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Implications of androgen receptor and FUS expression on tumor progression in urothelial carcinoma

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Summary. Androgen receptor (AR) interact with many pathways involved in bladder cancer development and progression. FUS (fused in liposarcoma), a multifunctional protein essential for different cellular processes, has been demonstrated as a key link between androgen receptor signaling and cell-cycle progression in prostate cancer but has not been examined in urothelial carcinoma (UC) despite an intimate association between prostate and bladder carcinogenesis.

Aim. to examine the immunohistochemical expression of AR and FUS in urothelial carcinoma in relation to prognostic parameters and to extrapolate any possible link between the expression of both markers and tumor progression.

Study design. Retrospective study using immuno-histochemical staining for AR and FUS on (88) cases of urothelial carcinoma.

Results. AR shows statistically significant relations with late tumor stage, high tumor grade, and non-papillary tumor pattern. On the other hand, FUS expression correlates with early tumor stage, low tumor grade and papillary pattern. An inverse relation is found between AR and FUS expression (p=0.001). Cases with high AR IHC expression show statistically significant shorter OS, RFS and PFS compared to cases with low AR expression. Cases with high FUS IHC expression reveal statistically significant longer OS, RFS and PFS compared to cases with low FUS expression.

Conclusion. FUS expression is associated with favorable prognostic parameters of UC. A possible interaction is suggested between FUS and AR pathways involved in urothelial cancer progression. Manipulating FUS levels and androgen deprivation therapy can provide new promising targets for treatment trials.

Key words: Urothelial carcinoma, Androgen receptors, FUS, Immunohistochemistry, Tumor progression

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Introduction

Urothelial carcinoma (UC) is a commonly diagnosed malignancy worldwide (Jemal et al., 2011). The incidence of bladder cancer is reported to be 3-4 times greater in men than in women (Dobruch et al., 2016). In Egypt, the male-to-female ratio is 3.5: 1 (El-Sharkawi et al., 2014).

The gender specific difference in incidence and aggressiveness of UC is believed to be influenced by environmental factors, such as cigarette smoking and industrial chemicals; however, it remains a preferental disease in men even after lowering these risk factors (Kamat et al., 2016). The male genital organs including the prostate, bulbourethral gland, and urothelium are derived from the urogenital sinus endoderm.

The androgen receptor (AR) has been documented to be a prerequisite for the differentiation of the prostate and its development (Thomas et al., 2008). Prostate cancer was also proven to be invariably dependent on the AR pathway. Accordingly, AR signaling was concluded to contribute in the development of bladder cancer (Wilson and Mc Phaul, 1996; Pelletier, 2000). Coupled with heat shock proteins, AR is located in the cell cytoplasm, and after being released from heat shock proteins, it translocates into the nucleus upon binding with androgens. In the nucleus, it binds with coregulators and androgen response elements (AREs), resulting in either activation or inhibition of gene transcription (Heinlein and Chang, 2004; Mudryj and Tepper, 2013). AR and related signaling pathways were found to be involved in the etiology and progression of bladder cancer (Li et al., 2012; Miyamoto et al., 2012a,b; Hsu et al., 2013). Although the precise mechanism of function of AR in urothelial cells is still poorly understood, several AR coregulators have been implicated in the modulation of urothelial tumorigenesis and tumor progression through a cross-talk between AR coregulators and other signaling pathways in bladder cancer cells (Li et al., 2017).

Fused in Ewing's sarcoma (FUS), also known as translocated in liposarcoma (TLS), is a member of the TET family of RNA-binding proteins. These proteins are

similar in structure and function and play a role in cellular processes such as gene expression, genome protection, and the mRNA and microRNA process (Trautmann et al., 2017). FUS was originally identified in human myxoid and round cell liposarcomas as an oncogenic fusion with the stress-induced DNA-binding transcription factor CCAAT/enhancer-binding protein homologous protein (Crozat et al., 1993; Rabbitts et al., 1993). It is a multifunctional protein essential for different cellular processes, such as genomic stability, RNA metabolism, and stress response (Sama et al., 2014). It was also noticed that FUS appears at DNA damage sites and was suggested to have a role in DNA repair response (Wang et al., 2013).

Recent studies have reported that FUS is directed to the regulatory regions of target genes by noncoding RNA transcripts tethered to the DNA, leading to the repression of transcription by binding to and inhibiting complexes bound to such elements (Wang et al., 2008). The studies of Brooke et al. (2011) and Ghanbarpanah et al. (2018) on prostate cancer highlighted that FUS exhibits certain characteristics of a tumor suppressor; it significantly retards androgen-induced prostate cancer cell growth in vitro and in vivo, regulates the expression of several factors involved in cell-cycle progression such as cyclin D1 and P27, and induces G1 arrest and apoptosis. Immunohistochemical (IHC) results performed on human tissue arrays revealed that FUS expression is inversely correlated with prostate tumor grade and bone metastases but directly correlated with survival and concluded that loss of FUS expression is important in disease progression (Brooke et al., 2011).

Many studies reported the expression pattern and the role of AR in the development and progression of UC (Chen et al., 2017; Li et al., 2017; Elzamy et al., 2018). However, to the best of our knowledge, there is no available data on the expression of FUS or its possible link with AR expression in UC. This study aimed to study the IHC expression of AR and FUS in UC, to correlate their expression with prognostic parameters of this tumor, and to educe any possible link between the expression of both markers and tumor progression.

Materials and methods

This retrospective study included 88 cases of UC collected from the archived files of the Pathology Department at El-Demerdash Hospital during the period from 2011 to 2018. Our cases included radical cystectomy specimens (followed by adjuvant therapy) and transurethral resection of the bladder tumor specimens with adequate deep muscle layer to assess tumor invasion. Cases with missing clinical data and follow-up data, cases with inadequate tissue for staining, superficial specimens with missing deep muscle layer, and cases who received neoadjuvant therapy before surgery were excluded from the study.

Clinical data such as age, sex, and history of treatment or recurrence were retrieved from the archived patient's files. Histopathologic data such as tumor size, grade, and TNM stage were obtained by reviewing the gross description and examining the hematoxylin and eosin-stained sections of each case. Tumors were classified into low-grade or high-grade UC according to the World Health Organization/International Society of Urological Pathology 2004/2016 (Compérat et al., 2019) and were staged into superficial (non-detrusor muscle [DM] invasive, NMI) and deep (DM invasive, MI) according to the American Joint Committee on Cancer, 8th edition (Amin et al., 2017).

Follow-up data of patients were reviewed to determine (a) overall survival (OS), which was calculated based on the date of major surgery and the date of the last follow-up or death; (b) recurrence free survival (RFS), which includes any recurrence (local or distant) and was calculated based on the date of major surgery or the last session of adjuvant therapy and the date of recurrence (local recurrence or distant metastasis) at the last follow-up; and (c) progression free survival (PFS), which includes metastatic tumor progression or death and was calculated based on the time between the date of major surgery and metastatic tumor progression or death.

Ethical approval

All materials were used in accordance with the ethical guidelines of the Pathology Department at the Ain Shams University, and the approval was granted by the institutional research ethics committee. All patients who participated in this study signed a written informed consent before biopsy procedure.

Immunohistochemical staining

IHC expressions of both AR and FUS were assessed using the streptavidin-biotin immunoperoxidase technique. Two tissue sections (5 mm each) were cut from selected formalin-fixed, paraffin-embedded tissue blocks. Deparaffinization in xylene and rehydration in a graded series of ethanol were performed. Endogenous peroxidase was blocked using 0.5% solution of hydrogen peroxide. Antigen retrieval was done by boiling the slides in a 0.01 μ buffer solution at PH 6.0 using a microwave. Then, the slides were incubated overnight at 4°C with primary Monoclonal Mouse Anti-Human AR (Catalog# YPA1811, Chongqing Biospes Co., Ltd. China, dilution 1:500) and Monoclonal Mouse Anti-Human FUS antibody (Catalog# YMA1152, Chongqing Biospes Co., Ltd. China, dilution 1:40) Avidin-biotin detection kit with diaminobenzidine as the chromogen was used to detect antibody reaction. According to the manufacturer's protocol, sections known to stain positively were included in each batch, and negative controls were prepared by replacing the primary antibody with Tris-buffered saline.

Interpretation of FUS immunostaining

A combined score of the percentage of positive

tumor nuclei per field and staining intensity was used for the evaluation of FUS immunostaining. The percent was scored as 0 (no staining), 1 (>0% to 25%), 2 (>25% to 50%), 3 (>50% to 75%), and 4 (>75%). The intensity was determined as 0 (none), 1 (weak), 2 (moderate), and 3 (strong). The final score was the summation of both parameters and was divided into negative (0), weak positive (>1-3), moderate positive (4-5), and strong positive (6-7). For simplification and statistical analysis, the negative and weak positive staining was designated as the FUS low expression level group, whereas the moderate and strong positive staining was designated as the FUS high expression level group (Xiong et al., 2018).

Interpretation of AR immunostaining

The immunoreactive score was calculated by multiplying the percentage of immunoreactive cells (0%=0; 1-10%=1; 11-50%=2; 51-80%=3; 81-100%=4) by staining intensity (0, negative; 1, weak; 2, moderate; 3, strong). Scores (range, 0-12) were considered negative (0; 0-1), weakly positive (1+; 2-4), moderately positive (2+; 6-8), and strongly positive (3+; 9-12) (Miyamoto et al., 2012a,b).

For simplification and statistical analysis, the negative and weak positive staining was designated as the low expression level group, whereas the moderate and strong positive staining were designated as the high expression level group for both markers.

Data management and analysis

Data were revised, coded, entered on the computer, and analyzed using the SPSS package version number 20. Quantitative data were tested for normality with Shapiro-Wilk test and expressed as mean (standard deviation [SD]) for parametric numerical data or median (interquartile range) for nonparametric numerical data. Qualitative data were expressed as frequencies (n) and percentage (%). Chi-square and Fisher exact tests were used to evaluate the association between qualitative variables. Kappa statistics was used to examine the agreement between AR and FUS IHC with values of <0 as indicating no agreement and 0-0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as almost perfect agreement. The Kaplan Meier curve was used to describe OS, RFS, and PFS, whereas the log-rank test was used to compare between groups. P≤0.05 was considered statistically significant.

Results

Patients

A total of 88 UC cases were included in this study, 66 of which were males (75%) and 22 were females (25%). The mean age was 60.25 years (SD, ±8.82) (range, 43-78 years). The details of clinicopathologic characteristics are presented in Table 1.

Table 1. Clinicopathologic characteristics among studied cases.

		Mean	±SD
Tumor size, cm		5.6	2.6
Detrusor muscle invasion	Negative	22	25.0%
	Positive	66	75.0%
Tumor pathologic stage	Ta	9	10.2%
	T1	13	14.8%
	T2	27	30.7%
	T3	32	36.4%
	T4	7	8.0%
Tumor pathologic grade	Low	9	11.3%
	High	79	89.7%
Lymph node stage*	N0	48	70.6%
	N1	12	17.6%
	N2	8	11.8%
Tumor pattern	Nonpapillary	57	64.8%
	Papillary	31	35.2%
Vascular invasion	Negative	76	86.4%
	Positive	12	13.6%

*Total number of UC cases with submitted lymph nodes=68 of the 88 cases.

Table 2. Relationship between AR expression and clinicopathologic characteristics.

	Androgen receptor IHC expression				P value
	High		Low		
	N	%	N	%	
Tumor stage					
Ta	1	11.1%	8	88.9%	0.003**
T1	3	23.1%	10	76.9%	
T2	11	40.7%	16	59.3%	
T3	22	68.8%	10	31.2%	
T4	5	71.4%	2	28.6%	
Tumor grade					
Low grade	1	11.1%	8	8.9%	0.031**
High grade	41	51.9%	38	48.1%	
Lymph node stage					
N0	20	41.7%	28	58.3%	0.39
N1	8	66.7%	4	33.3%	
N2	4	50%	4	50%	
Tumor size					
≤3 cm	16	40.0%	24	60.0%	0.185
>3 cm	26	54.2%	22	45.8%	
Tumor pattern					
Nonpapillary	38	66.7%	19	33.3%	0.0001*
Papillary	4	12.9%	27	87.1%	
Vascular invasion					
Absent	35	46.1%	41	53.9%	0.43
Present	7	58.3%	5	41.7%	

*Chi-square tests. **Fisher exact test

Immunohistochemical analysis

Notably, 42 of the 88 UC cases (47.7%) showed high AR nuclear expression, whereas the rest of the cases (52.3%) showed low AR nuclear expression. Focal nonspecific cytoplasmic staining of the muscle layer was identified in some cases (Fig. 1).

Of the 88 UC cases, 34 (38.6%) showed high FUS nuclear expression, whereas 54 cases (61.4%) showed low FUS nuclear expression (Fig. 2).

Correlation between AR, FUS, and clinicopathological parameters

Age and gender did not show any statistically significant relationships with the studied markers. Both AR and FUS showed statistically significant associations with tumor stage, tumor grade, and tumor pattern, such that high AR expression correlated with late tumor stage (P=0.003), high grade (P=0.001), and nonpapillary pattern (P=0.0001). In contrast, high FUS expression correlated with early tumor stage (P=0.002), low grade (P=0.001), and papillary pattern (P=0.0001). However, the association with tumor size was nonsignificant for both markers. Only FUS expression showed a significant association with vascular invasion (P=0.04), such that high FUS expression correlated with absent vascular expression (Tables 2, 3).

There was a moderate highly statistically significant agreement (kappa=0.424, P=0.001) between AR and FUS IHC expression, such that 83.3% of high AR cases showed low FUS expression and 59% of low AR cases showed high FUS expression (Fig. 3, Table 4). In this sense, the adjacent normal urothelium in the studied UC cases showed no AR expression, but high FUS nuclear expression (Fig. 3).
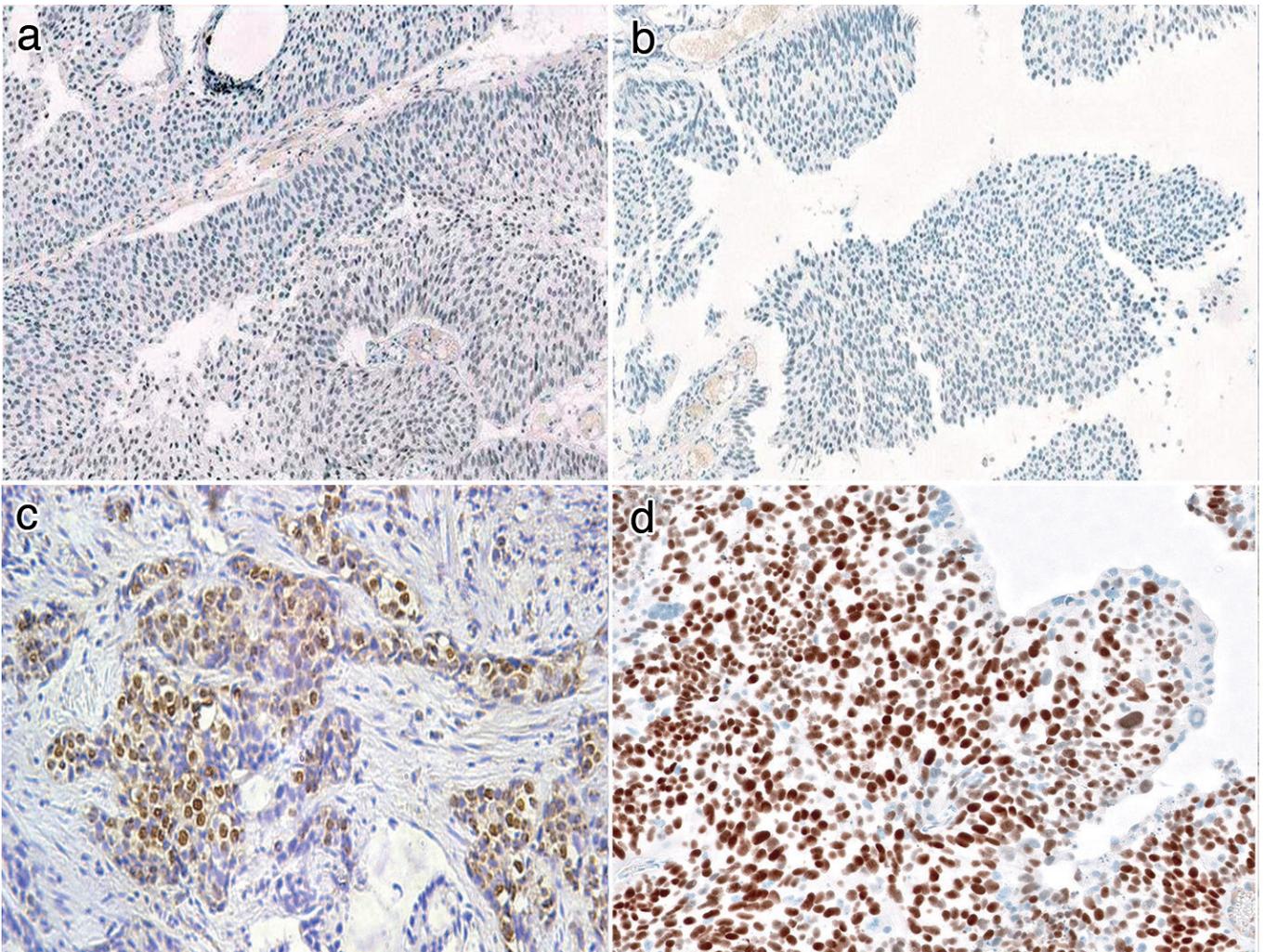
Fig. 1. Androgen receptor (AR) immunohistochemical expression in urothelial carcinoma. **a, b.** Low AR in low-grade urothelial carcinoma. **c, d.** High nuclear AR expression in most of the tumor cells in high-grade urothelial carcinoma. **c.** Lamina propria invasion. (IHC). a, b, x 100; c, d, x 200.

Survival analysis

The OS, RFS, and PFS among UC cases included in this study were 57.8% at 86 months, 27.2% at 85 months, and 57.8% at 86 months, respectively. Cases with high AR IHC expression showed statistically significant shorter OS, RFS, and PFS than cases with low AR expression, such that OS was 38% at 84 months vs 75% at 86 months, respectively (P=0.0001); RFS was 9.5% at 84 months vs 43.5% at 86 months, respectively (P=0.038); and PFS was 38.1% at 84 months vs 75.6% at 86 months, respectively (P<0.001) (Fig. 4).

In contrast, cases with high FUS IHC expression reveal statistically significant longer OS, RFS, and PFS than cases with low FUS expression, such that OS was 79.4% at 86 months vs 44.1% at 85 months, respectively (P=0.001); RFS was 49.6% at 84 months vs 13% at 85 months, respectively (P=0.001); and PFS was 75.6% at 86 months vs 38.1% at 84 months, respectively (P<0.001) (Fig. 5).

Meanwhile, cases with combined low AR and high FUS IHC expressions revealed statistically significant longer OS, RFS, and PFS than cases showing combined high AR and low FUS expressions, such that OS was 81.5% at 86 months vs 31.5% at 67 months, respectively (P=0.001); RFS was 48.1% at 86 months vs 0% at 24 months, respectively (P=0.001); and PFS was 81.5% at 86 months vs 31.4% at 67 months, respectively (P<0.001) (Fig. 6).

Discussion

The molecular mechanism of bladder cancer development is still poorly understood (Zhang et al., 2020). The identification of key molecules involved in bladder carcinogenesis is mandatory because it can provide novel targeted therapies and novel prognostic markers (Ide et al., 2017). Recent studies have proposed
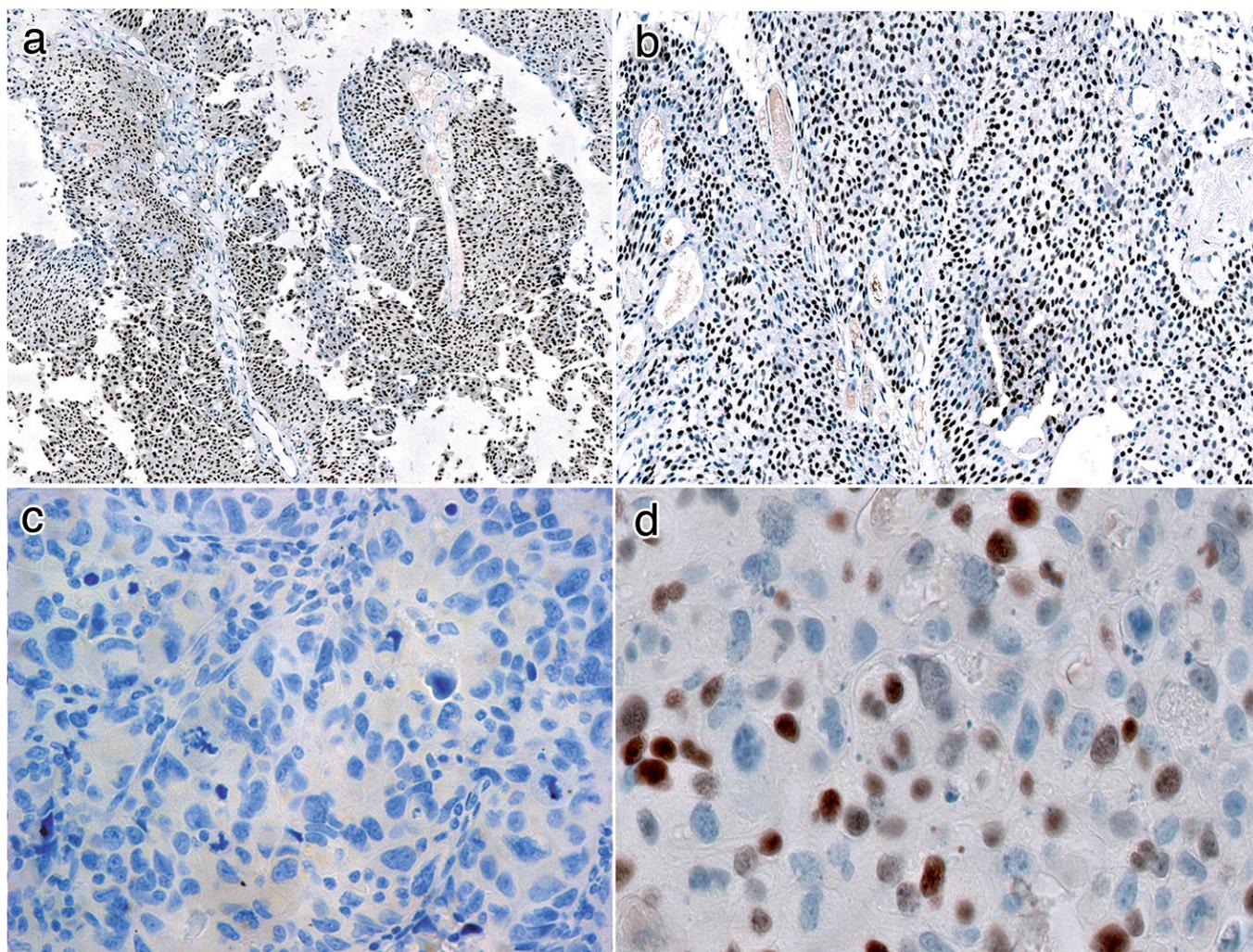
Fig. 2. FUS immunohistochemical expression in urothelial carcinoma. **a, b.** High FUS nuclear expression in low-grade urothelial carcinoma. **c.** Negative FUS expression in high-grade urothelial carcinoma. **d.** Focal FUS nuclear staining in high-grade urothelial carcinoma. (IHC). a, b, x 100; c, d, x 400.

that AR interacts with many pathways that are involved in the development and progression of bladder cancer (Miyamoto et al., 2012a,b). Downstream targets of the AR are important for characterizing the disease and identifying new therapeutic targets. FUS had been demonstrated as a key link between AR signaling and cell-cycle progression in prostate cancer (Brooke et al., 2011). Other studies had suggested an intimate association between prostate and bladder carcinogenesis (Marcinkiewicz et al., 2012). Hence, it is of interest to discover a similar link between AR and FUS expression in UC. However, there is no available data in the literature about the expression and the possible role of FUS in urinary bladder carcinogenesis. Therefore, this study was designed to examine the expression pattern of FUS in UC and to assess the possible link between AR and FUS and their implication on the progression of UC.

This objective was achieved by correlating the expression of each marker with the clinicopathologic characteristics and follow-up of disease progression of the tumors and then correlating the expression of both markers with each other.

In this study, AR showed high expression in 47.7% of the cases. This percentage was similar to other studies in which AR expression in UC ranged from 42% to 53.1% (Boorjian et al., 2004, 2009; Kauffman et al., 2011; Miyamoto et al., 2012a,b). The mean patients' age in this study was 60 years, and most of the patients (78.4%) were >50 years old. This was in accordance with the study of Pachauri et al. (2017) who attributed older age to the cumulative effects of long-time exposures to carcinogens and the defective DNA repair mechanisms with aging. The male-to-female ratio in the current study was 3:1, which was similar to previous
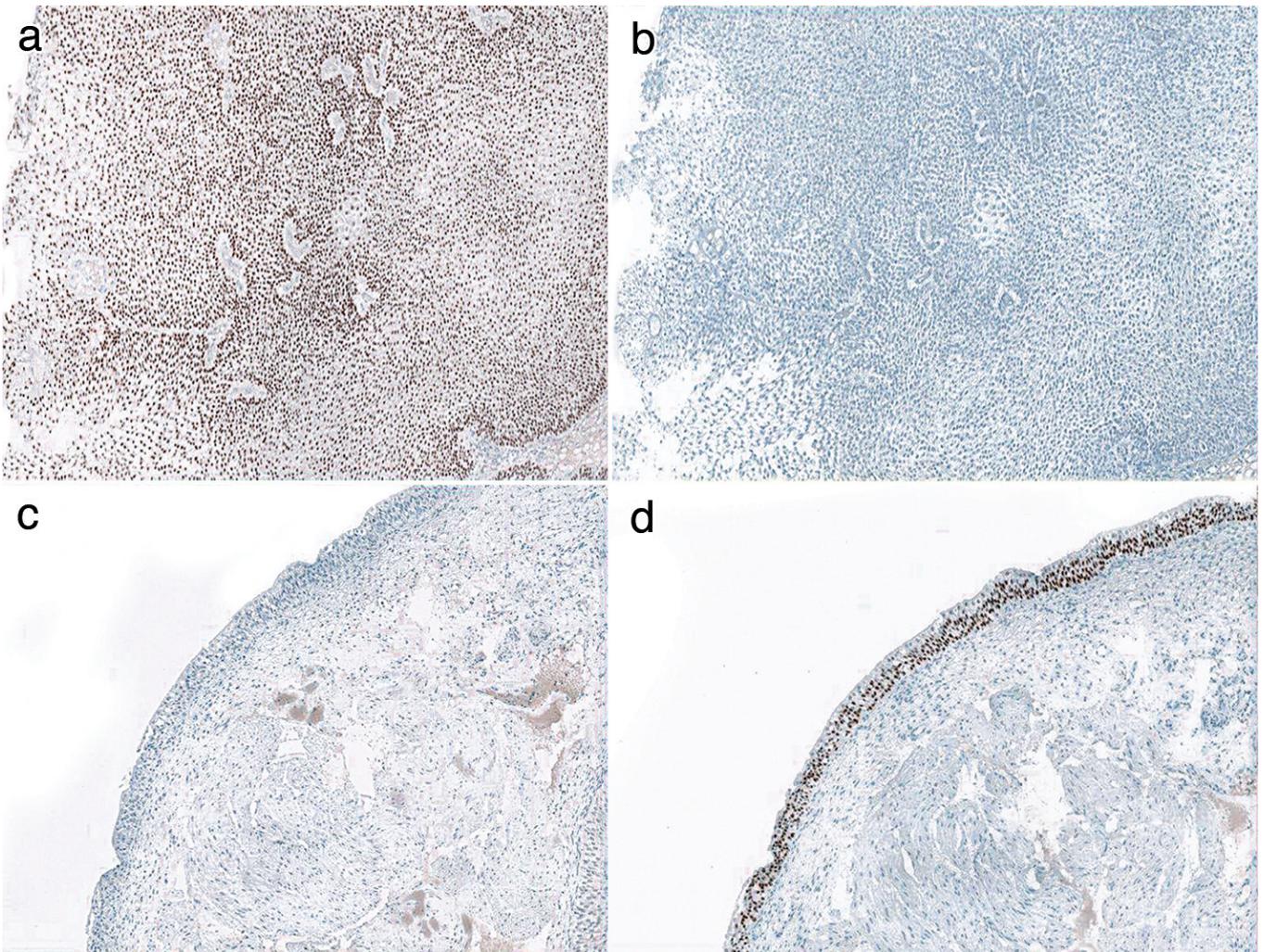
Fig. 3. Inverse relation between the immunohistochemical expression of AR and FUS. **a.** High AR nuclear expression in a case of high-grade UC. **b.** Negative FUS expression in the same case of high-grade UC. **c.** Negative AR expression in adjacent normal urothelium. **d.** High FUS nuclear expression in adjacent normal urothelium. (IHC). x 200.

Role of androgen receptor and FUS in progression of urothelial carcinoma
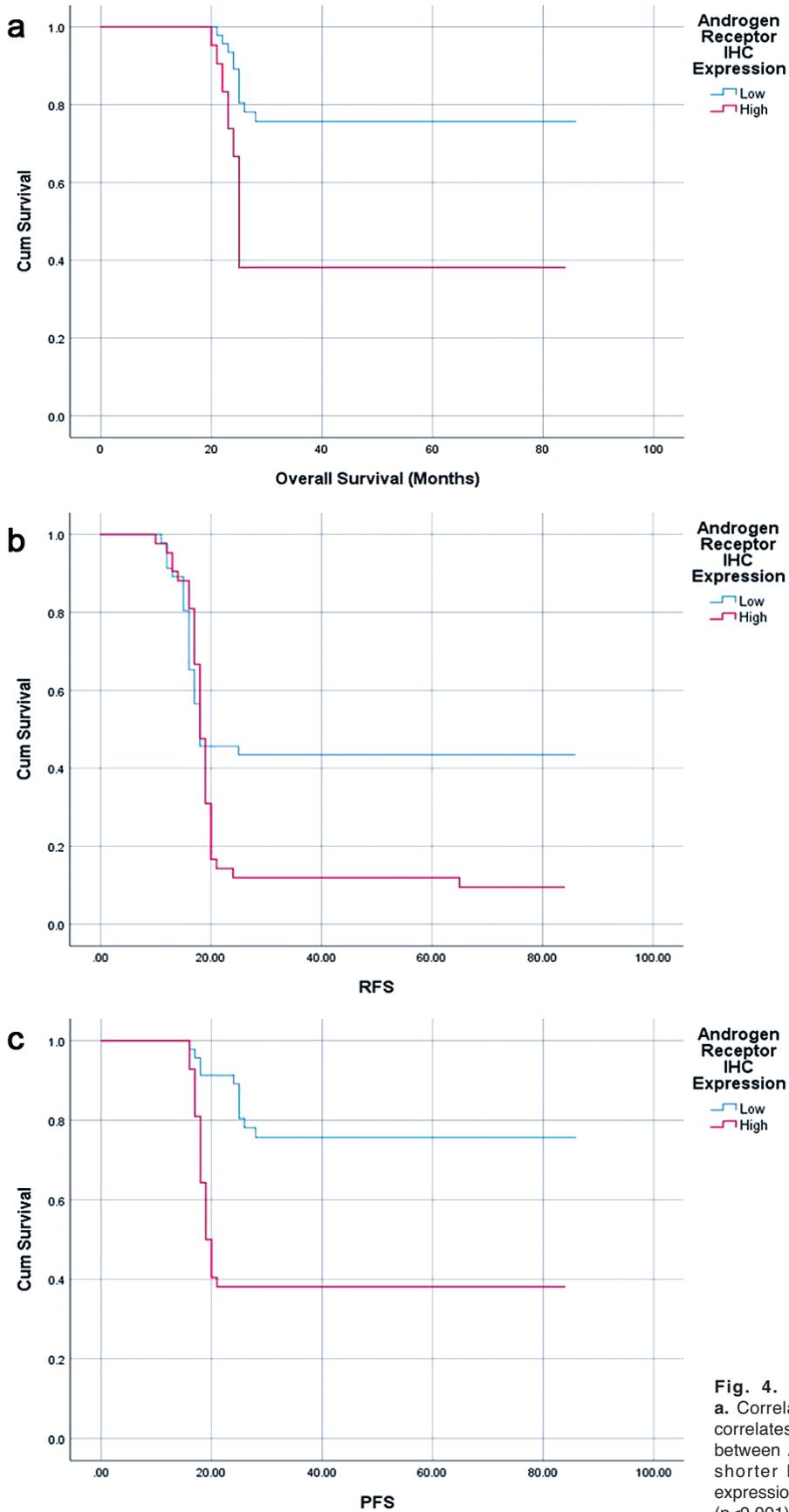
Fig. 4. Kaplan Meier analysis of AR expression. **a.** Correlation between AR expression and OS; high AR correlates with shorter survival (p=0.0001). **b.** Correlation between AR expression and RFS; high AR correlates with shorter RFS (p=0.038). **c.** Correlation between AR expression and PFS; high AR correlates with shorter PFS (p<0.001).

Role of androgen receptor and FUS in progression of urothelial carcinoma
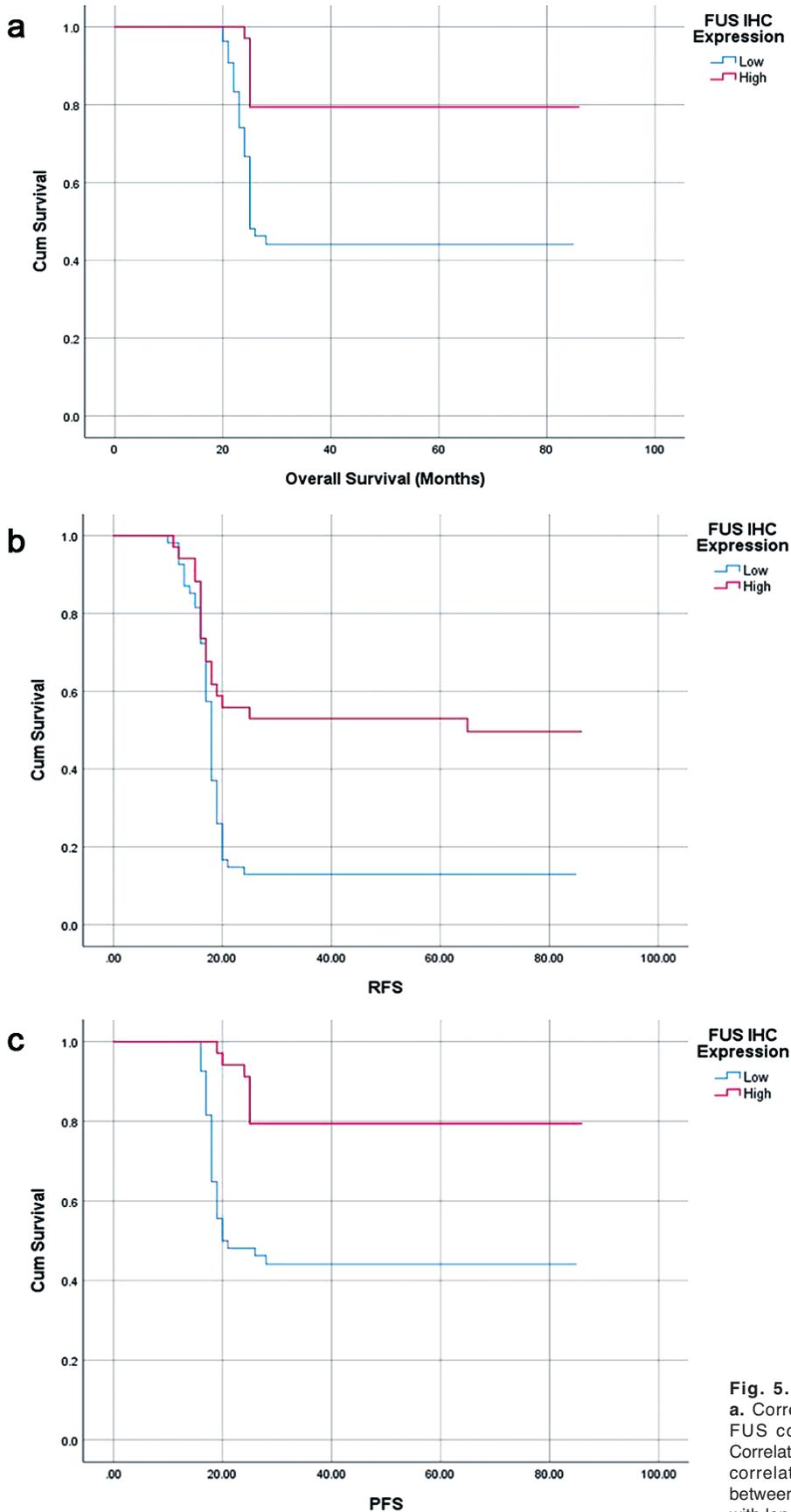
Fig. 5. Kaplan Meier analysis of FUS expression. **a.** Correlation between FUS expression and OS; high FUS correlates with longer survival (p=0.001). **b.** Correlation between FUS expression and RFS; high FUS correlates with longer RFS (p=0.001). **c.** Correlation between FUS expression and PFS; high FUS correlates with longer PFS (p<0.001).
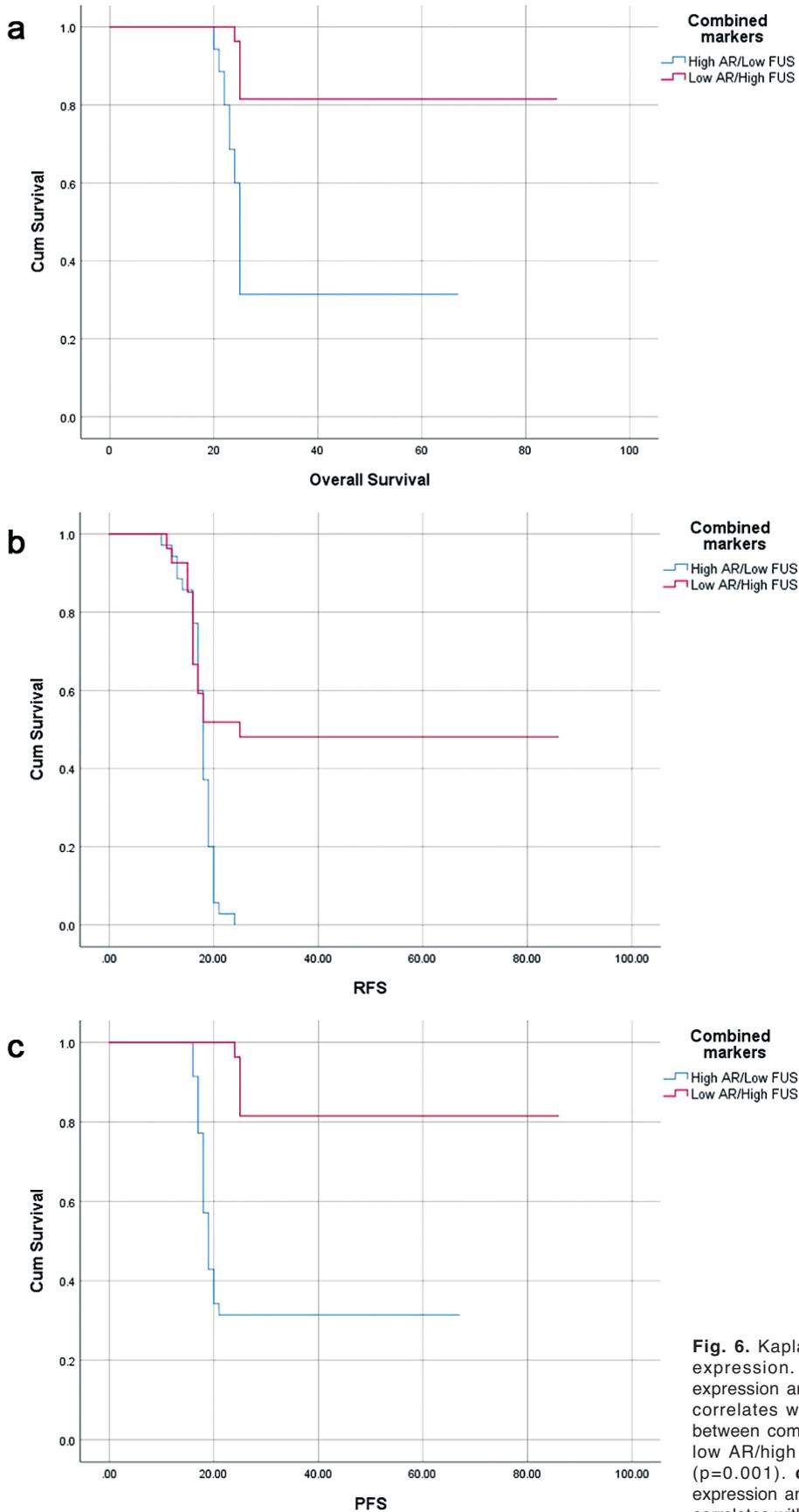
Fig. 6. Kaplan Meier analysis of combined AR and FUS expression. **a.** Correlation between combined AR/FUS expression and OS; combined low AR/high FUS expression correlates with longer survival (p=0.001). **b.** Correlation between combined AR/FUS expression and RFS; combined low AR/high FUS expression correlates with longer RFS (p=0.001). **c.** Correlation between combined AR/FUS expression and PFS; combined low AR/high FUS expression correlates with longer PFS (p<0.001).

studies (El-Sharkawi et al., 2014; Lombard and Mudryj, 2015). There was no statistically significant correlation between AR expression and either age or gender, although most of the positive cases were men. Lombard and Mudryj (2015) attributed the discrepancy between male and female incidence of bladder cancer to androgen signaling rather than AR expression and mentioned that the higher level of circulating androgen in men predisposes them more to bladder cancer, although AR shows variable expression and plays a role in disease progression.

Conflicting results exist among different studies regarding the possible role of AR in bladder cancer progression. Several studies demonstrated a positive association between AR expression and both high-grade and muscle-invasive tumors denoting a role of AR in tumor aggressiveness and invasion (Mir et al., 2011; Zheng et al., 2011; Jing et al., 2014; Elzamy et al., 2018). In contrast, few studies argued that AR expression decreases in higher grades and stages and concluded that low AR expression is associated with tumor progression and invasion (Tuygun et al., 2011; Gakis and Stenzl, 2013). In contrast, some studies reported that AR expression is upregulated in early disease stages with a subsequent decline in its expression with further disease progression and muscle invasion (Boorjian et al., 2004; Lombard and Mudryj,

2015; Li et al., 2017). Lombard and Mudryj (2015) explained the downregulation of AR with tumor progression and muscle invasion by the heterogeneity of UC like other carcinomas in the later stages, with a possible variation in AR dependence from patient to patient. They also mentioned that AR interacts with many regulatory pathways such as b-catenin, cyclin d1, and other growth factors, and these interactions can alter AR signaling. Gakis and Stenzl (2013) also suggested that AR sensitivity is lost in muscle-invasive tumors and that further disease progression and metastases can be directed by the activation of other genes, including genes with ARE in their promoter region, in an androgen-independent manner. Generally, the different results among studies can be attributed to differences in sample sizes, study protocols, techniques of staining, and scoring systems (Chen et al., 2017). The results of the current study were in accordance with the first group of studies where high AR IHC expression was found to be associated with high-grade, muscle-invasive tumors and correlated with other poor prognostic indicators as nonpapillary pattern. Furthermore, high AR expressing tumors showed statistically significant shorter OS, RFS, and PFS. This goes well with the study of Zheng et al. (2011) whose AR expressing cases of UC were significantly associated with tumor progression by Kaplan Meier analysis.

The study of Brooke et al. (2011) and that of Ghanbarpanah et al. (2018) on prostate cancer demonstrated that FUS exhibits some tumor suppressor features, because its overexpression was associated with apoptosis and tumor growth inhibition, whereas its suppression was associated with cell-cycle progression. Their studies also proved that its suppressor effect is mediated through a reduction in cell-cycle progression factors such as cyclin d1 and CDK6 and by increasing the level of P27, "an antiproliferative CDK inhibitor." In agreement with this observation, high FUS expression in our UC cases was associated with good prognostic parameters such as lower grade, lower tumor stage, absence of muscle invasion, and absence of vascular invasion denoting a suppressor role of this protein in UC. Moreover, high FUS was significantly associated with longer OS, RFS, and PFS by Kaplan Meier analysis. This was also in accordance with the study of Brooke et al. (2011) who suggested that loss of FUS

Table 3. Relationship between FUS expression and clinicopathologic characteristics.

	FUS expression				P value
	High		Low		
	N	%	N	%	
Tumor stage					
Ta	8	88.9%	1	11.1%	0.002**
T1	7	53.8%	6	46.2%	
T2	10	37.0%	17	63.0%	
T3	9	28.1%	23	71.9%	
T4	0	0.0%	7	100.0%	
Tumor grade					
Low	9	100%	0	0%	0.001**
High	25	31.6%	54	68.4%	
Lymph node stage					
N0	18	37.5%	30	62.5%	0.14
N1	3	25%	9	75%	
N2	1	12.5%	7	87.5%	
Tumor size					
≤3 cm	18	45.0%	22	55.0%	0.263*
>3 cm	16	33.3%	32	66.7%	
Tumor pattern					
Nonpapillary	10	17.5%	47	82.5%	0.0001*
Papillary	24	77.4%	7	22.6%	
Vascular invasion					
Absent	30	39.5%	46	60.5%	0.04**
Present	1	8.3%	11	91.7%	

*Chi-square tests. **Fisher exact test

Table 4. Agreement between androgen receptor IHC expression and FUS IHC expression.

	AR expression				Kappa	Significance
	High		Low			
	N	%	N	%		
FUS expression						
Low	35	83.3%	19	41.3%	0.424	0.001
High	7	16.7%	27	58.7%		

expression may contribute to cancer progression. Their patients with prostatic carcinoma, showing high levels of FUS expression, survived longer and were less likely to have bone metastases.

An inverse relationship was found between FUS and AR in prostatic carcinoma, and FUS was found to be downregulated in response to androgen at a transcriptional level (Brooke et al., 2011). Moreover, it was found that FUS can be regulated at protein level by c.jun. protein which has been found to be regulated by androgen (Perrotti et al., 2000; Velasco et al., 2004). From the above-mentioned results, it was concluded that a link exists between AR, FUS, and cell-cycle progression in prostate cancer in which AR and FUS act in an opposing way (Brooke et al., 2011). In parallel to this conclusion, our study revealed a significant inverse relationship between AR and FUS expression in the studied UC cases, suggesting an interaction between FUS and AR pathways involved in urothelial cancer progression. The expression pattern of low androgen/high FUS in low-grade tumors and high androgen/low FUS in high-grade tumors might be attributed to the fact that low-grade and high-grade UCs come from different molecular pathways (McConkey et al., 2010). Furthermore, Kaplan Meier analysis of combined low AR and high FUS showed significantly longer OS, RFS, and PFS than those associated with combined low AR and high FUS.

Previous studies, which were conducted on neurodegenerative disorders that may be mediated through a loss of FUS function, reported that it is possible to reverse the adverse effects of FUS depletion by replacing the FUS protein or by small-molecule intervention (Ward et al., 2014). Therefore, to elucidate the ability to apply this promising therapeutic choice in cancers, further studies involving cancers mediated through the loss of FUS function remain a compelling demand in this context. Furthermore, Boorjian et al. (2009) suggested that because bladder cancer progression involves a large number of signaling pathways that interact with AR, targeting these pathways can provide new effective treatment strategies. Based on the association between high AR expression with poor prognostic indicators and tumor recurrence and progression demonstrated in this study and because the currently used conventional treatments for bladder carcinoma such as intravesical and systemic chemotherapy fail to prevent tumor recurrence, using androgen deprivation therapy might offer a promising chemopreventive therapeutic option in positive cases (Li et al., 2017). However, owing to the conflicting results of AR role in tumor progression of UC among different studies, this suggestion needs to be validated by further studies.

Further IHC and molecular studies with larger patients' cohorts that include different histologic subtypes of UC are required to validate the current results and include control samples of normal urothelium in this context.

Conclusion

This study is the first IHC study that investigated the combined AR and FUS expression in UC. High AR expression is associated with poor prognostic indicators, tumor progression, and shorter survival of UC, whereas high FUS expression is associated with good prognostic indicators, late progression, and better survival. In that sense, AR and FUS IHC expression points toward their possibly important prognostic and promising therapeutic value.

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