RESEARCH ARTICLE

Can Recurrence and Progression be Predicted by HYAL-1 Expression in Primary T1 Bladder Cancer?

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Abstract

Background: Molecular prognostic markers have been under investigation for the last decade and no validated marker to date has been proven to be used in daily clinical practice for urinary bladder cancers. The aim of the present study is to evaluate the significance of HYAL-1 expression in prediction of recurrence and progression in pT1 urothelial carcinomas. Materials and Methods: Eighty-nine urothelial carcinoma cases staged as T1 according to 2004 WHO classification were studied. Representative sections from every case were stained immunohistochemically for HYAL-1 and scored between 0 and +3, according to staining density, and graded as low and high for the scores 0-1 and 2-3, respectively. Results: Of the 89 pT1 bladder cancer patients, HYAL-1 expression was high in 92.1% (82 patients; 72 patients +3 and 10 patients +2) and low in 7.9% (only 7 patients; 6 patients +1 and 1 patient 0) of the cases. Of the 89 patients, 38 (42.7%) had recurrence and 22 (24.7%) showed progression. HYAL-1 staining did not show significant characteristics for tumor grade, accompanying CIS, multiplicity, tumor size, age and sex. HYAL-1 expression did not have any prognostic value in estimating recurrence or progression. Conclusions: HYAL-1 expression was found to be high, but did not have any prognostic importance in T1 bladder urothelial carcinomas.

Keywords: Bladder cancer - HYAL-1 - expression - prognosis

Asian Pac J Cancer Prev, **15 (23)**, 10401-10405

Introduction

Non-muscle invasive bladder cancers (NMIBC) are non-invasive (Ta) in 70%, invasive to lamina propria (T1) in 20%, and flat (carcinoma in situ) in 10% of the cases (Kirkali et al., 2005). NMIBC invading lamina propria are heterogeneous that some patients are treated by transurethral resection (TUR), and some require aggressive treatment modalities such as radical cystectomy and close follow up. T1 tumors are generally high grade, with a high risk of progression and/or metastasis. Progression rate is 20 to 40% in low grade T1 tumors treated by TUR only (Nieder et al., 2005; Stein and Penson, 2008). Progression and recurrence rates of high-grade T1 tumors are reported to be 60% and 80%, respectively (Heney, 1992).

The prognosis of the patients with NMIBC which progressed during follow up is significantly poorer than T2 patients' prognosis with primary muscular invasion (Schrier et al., 2004). Solid or papillary pattern, grade, accompanying CIS, tumor size, multiplicity, lymphovascular invasion, depth of invasion (T1a or T1b), re-TUR pathology and recurrence in third month control are the clinical and pathological prognostic factors of T1 tumors (Nieder et al., 2005; Andius et al., 2007; Herr et al., 2007; Cho et al., 2009). However, the heterogeneous patient profile of the disease limits the value of prognostic factors. Therefore new molecular markers are needed to evaluate the prognosis of urothelial carcinomas (Tuna et al., 2003; Habuchi et al., 2005; Shariat et al., 2008; Bryan et al., 2010; Wang and Wang, 2012; Ghafouri-Fard et al., 2014).

Hyaluronoglycosidase 1 (HYAL-1) expressed by tumor cells is a member of hyaluronidase (HAase) enzymes family (Posey et al., 2003; Simpson and Lokeshwar, 2008). It is shown that HYAL-1 is a determinant of tumor growth, infiltration and angiogenesis (Lokeshwar et al., 2005). In bladder and prostate cancer, precluding HYAL-1 expression decreases the tumor cell proliferation 4 fold, by stopping cell cycle in G2/M phase (Lokeshwar et al., 2005). It is shown that HYAL-1 is an independent prognostic factor of biochemical recurrence after radical prostatectomy (Posey et al., 2003; Ekici et al., 2004). HYAL-1 expression is suggested to estimate biochemical recurrence in biopsy period and radical prostatectomy (Gomez et al., 2009). HYAL-1 was stated as an independent prognostic factor and reported that it has 78% sensitivity and 75% specifity in estimating muscular invasion in a heterogeneous urothelial carcinoma cases (Kramer et al., 2010).

In this study we aimed to evaluate the impact of HYAL-1 in predicting recurrence and/or progression in

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a homogeneous group of pT1 bladder urothelial cancer patients.

Materials and Methods

Patient characteristics

Data of 129 patients who were diagnosed as pT1 bladder cancer at our department between 2001 and 2009, was analyzed retrospectively. Tissue blocks of 112 patients were obtained from our pathology archive. Patients operated in other medical centers (n=17) were excluded from study. Nine patients with muscular invasion in re-TUR and fourteen patients whose tissue blocks could not be found in archive were also excluded. Thus, totally 89 T1 bladder cancer patients were included in the study.

All patients were initially treated with TUR. Patient and tumor characteristic are detailed in Table 1. After initial TUR, muscle samples were found in TUR specimens of 50 patients (56.2%). Patients with high grade urothelial carcinoma, incomplete resection, and TUR without muscle samples (total 41 patients, 46.1%) have all undergone re-TUR. Re-TUR histology was T1 in 11 patients, CIS in 1 patient and T0 (no tumor) in 29 patients. Following TUR, intravesical BCG therapy was administered to 68 patients (76.4%). Intravesical BCG was not used if the patients had low grade tumor and if CIS was not accompanying. After initial TUR, check cystoscopies were performed in at 3-month interval for the first 2 year, 4 months in the third year, every 6 months for 2 years and annually thereafter. Computerized tomography (CT) had been performed annually to detect the metastasis and to evaluate upper urinary tract for urothelial cancer.

Table 1. Patient and Tumor Characteristics of the Study Population

Characteristics	n=89	%
Number of patients		100
Male	72	80.9
Female	17	19.1
Age		
<65	39	43.8
>65	50	56.2
Number of tumor		
Solitary	34	38.2
Multiple	55	61.8
Grade		
Low grade	30	33.7
High grade	59	66.3
Accompanying CIS		
Yes	26	29.2
No	63	70.8
Re-TUR		
Yes	41	46.1
No	48	53.9
Tumor size		
<3 cm	58	65.2
>3 cm	31	34.8
Recurrence		
Yes	38	42.7
No	51	57.3
Progression		
Yes	22	24.7
No	67	75.3

Presence of bladder carcinoma in control cystoscopy was accepted as recurrence. Progression to muscular invasion, increased tumor grade or any metastases were considered as progression. During the follow up 38 patients (42.7%) showed recurrence, and 22 patients (24.7%) showed progression. Of the 22 patients with progression, 9 had radical cystectomy, 2 had radiotherapy and chemotherapy and 11 received chemotherapy only.

Immunohistochemistry

Representative sections from every case were stained immunohistochemically for HYAL-1 (Anti-HYAL-1 HPA002112 dilution 1:50, Atlas Antibodies). Immunohistochemical staining was performed with automatic stainer device (Dako Link Autostainer, Dako K8000) (Posey et al., 2003).

Immunohistochemical scoring

Two experienced uro-pathologists (BT and KY) graded all slides independently and blinded fashion. Any discrepancy in assigning staining intensity was resolved by both researchers reexamining those slides simultaneously. The staining for HYAL-1 was graded between 0 and 3+, according to the staining density. In the case of overall staining grade for each slide was assigned based on the staining intensity of the majority of the tumor tissue in the specimen. However, if 50% of the tumor tissue stained as 1+ and the other 50% as +3, the overall staining grade was accepted as +2. If the staining distribution was 50% of the tumor staining +2 and the remaining staining as +3, the overall staining was accepted as +3. Thereafter staining scale was divided as low expression (0 and +1) and high expression (+2 and +3) groups (Posey et al., 2003).

Statistical analysis

SPSS 15 was used for statistical analysis. Fischer exact test and chi-square tests were used and, $p<0.05$ value was accepted as statistically significant.

Results

Mean patient age was 68 (range 37-91) years. The follow up period ranged between 4 and 117 months with a median of 36 months. The average durations between TUR and recurrence, TUR and progression were 12 (range 3-56) and 11 months (range 3-43), respectively.

Of the 89 patients, HYAL-1 expression was high in 82 patients (92.1%; 10 patients +2, 72 patients +3) and low in only 7 patients (7.9%; 1 patient 0, 6 patients +1). Different staining features of HYAL-1 expression in T1 patients were shown in Figure 1. Of the 89 patients, 38 (42.7%) had recurrence and 22 (24.7%) showed progression.

There was no correlation between grade, the presence of CIS, multiplicity, age and sex and HYAL-1 expression (Table 2).

The expression of HYAL-1 did not correlate with recurrence in T1 tumors (p=1.0). On the other hand, the relationship was found between grade (p=0.034) and the presence of CIS (p=0.021) and recurrence (Table 3). HYAL-1 staining was not statistically effective to estimate progression in T1 tumors (p=0.186) (Table 3).

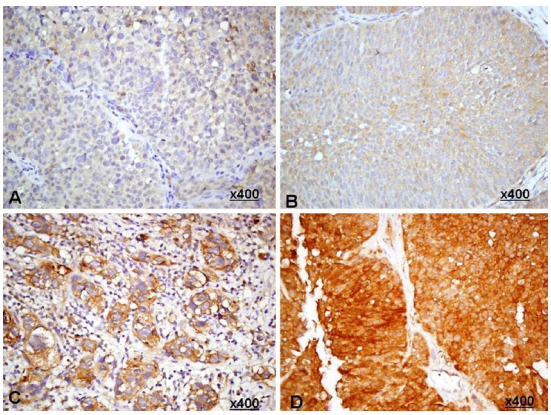

Figure 1. HYAL-1 Expression; Low Expression: Figure 1A-Grade 0, Figure1B-Grade +1; High Expression: Figure 1C-Grade +2, Figure 1D-Grade +3

Table 2. HYAL-1: Relationship with Staining Features and Parameters (Grade, Accompanying CIS, Tumor Size, Multiplicity, Age and Sex)

Parameter	HYAL-1 Low Expression (0 and +1) n=7	HYAL-1 High Expression (+2 and +3) n=82	p
Grade			0.09[a]
Low grade	0	30 (100)	
High grade	7 (11.9)	52 (88.1)	
Accompanying CIS			1.0[a]
Yes	2 (7.7)	24 (92.3)	
No	5 (7.9)	58 (92.1)	
Tumor size			0.232[a]
<3 cm	3 (5.2)	55 (94.8)	
> 3 cm	4 (12.9)	27 (87.1)	
Number of tumor			1.0[a]
Solitary	3 (8.8)	31 (91.2)	
Multiple	4 (7.3)	51 (61.8)	
Sex			0.615[a]
Male	5 (6.9)	67 (93.1)	
Female	2 (11.8)	15 (88.2)	
Age			0.223[a]
<65	5 (12.8)	34 (78.2)	
>65	2 (4.0)	48 (96)	

*Low grade: Low grade urothelial carcinoma; High grade: High grade urothelial carcinoma; CIS: Carcinoma in situ; a Fisher exact test

Table 3. Determinants of Recurrence and Progression

	Recurrence Positive n (%)	p	Progression Positive n (%)	p
HYAL-1 Low expression	3 (42.9%)	1.0	0	0.186
HYAL-1 High expression	35 (42.7%)		22 (26.8%)	
Low grade	9 (30%)	0.034*	2(6.7%)	0.005*
High grade	29 (49.2%)		20 (33.9%)	
CiS +	16 (61.5%)	0.021*	12 (46.2%)	0.003*
CiS -	22 (34.9%)		10 (15.9%)	
Tumor size<3 cm	23 (39.7%)	0.428	14 (24.1%)	0.862
Tumor size>3 cm	15 (48.4%)		8 (25.8%)	
Solitary	13 (38.2%)	0.503	7 (20.6%)	0.478
Multiple	25 (45.5%)		15 (27.3%)	
Male	30 (41.7%)	0.686	16 (22.2%)	0.348
Female	8 (47.1%)		6 (35.3%)	
Age <65	15 (38.5%)	0.476	9 (23.1%)	0.751
Age >65	23 (46%)		13 (26%)	

*Fisher exact test and Chi-square test were used, p<0.05 was deemed statistically significant

Multiplicity, tumor size, age and sex had no significant prognostic value for progression of T1 tumors but grade (p=0.005) and the presence of CIS (p=0.003) were found to have prognostic value.

Discussion

The optimal treatment in T1 bladder cancers is still controversial and unfortunately there is no reliable molecular marker estimating clinic prognosis of T1 tumors (Lascombe et al., 2006; Dalbagni et al., 2007). Ki67 and Vascular endothelial growth factor (VEGF) expression (Chen et al., 2012; Huang et al., 2013) , survivin expression (Srivastava et al., 2013) are all promising bladder cancer molecular prognostic markers which need validation. ppGalNAc T1 has recently been introduced as a novel molecular prognostic marker for bladder cancer (Ding et al., 2012), however repetitive studies are lacking with this novel marker.

HYAL-1 is a -55 - 60 kDa protein consisting of 435 amino acids (Lokeshwar et al., 1999) and HYAL-1 gene reside in the chromosome 3p21.3 locus (Csoka et al., 2001). RT-PCR and cDNA cloning, protein purification, immunoblotting, pH activity profile and immunohistochemistry have revealed that HYAL-1 is the major tumor-derived HAase expressed in prostate and bladder carcinoma cells with invasive/metastatic potential (Lokeshwar et al., 1999; Csoka et al., 2001; Lokeshwar et al., 2001; Eissa et al., 2005).

Recently it was shown that HYAL-1 could be a prognostic factor to estimate the muscle invasion and recurrence in heterogenous group of bladder cancer patients (Kramer et al., 2010). Kramer et al. reported that HYAL-1 expression has a correlation with grade, stage, accompanying CIS and multifocality. HYAL-1 expression was high in grade T1 and T2 tumors, but there wasn't any statistical difference for HYAL-1 expression between T1 and T2 tumors (Kramer et al., 2010). It was also demonstrated that HYAL-1 expression in bladder cancer significantly predicted metastasis and disease-specific survival in a study with majority of patients with high grade and muscle invasive disease (Kramer et al., 2011).

In the present study, we found that there is no correlation between HYAL-1 expression and clinical-pathological parameters in pT1 bladder cancer. In agreement with current literature HYAL-1 expression was high in 92.1% of the patients we studied (Kramer et al., 2010). The high expression of the HYAL-1 in T1 bladder tumors can be due to the similarity of its genetic features with bladder tumors showing muscle invasion (Lopez-Knowles et al., 2006).

We found that HYAL-1 expression was not a significant predictor for recurrence (p=0.91) and progression (p=0.186) of pT1 bladder cancers. Kramer et al. studied 111 bladder cancer patients without muscle invasion and found that HYAL-1 was a prognostic factor to determine the recurrence and muscle invasion development (Kramer et al., 2010). It is known that recurrence and progression probability is high for T1 tumors which are not invading the muscle. When we take into account that particularly T1 tumors had progressed and recurred in Kramer's study, those results are not correlated with our results (Kramer et al., 2010). This difference can be due to the difference of the sample size and patient selection (heterogenous and homogenous groups). On the other hand, it is shown that

HYAL-1 is a determinant of tumor growth, infiltration and angiogenesis (Lokeshwar et al., 2005; Lokeshwar et al., 2005). Our results demonstrated that HYAL-1 expression does not help in the prediction of the recurrence in T1 tumors. This result may be attributed to the fact that high levels of HYAL-1 expression may represent a later event in tumor progression rather an early event in urothelial tumorigenesis. The high expression of HYAL-1 in stage T1 bladder tumors together with insignificant role in determining the prognosis of stage pT1 tumors lead us to speculate if HYAL-1 is a tumor supressor or promoter.

Selection of cells expressing different HYAL-1 levels showed that the cells expressing the amounts similar to tumor tissues and cells promote tumor growth, invasion and angiogenesis. In contrast, cells with HAase levels exceeding 100 milliunits/10^6 cells, (i.e.; levels that are not naturally expressed by tumor cells) exhibit reduced tumor incidence and growth due to induction of apoptosis (Lokeshwar et al., 2005; Simpson and Lokeshwar, 2008). Therefore, the function of HAase as a tumor promoter or a suppressor is a concentration-dependent phenomenon and levels in genitourinary tumors are consistent with tumor cell derived HAase acting mainly as a tumor promoter (Simpson and Lokeshwar, 2008).

To our knowledge this is the first study evaluating prognostic value of HYAL-1 expression in recurrence and progression of solely pT1 bladder urothelial cancer. We have found high levels of HYAL-1 expression in our T1 bladder tumor patients but this finding did not show any significant prognostic value. We found that accompanying CIS may be a prognostic factor for recurrence and grade is related with recurrence and progression in our series. Currently grade, accompanying CIS, tumor size, multiplicity, re-TUR histology are still considered to have prognostic importance. We need more research to find new valid prognostic parameters in pT1 bladder cancer.

In the present study, we conclude that the tumor recurrence is related to tumor grade and the presence of CIS, while HYAL-1 expression does not seem to be related to the recurrence of the tumor. These results suggest that tumor grade and the presence of CIS may be helpful in predicting the tumor recurrence rather than other standard prognostic factors.

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