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Original Article

Relation of stem cell markers ALDH1 and CD44 with clinicopathological factors in urothelial carcinomas of urinary bladder

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Abstract: Molecular studies are ongoing in regards to superficial urothelial carcinoma of the bladder (UCB) either to define targeted therapy or to better select aggressive therapy candidates and also to delineate the outcome of the disease. In this study, we aimed to present the impact of ALDH1 and CD44 as stem cell markers in tumorigenesis and their prognostic value in urothelial carcinoma. We investigated ALDH1 and CD44 immunohistochemically in paraffin-embedded material of 125 non-muscle-invasive (NMI) cases in 163 UCB patients. In the NMI-UCB subgroup, we found ALDH1 to be significantly correlated with all poor prognostic factors, including high stage (≥pT2), high grade, recurrence and progression development and poor survey (P=0.001) in contrast to CD44 expression (P>0.05). Although ALDH1 expression had a good correlation with a poor clinical course of UCB, it could be used as a molecular marker to determine the best treatment strategy and could contribute to the development of targeted therapies.

Keywords: ALDH1, CD44, bladder carcinoma, prognostic value

Introduction

As one the most common human malignancies, urothelial carcinomas of the bladder (UCB) can present with non-muscle-invasive (NMI; pTa or pT1) tumors that can be treated with conservative approaches. However, recurrences and grade and/or stage progression are occasionally seen. Nonetheless, muscle-invasive (MI; ≥pT2) bladder cancers have high risks of disease progression and metastasis. These unfavorable prognoses of MI tumors have been used to propose additional prognostic indicators to identify the subset of NMI tumors that are likely to progress [1-3]. Within this context, the roles of stem cell markers have intensified in recent years in tumorigenesis and prognosis due to emerging cancer stem cell theory [4]. The cancer stem cells have the capacity for self-renewal and are also thought to be able to differentiate into other cancer stem cell types [5, 6].

Aldehyde dehydrogenase 1 (ALDH1) and CD44 expressions are related with stem cells and are supposed to be predictors of cancer stem cells in a variety of tumors [4, 6-9]. ALDH1 is an enzyme that takes part in the synthesis and regulation of retinoic acid (RA). It plays a crucial role in the differentiation and regulation of the self-renewing ability of either normal or cancer stem cells [10].

It has been reported that ALDH1 expression has a relationship with the prognostic factors and/or resistance to treatment in breast [11], colorectal [12], lung [13], ovarian [14], esophageal [15], stomach [16], and bladder [17] cancers. Therefore, in our study, we aimed to investigate the expression of CD44 and ALDH1 as

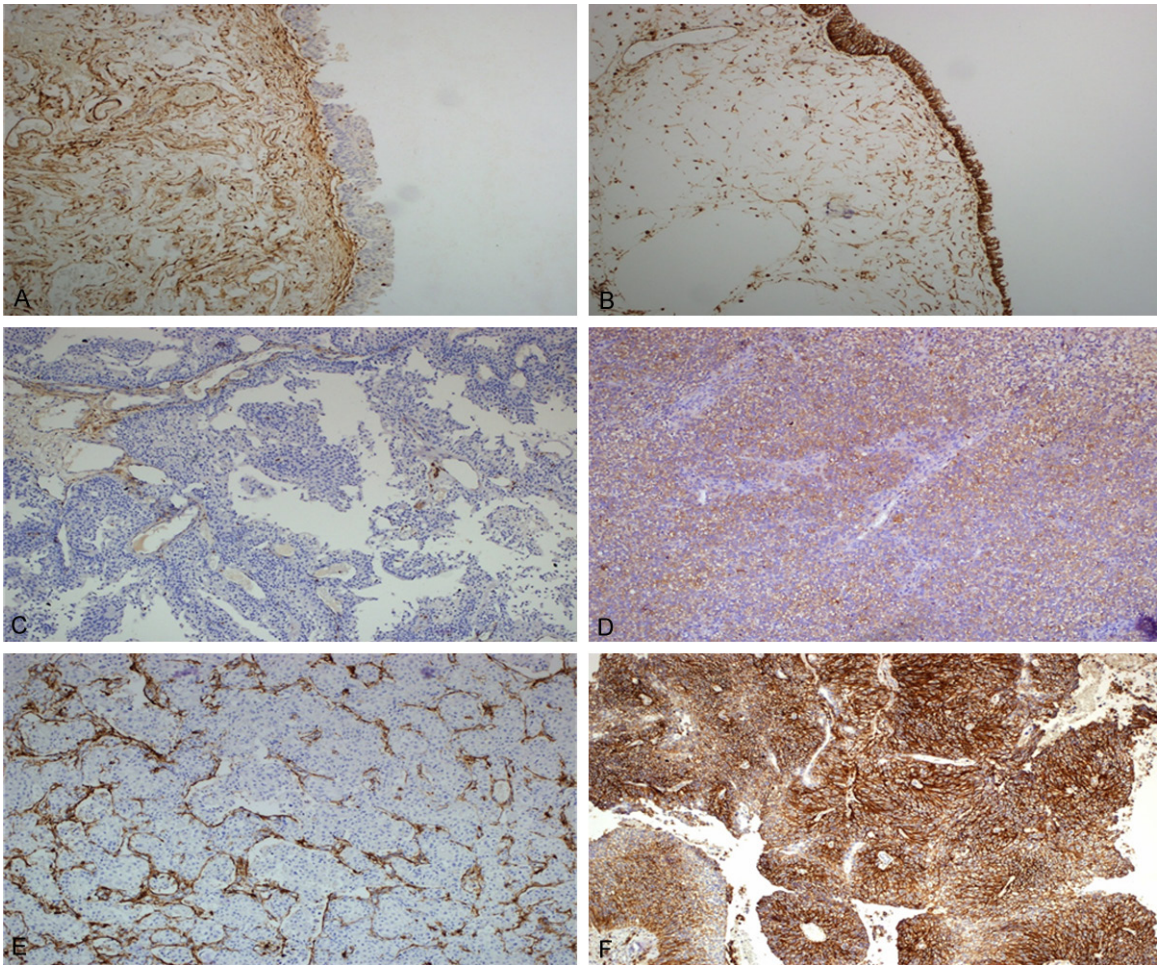

Figure 1. ALDH1 (A) and CD44 (B) expressions in normal urinary bladder (×100); positive immunohistochemical staining of ALDH1 in high grade UBC (×100) (C); negative immunohistochemical staining of ALDH1 in NMI-UCB (Low grade) (×100) (D); positive membranous immunohistochemical staining of CD44 in UBC (×100) (E); Loss of immunoreactivity of CD44 (×100) (F).

stem cell markers in UCB to determine the possible relationship between clinicopathological factors that have been proven to play a role in the prognosis in advance.

Materials and methods

This study included 163 cases of UCB that were diagnosed between 2006-2014 at the Istanbul Medeniyet University Goztepe Training and Research Hospital Pathology Department. All the sections were reviewed to confirm the original diagnosis, were staged according to the 2010 American Joint Committee on Cancer guidelines [18], and were graded according to the 2004 World Health Organization classification system [19] by two expert pathologists (SS and BC). All cases were graded according to the degree of nuclear pleomorphism, anaplasia, and architectural distortion [19].

Age, gender, grade, pT stage, presence of carcinoma *in situ*, recurrence rates, progression, and survey data of cases were compared with the immunohistochemical findings. Cases were divided into the two main groups of non-muscular invasion transitional cell carcinoma (NMI-UCB) or muscular invasion transitional cell carcinoma (MI-UCB) according to the depth of invasion and the pT stage (group 1: <pT2 and group 2: ≥pT2).

Tissue microarray construction (TMA)

Paraffin-embedded tumor tissues and hemotoxylin-eosin (H & E) slides were compared to

Table 1. The features of patients and immunohistochemical findings

	Total	NMI (Ta-T1)
	n (%)	n (%)
Gender		
Male	109 (66.9)	90 (72.0)
Female	54 (33.1)	35 (28.0)
Age		
<65 years	73 (44.8)	56 (44.8)
≥65 years	90 (55.2)	69 (55.2)
First Grade		
Low	75 (46.0)	69 (55.2)
High	88 (54.0)	56 (44.8)
CIS		
Absent	140 (85.9)	110 (88.0)
Present	23 (14.1)	15 (12.0)
Status		
Live	123 (75.5)	107 (85.6)
Exitus	40 (24.5)	18 (14.4)
Recurrence		
Absent	84 (51.5)	70 (56.0)
Present	79 (48.5)	55 (44.0)
Progression		
Absent	113 (69.3)	98 (78.4)
Present	50 (30.7)	27 (21.6)
ALDH1		
Negative	102 (62.6)	89 (71.2)
Positive	61 (37.4)	36 (28.8)
CD 44		
Negative	38 (23.3)	30 (24.0)
Positive	125 (76.7)	95 (76.0)

cylinder samples with a4-mm diameter, using a manual tissue microarrayer (Quick Ray; Unitma Co. Ltd., Seoul, Korea), and these blocks were prepared for immunochemical staining.

Immunohistochemistry and scoring

Sections 4 μm in thickness were taken from 163 UCB tissues and compared with the H & E sections. Immunohistochemical staining for ALDH1 and CD44 was performed on step sections of TMA blocks according to the Bond Max Autostainer (Leica Biosystems) manufacturer's procedure and protocol.

CD44 staining at the membrane of the tumor cells was evaluated with scores of zero to 3 + for 0-10, 10-25, 25-50, and more than 50 percent of tumor cell positivity, respectively. The staining intensity was classified from 1-3 (1, weak; 2, moderate; and 3, strong) [20, 21].

Cytoplasmic ALDH1 immunoreactivity was scored based upon the percent of tumor cell positivity from 0-100 and the staining intensity from 1-3 (1, weak; 2, moderate; and 3, strong). The product of the percentage of positive cells and the staining intensity was then divided by 4, making the reactivity score range from 0 to 100. The results of immunostaining were classified as negative or a score of 0, 1, 2 and 3 when the reactivity score was <3, 3-25, 26-50 and ≥50 percent, respectively [22].

Scores were calculated by multiplying the rate and intensity as follows: for CD44, 0-2 was scored as0 and 3-9 was scored as 1, while for ALDH1, 0-1 was scored as 0 and 2-9 received a score of 1, which were re-classified as semi-quantitative.

Statistical analysis

Data were analyzed by descriptive statistical methods (mean, standard deviation, median, frequency, and rate) and for the comparison of qualitative data using Pearson's chi-square test and Fisher Yates Continuity Correction, Freeman Halton (Monte Carlo) tests. For the immunohistochemical evaluation of variable cross-compliance, Spearman's correlation analysis was employed. Significance was set at $P<0.05$.

Clinicopathological data were evaluated using a Log Rank test and univariate and multivariate Cox regression analyses. Statistical analyses were performed using the Number Cruncher Statistical System (NCSS, 2007) and Power Analysis and Sample Size (PASS, 2008) statistical software (NCSS LLC, Kaysville, Utah, USA).

Results

According to the tumor stage, 125 patients (76.7%) were classified as NMI-UCB (Ta-T1), and 38 patients (23.3%) were placed in the MI-UCB (T2-T4) subgroup. The general follow-up period ranged from9 to72 months with an average of 33.50±14.39 months. ALDH1 immunoreactivity was observed mainly in the cytoplasm, whereas CD44 immunoreactivity was

Table 2. Clinicopathological parameters and cancer stem cell markers in total UCB group

UCB		ALDH1			CD 44		
		Absent	Present	*P* Value	Absent	Present	*P* Value
		n (%)	n (%)		n (%)	n (%)	
Age	<65 years (n=73)	49 (67.1)	24 (32.9)	*0.280*[a]	15 (20.5)	58 (79.5)	*0.572*[b]
	≥65 years (n=90)	53 (58.9)	37 (41.1)		23 (25.6)	67 (74.4)	
Gender	Male (n=109)	69 (63.3)	40 (36.7)	0.786[a]	26 (23.9)	83 (76.1)	*0.972*[b]
	Female (n=54)	33 (61.1)	21 (38.9)		12 (22.2)	42 (77.8)	
First pT	Ta-T1 (n=125)	89 (71.2)	36 (28.8)	*0.001*[**,b]	30 (24.0)	95 (76.0)	*0.875*[b]
	T2-T4 (n=38)	13 (34.2)	25 (65.8)		8 (21.1)	30 (78.9)	
First Grade	Low (n=75)	57 (76.0)	18 (24.0)	*0.001*[**,a]	15 (20.0)	60 (80.0)	*0.461*[b]
	High (n=88)	45 (51.1)	43 (48.9)		23 (26.1)	65 (73.9)	
CIS	Negative (n=140)	95 (67.9)	45 (32.1)	*0.001*[**,b]	34 (24.3)	106 (75.7)	*0.646*[b]
	Positive (n=23)	7 (30.4)	16 (69.6)		4 (17.4)	19 (82.6)	
Recurrence	Absent (n=84)	67 (79.8)	17 (20.2)	*0.001*[**,b]	18 (21.4)	66 (78.6)	*0.688*[b]
	Present (n=79)	35 (44.3)	44 (55.7)		20 (25.3)	59 (74.7)	
Progression	Absent (n=113)	87 (77.0)	26 (23.0)	*0.001*[**,b]	25 (22.1)	88 (77.9)	*0.735*[b]
	Present (n=50)	15 (30.0)	35 (70.0)		13 (26.0)	37 (74.0)	
Status (Mortality)	Live (n=123)	92 (74.8)	31 (25, 2)	*0.001*[**,b]	24 (19.5)	99 (80.5)	*0.072*[b]
	Exitus (n=40)	10 (25.0)	30 (75.0)		14 (35.0)	26 (65.0)	

[a]Pearson Ki-square Test; [b]Fisher-Freeman-Halton Test; [**]P<0.01.

Table 3. Clinicopathological parameters and cancer stem cell markers in NMI group

NMI (Ta-T1)		ALDH 1			CD 44		
		Absent	Present	*P* Value	Absent	Present	*P* Value
		n (%)	n (%)		n (%)	n (%)	
Age	<65 years (n=73)	42 (75.0)	14 (25.0)	*0.518*[c]	11 (19.6)	45 (80.4)	*0.414*[c]
	≥65 years (n=90)	47 (68.1)	22 (31.9)		19 (27.5)	50 (72.5)	
Gender	Male (n=109)	64 (71.1)	26 (28.9)	*1.000*[c]	23 (25.6)	67 (74.4)	*0.675*[c]
	Female (n=54)	25 (71.4)	10 (28.6)		7 (20.0)	28 (80.0)	
First Grade	Low (n=75)	57 (82.6)	12 (17.4)	*0.003*[**,c]	13 (18.8)	56 (81.2)	*0.198*[c]
	High (n=88)	32 (57.1)	24 (42.9)		17 (30.4)	39 (69.6)	
CIS	Negative (n=140)	83 (75.5)	27 (24.5)	*0.012*[*,b]	29 (26.4)	81 (73.6)	*0.116*[b]
	Positive (n=23)	6 (40.0)	9 (60.0)		1 (6.7)	14 (93.3)	
Recurrence	Absent (n=84)	59 (84.3)	11 (15.7)	*0.001*[**,c]	16 (22.9)	54 (77.1)	*0.899*[c]
	Present (n=79)	30 (54.5)	25 (45.5)		14 (25.5)	41 (74.5)	
Progression	Absent (n=113)	79 (80.6)	19 (19.4)	*0.001*[**,c]	23 (23.5)	75 (76.5)	*0.992*[c]
	Present (n=50)	10 (37.0)	17 (63.0)		7 (25.9)	20 (74.1)	
Status (Mortality)	Live (n=123)	83 (77.6)	24 (22.4)	*0.001*[**,c]	24 (22.4)	83 (77.6)	*0.372*[b]
	Exitus (n=40)	6 (33.3)	12 (66.7)		6 (33.3)	12 (66.7)	

[a]Pearson Ki-sqareTest; [b]Fisher-Freeman-Halton Test; [c]Yates' Continuity Correction Test; [*]P<0.05 [**]P<0.01.

seen in the cytoplasmic membrane. If focal staining on the basal layer were excluded, ALDH1 immunoreactivity was not observed in the 20 control cases with normal bladder transitional epithelium (**Figure 1A**). CD44 expression was represented in the normal bladder epithelium (**Figure 1B**).

In all UCB cases, ALDH1 expression was not observed in 102 (62.6%) patients (**Figure 1C**),

Table 4. ALDH1 and CD44 assessment using Cox regression analysis in UCB

	Recurrence			Progression			Overall Survival		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value	HR	95% CI	*P* Value
ALDH1									
Age>65	2.986	1.303-6.841	0.010*	2.990	1.330-6.718	0.008**	2.651	1.170-6.006	0.019*
Gender	1.022	0.455-2.293	0.958	0.916	0.408-2.056	0.832	1.100	0.477-2.536	0.823
First pT	3.693	1.778-7.670	0.001**	4.026	2,004-8.090	0.001**	3.139	1.563-6.301	0.001
Grade	1.138	0.463-2.800	0.778	1.858	0.826-4.179	0.134	1.962	0.874-4.404	0.102
CIS	0.974	0.395-2.407	0.955	0.871	0.346-2.193	0.769	1.006	0.409-2.477	0.990
ALDH1	4.590	2.042-10.319	0.001**	3.222	1.454-7.140	0,004**	3.109	1.383-6,985	0.006**
CD44									
Age>65	2.834	1.319-6.088	0.008**	3.110	1.427-6.780	0.004**	2.602	1.195-5.667	0.016*
Gender	1.129	0.554-2.343	0.745	1.134	0.540-2.382	0.740	1.463	0.687-3.116	0.324
First pT	5.306	2.526-11.144	0.001**	6.279	3.114-12.663	0.001**	4.832	2.393-9.760	0.001**
Grade	1.711	0.700-4.183	0.239	2.198	0.957-5.045	0.063	2.177	0.956-4.953	0.064
CIS	1.227	0.519-2.899	0.641	1.164	0.488-2.778	0.732	1,479	0.626-3.496	0.372
CD 44	0.548	0.283-1.059	0.074	0.516	0.266-1.00	0.050	0.508	0.260-0.995	0.058

*P<0.05; **P<0.01.

but 61 (37.4%) patients were positive (**Figure 1D**). In the NMU-UCB subgroups, these numerical values were 89 (71.2%) and 36 (28.8%), respectively. CD44 expression was negative in 38 (23.3%) patients (**Figure 1E**), while it was positive in 125 (76.7%) patients (**Figure 1F**). Assessment data for our clinicopathological and immunohistochemical findings are given in **Table 1**.

ALDH1 expression was significantly associated with carcinoma in situ (P<0.01) with a high stage (≥pT2) (P=0.001) and a higher grade (P<0.01) in UCB. In addition, ALDH1 expression in UCB as well as in the NMI-UCB subgroup was significantly associated with recurrence [P< 0.05 in the NMI-UCB subgroup], progression to a higher stage, and low survey (P=0.001). The immune expression of CD44 had no statistical correlation with any descriptive data or clinicopathological parameters. The association between ALDH1 and CD44 expression and clinicopathological characteristics are summarized in **Table 2** and **Table 3**.

The recurrence-free follow-up period ranged between 3 and 72 months, with an average of 23.60±16.88 months. Additionally, the progression-free follow-up period was between 3 and 72 months with an average of 29.58±16.26 months.

In the multivariate Cox regression analysis, ALDH1 expression both in the total UCB group and the NMU-UCB subgroup was identified as a significant predictor that could determine tumor recurrence, progression, and survival. However, CD44 expression was unrelated to the prognostic parameters in Cox regression analysis (P>0.05) (**Table 4**).

Kaplan-Meier analysis was carried out using the Long Rank test and revealed that the recurrence-free survival rate and average survival time were 50.8% and 34.73±4:50 months in ALDH1-positive patients, respectively, whereas they were 90.2% and 62.82±2.17 months in ALDH1-negative cases, respectively. The progression-free survival rate was 50.8%, and the average survival time was 40.76±3.94 months in ALDH1-positive cases. In negative cases, the survival rate was 90.2%, and the average survey length was 63.67±1.91 months, so a statistically significant difference was present. A significant difference was also seen for the ALDH1-positive patients whose overall survival rate was 50.8%; the average survival time was 44.85±3.35 months. In negative cases, the survival rate was 90.2% and the average survival time was 63.85±1.84 months (P<0.01). When compared with ALDH1 positivity, rates of recurrence-free time, progression-free time, and overall survival were statistically significantly increased in ALDH1 negativity (Kaplan-

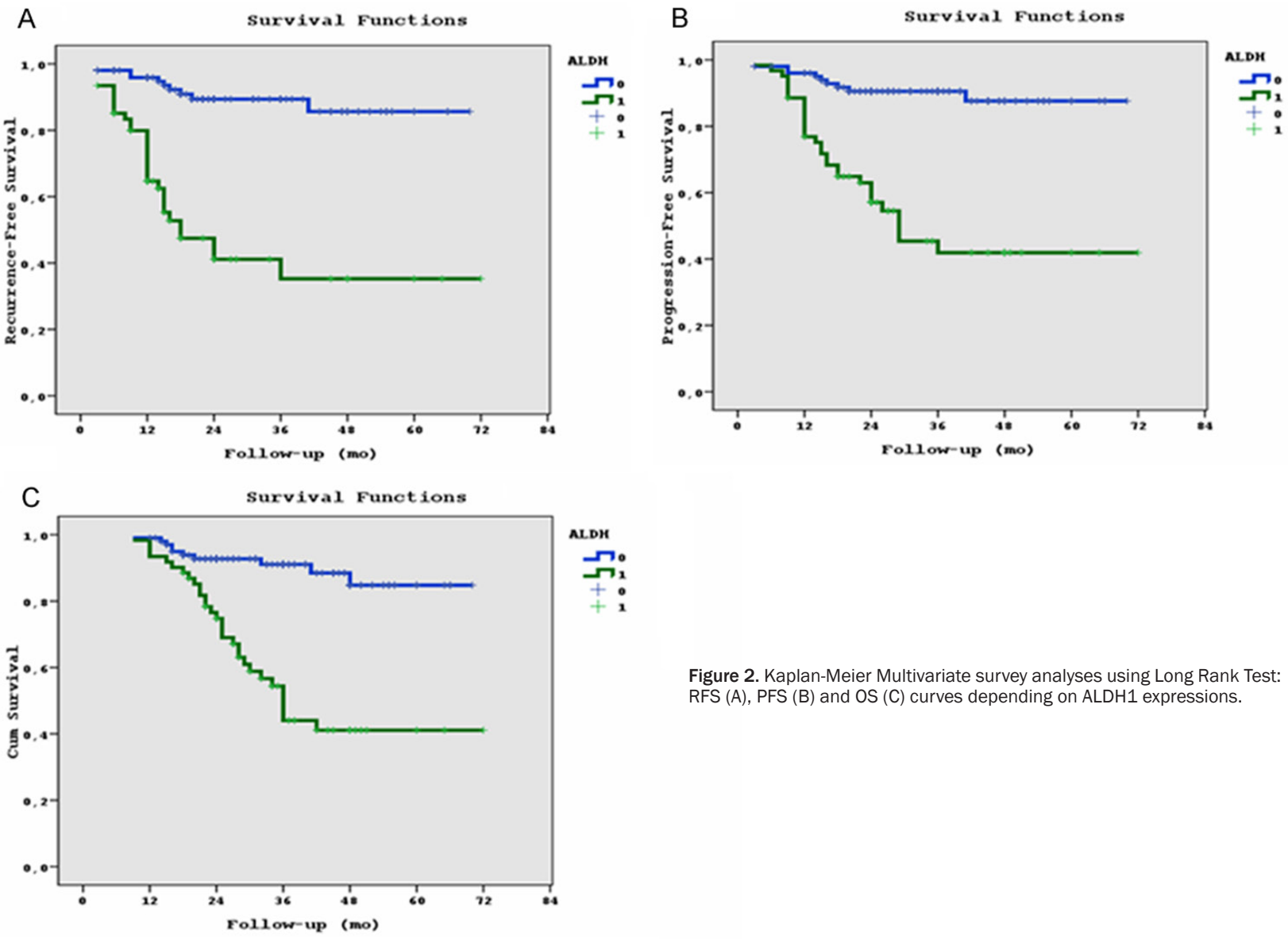

Figure 2. Kaplan-Meier Multivariate survey analyses using Long Rank Test: RFS (A), PFS (B) and OS (C) curves depending on ALDH1 expressions.

Meier, log-rank test =0.001; P<0.01) (**Figure 2**). No statistically significant difference with CD44 expression was observed (P>0.05).

Discussion

The parameters that should be used to determine the appropriate medical or surgical management of UCB are still controversial. The European Organization for Research and Treatment of Cancer (EORTC) scoring system currently assists professionals in determining the indications for cystectomy based upon parameters such as tumor diameter, grade, stage (pT), recurrence rate, and presence of carcinoma *in situ*. In addition to these parameters, ongoing immunohistochemical and molecular studies have attempted to identify the molecules that play a role in tumorigenesis [23]. In order to determine the risk of recurrence and progression of superficial/non-invasive bladder carcinoma, these new molecules might give rise to developments for new treatment modalities.

Our study had several limitations, including the probability of tumor heterogeneity, the effect of a residual tumor on recurrence, and the moderately short follow-up periods. Other investigations on stem cell markers have also been associated with poor prognostic factors and the development of resistance to chemo-radiotherapy [8, 9, 24-27]. In addition, while several studies have reported a statistically significant association between CD44 and poor prognosis-surveillance [28-30], we did not identify any significant prognostic association of CD44 with UCB in our study (P>0.05). However, Keymoosi *et al.* recently identified a correlation of CD44 in combination with ALDH1 with a poor prognosis in bladder cancer [31].

In contrast, ALDH1 expression has been correlated with a poor prognosis and resistance to therapy in tumors of the breast [20, 25], colon [12, 22], stomach [16], lung [13], and pancreas [26], and it has been suggested to be a negative prognostic factor [27]. For bladder carcinoma, Kitamura et al. [17] reported that ALDH1 has an effective role in bladder carcinoma development as its expression was negative in control bladder tissue. In our study, the results indicated that ALDH1 expression was significantly associated with poor surveillance and poor prognostic factors, such as high-stage, high grade, recurrence, progression, and presence of concomitant carcinoma in situ either in UCB (P<0.01) or in the NMU-UCB subgroup (P<0.01).

Moreover, Cox regression analyses and multivariate analyses detected a significant relationship with ALDH1 and survivals, such as the recurrence-free survival (RFS), progression-free survival (PFS), and the overall survival (OS), respectively. In light of these data, we suggest that ALDH1 might contribute to tumorigenesis in UCB. Additionally, the presence of ALDH1 could be a high risk factor for a poor prognosis, as ALDH1-positive tumors tend to be biologically aggressive. This hypothesis was also implied by our results that the expression of ALDH1 was correlated with indicators of poor prognosis, such as invasion into the muscularis propria and poorly differentiated tumors.

The present study showed that ALDH1 expression levels may be used as an indicator to determine the potential of tumor recurrence and progression within cases where T1 staged-TUR materials were present or the deep muscle layer was not seen.

Finally, we propose that inhibiting ALDH1 may provide new insights and can be an effective treatment for urothelial carcinoma eradication. This marker should be considered in the development of new diagnostic and therapeutic approaches for bladder cancer.

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Disclosure of conflict of interest

None.

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References

[1] Miyamoto H, Miller JS, Fajardo DA, Lee TK, Netto GJ and Epstein JI. Non-invasive papillary

urothelial neoplasms: the 2004 WHO/ISUP classification system. PatholInt 2010; 60: 1-8.
[2] Netto GJ. Molecular biomarkers in urothelial carcinoma of the bladder: are we there yet? Nat Rev Urol 2012; 9: 41-51.
[3] Pan CC, Chang YH, Chen KK, Yu HJ, Sun CH and Ho DM. Prognostic significance of the 2004 WHO/ISUP classification for prediction of recurrence, progression, and cancer-specific mortality of non-muscle-invasive urothelial tumors of the urinary bladder: a clinicopathologic study of 1,515 cases. Am J Clin Pathol 2010; 133: 788-795.
[4] Visvader JE and Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008; 8: 755-768.
[5] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL and Wahl GM. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res 2006; 66: 9339-9344.
[6] Rapp UR, Ceteci F and Schreck R. Oncogene-induced plasticity and cancer stem cells. Cell Cycle 2008; 7: 45-51.
[7] Lobo NA, Shimono Y, Qian D and Clarke MF. The biology of cancer stem cells. Annu Rev Cell Dev Biol 2007; 23: 675-699.
[8] Okudela K, Woo T, Mitsui H, Tajiri M, Masuda M and Ohashi K. Expression of the potential cancer stem cell markers, CD133, CD44, ALDH1, and beta-catenin, in primary lung adenocarcinoma--their prognostic significance. Pathol Int 2012; 62: 792-801.
[9] Podberezin M, Wen J and Chang CC. Cancer stem cells: a review of potential clinical applications. Arch Pathol Lab Med 2013; 137: 1111-1116.
[10] Koppaka V, Thompson DC, Chen Y, Ellermann M, Nicolaou KC, Juvonen RO, Petersen D, Deitrich RA, Hurley TD and Vasiliou V. Aldehyde dehydrogenase inhibitors: a comprehensive review of the pharmacology, mechanism of action, substrate specificity, and clinical application. Pharmacol Rev 2012; 64: 520-539.
[11] Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS and Dontu G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1: 555-567.
[12] Huang EH, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, Fields JZ, Wicha MS and Boman BM. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Res 2009; 69: 3382-3389.
[13] Jiang F, Qiu Q, Khanna A, Todd NW, Deepak J, Xing L, Wang H, Liu Z, Su Y, Stass SA and Katz RL. Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. Mol Cancer Res 2009; 7: 330-338.
[14] Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, Li C, Wang LP, Roby KF, Orsulic S, Connolly DC, Zhang Y, Montone K, Butzow R, Coukos G and Zhang L. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. PLoS One 2010; 5: e10277.
[15] Wang D, Miyamoto R, Shiraishi Y and Hirai T. BODIPY-conjugated thermoresponsive copolymer as a fluorescent thermometer based on polymer microviscosity. Langmuir 2009; 25: 13176-13182.
[16] Wakamatsu Y, Sakamoto N, Oo HZ, Naito Y, Uraoka N, Anami K, Sentani K, Oue N and Yasui W. Expression of cancer stem cell markers ALDH1, CD44 and CD133 in primary tumor and lymph node metastasis of gastric cancer. Pathol Int 2012; 62: 112-119.
[17] Kitamura H, Torigoe T, Hirohashi Y, Asanuma H, Inoue R, Nishida S, Tanaka T, Fukuta F, Masumori N, Sato N and Tsukamoto T. Prognostic impact of the expression of ALDH1 and SOX2 in urothelial cancer of the upper urinary tract. Mod Pathol 2013; 26: 117-124.
[18] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL and A. T. Urinary bladder. In: Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, A. T, editors. AJCC Cancer Staging Manual. 7th edition. New York: Springer; 2010. pp. 497-505.
[19] Eble J, Sauter G, Epstein J and Sesterhenn I. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon: IARC Press; 2004.
[20] Ricardo S, Vieira AF, Gerhard R, Leitao D, Pinto R, Cameselle-Teijeiro JF, Milanezi F, Schmitt F and Paredes J. Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. J Clin Pathol 2011; 64: 937-946.
[21] Pitule P, Cedikova M, Daum O, Vojtisek J, Vycital O, Hosek P, Treska V, Hes O, Kralickova M and Liska V. Immunohistochemical detection of cancer stem cell related markers CD44 and CD133 in metastatic colorectal cancer patients. Biomed Res Int 2014; 2014: 432139.
[22] Avoranta ST, Korkeila EA, Ristamaki RH, Syrjanen KJ, Carpen OM, Pyrhonen SO and Sundstrom JT. ALDH1 expression indicates chemotherapy resistance and poor outcome in node-negative rectal cancer. Hum Pathol 2013; 44: 966-974.

[23] Clairotte A, Lascombe I, Fauconnet S, Mauny F, Felix S, Algros MP, Bittard H and Kantelip B. Expression of E-cadherin and alpha-, beta-, gamma-catenins in patients with bladder cancer: identification of gamma-catenin as a new prognostic marker of neoplastic progression in T1 superficial urothelial tumors. Am J Clin Pathol 2006; 125: 119-126.

[24] Rahadiani N, Ikeda J, Mamat S, Matsuzaki S, Ueda Y, Umehara R, Tian T, Wang Y, Enomoto T, Kimura T, Aozasa K and Morii E. Expression of aldehyde dehydrogenase 1 (ALDH1) in endometrioid adenocarcinoma and its clinical implications. Cancer Sci 2011; 102: 903-908.

[25] Charafe-Jauffret E, Ginestier C, Iovino F, Tarpin C, Diebel M, Esterni B, Houvenaeghel G, Extra JM, Bertucci F, Jacquemier J, Xerri L, Dontu G, Stassi G, Xiao Y, Barsky SH, Birnbaum D, Viens P and Wicha MS. Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. Clin Cancer Res 2010; 16: 45-55.

[26] Kahlert C, Bergmann F, Beck J, Welsch T, Mogler C, Herpel E, Dutta S, Niemietz T, Koch M and Weitz J. Low expression of aldehyde dehydrogenase 1A1 (ALDH1A1) is a prognostic marker for poor survival in pancreatic cancer. BMC Cancer 2011; 11: 275.

[27] Chang B, Liu G, Xue F, Rosen DG, Xiao L, Wang X and Liu J. ALDH1 expression correlates with favorable prognosis in ovarian cancers. Mod Pathol 2009; 22: 817-823.

[28] Ross JS, del Rosario AD, Bui HX, Kallakury BV, Okby NT and Figge J. Expression of the CD44 cell adhesion molecule in urinary bladder transitional cell carcinoma. Mod Pathol 1996; 9: 854-860.

[29] Lipponen P, Aaltoma S, Kosma VM, Ala-Opas M and Eskelinen M. Expression of CD44 standard and variant-v6 proteins in transitional cell bladder tumours and their relation to prognosis during a long-term follow-up. J Pathol 1998; 186: 157-164.

[30] Toma V, Hauri D, Schmid U, Ackermann D, Maurer R, Alund G, Knonagel H, Rist M, Gasser TC, Sauter G and Roth J. Focal loss of CD44 variant protein expression is related to recurrence in superficial bladder carcinoma. Am J Pathol 1999; 155: 1427-1432.

[31] Keymoosi H, Gheytanchi E, Asgari M, Shariftabrizi A and Madjd Z. ALDH1 in combination with CD44 as putative cancer stem cell markers are correlated with poor prognosis in urothelial carcinoma of the urinary bladder. Asian Pac J Cancer Prev 2014; 15: 2013-2020.