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DIFFERENTIAL EXPRESSION OF STROMA DERIVED FACTOR-1 ISOFORMS IN BLADDER CANCER

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Abstract

Purpose—Stroma-Derived Factor (SDF)-1 is a ligand for chemokine receptors CXCR4 and CXCR7. The six known SDF-1 isoforms are generated by alternative mRNA splicing. While SDF-1 expression has been detected in various malignancies, only a few studies have reported differential expression of SDF-1 isoforms and its clinical significance. In this study we evaluated the expression three SDF-1 isoforms (α , β , γ) in bladder cancer (BCa).

Methods—Using quantitative PCR, mRNA levels of SDF-1 α , SDF-1 β and SDF-1 γ were measured in bladder tissues (normal: 25; BCa: 44) and urine specimens (n=210; normal: 28; benign conditions: 74; BCa: 57, history of BCa (HxBCa): 35, Hx other Ca: 8; other Ca: 8) from consecutive patients. These levels were correlated with clinical outcome.

Results—Among SDF-1 isoforms, only SDF-1 β mRNA was significantly overexpressed by 2.5-6-fold in BCa tissues when compared to normal bladder tissues. While SDF-1 α was expressed in bladder tissues, SDF-1 γ expression was undetectable. In multivariate analysis, SDF-1 β (P=0.017) was an independent predictor of metastasis and disease specific mortality (P=0.043). In exfoliated urothelial cells, only SDF-1 β mRNA levels were differentially expressed and having a 91.2% sensitivity and 73.8% specificity for detecting BCa. In patients with HxBCa, elevated SDF-1 β levels indicated 4.3-fold increased risk (P=0.0001) for developing recurrence within 6-months.

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Conclusion—SDF-1 isoforms are differentially expressed in bladder tissues and exfoliated urothelial cells. SDF-1 β mRNA levels in BCa tissues predict poor prognosis. Further, SDF-1 β mRNA levels in exfoliated cells detect BCa with high sensitivity and are potential predictors of future recurrence.

Keywords

Prognostic markers; Stroma derived Factor-1 isoforms; bladder cancer diagnosis; bladder cancer metastasis; bladder tumor markers; SDF1- α ; SDF1- β

INTRODUCTION

Several molecular signatures have been proposed as potential biomarkers for BCa; however, the expression and levels of CXCL12 or Stroma-Derived Factor (SDF)-1 have not been examined. SDF-1 is a member of the cysteine-X-cysteine class of chemokines but lacks the glutamine-leucine-arginine or ELR-motif (1–3). It promotes angiogenesis and metastasis by binding to one of two 7-span transmembrane G-protein coupled receptors, CXCR4 and CXCR7 (1–4). Others and we have shown that CXCR7 but not CXCR4 expression is elevated in bladder tumors and correlates with high-grade and metastasis (5,6). Treatment of BCa cells with SDF-1 promotes cell proliferation and motility and although it is presumed that these effects of SDF-1 are mediated through CXCR4 (7).

SDF-1 mRNA is alternatively spliced to generate splice variants of which 5 are described in the NCBI Genome Browser - α , β , γ , δ , and 5-precursor respectively. All SDF-1 isoforms share the first three exons, and therefore, the first 88 amino acids in all isoforms are common (8–11). Functional differences among these isoforms have been reported regarding hematopoietic progenitor cell survival, chemotaxis, and anti-HIV activity (8–10). Furthermore, while SDF1- α activates NF κ B and promotes invasion, SDF1- β has more angiogenic properties (12–14). Contrarily, SDF1- γ has potent anti-HIV properties and may localize within the nucleus (15). SDF-1 expression has been associated with metastasis and survival in gastric, gallbladder, cervical and ovarian cancers, as well as, mesothelioma (16–21). SDF-1 gene polymorphism may also predict clinical outcome in ER positive breast cancer (22). However, in very few studies, the expression of various isoforms of SDF-1 has been differentially measured (23,24). This is because protein sequences of the SDF-1 isoforms SDF1- α and SDF1- β differ only in 4 amino acids and currently there are no antibodies that can selectively distinguish one isoform from the other in immunohistochemistry or ELISA assays. Since the isoforms are generated due to alternative splicing, containing different non-coding intron/exon sequences, by designing isoform-specific primer it is possible to perform Q-PCR to selectively measure the expression of each SDF-1 isoform transcript. In this study, we examined the expression of SDF1- α , SDF1- β and SDF1- γ transcripts in bladder tissues and exfoliated urothelial cells. Our data shows that SDF1- β expression is elevated in BCa tissues and exfoliated cells and detects BCa, as well as, predicts metastasis and BCa recurrence with high accuracy.

MATERIALS AND METHODS

Tissue specimens

All specimens (n = 69; normal bladder (NBL): 25; BCa: 44) were obtained based on their availability for research purpose and under a protocol approved by University of Miami's Institutional Review Board (Table 1). To determine whether SDF-1 isoform levels might be different in normal urothelial tissues from a BCa patient, NBL tissues from organ donors (NBL-O; n=17) or from patients who underwent cystectomy for muscle invasive BCa (NBL-T; n=8), were assayed. A portion of each tissue was flash frozen. Total RNA was isolated from tissues (~ 30 mg) using the RNeasy Mini kit.

Urine specimens

Urine specimens were collected from 210 individuals (Table 1); except for normal individuals, urine from all study individuals were collected at urology clinic and no other inclusion or exclusion criteria were applied. Diagnosis was based on the routine clinical workup for all cases, and for BCa patients it included cystoscopy and biopsy (if indicated for their clinical condition). There were no patients with upper track lesions, in the study cohort. Clinical follow-up was collected on patients with a history of BCa (HxBCa); mean follow-up 15 months. All urine specimens were brought to the laboratory within two hours of collection and processed for total RNA isolation using the ZR urine isolation kitTM (25).

Q-PCR

Total RNA isolated from tissues or exfoliated cells was subjected to Q-PCR in a Bio-Rad iCycler iQ real time PCR system (25) using the following primers: SDF1- α : Forward: 5'CACAGAAGGTCCTGGTGGTA3'; Reverse: 5'CATTGAAAAGCTGCA ATCACA3'; SDF1- β : Forward: 5'CGCCTTTCCAGGTGCTAAC3'; Reverse: 5'TGG TCTGCTTAGGGGATTTGG3'; SDF-1 γ : Forward: 5' GTGCCCTTCAGATTGTAGCC3'; Reverse: 5' GGGCAGCCTTTCTCT TCTTC3'. Each cDNA sample was simultaneously subjected to β -actin (for tissue samples) 18S-RNA (for exfoliated cells) Q-PCR and the normalized transcript levels for each gene were calculated as $(1/2^{-Ct} \times 100)$; $Cq = Cq$ (transcript) – Cq (β -actin or 18S-RNA). β -actin was chosen for normalization of SDF-1 isoform levels in tissues, since, the Cq values in NBL (19.8 ± 2.4) and TBL (19 ± 1.4) were not significantly different ($P=0.13$)

Statistical analysis

SDF1- α and SDF1- β levels among bladder tissues (e.g., NBL versus low-grade, NBL versus high-grade) and among various categories of urine specimens were compared using the Mann-Whitney U-test, because the data showed a non-normal distribution. All of the P-values reported in this study are two-tailed. Logistic regression model (univariate analysis) was used to determine: 1. the association of clinical parameters, and SDF1- α or - β levels with metastasis and disease-specific survival; 2. the association of urinary SDF1- α or - β levels with BCa. Cox-proportional hazards model (multivariate analysis) was used to determine which of the pre- and post-operative parameters and/or tissue SDF1- α or - β levels predict metastasis and disease-specific mortality. Kaplan-Meier plots were created by

grouping the data by parameters that were found to be significant on multivariate analysis. Log-rank test was performed to determine whether differences in various groups were significant.

Cut-off values were selected from the ROC curve for calculating sensitivity and specificity of SDF1- α or - β to predict metastasis, disease-specific survival (tissue specimens) and the presence of BCa (urine specimens). A biomarker level that yielded the highest efficacy (i.e., sensitivity – (1-specificity)) was selected by the statistical program as the cut-off limit. JMP® Software Program (SAS Institute, Cary, NC) was used for statistical analyses.

RESULTS

SDF-1 expression is increased in BCa tissues

To visually evaluate the differences between the 5 transcript isoforms of SDF-1 on Chr10 q11.21, we utilized the UCSC Genome Browser. Figure 1 shows that the first 3 exons in the SDF-1 transcript are highly conserved between isoforms (8–11). The levels of SDF1- α , SDF1- β and SDF1- γ transcripts were measured in bladder tissues using variant-specific Q-PCR primers and were normalized to β -actin. β -actin was chosen for normalization of SDF-1 isoform levels in tissues, since, the mean Cq values in NBL-O (19.1 ± 1.7), NBL-T (19.9 ± 2.7), low-grade (LG; 19.1 ± 1.8) and high-grade (HG; 19.2 ± 1.4) were not significantly different ($P=0.41$). Figure 2A shows that when compared to NBL tissues (NBL-O; 0.66 ± 1.18 NBL-T: 0.36 ± 0.44), the mean SDF1- α mRNA levels were 3–4-fold lower in LG (0.08 ± 0.12) and comparable in HG (0.42 ± 0.11) BCa tissues. However, the differences in SDF1- α mRNA levels between NBL-O and NBL-T ($P=0.79$), LG ($P=0.25$) or HG ($P=0.39$) tissues were not significant and SDF1- α levels did not correlate with tumor grade or stage. The mean SDF1- β levels were elevated 2.5–6-fold in both low-grade (0.55 ± 0.8) and high-grade (1.4 ± 2.3) BCa tissues when compared to NBL-O (0.17 ± 0.21) and NBL-T (0.18 ± 0.15) tissues (Figure 2B). However, the differences between NBL-O or NBL-T and LG tissues were not significant ($P=0.09$, 0.24 , respectively). The difference between NBL-O or NBL-T and HG tissues in SDF1- β transcript levels were significant ($P<0.01$). The differences in SDF1- β levels in patients with HG and LG BCa, as well as, among patients with non-muscle invasive (stages Ta, T1) and muscle invasive (stages T2) disease were not significant ($P>0.05$). The expression of SDF1- γ was undetectable in both normal and tumor tissues. Furthermore, the levels of none of the SDF-1 isoforms correlated with response to BCG, neo-adjuvant chemotherapy or radiation therapy ($P>0.05$).

Association of SDF1- α and SDF1- β expression with metastasis

In this study, the majority of patients had high-grade ($n = 35$) and muscle invasive ($n = 32$) BCa. The mean SDF1- α levels among patients with metastasis (0.7 ± 1.47) were elevated when compared to patients without metastasis (0.14 ± 0.2). The mean SDF1- β levels among patients with metastasis (1.8 ± 2.1) were 2.5-fold higher than the levels among patients who did not metastasize (0.7 ± 0.95) during follow-up. In univariate analysis pathologic stage, lymph node positivity for tumor, SDF1- α and SDF1- β transcript levels significantly associated with metastasis (Table 2). In the multivariate model, only stage and SDF1- β mRNA levels significantly associated with metastasis. While age, gender, grade, stage and

lymph node status significantly associated with disease specific mortality (DSM) in univariate analysis, only age and SDF-1 β mRNA levels were significant in the multivariate model. Kaplan-Meier plots showed significant differences in groups based on stage and SDF-1 β status with respect of developing metastatic disease (Figure 3). Although, the number of specimens was limited, SDF-1 β transcript levels (cut-off 0.45) had 70% sensitivity and 76.4% specificity for predicting metastasis, respectively. The sensitivity (58.8%) and specificity (59.1%) of SDF-1 β were low for predicting disease specific mortality.

SDF-1 α and SDF-1 β mRNA expression in exfoliated urothelial cells

We used the same Q-PCR assays to measure transcript levels of SDF-1 isoforms in exfoliated urothelial cells present in urine specimens from 210 subjects. 18S RNA was used to normalize the SDF-1 levels, as the Cq values for 18S RNA among BCa patients (17.4 ± 1.4) and control categories (17.8 ± 1.6) were not significantly different ($P=0.1$). As shown in Fig. 4A, SDF-1 α levels in patients with low-grade BCa were significantly lower than the levels in control categories of individuals ($P=0.003$). However, the difference in SDF-1 α levels among patients with low- or high-grade BCa was not significantly different ($p=0.09$). Contrarily, SDF-1 β levels were significantly higher in high-grade BCa patients when compared to various control categories ($P=0.001$) and low-grade BCa patients ($P=0.022$; Figure 4B). The differences in SDF-1 β levels between patients with low-grade BCa and all control categories, except normal individuals were not significant ($P>0.05$). SDF-1 γ mRNA levels were undetectable in exfoliated cells.

Efficacy of SDF-1 β levels to detect BCa

Based on the cut-off values generated by ROC curves, SDF-1 α transcript levels had high sensitivity but poor specificity to detect BCa (Table 3). SDF-1 β levels had high sensitivity to detect BCa, further the sensitivity was higher for high-grade than low-grade BCa (Table 3). The specificity of SDF-1 β levels among various non-BCa categories was reasonable (75–82%; Table 1), except in the HxBCa category (specificity: 57.1%). This is because 11 of these patients recurred within 6 months. While 10 out of these 11 patients had SDF-1 β levels above the cut-off limit, only 5 out of the 24 HxBCa patients who did not recur within 6-months had levels above the cut-off limit (*chi-square*: 15.1; $P=0.0001$; relative risk = 4.3, 95% CI: 1.4 – 5.6). In this study 8 patients had cancers other than BCa; SDF-1 α levels were higher in all 8 patients, whereas, SDF-1 β levels were higher 6 of the patients.

DISCUSSION

This is the first study that demonstrates differential expression of SDF-1 isoforms in BCa tissues and in exfoliated urothelial cells from BCa patients. The key findings of our study are: 1. SDF-1 β , mRNA levels differentially overexpressed in BCa tissues and exfoliated urothelial cells; 2. SDF-1 β mRNA levels correlated with metastasis and had high sensitivity and reasonable specificity to detect BCa, especially HG BCa; 3. Elevated SDF-1 β mRNA levels among patients with HxBCa indicated a 4-fold increased risk for recurrence within 6-months.

In different carcinomas, elevated SDF-1 expression has been shown to either associate with metastasis and poor survival or with improved survival (16–21; 26). Such contradictory observations may be because these studies detected SDF-1 expression by immunohistochemistry and hence, did not distinguish which of the SDF-1 isoforms was responsible for the observed higher SDF-1 protein expression. SDF-1 expression in cancer is also epigenetically regulated by promoter methylation and SDF-1 gene polymorphism probably increases SDF-1 expression (22, 27–29). Our study shows that differential expression of SDF-1 isoforms is another layer of regulation for SDF-1 expression in tumor tissues. Furthermore, although the sample size was small, a significant limitation of the study, SDF1- β expression independently correlated with metastasis. Others and we have previously shown that in renal cell carcinoma, SDF-1 α and SDF-1 β mRNA levels correlate with metastasis (22, 23).

In BCa, CXCR4 and SDF-1 α interaction promotes proliferation and motility (7,30). However, we recently demonstrated that CXCR7 is the major chemokine receptor in BCa tissues and cells (5). The functional significance of CXCR7 and SDF-1 β expression in BCa cells and tissues remains to be demonstrated. However, in our study, a combined CXCR7 and SDF-1 β biomarker did not improve the efficacy of BCa detection. This appears to be consistent with the finding that CXCR7 may be acting in a ligand-independent manner, although it has higher affinity for SDF-1 than for CXCR4 (1–3).

Although SDF-1 is a C-X-C inflammatory chemokine, the expression of SDF-1 β mRNA was not consistently elevated in the exfoliated cells of patients with nephrolithiasis; the latter being the case regarding CXCR7 expression (5). In our study 35 patients were being monitored for HXBCa and in this group, elevated SDF-1 β mRNA levels were highly predictive of BCa recurrence within 6-months. Since BCa patients undergo cystoscopy for monitoring recurrence at 3 to 6 month intervals, measurement of SDF-1 β may potentially detect recurrence even before the tumors are detectable on cystoscopy. A limitation of any study that examines the differential expression of SDF-1 isoforms is that such studies are limited to mRNA expression only. This is because, SDF-1 α and SDF-1 β differ in their sequences by four amino acids, and hence, antibodies that distinguish between these isoforms are not available. A limitation of studies involving tissues and exfoliated cells is tumor heterogeneity. However, this limitation may not have appreciable impact because only SDF-1 β levels correlated with metastasis. Single institution is another limitation. However, a sample size of over two hundred consecutive patients is fairly large. Nevertheless, given that Q-PCR assays can be conducted in a reference laboratory, if multi-center studies confirm our initial observation, SDF-1 Q-PCR test can be potentially developed for diagnosing BCa and for monitoring its recurrence. Taken together, the expression of SDF-1 β isoform is a potential predictor of metastasis, survival and recurrence. An investigation into the function of SDF-1 β in BCa cells is warranted.

Take home message

This is the first study that demonstrates differential expression of SDF1-isoforms in bladder tissues and that elevated SDF1- β mRNA levels are independently associated with BCa

metastasis and disease-specific mortality. Furthermore, SDF1- β mRNA levels in exfoliated cells detect bladder cancer with high sensitivity and predict future BCa recurrence.

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Abbreviations used

SDF	Stroma derived factor
Q-PCR	quantitative polymerase chain reaction
RT	reverse transcription

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Figure 1. Discerning SDF-1 transcript isoforms

All 5 SDF-1 isoforms were found from a RefSeq search on the UCSC Genome Browser.

The figure shows the chromosomal position based on Human Genome version 19 (hg19) and exon-intron boundaries in the 5 isoforms. RefSeq accession mRNA numbers for the SDF-1 isoforms α , β , γ , δ , and 5-precursor are - NM_199168.3; NM_000609.6; NM_001033886.2; NM_001178134.1; NM_001277990.1, respectively.

Figure 2. Analysis of SDF-1α and SDF-1β expression in bladder tissues

NBL: normal bladder; NBL-O: NBL tissue obtained from organ donors; NBL-T: NBL tissue obtained from BCa patients at the time of cystectomy. LG: low-grade BCa; HG: high-grade BCa. Y-axis: SDF-1 mRNA levels normalized to β-actin mRNA levels as described in “Materials and Methods”. Data on Mean ± sd levels are shown.

Figure 3. Kaplan-Meier plots for metastasis and survival

Kaplan-Meier plots. Kaplan-Meier plots were created to evaluate the effect of variables that were found to be independently associated with metastasis or survival. Stage was stratified as $\geq T2$ (high or H) and $< T2$ (low or L). Normalized SDF-1 β mRNA levels were stratified as ≥ 0.32 (H) or < 0.32 (L); 0.32 was used as the cut-off limit for calculating efficacy.

Figure 4. Analysis of SDF-1 α and SDF-1 β expression in exfoliated cells
 SDF1-isoform mRNA levels were measured in exfoliated cells. Y-axis: SDF-1 mRNA levels normalized to 18S RNA, as described in “Materials and Methods” Mean $\pm$ sd levels are shown.

Table 1

Specimen and patient characteristics. Characteristics of bladder tissue and urine specimens are shown.

BGU: patients with benign genitourinary conditions. For patients undergoing cystectomy (stages T2), urine specimens were collected prior to scheduling the patient for cystectomy. Presence of metastasis was determined based on CT scan inference.

Tissue specimens n= 69 (NBL = 25) Organ donors (NBL-O) = 17 BCa patients undergoing cystectomy (NBL-B) = 8		Urine Specimens (n = 210)	
BCa = 44; Transurethral resection (TURBT) = 12 Cystectomy = 32		Normal	n = 28 Group 1: n = 17; Age: 38.3 ±13.9; Median: 30.5 yrs Gender: Female = 6; Male = 11 Group 2: n = 11 Age: 63.2 ± 10.1; Median: 61 yrs Gender: Female = 6; Male = 5
Gender	Female n = 10 Male, n = 34	BCa	n = 57 LG = 17; HG = 40 Stage: Ta = 19; CIS = 1 ; T1 = 15; T2 = 13 ; T3 = 8 ; T4 = 1 Age: 68.9 ± 9.2; 69 Gender: Female = 20; Male = 37
Smoker	(+) = 25 (-) = 2 Unknown: 17	BGU	n = 74 Urinary tract infection = 8 Cystitis: 2 Benign prostatic hyperplasia = 13 Hematuria = 20 Hydronephrosis = 2 Nephrolithiasis = 13 Prostatitis = 5 Hydrocele = 1 Dysuria = 1 Urethral stricture = 4 Impotence = 1 Renal cyst = 3 Adrenal mass = 1 Age = 54.3 ± 11.9; 55.5 Gender: Female = 32; Male = 42
Grade	LG = 8 (all, stage Ta) HG = 36	HXBCa	n = 35 Age: 64.2 ±8; 63 Gender: Female = 7; Male = 28

		Hx other cancers	n = 8 Hx Renal cancer n = 2 Hx prostate cancer = 6 Age: 62.9 ± 12.9; 62.5 Gender: Female = 3; Male = 5
Stage	Ta = 8; T1 = 3 T2 = 13; T3 = 15; T4 = 5 Concomitant CIS present = 4	Other cancers	N = 8 Prostate cancer n = 4 Prostate cancer metastatic to bone: 2 Rectal cancer metastatic to bladder = 1 Cervical cancer = 1 Age: 65.7 ± 9.6; 64 Gender: Female = 2; Male = 6
LN	(+) = 11 (-) = 26 Unknown = 7		
Metastasis (all BCa patients)	(-) = 22 (+) = 16 Unknown = 6		
Metastasis in patients with stage T2 tumor	(-) = 13 (+) = 16 Unknown = 4		
Age	Metastasis (+): 64.5 ± 11.9 yrs Median: 67 yrs Metastasis (-): 64.3 ± 10.6 yrs Median: 63 yrs		
BCG	(+) 8 (-) 26 Unknown = 10		
Neoadjuvant Chemotherapy	(+) = 9 (-) = 23 Unknown = 12		
Adjuvant Chemotherapy	(+) = 6 (-) = 22 Unknown = 16		
Radiation	(+) = 5 (-) = 20 Unknown = 19		
Death	(-) = 23 (+) = 18; BCa specific = 16		

	Unknown = 3	
Mean Follow-up	23.6 ±4; median = 18 months (1*-120 months) * All stage T4 patients were considered as positive for metastasis at the time of diagnosis.	

Table 2

Determination of the association between clinical outcome and SDF-1 expression. Pre- and post-operative parameters included age, gender, prior recurrence, tumor grade, stage, lymph node status and concomitant presence of CIS. **A:** Univariate analysis: Logistic regression single parameter analysis was used to determine the association of pre- and post-operative parameters and SDF-1 α and SDF-1 β levels with metastasis. ND: Not determined for parameters that did not reach significance. **B:** Cox proportional hazard model was used for performing multivariable analysis. Age, gender, grade, stage, lymph node status, concomitant presence of carcinoma *in situ* (CIS), SDF-1 α , and SDF-1 β mRNA levels were included in the model. Only significant parameters are shown in the Table.

	Metastasis				DSM			
Univariate analysis								
Parameter	P-value	Chi-sq	Odds Ratio	95% CI	P-value	Chi-sq	Odds Ratio	95% CI
Age	0.769	0.09	ND	ND	0.043	4.1	ND	ND
Gender	0.967	0.00	ND	ND	0.049	3.87	3.0	1.2–13.5
Grade	0.94	0.01	ND	ND	0.041	4.2	5.6	1.6–78.7
Stage	0.0031	8.72	6.6	2.5–32.3	0.003	8.55	3.5	1.7–9.1
Lymph node	0.015	5.94	4.6	1.6–20.4	0.036	4.4	3.0	1.2–10
CIS	0.389	0.75	ND	ND	0.397	0.72	ND	ND
SDF1-α	0.044	3.45	2.2	1.1–5.0	0.987	0.0	ND	ND
SDF1-β	0.02	5.42	2.6	1.2–7.2	0.79	0.07	ND	ND
Multivariate analysis								
Parameter	P-value	Chi-sq	Risk Ratio	95% CI	P-value	Chi-sq	Risk Ratio	95% CI
Stage	0.005	7.9	2.9	1.3–8.0				
SDF1-β	0.017	5.74	1.6	1.1–2.4	0.043	4.1	2.1	1.0–5.1
Age					0.043	4.1	1.1	1.0–1.25

Table 3

Determination of the efficacy of SDF-1 expression to detect BCa. Analysis of sensitivity by tumor grade and of specificity by non-BCa conditions is also included.

Parameter	P-value	Chi-sq	AUC	Cutoff	Sensitivity	Specificity	Accuracy
SDF-1 α	0.23	1.44	0.58	0.05	84.2% (48/57) LG: 66.7% (12/18) HG: 92.4% (36/39)	29.9% (43/144) Normal: 39.3% (11/28) BGU: 27% (20/74) HXBCa: 31.4% (11/35) HX other Ca: 12.5% (1/8)	41.3% (83/201)
SDF-1 β	<0.0001	15.7	0.83	0.32	91.2% (52/57) LG: 77.8% (14/18) HG: 97.4% (38/39)	73.8% (107/145) Normal: 85.7% (24/28) BGU: 77% (57/74) HXBCa: 57.1% (20/35)* HX other Ca: 75% (6/8)	78.6% (158/201)

* : 11 patients with HXBCa had a recurrence within 6 months: 10 out of these 11 were false positive for SDF-1 β . Contrarily, 5 out of the remaining 24 HXBCa patients who did not recur were false positive for SDF-1 β .