Impact of reduced levels of *APE1* transcripts on the survival of patients with urothelial carcinoma of the bladder

MARIANA CHANTRE-JUSTINO[1-3], GILDA ALVES[2,3], CONSTANÇA BRITTO[4], ANGÉLICA CARDOSO[4], LUCIANO SCHERRER[5], ALINE DOS SANTOS MOREIRA[6], RAUL QUIRINO[7], ANTONIO ORNELLAS[7,8], ALVARO LEITÃO[1] and CLAUDIA LAGE[1]

[1]Carlos Chagas Filho Institute of Biophysics, Rio de Janeiro Federal University; [2]Research Coordination, National Institute of Cancer (INCA); [3]Circulating Biomarkers Laboratory, Department of Pathology, State University of Rio de Janeiro (UERJ); [4]Molecular Biology and Endemic Diseases Laboratory, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro; [5]Brazilian Society of Clinical Oncology, Belo Horizonte, Minas Gerais; [6]DNA Sequencing Platform, PDTIS/FIOCRUZ; [7]Department of Urology, National Institute of Cancer; [8]Department of Urology, Hospital Mário Kröeff, Rio de Janeiro, Brazil

Abstract. Molecular evidence indicates that alterations in genes involved in the maintenance of genome stability may be related to susceptibility to bladder carcinoma. Our goal was to evaluate the prognostic role of base excision repair (BER) genes in a cohort of patients diagnosed with primary urothelial carcinoma of the bladder (UCB). The levels of all *APE1*, *XRCC1* and *POLB* transcripts were detected by quantitative real-time PCR (qPCR) technique in tumor samples from 52 patients undergoing transurethral resection (TUR) for primary UCB at the Department of Urology, Brazilian National Cancer Institute, Rio de Janeiro. Increased levels of *APE1*, *XRCC1* and *POLB* transcripts were significantly associated with high-grade tumors when compared to these levels in low-grade tumors (p<0.01) and could be attributed to different mechanisms of transcriptional regulation as a response to tumorigenesis and oxidative stress. By analyzing the collected data in the present study, regardless of pathological grade or stage, univariate analysis revealed that the reduced levels of *APE1* transcripts were significantly associated with cancer-specific mortality (p=0.032). Furthermore, the variant genotype (TG/GG) of the APE1 T1349G polymorphism was observed in 75% of a subset of patients who concomitantly experienced reduced levels of the *APE1* transcript and death and/or recurrence events. Taken together, our data reinforce

the idea that human DNA repair mechanisms must be finely regulated in order to avoid instability leading to tumorigenesis and poor clinical outcomes in UCB patients.

Introduction

Urothelial carcinoma of the bladder (UCB) is the second most common genitourinary tumor in men, significantly increasing worldwide cancer statistics. The overall survival rate for bladder cancer recorded for a five-year follow-up period is ~78% (1). It is a heterogeneous neoplasm of unpredictable clinical course. Approximately 75% of cases are classified as non-muscle invasive bladder cancer (NMIBC; pTa/pT1 stage) at initial diagnosis. The remaining 25% of cases consist of tumors displaying the ability of muscular invasion and are classified as muscle invasive bladder cancer (MIBC; pT2-pT4 stage). Genetic studies suggest that the events of initiation, promotion, and progression of UCB can be described by a model based on two distinct molecular alteration patterns showing divergent clinical behavior (2-4). Tobacco smoking and occupational exposure to aromatic amines are considered well-established exogenous risk factors for UCB (5). Furthermore, genetic and epigenetic alterations also contribute to UCB risk (6-9). Thus, interaction between environmental exposure and genetic susceptibility can greatly increase DNA damage and thus trigger urothelial precancerous events; therefore, the prompt elimination of such damage counteracts malignant transformation (10).

DNA repair mechanisms are multistep processes essential for maintaining genomic stability. Distinct DNA repair pathways recognize different types of DNA damage (11,12). The base excision repair (BER) pathway plays a critical role by removing nucleobase modifications by oxidation, alkylation, deamination, and also backbone single-strand DNA (ssDNA) breaks (13). A BER malfunction could thus lead to accumulation of DNA lesions with a direct impact on the risk of tumorigenesis, including UCB (14). The enzyme APE1 has

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Correspondence to: Dr Mariana Chantre-Justino or Dr Gilda Alves, Circulating Biomarkers Laboratory, Department of Pathology, State University of Rio de Janeiro (UERJ), Av. Prof Manuel de Abreu, 444, 4° andar, Vila Isabel, Rio de Janeiro 20550-170, Brazil
E-mail: mari.chantre@gmail.com
E-mail: galvesbrown@gmail.com

a mainframe role in BER by correcting apurinic/apyrimi-dinic (AP) sites, which are pre-mutagenic lesions able to stall DNA replication forks. Besides its role in DNA repair, APE1 is also a transcriptional regulator of gene expression (15). The protein XRCC1 plays a crucial role in BER. Although no direct catalytic activity has been observed for XRCC1, it operates as a scaffold protein for other BER enzymes (16,17). DNA polymerase β (Pol β), a member of the X family DNA polymerases, is the major polymerase in BER due to its dRP-lyase activity and the main gap-filling enzyme (18).

Several molecular markers have been investigated to improve UCB diagnosis and to provide potential therapeutic targets. Gene expression profiling has been used as a strategy to identify molecular subtypes, which could refine the classification of bladder tumors (19-21). However, to date, no analyzed marker has appeared conclusive for clinical practice. The aim of the present study was to investigate whether variations in the levels of transcripts *APE1*, *XRCC1* and *POLB* could influence the clinical outcomes of patients diagnosed with primary UCB. The analyses were performed by quantitative real-time PCR (qPCR) methodology (22). Our findings suggest a significant correlation between the reduced levels of *APE1* transcripts and the poor survival of UCB patients.

Materials and methods

Patient database. Fifty-two patients diagnosed with primary UCB submitted to transurethral resection (TUR) at the Department of Urology, Brazilian National Cancer Institute, Rio de Janeiro, between 2004 and 2009 were eligible for this study. After TUR, fresh tissue samples were immediately stored in liquid nitrogen until further analysis. This project was approved by the Ethics Committee of the Brazilian National Cancer Institute. At recruitment, informed consent was obtained from each eligible subject. Patient records were accessed to collect the following information: date of registration at the hospital, age at diagnosis, gender, pathological grade and stage, recurrence, and disease-specific mortality (DSM).

The specimens obtained were classified as 25 low-grade papillary urothelial carcinoma and 27 high-grade papillary urothelial carcinoma. Pathological staging consisted of 42 NMIBC specimens (Ta/T1 stage) and 10 MIBC specimens (T2-T4 stage). The study group included 43 men and 9 women with a mean age of 68 years (range 48 to 92). Median follow-up was 55.5 months (range 2 to 101 months). Disease recurred in 51.9% of all patients (range 1 to 33 months) and 23.1% died (range 3 to 95 months) of UCB. Table I shows the major clinicopathologic characteristics of the UCB patients in this study.

RNA isolation and cDNA synthesis. Total RNA was isolated from tissue samples using TRIzol® reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). After the extraction process, all samples were treated with DNase I (RNase-free) [Uniscience, New England Biolabs (NEB), Ipswich, MA, USA] to remove any residual DNA from the previous step. RNA quality was evaluated using 1% agarose gel electrophoresis in 1X TBE buffer (Tris-borate-EDTA in DEPC water). Subsequently, RNA quantification was performed on the NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and the

Table I. Characteristics of the investigated UCB patients.

Characteristics	Data
No. of patients with primary tumor diagnosis	52
Mean age (years)	68
Gender, n (%)	
Female	9 (17)
Male	43 (83)
Grade, n (%)	
Low-grade (LG)	25 (48)
High-grade (HG)	27 (52)
Stage, n (%)	
Ta/T1	42 (81)
T2-T4	10 (19)
Follow-up (months)	55.5
Disease-specific mortality (DSM), n (%)	
Low-grade (LG)	5 (20)
High-grade (HG)	7 (26)
Ta/T1	7 (17)
T2-T4	5 (50)
Recurrence, n (%)	
Low-grade (LG)	12 (48)
High-grade (HG)	15 (55.6)
Ta/T1	19 (45.2)
T2-T4	8 (80)

260/280 nm ratio was measured to obtain RNA purity. Total RNA (1 µg) was converted by reverse transcription to cDNA using the SuperScript™ II reverse transcriptase and random primers (both from Invitrogen), according to the manufacturer's instructions. Following cDNA synthesis, the samples were stored at -20°C.

Quantitative real-time PCR analysis. The relative expression of target transcripts was compared with cDNA pools from non-cancerous tissues adjacent to the tumor, which were used as reference samples. Quantitative real-time PCR (qPCR) reactions were performed with 100 ng cDNA samples in uniplex reactions by using a mix of Platinum® SYBR®-Green qPCR SuperMix-UDG (Invitrogen) in Platform Applied Biosystems (ABI PRISM® 7000 sequence detection system; Applied Biosystems, Foster City, CA, USA). Details regarding GenBank accession numbers, primer sequences, Tm values, and amplicon length are listed in Table II. The *GAPDH* gene was used as reference and its expression level was measured in all samples individually to normalize *APE1*, *XRCC1* and *POLB* expression. The relative quantification of the results obtained by qPCR was based on the comparative Ct method ($2^{-\Delta\Delta Ct}$).

The qPCR conditions were conducted as follows: 1 cycle at 50°C (5 min) and 94°C (3 min) for enzyme activation, followed by 40 cycles at 94°C (45 sec), 50°C (30 sec), 72°C (30 sec), and a subsequent final extension at 72°C (5 min). Net gene

Table II. GenBank accession no., primer sequences, amplicon length and the expected Tm value for the qPCR assays.

Gene	GenBank accession no.	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon length (bp)	TM value (°C)
APE1	NM_001641	CAATACTGGTCAGCTCCTTCGG	TCATGCTCCTCATCGCCTATG	127	83
XRCC1	NM_006297	GACACTTACCGAAAATGGCGG	GCCATCATTCCCAATGTCCA	111	83
POLB	NM_002690	CAATGAGTACACCATCCGTCCC	GTTCCCGGTATTTCCACTGGA	110	81
GAPDH	NM_001256799	GCAAATTCCATGGCACCGT	TCGCCCCACTTGATTTTGG	106	82

APE1, apurinic/apyrimidinic (AP) endonuclease; *XRCC1*: X-ray repair cross-complementing protein 1; *POLB*, DNA polymerase β; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

expression for each patient was then compared, as a panel, for low- and high-grade bladder tumors.

Genotyping. Genotyping assays to evaluate the APE1 T1349G polymorphism were performed by sequencing cDNA samples. The PCR amplification was carried out in the Veriti® Thermal Cycler (Applied Biosystems) by using the primers 5'-GCTTCGAGCCTGGATTAAGAA-3' (forward) and 5'-GGCCTGCATTAGGTACATATGCT-3' (reverse) to amplify the target fragment of APE1. PCR conditions were 94°C (10 min), followed by 40 cycles of 94°C (45 sec), 50°C (30 sec), and 72°C (30 sec), and subsequent final extension at 72°C (5 min). PCR products were purified by using GFX PCR DNA and Gel Band Purification kit (GE Healthcare Life Sciences, Chalfont, UK), according to the manufacturer's instructions. The cDNA sequencing was carried out on the Sequencer 3130 Genetic Analyzer (Applied Biosystems) and the results were analyzed with the Sequencher Program (Gene Codes Corporation, Ann Arbor, MI, USA) using the reference sequence from NCBI (NM_001244249.1).

Statistical analyses. Contingency tables were used to associate UCB tumor grade (low-grade vs. high-grade) and stage (pTa/pT1 vs. pT2-pT4) with clinical outcomes of recurrence and DSM. The Fisher's exact test was adopted to test the statistical significance of the association between these parameters. Statistical analysis was carried out with statistical program R (v.2.15.1). The association between tumor grade and stage with expression profile was evaluated by non-parametric Mann-Whitney test with SPSS software (v.17.0). The optimal cut-off point for sensitivity and specificity of the transcripts was estimated by receiver operating characteristic (ROC) curves. Univariable recurrence and survival probabilities were estimated by using the Kaplan-Meier method. Univariable and multivariable Cox regression models addressed time to recurrence and mortality. For all statistical tests, p<0.05 was considered to indicate a statistically significant result.

Results

Association of APE1, XRCC1 and POLB transcript levels and clinicopathological parameters. All samples from MIBC patients (T2-T4 stage) were also classified as high-grade tumors and are referred to in Fig. 1 as HG5, HG8, HG9, HG10, HG11, HG13, HG22, HG23, HG24, and HG25. As shown in Table III, *APE1*, *XRCC1* and *POLB* transcript levels were similar between the NMIBC (Ta/T1) and MIBC (T2-T4) groups and therefore were not associated with stage (p>0.05).

However, as summarized in Table III, statistical analysis revealed highly significant differences in BER transcript levels between low- and high-grade groups (p<0.01). In particular, increased levels of *APE1*, *XRCC1* and *POLB* transcripts were detected in high-grade tumors (Fig. 1 and Table III).

According to Fig. 1, reduced levels of *XRCC1* transcripts were observed in 92% of low-grade tumors, followed by *APE1* (64%) and *POLB* (60%). In addition, the triple combination of reduced levels of *APE1/XRCC1/POLB* transcripts was observed in 40% of this tumor group. With regard to high-grade tumors, high levels of *POLB* transcripts were detected in nearly 77% of these tumors, followed by *APE1* (52%) and *XRCC1* (52%). Likewise, our data revealed that 33.3% of high-grade tumors exhibited combined increased levels of the *APE1/XRCC1/POLB* transcripts.

By observing the panel displayed in Fig. 1, it can be seen that expression of at least one of the BER genes varied among tumor samples of all UCB patients investigated in the present study, appearing different from the levels detected in the noncancerous samples. These findings place BER gene transcriptional activity as a common genetic alteration in UCB pathogenesis.

Correlation of clinicopathological characteristics with clinical outcomes. To assess whether tumor grade or stage has an influence on clinical outcomes, we investigated the relationship between these parameters. The presence of the first recurrence episode was associated with neither tumor stage nor tumor grade (Table IV). Similarly, there was no association between tumor grade and DSM (p=0.746). However, the mortality rate was higher and positively associated with advanced pathological stage (p=0.039).

Regarding the overall survival rate recorded at two and five years after diagnosis, no significant difference between low- and high-grade groups was observed (p=0.596, Table IV). However, MIBC patients had significantly shorter overall survival compared with the NMIBC patients (p=0.013). In relation to recurrence-free survival, we did not observe a significant difference between tumor grade or stage (p>0.05).

Association of reduced levels of APE1 with cancer-specific mortality. The levels of *APE1*, *XRCC1* and *POLB* transcripts

Low-grade (LG)				High-grade (HG)			
Cases	Expression values of transcripts			Cases	Expression values of transcripts		
	APE1	*XRCC1*	*POLB*		*APE1*	*XRCC1*	*POLB*
LG 1	2.07	0.11	0.27	HG 1	4.87	3.55	4.14
LG 2	1.85	0.20	0.33	HG 2	3.27	2.50	2.90
LG 3	1.70	0.30	0.97	HG 3	1.52	4.67	2.58
LG 4	0.79	0.24	0.40	HG 4	2.13	1.31	0.33
LG 5	1.03	0.13	0.57	HG 5	1.12	0.30	2.97
LG 6	0.61	0.19	0.13	HG 6	2.16	0.58	2.13
LG 7	0.85	0.22	0.77	HG 7	1.75	0.80	2.27
LG 8	0.61	0.19	0.33	HG 8	0.10	1.80	0.62
LG 9	0.51	0.19	0.57	HG 9	1.63	1.44	1.73
LG 10	0.37	0.51	0.30	HG 10	1.44	0.58	3.27
LG 11	0.43	1.70	0.73	HG 11	1.28	0.38	-
LG 12	0.65	0.29	0.27	HG 12	0.84	0.61	1.05
LG 13	1.83	0.85	0.86	HG 13	4.85	2.36	1.35
LG 14	0.17	0.09	0.80	HG 14	2.00	1.82	5.31
LG 15	0.53	0.21	0.40	HG 15	2.09	2.18	2.70
LG 16	0.61	0.20	0.74	HG 16	0.15	0.13	0.62
LG 17	0.04	0.33	1.55	HG 17	0.51	0.69	2.48
LG 18	0.45	0.26	0.63	HG 18	0.29	0.49	1.66
LG 19	0.61	0.17	0.52	HG 19	0.81	2.02	3.64
LG 20	0.35	0.40	0.16	HG 20	1.10	0.64	1.39
LG 21	0.36	0.26	1.17	HG 21	0.85	4.03	1.30
LG 22	0.43	0.25	0.61	HG 22	1.68	0.63	3.10
LG 23	0.73	0.33	0.93	HG 23	0.21	0.18	0.33
LG 24	0.49	0.10	2.07	HG 24	0.98	1.06	1.70
LG 25	0.90	0.05	0.13	HG 25	0.50	2.48	0.54
				HG 26	3.38	2.71	3.14
				HG 27	2.74	1.49	2.77

Figure 1. Panel of variations in transcripts levels for *APE1*, *XRCC1* and *POLB* in low- and high-grade bladder tumors. The levels of all transcripts were normalized against *GAPDH* levels. Ratios <0.70 indicate reduced levels and are highlighted in green; ratios of 0.70-1.29 are in gray and represent similar levels to reference samples; ratios ≥1.30 are in red and show elevated levels. The symbol (-) represents a transcript not analyzed due to insufficient quantity of sample from patient HG11. The cut-off values of transcripts levels were arbitrarily chosen at 30% above or below the levels of normal mucosa adjacent to the tumor. *APE1*, apurinic/apyrimidinic (AP) endonuclease; *XRCC1*: X-ray repair cross-complementing protein 1; *POLB*, DNA polymerase β.

Table III. Median variation in transcript levels in each UCB group.

	Median value of transcript expression			Median value of transcript expression		
Transcripts	Low-grade (n=25)	High-grade (n=27)	P-value	Ta/T1 stage (n=10)	T2-T4 stage (n=42)	P-value
APE1	0.61	1.44	0.005[a]	1.18	1.38	0.745
XRCC1	0.22	1.31	0.000[a]	0.90	1.12	0.150
POLB	0.57	2.20	0.000[a]	1.35	1.73	0.235

[a]p<0.01 represents a significant difference. *APE1*, apurinic/apyrimidinic (AP) endonuclease; *XRCC1*, X-ray repair cross-complementing protein 1; *POLB*, DNA polymerase β.

were compared with the selected clinical outcomes (DSM or recurrence) in each tumor grade and stage group to assess predictive value. Overall, no significant correlation was observed between transcript levels and DSM or recurrence, in either pathological grade or stage (data not shown). Despite that, by analyzing all patients in the study regardless of tumor stage or grade, univariable analysis revealed that reduced levels of *APE1* transcripts were significantly associated with DSM (p=0.032, Fig. 2). According to the ROC plot, specificity and sensitivity for the *APE1* gene were, respectively, 60% and

75%. Therefore, these results revealed that *APE1* expression may be an indicator of poor prognosis in UCB patients.

APE1 T1349G polymorphism. Genotyping assays to evaluate the *APE1* T1349G polymorphism were performed by preferentially sequencing the cDNA samples from a subset of patients who concomitantly presented reduced levels of *APE1* transcripts and death and/or recurrence events (Fig. 3). As summarized in Table V, the G allele variant was observed in 75% (9/12) of this subset, being present in 67% (6/9) among

Table IV. Correlation of the pathological grade and stage with overall survival and recurrence-free survival in the UCB patients.

| | Grade | | | | Stage | | |
Parameters	Low-grade (n=25)	High-grade (n=27)	P-value	Parameters	Ta/T1 (n=42)	T2-T4 (n=10)	P-value
First recurrence, n (%) episode[a]	12 (48)	15 (55.6)	0.786	First recurrence, n (%) episode[a]	19 (45.2)	8 (80)	0.078
DSM[a], n (%)	5 (20)	7 (25.9)	0.746	DSM[a], n (%)	7 (16.7)	5 (50)	**0.039[c]**
Overall survival rate[b]				Overall survival rate[b]			
2-year	95.2%	77.3%	0.596	2-year	92.3%	57.1%	**0.013[c]**
5-year	78.1%	73.2%		5-year	82.9%	45.7%	
Recurrence-free survival[b]				Recurrence-free survival[b]			
2-year	95.2%	85%	0.457	1-year	31.6%	25%	0.585
5-year	65.1%	63.6%		2-year	10.5%	12.5%	

[a]The Chi-square test was adopted to test the statistical significance of the association between these variables. [b]Statistical differences evaluated by the log rank test. [c]P<0.05 represents a significant difference. DSM, disease-specific mortality.
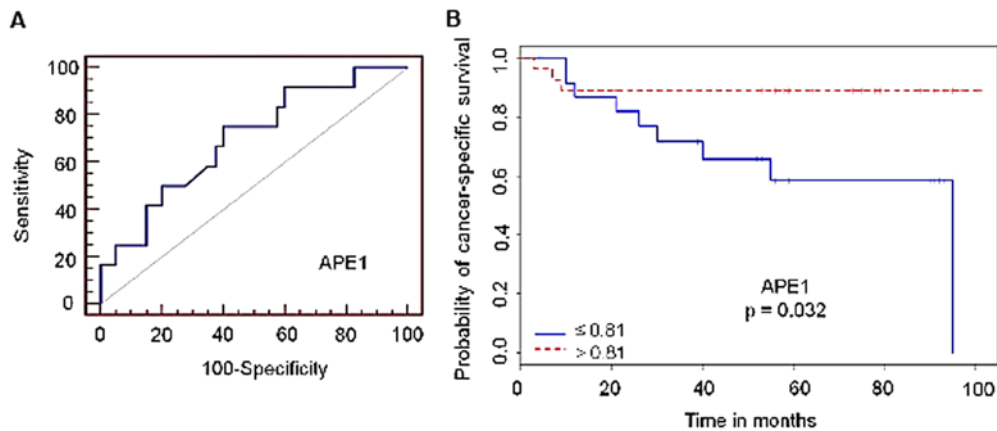

Figure 2. Probability of bladder cancer-specific mortality relatively to APE1. (A) Receiver operating characteristic (ROC) curve for APE1 levels in all UCB patients achieved 75% sensitivity and 60% specificity. The optimal cut-off value was 0.81 and the calculated area under the curve (AUC) was 0.697 (95% CI 0.554-0.817). (B) Kaplan-Meier survival curves depicting cancer-specific survival (%) of bladder cancer patients for APE1 (red line, altered when combined score was >0.81). APE1, apurinic/apyrimidinic (AP) endonuclease.
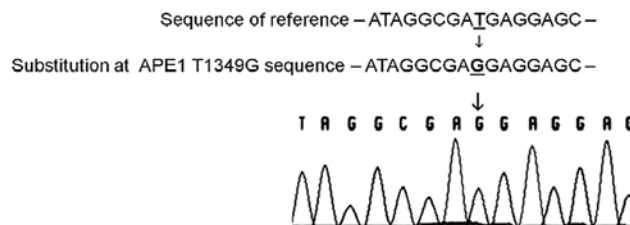

Figure 3. APE1 T1349G polymorphism in the UCB patients. Representative chromatogram of the sense sequence from patient LG19 showing the T to G transversion at APE1 T1349G, as indicated by arrows.

the low-grade cases analyzed. In addition, the variant geno-type (TG/GG) was found in 80% (4/5) of the low-grade cases, whose outcome was either death and/or recurrence. All high-grade patients analyzed had variant genotype (TG/GG), while only individuals with the variant genotypes TG (T2-T4 stage) succumbed to the disease.

Discussion

Urothelial carcinoma of the bladder (UCB) is a heterogeneous disease comprising multiple and complex molecular changes associated with initiation and tumor progression. Previous indications that pro-oxidant status appears to play a role in

Table V. Genotype frequencies of the APE1 T1349G polymorphism among the UCB patients.

	Low-grade (LG), n=9			High-grade (HG), n=3		
Genotype	n (%)	Death	Recurrence	n (%)	Death	Recurrence
TT	3 (33)	LG17	LG17	0 (0)	-	-
TG	3 (33)	LG23	LG23	2 (67)	HG8; HG23	HG8
GG	3 (33)	LG11; LG19; LG22	LG11; LG19; LG22	1 (33)	-	HG17

genitourinary carcinogenesis and its correlation with tumor invasion and metastasis encouraged our team to screen for molecular markers of genetic stability as an attempt to improve UCB prognosis (23,24). DNA repair mechanisms act as a barrier to prevent genetic instability and cancer susceptibility. A few studies have evaluated the association between dysfunction/dysregulation in particular DNA repair genes and UCB risk (25,26). Since APE1, XRCC1 and POLB proteins operate downstream the same DNA repair pathway to eliminate DNA oxidative damage, their expression above basal levels found in our study could reveal excessive oxidative stress in high-grade bladder tumors.

XRCC1 protein is required for efficient DNA repair of other BER-involved enzymes. Notwithstanding its core interplay in BER, a recent meta-analysis did not associate a dysfunctional *XRCC1* polymorphism to UCB risk (27). Increased levels of *XRCC1* transcripts were also associated with chemoresistance to cisplatin in non-small cell lung cancer (28). The set of results presented in this study revealed that *XRCC1* transcripts were increased in 52% of high-grade tumors, whereas 92% of low-grade tumors displayed reduced levels of *XRCC1*. Since single nucleotide polymorphisms (SNPs) cannot be ascribed to such status, transcriptional alterations for *XRCC1* were revealed in this study to better characterize the histopathological grade.

In higher eukaryotes, DNA polymerases participate in replication, repair and recombination. Pol β is the major BER DNA polymerase. Pol β is minimally expressed in normal cells but has been shown to be overexpressed in different tumors both at the transcriptional and protein levels, including bladder tumors (29). Approximately 30% of all human tumors express variant Pol β (30). As previously described in other studies, Pol β destabilization plays an important role in disrupting cell cycle control and increasing mutation rates. Being an error-prone DNA polymerase, excess Pol β could disturb the processivity of replication forks and increase genetic instability and tumorigenesis by curtailing the activity of error-free DNA polymerases during replication (31,32). Furthermore, Pol β can also enhance mutagenesis by incorporation of ribonucleotides into DNA and by competition with replicative DNA polymerases (33,34). Our results revealed that the *POLB* transcripts had increased levels in ~77% of high-grade tumors, suggesting a strong correlation of *POLB* dysregulation with tumorigenesis and progression events.

APE1 is a multifunctional protein essential in BER and a redox transcriptional co-activator. APE1 participates in the initial steps of BER by recognizing and nicking potentially genotoxic and mutagenic abasic (AP) sites in different substrates, such as double-strand DNA, hybrid DNA/RNA and RNA molecule (35). APE1 elevated levels have been reported to occur in a group of tumors (36-38). In general, its increased levels provide resistance to chemotherapeutic drugs and ionizing radiation and, as expected, marked cisplatin sensitivity was observed to occur *in vitro* in tumor cells knocked out for APE1 (39,40). Nonetheless, Sak *et al* reported that increased APE1 protein levels were correlated with improved cancer-specific survival in patients with muscle-invasive bladder cancer following radical radiotherapy (41). In the present study, reduced *APE1* levels at initial diagnosis were significantly associated with poor clinical outcome, regardless of the chosen therapeutical approach, pathological grade, or stage. Moreover, reduced levels of *APE1* also compromise the regulation of p53 target genes through its redox activity and may result in extensive genomic instability. All of these factors could explain why reduced levels of *APE1* transcripts resulted in poorer UCB patient survival, causing it to be an important gene for clinical cancer-specific survival prediction.

In our study, differences in clinical outcomes such as recurrence and DSM were not correlated with pathological grade. However, we identified higher mortality rates and reduced overall survival in patients with advanced tumor stage. Since all MIBC patients analyzed were also designated as high-grade tumors, the characteristic highly aggressive profile of these tumors could explain the increased mortality and poor survival in this subset of UCB patients. The comprehensive profiling of BER transcripts in each pathological grade group highlights meaningful differences in the molecular signature of low- and high-grade bladder tumors that could be attributed to differential transcriptional regulation ensued by tumorigenesis and oxidative stress. Therefore, these differences in BER transcript levels able to characterize two distinct histologic UCB groups found in our study could complement molecular data from previous studies associating low- and high-grade UCB groups with distinct genetic alteration patterns (2-4). Moreover, *APE1* analyses presented good specificity and sensitivity parameters (60% specificity and 75% sensitivity) which represent important information for a suitable clinical test.

In carcinogenesis events, the most extensively studied polymorphism in the *APE1* gene is the T to G transversion (T1349G, also known as Asp148Glu, rs3136820) (42). Functional studies on this polymorphism have shown that the G variant allele may have an impact on APE1 endonuclease and reduce the ability to communicate with other BER proteins (43). In our study, some patients with reduced levels of *APE1* transcripts also experienced death and/or recurrence

events. In this context, we performed an additional analysis to investigate the presence of the *APE1* T1349G polymorphism from this group of patients. Our data pointed out that all high-grade patients analyzed had variant genotypes (TG/GG), but only individuals with genotype TG (T2-T4 stage) succumbed to the disease. With regard to the low-grade group, the G allele variant occurred in 80% (4/5) of the cases, whose outcome was mortality and/or recurrence events. In a study for functional characterization of polymorphisms in DNA repair genes and chromosome aberrations by using X-rays or ultraviolet (UV) light to irradiate blood lymphocytes, samples from individuals with the TG or GG genotype showed higher levels of damage, including aberrant cells, chromatid breaks, chromatid exchanges, deletions, and dicentrics (44). A meta-analysis study showed that individuals carrying at least one G allele were associated with a higher cancer risk than subjects with the TT genotype (45). However, a meta-analysis study performed in 2013 suggested that the *APE1* T1349G polymorphism was not associated with bladder cancer risk among Asians or non-Asians (46). Consistent with these observations, our data suggest that besides interfering with APE1 enzymatic activity, as shown in other studies, the G allele may also modify the *APE1* transcriptional levels and result in worse clinical outcome for bladder cancer patients. Our small sample size for this polymorphism analysis (n=9 for low-grade and n=3 for high-grade groups) should be interpreted with caution, as more detailed data are needed to verify these findings. However, taken together, our results indicate that *APE1* is an attractive candidate gene to open new perspective studies for evaluating clinical outcomes of UCB patients. Further analyses are required to validate *APE1* as a reliable predictive molecular marker for UCB.

Finally, the present study thus reinforces the notion that DNA repair gene expression must be finely tuned in order to avoid genetic instability and the process of tumorigenesis (47). Despite current effective treatments to ensure better results for UCB patients, diagnostic and prognostic exams are still partially accurate and should be complemented with auxiliary tests. Therefore, more clinical research on UCB should be encouraged to allow a significant increase in clinical data, better comprehension of the biology of the disease, and optimization of diagnostic and prognostic tests (48).

In conclusion, evaluation of DNA repair transcripts revealed that high-grade tumors exhibited elevated levels of *APE1*, *XRCC1*, and *POLB* as compared with low-grade tumors. Taken together, this panel adds information on the histopathology of bladder tumors. By analyzing all patients, only *APE1* reduced levels were an independent predictor of cancer-specific mortality in primary UCB regardless of pathological grade or stage, and *APE1* may represent a possible candidate gene for evaluating clinical outcomes in UCB. Despite this, evidence suggests that dysregulated BER transcription may promote bladder carcinogenesis.

Acknowledgements

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