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Correlation between p53 and Rb protein expression and the Ki-67 proliferative index in urothelial carcinoma of the urinary bladder

Sabit Mehmeti[1], Rinë Limani[2], Suzana Manxhuka-Kërliu[2]

[1] Faculty of Medicine, University of Prishtina "Hasan Prishtina" and Clinic of Urology, University Clinical Center of Kosovo, 10, 000 Prishtina Kosovo

[2] Faculty of Medicine, University of Prishtina "Hasan Prishtina" and Institute of Anatomical Pathology University Clinical Center of Kosovo, 10,000 Prishtina Kosovo

Abstract

Genetic and environmental factors are considered to play a significant role in the development of Urothelial Carcinoma (UC) of the urinary bladder, with various molecular markers being used for diagnostic and prognostic purposes. The aim of this study was to evaluate the expression of p53, Rb and Ki-67 in UC of the urinary bladder in our population group. Additionally, we investigated potential correlations in expression between p53 and Rb, as well as their associations with the Ki-67 proliferative index in urinary bladder UC. Tissue specimens of 70 samples of UC and 20 samples of non-tumorous bladder tissue were immunohistochemically stained with primary monoclonal antibodies against p53, Rb, and Ki-67 in our single-center study. When correlating expression levels of p53, Rb, and Ki-67 statistically significant higher expression for p53 and Ki-67 ($p=0.001$; $p<0.001$, respectively) was found with increasing grade and stage in UC of the bladder. Rb expression was decreased with increasing grade and stage but not statistically significant in our study group. Our study confirms the role of p53 and Rb alterations in UC of the urinary bladder. This is the first report of p53 and Rb alterations in UC of the urinary bladder in Kosovo population.

Key Words: urothelial carcinoma; urinary bladder; p53; Rb; Ki-67

Address for correspondence:
Rinë Limani,
Faculty of Medicine, University of Prishtina "Hasan Prishtina" and Institute of Anatomical Pathology University Clinical Center of Kosovo, 10, 000 Prishtina Kosovo
E-mail: rine.limani@uni-pr.edu

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INTRODUCTION

Urinary bladder cancer is the 10th most common cancer worldwide with the highest number of cases in men [1]. Its management relies on pathologic staging and despite advances in surgical techniques, perioperative therapies, and postoperative management the prognosis has largely remained unchanged. Urothelial Carcinoma (UC) is the most common histological type of urinary bladder cancer [2]. Historically, bladder carcinoma has been categorized into two main groups: Non Muscle-Invasive Blad der Cancer (NMIBC), which encompasses AJCC stage categories Ta, T1, and urothelial carcinoma in situ, and Muscle-Invasive Bladder Cancer (MIBC), comprising T2-T4 disease [3]. UC of the urinary bladder is prone to recurrence and in some patients to progression. There is a reported 30%-70% rate of same stage recurrence at 5 years in NMIBC, of which 10%-15 % develop MIBC [4, 5]. Considering its high incidence and its relatively high recurrence and progression rate there is much interest on the molecular processes underlying the development and progression of UC of the urinary bladder. On a molecular level, UC exhibits heterogeneity, marked by genomic instability and a heightened mutation rate. Numerous molecular biomarkers have been extensively studied in relation to UC of the urinary bladder to identify the less favorable group of cancer and as potential predictors of progression, aggressiveness and its response to treatment.

TP53 gene is a tumor suppressor gene located on chromosome 17 p13. It encodes the tumor suppressor protein p53 which has an active role in genomic stability, cell cycle, and apoptosis [6]. When TP53 gene is mutated, it loses its function as a tumor suppressor gene and promotes unlimited proliferation of tumor cells and tumorigenesis [6]. The frequent occurrence of altered TP53 status in bladder cancer has been documented and is known to hold prognostic significance. A strong correlation is reported between the p53 status and tumor stage and grade in bladder cancer,

with alterations being more prevalent in high-stage and high-grade tumors.
The Retinoblastoma-1 (RB1) gene, situated on chromosome 13q 14.2, is a recessive tumor suppressor gene. Mutations within the RB1 gene lead to dysfunction in the Retinoblastoma (RB) protein family, which includes Rb, p107, and p130. As a consequence, these mutations contribute to the development of advanced-stage bladder cancers, which exhibit a high frequency of recurrences and reduced survival rates [7-12].

Ki-67, a nuclear protein encoded by a gene found on chromosome 10, plays a role in ribosomal RNA transcription and serves as an indicator of cellular proliferation. It holds significance as a prognostic factor for both tumor recurrence and progression. In the context of Urothelial Carcinoma (UC) in the urinary bladder, elevated Ki-67 expression has been linked to more advanced tumor stage, higher grade, and an increased likelihood of recurrence [13-15].

In our research, we assessed the p53, Rb, and Ki-67 immunohistochemical expression, as well as their association with the histological grade and stage of urinary bladder cancer within our specific patient population.

PARTICIPANTS AND METHODS

Participants

Tissue samples of UC and urinary bladder non-tumorous tissue were obtained through Transurethral Resection (TUR) of the urinary bladder. In total, 90 tissue samples were analyzed. Patients with multifocal tumors, recurrent tumor and patients with severe inflammatory changes of the mucosa were excluded from the study.

The study was conducted at the Clinic of Urology and the Institute of Anatomical Pathology at the University Clinical Center of Pristina, Republic of Kosovo. Informed consent was obtained, and the Institutional Review Board approved this study under the 2013 Helsinki Declaration guidelines.

Hematoxylin-Eosin Staining

After resection, tissue samples were immersed in 10% buffered formalin and fixed for 24 hours, following which they were dehydrated in ethanol, embedded in paraffin cut into 5 μm thin sections, and stained with the standard hematoxylin-eosin, H&E method. Representative tumor tissue and non-tumorous tissue were marked on H&E slides for further immunohistochemical analysis and reviewed by two experienced pathologists on light microscopy.

Histological diagnosis, grading and the TNM were determined according to the WHO last edition Urinary Bladder tumor classification and the 8th edition of AJJC - TNM classification and staging [2, 3].

Immunohistochemistry

From the selected paraffin blocks, 3 to 4 μm thick sections were taken for Immunohistochemical (IHC) staining. Primary monoclonal antibodies against p53 (clone DO-7, DAKO, dilution 1:100), Rb (clone NCL-RB- 358, Novocastra, dilution 1:50), and Ki-67 (clone MIB-1, DAKO, dilution 1:100) were applied using the immunoperoxidase avidin–biotin method in an automatic stainer (Autostainer, Dako, Glostrup, Denmark). IHC analysis was performed with the EnVision™FLEX (K800 Dako, Glostrup, Denmark) detection system. Tonsil tissue was used as positive control for all antibodies as per manufacturers' instructions. Negative controls consisted in the same immunohistochemical method with omission of the primary antibody.

Nuclear staining in cells was considered as positive staining. All slides were analyzed using light microscopy. We assessed nuclear p53, Rb and Ki-6 immunohistochemical expression as the percentage of positively stained cells relative to total cells.

p53, Rb and Ki-67 immunostaining in the tumor and non-tumorous urinary bladder tissue were evaluated semi-quantitatively as follows below and presented in Figure 1.

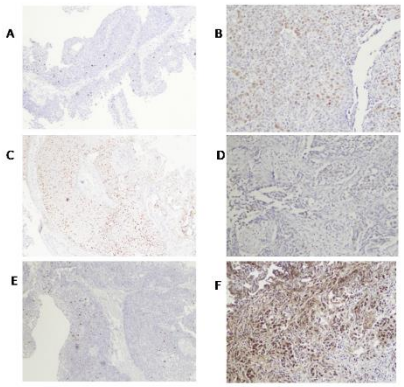

Fig. 1. A. Low IHC expression of p53 in noninvasive low grade urothelial carcinoma x 200 **B.** High IHC expression of p53 in invasive high grade urothelial carcinoma x 200 **C.** High IHC expression of Rb in noninvasive low grade urothelial carcinoma x 100 **D.** Low IHC expression of Rb in invasive high grade urothelial carcinoma x 200 **E.** Low IHC expression of Ki-67 in noninvasive low grade urothelial carcinoma x 100 **F.** High IHC expression of Ki-67 in invasive high grade urothelial carcinoma x 200

p53: 0, low (<10% of cells positive); 1+, weak (≥ 10% to <25% of cells positive); 2+, moderate (≥ 26 to <50% of cells positive); and 3+, high (≥ 50% of cells positive).

Rb: 0, low (< 1%); 1+, weak (≥ 1% to <10% of cells positive); 2+, moderate (≥10% to < 50% of cells positive); and 3+, high (≥ 50% of cells positive).

Ki-67: 0, low (< 20%); 1+, moderate (≥ 20 to < 50%); 2 high, (≥ 50% of cells positive).

Statistical analysis

Statistical analysis was performed using the software package SPSS 21 (IBM, New York, USA) and Microsoft Excel (Microsoft, USA).

The expression of p53, Rb, and Ki-67 were specified as absolute numbers and proportions of expression were calculated. One-way ANOVA test was used for comparisons between age and other variables. In case of correlations and qualitative variables the differences were analyzed by the Pearson's chi-square test and Spearman correlation. Results were considered as statistically significant when $p<0.05$.

RESULTS

90 tissue samples were included in the study. Descriptive statistics including gender, histological type, and grade are presented in Table 1.

Tab. 1. Descriptive statistics of patients including histological diagnosis and tumor grade

	All patients (N=90)	
Age [years]	**69.2 ± 10.3**	
Gender		
Male/female	70/20	77.8%/22.2%
IUC		
IUC, LG	15	16.70%
pT1	14	-
pT2	1	-
IUC, HG	20	22.20%
pT1	11	-
pT2	8	-
pT3	0	-
pT4	1	-
NIUC		
NIUC, LG	28	31.10%
NIUC, HG	7	7.80%
Non-tumorous mucosa	20	22.20%

IUC LG: invasive urothelial carcinoma low grade;
IUC HG: invasive urothelial carcinoma high grade;
NIUC LG: noninvasive urothelial carcinoma low grade;
NIUC HG: noninvasive urothelial carcinoma high grade;

The mean age of the patients was 69.2 years, with a standard deviation (SD) of ± 10.3. Out of the 90 patients, 70 (77.8%) were male, and 20 (22.2%) were female. Among the UC group, there were 56 male patients and 14 female patients, evenly divided between NIUC and IUC samples. In the control group, there were 20 subjects with non-tumorous mucosa.

While the majority of participants were male, our analysis revealed no statistically significant differences between male and female individuals concerning the

expression levels of p53, Rb, or Ki-67 (see Table 2). Furthermore, gender did not display any correlations with other variables.

Tab. 2. Correlation of p53, Rb and Ki67 expression in relation to gender

	Gender		
	Male (n=70)	**Female (n=20)**	**p-value**
p53			
<10% (low)	18	7	0.61
≥ 10% to <25% (weak)	34	9	
≥ 26% to <50% (moderate)	11	1	
≥ 50% (high)	7	3	
Rb			
<1% (low)	12	5	0.815
≥1% to < 10% (weak)	39	9	
≥ 10% to <50% (moderate)	11	4	
≥ 50% (high)	8	2	
Ki-67			
<20% (low)	34	10	0.36
>20% to <50% (moderate)	30	10	
≥ 50% (high)	6	0	

We observed a positive correlation between p53 expression and tumor grade and stage (p=0.001), as detailed in Tables 3 and 4.

Tab. 3. p53 immunohistochemical expression in study groups

	Non tumorous mucosa (n=20)	**NIUC LG (n=28)**	**NIUC HG (n=7)**	**IUC LG (n=15)**	**IUC HG (n=20)**	**p-value**
p53						
<10% (low)	13	5	1	3	3	0.001
≥ 10% to <25% (weak)	7	21	3	8	4	
≥ 26% to <50% (moderate)	0	2	3	2	5	
≥ 50% (high)	0	0	0	2	8	

NIUC LG: noninvasive urothelial carcinoma low grade;
NIUC HG: noninvasive urothelial carcinoma high grade;
IUC LG: invasive urothelial carcinoma low grade;
IUC HG: invasive urothelial carcinoma high grade;

Tab. 4. p53 expression in correlation with tumor grade and stage

p53 (N=70)		
	IUC, LG, pT1 (n=14)	**IUC, HG, pT1 (n=11)**
<10% (low)	3 (21.4%)	2 (18.2%)
≥ 10% to <25% (weak)	8 (57.1%)	3 (27.3%)
≥ 26% to <50% (moderate)	1 (7.1%)	4 (36.4%)
≥ 50% (high)	1 (7.1%)	2 (18.2%)
Total	13	11
	IUC, LG, pT2 (n=1)	**IUC, HG, pT2 (n=8)**
<10% (low)	1 (100%)	1 (12.5%)
≥ 10% to <25% (weak)	0	1 (12.5%)
≥ 26% to <50% (moderate)	0	1 (12.5%)
≥ 50% (high)	0	5 (62.5%)
Total	1	8
	IUC, HG, pT3 (n=0)	**IUC, HG, pT4 (n=1)**
<10% (low)	0	0
≥ 10% to <25% (weak)	0	0
≥ 26% to <50% (moderate)	0	1 (100%)
Total	0	1
	NIUC, LG (n=28)	**NIUC, HG (n=7)**
<10% (low)	5 (17.9%)	1 (14.3%)
≥ 10% to <25% (weak)	21 (75.0%)	3 (42.9%)
≥ 26% to <50% (moderate)	2 (7.1%)	3 (42.9%)
Total	28	7

IUC LG: invasive urothelial carcinoma low grade;
IUC HG: invasive urothelial carcinoma high grade;
NIUC LG: noninvasive urothelial carcinoma low grade;
NIUC HG: noninvasive urothelial carcinoma high grade;

In the case of Rb expression in UC, the results presented in Tables 5 and 6 show that Rb expression did not exhibit a statistically significant correlation with tumor grade and stage in our patient population (p=0.094).

Tab. 5. Rb immunohistochemical expression in study groups

	Non tumor	**NIUC**	**NIUC**	**IUC LG**	**IUC HG**	**p-value**

	ous mucosa (n=20)	LG (n=27)	HG (n=8)	(n=15)	(n=20)	
Rb						
<1% (low)	2	3	0	1	5	0.094
≥ 1% to < 10% (weak)	7	5	6	10	9	
≥ 10% to <50%(moderate)	11	19	2	3	5	
≥ 50% (high)	0	0	0	1	1	

NIUC LG: noninvasive urothelial carcinoma low grade;
NIUC HG: noninvasive urothelial carcinoma high grade;
IUC LG: invasive urothelial carcinoma low grade;
IUC HG: invasive urothelial carcinoma high grade.

Tab. 6. Rb expression in correlation with grade and stage

Rb (N=70)		
	IUC, LG, pT1 (n=14)	**IUC, HG, pT1 (n=11)**
<1% (low)	1 (7.1%)	1 (9.1%)
≥ 1% to <10% (weak)	9 (64.3%)	7 (63.63%)
≥ 10% to <50% (moderate)	3 (21.4%)	2 (18.2%)
≥ 50% (high)	1 (7.1%)	1 (9.1%)
Total	14	11
	IUC, LG, pT2 (n=1)	**IUC, HG, pT2 (n=8)**
<1% (low)	0	3 (37.5%)
≥ 1% to <10% (weak)	1 (100%)	2 (25.0%)
≥ 10% to <50% (moderate)	0	3 (37.5%)
≥ 50% (high)	0	0
Total	1	8
	IUC, HG, pT3 (n=0)	**IUC, HG, pT4 (n=1)**
<1% (low)	0	1 (100%)
≥ 1% to <10% (weak)	0	0
≥ 10% to <50% (moderate)	0	0
Total	0	1
	NIUC, LG (n=28)	**NIUC, HG (n=7)**
<1% (low)	3 (10.7%)	2 (28.57%)
≥ 1% to <10% (weak)	5 (21.4%)	5 (71.42%)
≥ 10% to <50% (moderate)	19 (67.9%)	1 (14.3%)
≥ 50% (high)	0	0
Total	28	7

IUC LG: invasive urothelial carcinoma low grade;
IUC HG: invasive urothelial carcinoma high grade;
NIUC LG: noninvasive urothelial carcinoma low grade;
NIUC HG: noninvasive urothelial carcinoma high grade.

However, we did note a decreasing trend in Rb expression as the grade and stage increased within our sample of UC patients. Higher Ki-67 expression correlated with higher tumor grade and invasion in UC as presented in Table 7 and 8 ($p<0.001$).

Tab. 7. Ki-67 immunohistochemical expression in study groups

	Non tumorous mucosa (n=20)	**NIUC LG (n=27)**	**NIUC HG (n=8)**	**IUC LG (n=15)**	**IUC HG (n=20)**	**p-value**
Ki-67 stage						
<20% (low)	20	19	1	3	1	<0.001
>20% to <50% (moderate)	0	8	7	12	13	
≥ 50% (high)	0	0	0	0	6	

NIUC LG: noninvasive urothelial carcinoma low grade;
NIUC HG: noninvasive urothelial carcinoma high grade;
IUC LG: invasive urothelial carcinoma low grade;
IUC HG: invasive urothelial carcinoma high grade.

Tab. 8. Ki-67 expression in correlation with grade and stage

	Ki67 (N=70)	
	IUC, LG, pT1 (n=14)	**IUC, HG, pT1 (n=11)**
<20% (low)	3 (21.4%)	1 (9.1%)
>20% to 50% (moderate)	11 (78.6%)	8 (72.7%)
≥ 50% (high)	0	2 (18.2%)
Total	15	11
	IUC, LG, pT2 (n=1)	**IUC, HG, pT2 (n=8)**
<20% (low)	0	0
>20% to <50% (moderate)	1 (100%)	5 (62.5%)
≥ 50% (high)	0	3 (37.5%)
Total	1	8
	IUC, HG, pT3 (n=0)	**IUC, HG, pT4 (n=1)**
<20% (low)	0	0
>20% to <50% (moderate)	0	0
≥ 50% (high)	0	1 (100%)
Total	0	1

	NIUC, LG (n=28)	NIUC, HG (n=7)
<20% (low)	19 (67.9%)	1 (14.3%)
>20% to <50% (moderate)	9 (32.1%)	6 (85.7%)
≥ 50% (high)	0	0
Total	28	7

IUC LG: invasive urothelial carcinoma low grade;
IUC HG: invasive urothelial carcinoma high grade;
NIUC LG: noninvasive urothelial carcinoma low grade;
NIUC HG: noninvasive urothelial carcinoma high grade.

P53 and Ki-67 exhibited low and weak expression in low-grade tumors and non-tumorous tissue. Specifically, the correlations were positively associated with higher tumor grade and invasion (p53: rho=0.473, p<0.001; Ki-67: rho=0.429, p=0.010) and with advanced cancer stages (Spearman correlation p53: rho=0.539, p<0.001; Ki-67: rho=0.749, p<0.001), as illustrated in Table 9. Additionally, age was positively correlated with increasing cancer stage (rho=0.338, p=0.001) and with increased marker expression (p53: rho=0.215, Ki-67: rho=0.366, p<0.001) as detailed in Table 9.

Tab. 9. Spearman correlations of p53, Rb and Ki-67 expression with independable variables

	p53	Rb	Ki67
Cancer stage	rho=0.539, p<0.001	rho=0.219, p=0.042	rho=0.749, p<0.001
Tumor Grade	rho=0.473, p<0.001	rho=0.393, p=0.019	rho=0.429, p=0.010
Age	rho=0.215, p=0.042	rho=0.210, p=0.047	rho=0.366, p<0.001
p53	NA	rho=0.323, p=0.002	rho=0.424, p<0.001
Rb	rho=0.323, p=0.002	NA	rho=0.211, p=0.045
Ki67	rho=0.424, p<0.001	rho=0.211, p=0.045	NA

We identified a positive correlation between the expression of p53 and Ki-67, with a Spearman correlation of p53 *vs.* Ki-67, rho=0.424, p<0.001. Post-hoc analysis of the respective study groups and stages did not reveal any significant differences in marker expression (p>0.05, respectively).

DISCUSSION

Understanding the molecular mechanisms underlying the development, progression, and treatment of bladder disorders is of paramount importance in the field of urology and oncology. These molecular mechanisms encompass a wide spectrum of genetic, epigenetic, and biochemical processes that dictate the behavior of bladder cells, impacting not only the pathogenesis of diseases but also the design of therapeutic strategies.

Kamoun *et al.* in their study of a global collaborative effort aimed at establishing a unanimous classification for molecular subtypes in IUC [16]. They have defined as they call a consensus grouping of six distinct molecular categories: Luminal papillary (comprising 24% of cases); Luminal no specified (constituting 8% of cases); Luminal unstable (accounting for 15% of cases); Stroma-rich (representing 15% of cases); Basal/squamous (making up 35% of cases); Neuroendocrine-like (comprising 3% of cases) [16]. These consensus categories were shown to exhibit variations in terms of the fundamental oncogenic mechanisms, levels of infiltration by immune and stromal cells, as well as differences in histological and clinical characteristics, which also impact patient outcomes. Additionally, they have reported a classifier that can assign a consensus class label to a tumor sample's transcriptome, providing a valuable tool for further research and clinical applications.

Lopez-Beltran A et al in a set of 91 bladder cancer cases using the NanoString-based four-gene panel gene expression analysis, typically relying on luminal markers (GATA3+/KRT20+) and basal markers (KRT14+/KRT5+/GATA3low/-/KRT20low/-), categorized urothelial bladder carcinoma samples into three distinct groups: luminal, basal, and a third category labeled as null/double negative (non-luminal/non-basal) [17]. These categories demonstrated significant relevance in terms of overall cancer-specific survival (p<0.0001) when considering conventional urothelial carcinoma versus variant histology urothelial carcinoma (p<0.0001), NMIBC versus IUC (p<0.001), or according to AJCC stage categories Ta (p=0.0012) and T1 (p<0.0001) [17]. However, the significance was not observed in the case of T2-T4 (p=0.563). Furthermore, the luminal subtype was more prevalent in NIUC cases with favorable cancer-specific survival (p<0.0001). Conversely, the basal and null

subtypes were linked to aggressive MIBC tumors with shorter cancer-specific survival ($p<0.0001$), some of which exhibited variant histology [17].

The expression of p53, Rb, and Ki-67 has been a subject of investigation in several studies, with a particular focus on their correlation with UC grade, stage, recurrence, and prognosis. The findings from these studies have varied, and at times, they have presented conflicting results.

Both p53 and Rb proteins hold significant roles in the regular cell cycle and the maintenance of cellular homeostasis. Mutations in the TP53 gene have been linked to the advancement of bladder cancer in terms of both stage and grade [18-21]. Kamoun et al. have reported that all molecular subtypes within MIBC exhibit mutations in TP53 and RB1 to varying degrees: Luminal papillary (TP53 32%, RB1 5%), luminal nonspecified (TP53 45%, RB1 5%), luminal unstable (TP53 76%, RB1 22%), stroma-rich (TP53 28%, RB1 21%), and basal/squamous (TP53 61%, RB1 25%) [16].

Additionally, the nuclear accumulation of the p53 protein, as detected by immunohistochemistry, has been shown to align with stage and grade in bladder cancer [18-22]. Moreover, TP53 mutations are considered to be a significant factor in the increased tendency of carcinoma in situ to invade the bladder wall [19].

The assessment of p53 immunohistochemical expression in bladder cancer has employed various cutoff values for immunohistochemical staining (most commonly 10%, but others, including 20% and even up to 50%, have also been used) to distinguish between p53 "positive" and "negative" cases [22]. Cases that exhibited no expression, essentially presenting a null phenotype, were designated as "negative." An updated model for p53 assessments has been introduced [22]. This model distinguishes between "abnormal" and "wild type" designations. According to this approach, abnormal tumors are characterized by either 0% or more than 50% of tumor nuclei exhibiting p53 expression, while wild-type cases fall within the range of 1% to 49% staining [22]. This revised approach to assessing p53 closely aligns with evaluation methods utilized in other organ systems and takes into account the underlying biology of most TP53 mutations. This includes missense mutations resulting in protein overexpression or nonsense mutations leading to a complete absence of protein expression. Theoretically, this model promises a more precise correlation with TP53 mutations in tumor cells. Importantly, this updated method for assessing p53 immunohistochemistry in bladder cancer has been reported to have a strong association with oncologic outcomes.

In our study, we applied a cutoff of 50% between low, weak, moderate, and high p53 expression, and we reported a higher p53 nuclear accumulation in UC with the increasing rate in higher grade and stage tumors ($p=0.001$).

Rb protein is a tumor suppressor that is considered to control cellular responses to oncogenic stimuli and its processes, including DNA damage, cell division, as well as improper mitogenic signals [11].

Robertson et al. in their study of molecular characterization of 412 MIBC patients reported that approximately 89% showed inactivation of the TP53/RB1 signaling pathway, with nearly 50% of these cases having a direct mutation in the TP53 gene, while RB1 mutations were the second most common, accounting for 17% of MIBC cases [23].

RB-1 gene encoded protein expression has been reported to decrease with increasing rate of tumor progression and lower survival rates in UC [24]. In our study however, we did not find any correlation with tumor grade and stage. We consider that if we were to increase the number of our sample, we could potentially confirm decreased Rb expression to correlate with tumor grade and stage.

Ki-67 presents the G1 stage of the cell cycle and is a cell proliferation marker [25]. Various previous studies have reported a correlation between Ki-67 high expression and higher tumor stage and grade as well as with lymph node invasion, lymphovascular invasion, and worse prognosis in bladder cancer [14, 26-29]. He et al. in their meta-analysis study have concluded that Ki-67

increased expression was a risk factor for progression-free survival in NUIC patients that were treated with BCG [14]. Moreover, Tian et al. in their meta-analyses have revealed a strong correlation between elevated Ki-67 expression and reduced survival in bladder cancer patients among non-Asian populations; however, the data indicated no significant link between Ki-67 expression and bladder cancer prognosis in Asian patients [29].

We report a higher proliferative index in higher grade and stage UC of the urinary bladder in our study ($p<0.001$) (Table 5). Furthermore, we report a positive correlation between p53 and Ki- 67 expression ($p<0.0001$) (Table 9).

Our research was focused exclusively on conventional UC of the urinary bladder and the obtained results might differ if we include other possible histological subtypes and reported predictive factors for UC of the urinary bladder, which could be a limitation of our study.

Our study and control groups are limited in size, comprising only patients who underwent transurethral resection at our institution. As a result, our data reflects a limited subset of information and is not generalizable to the broader population. Moreover, testing for TP53 and RB-1 gene alterations could raise the pool of TP53 and RB-1 in our study group when including potential nonsense TP53 alterations as well as RB-1 alterations that could not be detected through the immunohistochemical protein analyses.

Nevertheless, this is the first report on p53, Rb and Ki-67 expression in UC of the urinary bladder of our Kosovo population and our results confirm that p53 protein plays a role in the development and progression of UC of the urinary bladder in our group population as well. Rb protein alterations were not statistically significant in our study; however, there was a decreased tendency of expression for Rb with increasing grade and stage in our population group of UC of the urinary bladder.

We report that p53 and Ki-67 are statistically significantly more expressed in higher grade and stage UC carcinoma of the urinary bladder. Their expression correlates with grade and stage ($p<0.001$ and $p=0.010$, respectively).

Continued investigation into the discovery of potential targeted therapies for p53 and Rb, tailored to various stages of urinary bladder cancer progression, has the potential to enhance treatment strategies and ultimately lead to improved prognoses for this cancer, which currently carries a challenging outlook.

Consequently, gaining a more profound insight into the molecular interactions of these genes and their correlation with other genomic predictors of response holds significant clinical implications. Such understanding may help elicit, at least in part, the responsiveness to therapeutic agents.

CONCLUSION

Our research contributes to the growing body of knowledge on the molecular aspects of UC in the urinary bladder, focusing on the immunohistochemical expression of key proteins-p53, Rb, and Ki-67 proliferative index. The robust correlation observed between p53 and Ki-67 expression and tumor grade and stage underscores their potential as valuable prognostic indicators in UC. While Rb expression did not exhibit a statistically significant correlation with grade and stage in the studied population, the decreasing trend suggests its potential relevance in the progression of UC and warrants further exploration.

FUNDING

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AVAILABILITY OF DATA AND MATERIAL

All original materials and data are available upon request.

AUTHORS' CONTRIBUTIONS

Conceptualization and methodology S.M and S.K. Investigation S.M, S.K and R.L. Original draft preparation and writing R.L and S.M. Review and editing: S.K.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki, and approved by Ethics Committee of Faculty of Medicine, University of Prishtina.

PATIENT CONSENT FOR PUBLICATION

Informed consent was obtained from all subjects involved in the study.

COMPETING INTERESTS

The authors declare no competing interests.

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Not applicable.

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