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Original contribution

The coexpression of fibroblast activation protein (FAP) and basal-type markers (CK 5/6 and CD44) predicts prognosis in high-grade invasive urothelial carcinoma of the bladder☆,☆☆

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Summary High-grade urothelial carcinoma (UC) of the bladder is a heterogeneous disease with dismal prognosis. Bladder tumors with basal phenotype are intrinsically aggressive, and morphological parameters that define disease staging remain main prognosticators. We intend to evaluate the role of cancer-associated fibroblasts (CAFs) in the prognosis of bladder cancer and its association with basal and luminal phenotypes. Clinical and pathological parameters, including the immunohistochemical expression of fibroblast activation protein (FAP) and markers of basal (CK5/6, CD44) and luminal (CK20, GATA3) phenotypes, have been investigated in a series of 121 patients with UC of the bladder treated by radical cystectomy with lymph node dissection, and their implication in long-term cancer-specific survival has been evaluated. A cytoplasmic immunostaining of FAP in CAFs implies worse disease-specific survival (hazard ratio [HR] = 1.68; $P = .048$). FAP expression is associated with tumor staging ($P < .0001$), with best discrimination at T2a/T2b level, and with negative expression of markers of

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luminal phenotype, such as CK20 ($P < .0001$) and GATA3 ($P = .005$). In the multivariate analysis, simultaneous expression of FAP, CK5/6, and CD44 is a strong prognosticator of disease-specific survival (HR = 2.3; $P = .001$), together with nodal invasion (HR = 3.47; $P < .0001$) and bladder infiltration up to deep muscle or beyond (HR = 2.47; $P = .02$). There is no association between positive FAP expression in primary tumor and nodal disease ($P = .22$). FAP expression in CAFs favors tumor invasion in high-grade invasive UC of the bladder with basal phenotype. This new immunohistochemical marker could be added to the routine immunohistochemical protocol to predict clinical behavior in these patients.
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1. Introduction

Bladder cancer is the second most common malignancy of the genitourinary tract and a leading cause of cancer death in Western countries. According to cancer registry data, the highest incidence rates in men are in Southern Europe, particularly in Spain [1]. Incidence is also increasing in the United Sates, with 80 470 new cases and 17 670 deaths estimated in 2019 [2]. Despite therapeutic efforts, tumor control fails in a large proportion of patients because of tumor recurrence and distant metastases [3]. Classical histopathologic parameters such as tumor stage and lymph node involvement remain for decades main prognosticators after cystectomy, and many new candidate biomarkers often correlate with tumor-node-metastasis staging [4,5].

Tumor tissue is composed not only of cancer cells but also of various types of stromal elements, such as fibroblasts, macrophages, and endothelial cells. These tumor-associated stromal cells are key contributors to the tumor microenvironment, promoting cancer cell migration and metastasis [6]. On the other hand, basal and luminal phenotypes of muscle-invasive bladder cancer have been identified following the example of breast cancer. Basal-type bladder cancers are enriched with biomarkers associated with stem cells and epithelial-to-mesenchymal transition (EMT), and are associated with shorter disease-specific survival than luminal-type ones [7,8].

Cancer-associated fibroblasts (CAFs) are activated fibroblasts characterized by the expression of α-smooth muscle actin, fibroblast activation protein-α (FAP), fibronectin, and vimentin [9].CAFs in the stromal microenvironment support growth and invasion of epithelial cells through secretion of cytokines, chemokines, and extracellular matrix components that promote EMT and favor metastasis [6,10]. Specifically, Schulte et al reported a correlation between CAFs and EMT markers in bladder cancer by immunohistochemistry (IHC) and confirmed the relationship between stromal fibroblast activation and invasive behavior of carcinoma cells [11].

FAP is a transmembrane peptidase which plays a major role on the tumor microenvironment by remodeling the extracellular matrix. A meta-analysis has revealed that FAP overexpression in the fibroblasts surrounding several malignancies has important implications [12]. We have unveiled FAP as an important marker to predict prognosis in clear cell renal cell carcinoma [13,14]. Now, we evaluate the role of FAP expression together with the expression of markers of luminal and basal phenotyping and clinical-pathological features for the prediction of prognosis in a series of patients with high-grade invasive urothelial carcinoma (UC) of the bladder.

2. Materials and methods

2.1. Patients and samples

A retrospective study was carried out on a series of 121 patients with nonmetastatic high-grade transitional cell carcinoma (TCC) of the bladder treated in a single institution with radical cystectomy including lymph node dissection by the same surgeon (J. A. C.) between 2000 and 2015. Open metastatic disease at the time of surgery, treatment with neoadjuvant chemotherapy or radiation before cystectomy, and pelvic lymph node dissection not performed at the time of cystectomy were exclusion criteria. Also, cases with scarce neoplastic tissue in the specimen and cases with histology different to conventional high-grade transitional cell carcinoma (TCC) were excluded in this series. All the cases included were registered in a database with the approval of the Institutional Review Board (A06/16). Patients with positive lymph nodes and locally advanced disease were offered cisplatinum-based adjuvant chemotherapy (gemcitabine or mitomycin, vincristine, adriamycin, and cisplatin). All patients were followed up until death or until the data were censored on December 2016. All living patients were informed about the potential use for research of their surgically resected tissues and accepted this eventuality by\ signing a specific Institutional Review Board–approved document.

Primary end point of the study was evaluation of disease-specific mortality, so cause of mortality was registered in all deceased patients. Clinical parameters before cystectomy (age, American Society of Anesthesiologists [ASA] score, Charlson comorbidity index, and preoperative hemoglobin) were also evaluated. Two pathologists collected representative

formalin-fixed and paraffin-embedded tissue blocks for both primary tumor and lymph nodes, and a third pathologist (J. I. L.) reviewed all the specimens; confirmed histological type, histological grade, and tumor stage (American Joint Committee on Cancer/tumor-node-metastasis 2017); and performed IHC evaluation.

2.2. Tissue microarray construction and IHC staining

Tissue microarrays (TMAs) were performed selecting tissue samples with abundant tumor tissue without artifact and from the leading fronts of invasive tumor, when possible. For each case, 2 tumor samples (2.5 mm in diameter) were transferred from the original paraffin block to the recipient TMA block. Whenever allowed by the size of the tumor seed within lymph nodes, 2 additional samples were obtained from lymph node metastases and transferred following the same process. Consecutive 4-μm sections were performed from TMA blocks, and the first one was stained with hematoxylin-eosin to verify the proper construction of the blocks and that representative material was present in all cases.

An IHC study was carried out with FAP, GATA3, CK20, CK5/6, and CD44 antibodies (Cambridge, UK). FAP antibody (Abcam, ref. ab53066, dilution 1:70, cytoplasmic staining) was evaluated in the stromal fibroblasts adjacent to neoplastic nests. GATA3 (Ventana Medical Systems, Tucson, AZ; ref. L50-823, ready to use, nuclear staining), CK20 (Ventana ref. SP-33, ready to use, cytoplasmic staining), CK5/6 (Ventana, ref. D5/16B4, ready to use, cytoplasmic staining), and CD44 (Ventana, ref. SP-37, ready to use, cytoplasmic staining) antibodies were evaluated in tumor cells. IHC immunostainings were performed in automated immunostainers (EnVision FLEX, Dako Autostainer Plus, Dako, Glostrup, Denmark, and BenchMark Ultra, Ventana Medical Systems) following routine methods. Tris-EDTA was used for antigen retrieval. Negative controls were slides not exposed to the primary antibody, and these were incubated in phosphate-buffered saline and then processed under the same conditions as the test slides. The analysis was performed using a Nikon Eclipse 80i microscope (Tokyo, Japan). There were no equivocal stain results, and focal weak staining positivity was not observed either. Cutoff points or automated scoring system were not used. Results of the 2 cores were combined as positive when at least 1 core was positive.

2.3. Statistical analysis

Descriptive study and survival analysis were carried out. The association between variables was studied using the Cochran-Armitage, χ^2, and Fisher exact tests. The risk of death due to the disease was estimated (cancer-specific mortality) using a univariate analysis, evaluating the survival function according to the Kaplan-Meier method and the log-rank test for the different variables studied: molecular, histopathological, or clinical. A multivariate analysis was then performed using Cox proportional hazards regression model with a threshold entry $P = .15$ and stay criterion $P = .1$. The estimated hazard ratios (HRs) were defined with their respective 95% confidence intervals for each variable analyzed, detecting those which independently predicted survival in this model. The statistical analysis was developed using SAS 9.4 (2002-2010, SAS Institute Inc, Cary, NY).

3. Results

Main clinical and histopathological characteristics of the series analyzed are shown in Table 1. Mean age of the patients at the time of cystectomy was 68.1 ± 9.25 (range 44-

Table 1 Characteristics of the series analyzed (N = 121)

	n (%)
Sex	
Male	118 (97.5)
Female	3 (2.5)
Age, y [a]	68.1 ± 9.25
ASA score	
I	9 (7.4)
II	76 (62.8)
III	32 (26.5)
IV	4 (3.3)
Charlson comorbidity index	
1	8 (6.6)
2	36 (29.7)
3	40 (33.1)
4	19 (15.7)
5	4 (3.3)
≥6	14 (11.6)
pT category	
pT1	13 (10.7)
pT2	28 (23.1)
pT3	51 (42.2)
pT4	29 (24)
pN category	
pN0	75 (59.5)
pN1	21 (17.4)
pN2	27 (22.3)
pN3	1 (.8)
Associated pTis	
Yes	42 (34.7)
No	79 (65.3)
Preoperative hemoglobin, g/dL [a]	13.2 ± 2.1
Adjuvant chemotherapy	
Yes	31 (25.6)
No	90 (74.4)
Dead of bladder cancer	
Yes	65 (53.7)
No	56 (46.3)

[a] Mean ± SD.

89) years. Globally, 25.6% of the patients received adjuvant systemic chemotherapy, and 53.7% of the patients died of disease progression during a mean follow-up of 51.4 ± 48.8 (range 1-192) months. Histopathological staging revealed locally advanced disease, including perivesical infiltration (pT3) or invasion of neighboring organs (pT4), in 39.7% of the patients and positive nodal disease (pN1-3) in 40.5%. Median preoperative hemoglobin was 13.2 ± 2.1 (range 7.8-17.3) g/dL, and transfusion rate, including intra- and postoperative transfusion, was 32.2%.

FAP expression is positive in 76 (62.8%) of the primary tumors, always restricted to stromal fibroblasts adjacent to epithelial tumor cells. No immunostaining with this antibody was detected in neoplastic epithelial cells. FAP expression within CAFs is not related to patient age ($P = .5$), associated carcinoma in situ ($P = .59$), use of adjuvant chemotherapy ($P = .29$), preoperative hemoglobin level ($P = .93$), ASA score ($P = .83$), or Charlson comorbidity index ($P = .57$). However, FAP expression appears associated to higher pT category ($P = .0001$), at a discrimination level between pT2a and pT2b, but not to pN category ($P = .2$). FAP expression is inversely associated to GATA3 and CK20 expressions ($P = .005$ and $P < .0001$, respectively), both markers of luminal phenotype. However, the association is not so firm between FAP expression and markers of basal phenotype, such as CK5/6 and CD44 ($P = .07$ and $P = .06$, respectively) (Table 2).

Lymph nodal invasion is the histopathological factor of worst disease-specific survival (HR 3.425; $P < .0001$) in the univariate analysis, together with bladder wall infiltration beyond deep muscle, that is, pT2b or beyond (HR 3.61; $P = .003$). In other words, elements that define American Joint Committee on Cancer staging systems, nodal status, or tumor infiltration of the organ are major predictors. Among the clinical factors evaluated, only preoperative hemoglobin at 13 g/dL or higher cutoff presents as protective factor but does not reach statistical significance (HR .639; $P = .067$). Administration of adjuvant chemotherapy was also protective but far from being statistically significant (HR .85; log-rank, $P = .56$). FAP positive immunostaining in CAFs within the primary UC implies worse prognosis (HR 1.68; $P = .048$) (Table 3). Disease-specific survival is 73.3% for FAP-negative tumors compared to 48.7% for FAP-positive ones (Fig. 1). Luminal-type markers GATA3 (HR .745; $P = .27$) and CK20 (HR .82; $P = .47$) behave as protective factors but without statistical significance. Conversely, basal-type markers CK5/6 (HR 1.58; $P = .06$) and CD44 (HR 1.45; $P = .14$) are factors of adverse prognosis but do not reach statistical significance either.

Individual markers (statistically significant or with a tendency toward significance) were pooled together. The series evaluating simultaneous expression is based on 110 patients because assessment of at least 1 of the markers FAP, CD44, or CK5/6 is missing in 11 cases. Positive expression of the

Table 2 Association between FAP expression and other histopathological features

Variables	FAP positive (%) n = 76	FAP negative (%) n = 45	P [a]	Cramer's V
pT category				
pT1	2.6	24.4		
pT2a	5.3	26.7		
pT2b	13.2	4.4		
pT3a	34.2	13.3		
pT3b	18.4	11.1		
pT4	26.3	20	.0001 [a]	.50
pN category				
pN0	52.6	71.1		
pN1	21.1	11.1		
pN2	25	17.8		
pN3	1.3	0	.2 [b]	.19
GATA3 expression				
Positive	62.2	86.4		
Negative	37.8	13.6	.005 [c]	−.25
CK20 expression				
Positive	20.8	59.5		
Negative	79.2	40.5	<.0001 [c]	−.39
CK5/6 expression				
Positive	46.6	29.3		
Negative	53.4	70.7	.07 [c]	.16
CD44 expression				
Positive	60.8	42.9		
Negative	39.2	57.1	.06 [c]	.17

[a] Cochran-Armitage.
[b] Fisher.
[c] χ^2.

Table 3 Cox regression model to predict cancer-specific survival in the series analyzed

	HRs			
	Point estimate	95% confidence limits	Wald	*P*
Univariate variable				
Bladder infiltration: pT2b-4 *vs* pT1-2a	3.61	1.716	7.595	.003
Nodal invasion: pN1–3 *vs* pN0	3.425	2.075	5.65	<.0001
Associated carcinoma in situ: yes *vs* no	1.376	.836	2.262	.202
FAP: positive *vs* negative	1.681	.991	2.849	.048
GATA3: positive *vs* negative	.745	.439	1.266	.269
CK20: positive *vs* negative	.823	.482	1.403	.468
CK5/6: positive *vs* negative	1.58	.962	2.597	.064
CD44: positive *vs* negative	1.453	.879	2.404	.137
Simultaneous FAP, CK5/6, and CD44: positive *vs* negative	1.902	1.055	3.077	.0265
Age: ≥65 *vs* <65 years	1.125	.673	1.883	.649
Charlson comorbidity index: ≥3 *vs* 1-2	1.513	.893	2.571	.117
Preoperative hemoglobin: ≥13 g/dL *vs* <13 g/dL	.639	.392	1.043	.067
Multivariate variable				
Adjuvant chemotherapy: yes *vs* no	.852	.495	1.467	.559
Bladder infiltration: pT2b-4 *vs* pT1-2a	2.475	1.025	5.977	.02
Nodal invasion: pN1-3 *vs* pN0	3.472	2.004	6.024	<.0001
Simultaneous FAP, CK5/6, and CD44: positive *vs* negative	2.304	1.304	4.065	.001
Preoperative status: hemoglobin level ≥13 g/dL *vs* <13 g/dL	.634	.377	1.069	.085

3 markers (FAP, CK5/6, and CD44) enhances the predictive value of individual stains and implies worse disease-specific survival in univariate analysis (HR 1.9, $P = .0265$; Figs. 1 and 2). Besides, combined FAP, CK5/6, and CD44 expression, regardless the status of GATA3 and/or CK20, is the only immune-histochemical marker that stands in the multivariate analysis as independent factor.

Cox regression multivariate analysis shows nodal invasion is the independent variable with highest significance (HR 3.47; $P < .0001$). As already mentioned, simultaneous expression of FAP, CD44, and CK5/6 is also a major independent factor to predict worse cancer-specific survival (HR 2.3; $P = .001$). The next independent variable identified, although to a lesser extent, is bladder wall tumor infiltration

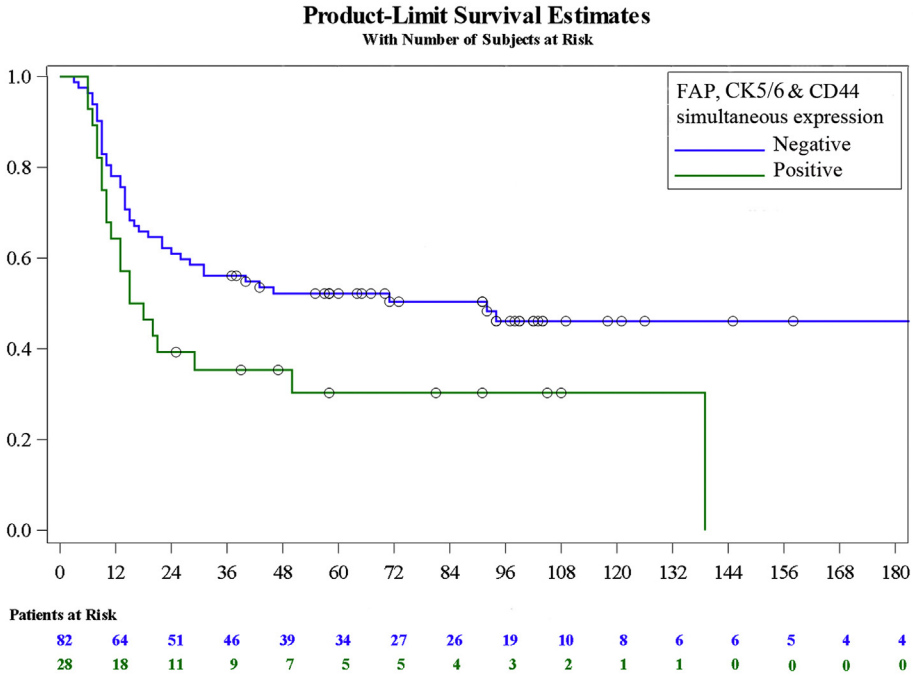

Figure 1 Kaplan-Meier disease-specific survival curve for simultaneous combined FAP, CK5/6, and CD44 expression.

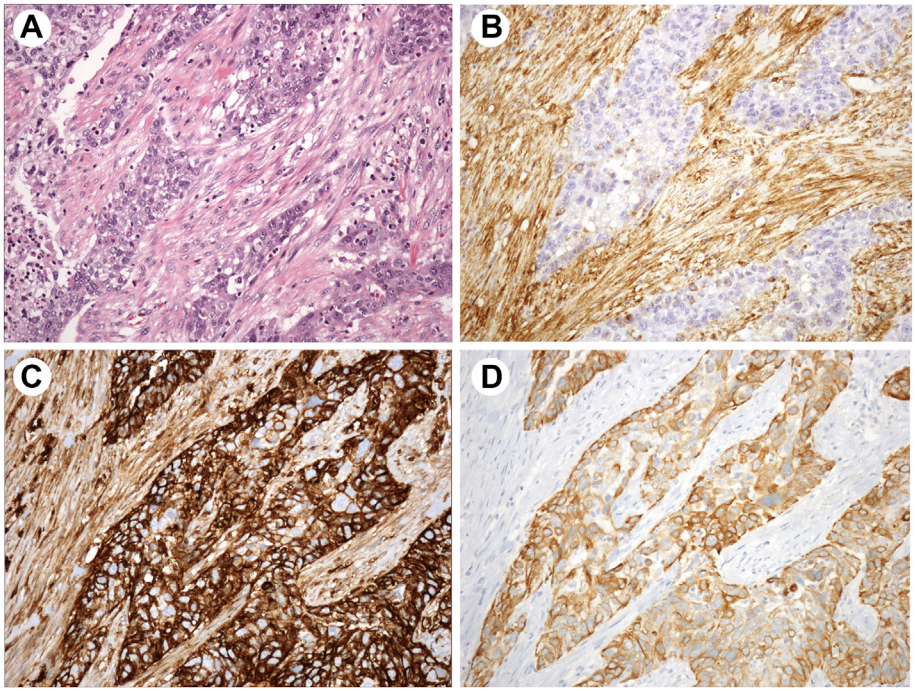

Figure 2 High-power detail of the front of invasion in a high-grade UC showing stromal fibroblasts intermingled with tumor cells on hematoxylin-eosin stain (A), as well as positive cytoplasmic immunostaining with membranous enhancement of FAP in CAFs (B) and positive immunostaining for CD44 (C) and CK5/6 (D) in tumor cells.

of deep muscle or beyond (HR 2.475; $P = .02$). Finally, preoperative hemoglobin value is the last independent predictive variable acting in this series as a protector (HR .634; $P = .085$) (Table 3). Harrel's concordance statistic for the multivariate model presented is 0.74.

4. Discussion

Therapeutic decisions for bladder cancer are largely based on histopathologic features, but identification of molecular subtypes of UC has the potential to guide patient stratification for prognosis and treatment, and a more rational selection of patients for chemotherapy [15,16]. High-grade UC is a life-threatening heterogeneous neoplasia containing distinct cell populations, some exhibiting differential gene expression pattern related with basal stem cells [17,18].

A molecular taxonomy of bladder cancer segregates tumors that express cytokeratins characteristic of basal (CK5/6) or luminal (CK20) tumors and a third signature associated with resistance to cisplatin-induced apoptosis [7]. However, some tumors exhibit basal and luminal signatures consistent with intratumor heterogeneity. Basal bladder cancers are intrinsically aggressive, enriched with squamous and sarcomatous features, and associated with metastases and advanced stage at presentation. Basal bladder tumors express cancer stem cell (CSC) biomarkers characteristic of EMT, like cytokeratins 5, 6, and 14; cadherin 3; and CD44 antigen, a cell-surface glycoprotein involved in cell adhesion and migration [7]. Potential use of CK5/6 [8] and CD44 [10] in the prognostic stratification of the patients with bladder cancer has been recently recognized.

FAP is a cell surface glycoprotein with dipeptidyl peptidase and collagenolytic activity highly expressed on the surface of CAFs surrounding different epithelial cancers, including carcinomas of the oral cavity, esophagus, stomach, pancreas, colon, breast, ovary, endometrium, lung, melanoma, prostate, and kidney [12,13]. Some mesenchymal cancers including bone and soft tissue sarcomas also express FAP [19].

Cancers are not only composed of malignant cells. Cancer-associated stromal cells, including fibroblasts, endothelial cells, and immune cells, also play a crucial role in the interaction between neoplasia and tumor microenvironment [20]. EMT is a physiological process in embryogenesis and tissue repair with topographical specificity but also a mechanism involved in neoplasia. Tumor invasiveness and metastases development mainly occur through the acquisition of a mesenchymal phenotype by neoplastic epithelial cells that allows them to invade and migrate [21]. CAFs originating from local fibroblasts or bone marrow–derived cells are recruited into the tumor and adopt a fibroblastic phenotype [22]. FAP promotes angiogenesis, cell adhesion, motility, and invasion by degrading and remodeling the extracellular matrix, thus enhancing stromal cell proliferation

and tumor invasiveness [23,24]. Consequently, a relation between FAP expression in CAFs, poor overall survival, and lymph node metastases in solid tumors has been confirmed in a meta-analysis [12].

Despite the limited number of cases evaluated and the retrospective nature of the study, this is the first study to address the clinical implications of FAP expression in bladder cancer. We do not consider FAP expression as a surrogate itself of desmoplasia. In fact, not all fibroblasts within cancer-associated desmoplasia are FAP positive, and FAP positivity is sometimes present in a nondesmoplasic environment, as already described in clear cell renal cell carcinoma [13]. This molecular marker is often negative in non–muscle-invasive (pT1) or superficially muscle-invasive tumors (pT2a) and positive when bladder cancer invades beyond deep muscularis propria (≥pT2b), and it is not associated to lymph node status. Preclinical evidence sustains that FAP promotes tumor progression in bladder CAFs, and likely mechanisms involve CXCL1-mediated interaction between cancer cells, tumor-associated macrophages, and CAFs [25] and increased Kindlin-2 expression, a focal adhesion protein expressed in CAFs [26]. FAP could also be a potential therapeutic target because the specific inhibition of FAP prevents tumor progression in vitro [27] and FAP-activated prodrugs have already been tested in prostate and breast cancer with promising results [28,29].

The importance of preoperative hemoglobin level as a pure clinical prognostic variable was recently recognized in the urological literature [30,31], and this finding deserves consideration in daily practice. Positive FAP immunostaining implies worse prognosis in this series, but it is not an independent factor on multivariate analysis. However, when considered simultaneously with basal-type markers CK5/6 and CD44, the predictive value of FAP expression is enhanced to become a first-line independent prognostic factor of cancer-specific survival, together with well-known parameters such as lymph node invasion and bladder wall infiltration [4].

Cancer development and progression are still complex processes in which both neoplastic cells and their local microenvironment are intimately involved [6]. Strong experimental evidence has shown that CAFs can promote tumorigenesis and tumor progression through multiple mechanisms, including induction of proliferation, survival, angiogenesis, EMT, and suppression of immune cells [32,33]. In the context of multidirectional signals between host and cancer cells, CAFs regulate CSC plasticity through promoting cell dedifferentiation and providing a supportive collagen-enriched niche for their colonization and chemoresistance features [34]. Genomic data from TCGA platform and the induction of healthy primary bladder fibroblasts into CAFs by bladder cancer–derived exosomes suggest that interleukin-6 is necessary for CAF-induced EMT in the progression of human bladder cancer [35].

Our results describing a coexpression of FAP-positive activated fibroblasts and CD44-positive CSCs in more aggressive tumors suggest an interaction between CAFs and CSCs in bladder cancer microenvironment that requires further investigation.

5. Conclusions

The simultaneous IHC evaluation of FAP in CAFs and basal-type markers CK5/6 and CD44 in neoplastic cells could be a major tool to predict the clinical behavior of patients with high-grade UC of the urinary bladder treated with radical cystectomy. Taking into consideration the heterogeneity of bladder cancer and the increasing evidence to consider the importance of CAFs in solid malignancies, we propose the combined use of FAP, CK5/6, and CD44 immunomarkers in the routine of pathologists to predict clinical behavior in these patients. The role of these combined markers to predict response to cisplatinum-based chemotherapy in a neoadjuvant setting or immune checkpoint inhibitors in second-line therapy should also be evaluated.

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References

[1] Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: a global overview and recent trends. Eur Urol 2017;71:96-108.
[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7-34.
[3] Chamie K, Litwin MS, Bassett JC, et al. Urologic Diseases in America Project. Recurrence of high-risk bladder cancer: a population-based analysis. Cancer 2013;119:3219-27.
[4] Welty CJ, Sanford TH, Wright JL, et al. The Cancer of the Bladder Risk Assessment (COBRA) score: estimating mortality after radical cystectomy. Cancer 2017;123:4574-82.
[5] Giulietti M, Occhipinti G, Righetti A, et al. Emerging biomarkers in bladder cancer identified by network analysis of transcriptomic data. Front Oncol 2018;8:450.
[6] Clark AG, Vignjevic DM. Modes of cancer cell invasion and the role of the microenvironment. Curr Opin Cell Biol 2015;36:13-22.
[7] Choi W, Czerniak B, Ochoa A, et al. Intrinsic basal and luminal subtypes of muscle-invasive bladder cancer. Nat Rev Urol 2014;11:400-10.
[8] Hurst CD, Knowles MA. Molecular subtyping of invasive bladder cancer: time to divide and rule? Cancer Cell 2014;25:135-6.
[9] Xing F, Saidou J, Watabe K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. Front Biosci 2010;15:166-79.
[10] Wu CT, Lin WY, Chen WC, Chen MF. Predictive value of CD44 in muscle-invasive bladder cancer and its relationship with IL-6 signaling. Ann Surg Oncol 2018;25:3518-26.
[11] Schulte J, Weidig M, Balzer P, et al. Expression of the E-cadherin repressors snail, slug and Zeb1 in urothelial carcinoma of the urinary bladder: relation to stromal fibroblast activation and invasive

behaviour of carcinoma cells. Histochem Cell Biol 2012;138: 847-60.
[12] Liu F, Qi L, Liu B, et al. Fibroblast activation protein overexpression and clinical implications in solid tumors: a meta-analysis. PLoS One 2015;10:e0116683.
[13] López JI, Errarte P, Erramuzpe A, et al. Fibroblast activation protein predicts prognosis in clear cell renal cell carcinoma. Hum Pathol 2016;54:100-5.
[14] Errarte P, Guarch R, Pulido R, et al. The expression of fibroblast activation protein in clear cell renal cell carcinomas is associated with synchronous lymph node metastases. PLoS One 2016;11:e0169105.
[15] Choi W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 2014;25:152-65.
[16] Seiler R, Ashab HAD, Erho N, et al. Impact of molecular subtypes in muscle-invasive bladder cancer on predicting response and survival after neoadjuvant chemotherapy. Eur Urol 2017;72:544-54.
[17] Ferreira-Teixeira M, Parada B, Rodrigues-Santos P, et al. Functional and molecular characterization of cancer stem-like cells in bladder cancer: a potential signature for muscle-invasive tumors. Oncotarget 2015;6:36185-201.
[18] Hashmi AA, Hussain ZF, Irfan M, et al. Cytokeratin 5/6 expression in bladder cancer: association with clinicopathologic parameters and prognosis. BMC Res Notes 2018;11:207.
[19] Yuan D, Liu B, Liu K, Zhu G, Dai Z, Xie Y. Overexpression of fibroblast activation protein and its clinical implications in patients with osteosarcoma. J Surg Oncol 2013;108:157-62.
[20] Orimo A, Weinberg RA. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. Cell Cycle 2006;5:1597-601.
[21] Potenta S, Zeisberg E, Kalluri R. The role of endothelial-to-mesenchymal transition in cancer progression. Br J Cancer 2008;99:1375-9.
[22] Ostman A, Augsten M. Cancer-associated fibroblasts and tumor growth-bystanders turning into key players. Curr Opin Genet Dev 2009;19:67-73.
[23] Zi F, He J, He D, Li Y, Yang L, Cai Z. Fibroblast activation protein α in tumor microenvironment: recent progression and implications. Mol Med Rep 2015;11:3203-11.
[24] Kuzet SE, Gaggioli C. Fibroblast activation in cancer: when seed fertilizes soil. Cell Tissue Res 2016;365:607-19.
[25] Miyake M, Hori S, Morizawa Y, et al. CXCL1-mediated interaction of cancer cells with tumor-associated macrophages and cancer-associated fibroblasts promotes tumor progression in human bladder cancer. Neoplasia 2016;18:636-46.
[26] Wu J, Yu C, Cai L, et al. Effects of increased Kindlin-2 expression in bladder cancer stromal fibroblasts. Oncotarget 2017;8:50692-703.
[27] Teichgräber V, Monasterio C, Chaitanya K, et al. Specific inhibition of fibroblast activation protein (FAP)-alpha prevents tumor progression in vitro. Adv Med Sci 2015;60:264-72.
[28] Brennen WN, Rosen DM, Wang H, Isaacs JT, Denmeade SR. Targeting carcinoma-associated fibroblasts within the stroma with a fibroblast activation protein-activated prodrug. J Natl Cancer Inst 2012; 104:1320-34.
[29] Brennen WN, Rosen DM, Chaux A, Netto GJ, Isaacs JT, Denmeade SR. Pharmacokinetics and toxicology of a fibroblast activation protein (FAP)–activated prodrug in murine xenograft models of human cancer. Prostate 2014;74:1308-19.
[30] Schubert T, Todenhöfer T, Mischinger J, et al. The prognostic role of pre-cystectomy hemoglobin levels in patients with invasive bladder cancer. World J Urol 2016;34:829-34.
[31] Huang P, Lan M, Peng AF, et al. Serum calcium, alkaline phosphotase and hemoglobin as risk factors for bone metastases in bladder cancer. PLoS One 2017;12:e0183835.
[32] Kelly T, Huang Y, Simms AE, Mazur A. Fibroblast activation protein-α: a key modulator of the microenvironment in multiple pathologies. Int Rev Cell Mol Biol 2012;297:83-116.
[33] Fearon DT. The carcinoma-associated fibroblast expressing fibroblast activation protein and escape from immune surveillance. Cancer Immunol Res 2014;2:187-93.
[34] Najafi M, Farhood B, Mortezaee K. Cancer stem cells (CSCs) in cancer progression and therapy. J Cell Physiol 2018;234:8381-95.
[35] Goulet CR, Champagne A, Bernard G, et al. Cancer-associated fibroblasts induce epithelial-mesenchymal transition of bladder cancer cells through paracrine IL-6 signalling. BMC Cancer 2019 Feb 11;19(1): 137.