABC, original magnification × 400)

Table 3

Alteration of cell cycle regulatory proteins in upper urothelial carcinoma (UUC)

One marker	n	Two markers	n	Three markers	n	Four markers	n
Cyclin D1	5	Cyclin D1+ HER2	6	Cyclin D1+ p16+ HER2	5	Cyclin D1+ p16+p53+HER2	7
p16	4	Cyclin D1+p16	4				
HER 2	3	Cyclin D1+p53	1				
		p16+ HER2	4				
		p16+p53	1				
Total	12 (27%)		16 (37%)		5 (11%)		7 (16%)

Note: 4 (9%) UUC have wild type of molecular markers

Table 4

Association of p53 and p16 in upper urothelial carcinoma (UUC)

Marker	p53		*p*
	low	high	
p16 normal	18	1	< 0.05
p16 altered	17	8	

Table 5

Ki-67, p53 and cyclin D1 index in relation to grade, stage, growth, lymphovascular invasion (LVI) and multifocality

Fenotipic characteristics	Total (n = 44)	Ki-67		*p*	p53		*p*	CyclinD1		*p*
		low	high		low	high		low	high	
Grade										
low grade	27	23	4	< 0.05	23	4	N.S.	9	18	N.S.
high grade	17	10	7		12	5		7	10	
Stage										
low stage	15	15	0	< 0.005	13	2	N.S.	5	10	N.S.
high stage	29	18	11		22	7		11	18	
Growth										
papillary	24	22	2	< 0.01	22	2	< 0.05	10	14	N.S.
solid	20	11	9		13	7		6	14	
LVI										
yes	12	7	5	N.S.	8	4	N.S.	4	8	N.S.
no	32	26	6		27	5		12	20	
Multifocality										
yes	9	4	5	< 0.05	8	1	N.S.	2	7	N.S.
no	35	29	6		27	8		14	21	

N.S. –no significance

Table 6

p16 and HER-2 expression in relation to grade, stage, growth, lymphovascular invasion (LVI) and multifocality

Fenotipic characteristics	Total (n = 44)	p16 altered n (%)	*p*	HER-2 score ≥ 2 n (%)	*p*
Grade					
low grade	27	13 (30)	N.S.	17 (39)	N.S.
high grade	17	12 (27)		8 (18)	
Stage					
low stage	15	9 (20)	N.S.	10 (23)	N.S.
high stage	29	16 (36)		15 (34)	
Growth					
papillary	24	15 (34)	N.S.	14 (32)	N.S.
solid	20	10 (23)		11 (25)	
LVI					
yes	12	8 (18)	N.S.	4 (9)	N.S.
no	32	17 (39)		21 (48)	
Multifocality					
yes	9	5 (11)	N.S.	6 (14)	N.S.
no	35	20 (45)		19 (43)	

N.S. – no significance

staining of Ki-67, p53, cyclin D1, p16 and HER-2 in UUC. The Ki-67 labeling index significantly correlated with the grade ($\chi^2 = 3.87$, $p < 0.05$), stage (Fisher = 0.0045, $p < 0.005$), growth ($\chi^2 = 7.82$, $p < 0.01$) and multifocality of tumor ($\chi^2 = 5.63$, $p < 0.05$). The p53 labeling index correlated only with the growth pattern (Fisher = 0.0031, $p < 0.05$). Expression of p16, cyclin D1, and HER-2 was not significantly associated with the conventional pathological parameters and multifocality of tumor. Also, tumors which showed HER-2 score 3 were more often in high than in low stage [7/29 (24%) *vs* 2/15 (13%)], while strong expression of cyclin D1 (> 25%) had a similar distribution in dependence of the stage [5/29 (17%) *vs* 3/15 (20%)].

A possible correlation between molecular markers (p53, p16, cyclin D1, HER-2, Ki-67) and standard pathological features was investigated. Regression analysis showed that proliferative marker Ki-67 correlated with the growth ($p < 0.0001$), stage ($p < 0.001$), grade ($p < 0.05$), and multifocality ($p < 0.05$) of UUC on the best way. Ki-67 and HER-2 expression correlated with the lymphovascular invasion ($p < 0.05$) (Table 7).

Also, we found that the expression of cell cycle regulators is commonly altered in UUC patients, with 91% of patients having altered expression of at least one marker, and with 16% exhibiting altered expression of p53 and p16. The association of p53 and p16 tumor suppressor pathway was significant in UUC.

P16 is specific CDK inhibitor, and negatively regulates cell-cycle progression in a p53-independent manner [4].

Shariat et al. [24] have found alteration of at least one of the three tumor markers in 83% of patients, and alteration of p53, p21, and pRB/p16 in 26% of patients with bladder cancer. Also, the authors have found that tumors with alterations of p53 and pRB/p16, were associated with muscle-invasive disease. They had significantly higher risk for unfavorable clinical outcome than patients with only one of the two markers altered.

The role of cell cycle regulators in bladder cancer progression seems to be a complex accumulation of genetic alteration, from which p53 and p16 seem to be associated with the later stages of bladder cancer clinical progression [24].

Table 7

Influence of Ki-67 and HER-2 on fenotypic characteristics of tumor

Constant	Dependent variable	Standardized coefficients β	t	*p*
Ki-67	Grade	0.309	2.061	< 0.05
Ki-67	Stage	0.448	3.054	< 0.01
Ki-67	Growth	0.596	4.606	< 0.0001
Ki-67	Multifocality	0.387	2.622	< 0.05
Ki-67	LVI	0.364	2.621	< 0.05
HER-2	LVI	-0.381	-2.522	< 0.05

LVI – lymphovascular invasion

Discussion

Various histopathological and clinical parameters are known to have prognostic significance in UUC. These parameters include tumor stage, histological grade, multicentricity, tumor growth pattern (papillary *vs* solid), LVI, and tumor cell proliferation. While histopathological criteria can provide important morphological information about tumors, they are not reliable to specify the risk for progression or response to treatment for a patient with UUC [27, 28].

The mutations of tumor suppressor gene p53 of chromosome 17 have already been reported in urothelial carcinoma. Several studies have demonstrated a positive correlation between p53 abnormalities and higher tumor stage [7, 29]. Esring et al. [30] observed that p53 overexpression correlated significantly with recurrence and crude survival, and in multivariate regression analysis, it was a factor independent of pathological stage and histological grade.

This study showed that overexpression of p53 was detected in 20% of tumors. UUC with high grade, high stage, solid growth and LVI had more common overexpression of p53, however statistically significant only in solid tumors. The sample size may have limit of our ability to detect other differences. Our finding is similar to investigation of Kamijima et al. [9].

In this study, nuclear expression of cyclin D1 in more than 5% of tumor cells was found in 64% of the cases and was not associated with the growth, differentiation and stage. Very strong expression of cyclin D1 (> 25%) was detected in 18% of UUC.

Cyclin D1 overexpression may be an important early event in the progression of TCCs. Its association with well-differentiated, papillary tumors suggests that cyclin D1 may also play a role in tumor differentiation [31, 32]. Khan et al. [31] suggested that a cyclin D1 dependent pathway determines the evolution of a group of well-differentiated low-stage papillary TCCs, whereas tumors that evolve *via* cyclin D1 independent mechanisms are less differentiated and pathologically more aggressive. These observations are consistent with the reported accumulation of Rb and p53 mutations in advanced TCCs [33].

Deregulation of HER-2 was present in 57% of UUC, where 20% of those tumors had HER-2 score 3 and more often in high stage of the disease. No significant association was present with the expression of HER-2 and other conventional pathological parameters.

The level of expression and the prognostic significance of HER-2 protein in urothelial cancer varies among different studies, with some revealing no prognostic relevance and other suggesting a better or worse prognosis [34, 36].

Strong HER-2 overexpression was detected using immunohistochemistry in 23% of bladder urothelial cancer, and in 33% of patients with metastases. HER-2 overexpression is strongly associated (95%) with gene amplification assessed using FISH [33].

Inoue et al. [18] did not found a correlation between the relative increase in a HER-2 copy number and tumor stage. HER-2 amplification, found more frequently in pT2-T4 tumors than in pTa-T1, did not correlate with tumor grade. The relative increase of the HER-2 copy number may be associated with the number of recurrences and the presence of CIS.

In our study, high proliferative Ki-67 index had 25% of UUC. Overexpression of Ki-67 was significantly associated with the advanced pathological stage, higher tumor grade, solid growth and multifocality of UUC. The regression analysis showed that proliferative marker Ki-67 correlated with phenotypic characteristics of UUC in the best way. Also, Ki-67 activity and HER-2 oncogene correlated with the lymphovascular invasion.

Proliferative marker Ki-67 has a prognostic value in renal cell carcinoma and urothelial neoplasms of the urinary bladder [37], being associated with tumor grade, stage, recurrence and prognosis of urothelial carcinoma [9, 38–40], as well as with LVI, and metastases to lymph nodes. Ki-67 is the best predictor of recurrence in noninvasive bladder tumors, but also is an independent predictor of disease recurrence, progression, and response to intravesical therapy in patients with nonmuscle-invasive bladder cancer. Its high expression is related to a poor survival. The availability of Ki-67 and MIB1 antibodies in most laboratories makes it an ideal marker to be used in the daily evaluation of urothelial tumors [9, 12, 41].

Conclusion

This investigation showed that only negative regulatory proteins of the cell cycle, p53 and p16, were significantly associated in UUC, while the proliferative marker Ki-67 was in relation to the key phenotypic characteristics of UUC in the best way.

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