Comparative Clinical Pathology (2021) 30:559–564
https://doi.org/10.1007/s00580-020-03188-8

ORIGINAL ARTICLE

Immunohistochemical expression of stem cell markers ALDH1 and CD44 in urothelial carcinoma of the urinary bladder

Suzan M. Talaat[1] · Sabrine A Ahmad[2] · Nour El Hoda S. Ismael[3] · Lubna O. Abdel-Salam[3]

Received: 8 April 2020 / Accepted: 8 December 2020 / Published online: 9 January 2021

Abstract

Bladder cancer is the 10th most common cancer. Egyptian men with bladder cancer have the greatest mortality rates, twice as high as the greatest rate in Europe and 4 times greater than in the USA. The existence of bladder CSCs has been suggested as a possible reason for the failure of adjuvant treatment and poor oncological control outcomes. The discovery of therapies targeting CSCs will achieve better treatment effects at a lower cost. This study aimed to study ALDH1A1 and CD44 expression among urothelial cancer patients. Sixty cases of urothelial carcinoma were studied using ALDH1A1 and CD44 immunohistochemistry, and their expression was correlated with different clinicopathologic parameters. There was only a statistically significant relationship between presence of bilharziasis and expression of ALDH1. No statistically significant relationship is found between ALDH1 or CD44 expression and age, gender, tumor grade, histological variants of urothelial carcinoma, depth of invasion (T), presence of carcinoma in situ in adjacent mucosa, lymphovascular invasion, lymph node involvement, and tumor recurrence. ALDH1 and CD44 stem cell marker expression is related to at least one clinicopathological parameter of urinary bladder urothelial carcinoma, and so they could be considered as prognostic markers. This is worthy to be further evaluated and studied on a larger number of patients.

Keywords Urothelial carcinoma · Cancer stem cells · ALDH1 · CD44

Introduction

Bladder cancer is the 10th most common cancer worldwide, with an estimated 549,000 new cases and 200,000 deaths. It is more common in men than in women, with respective incidence and mortality rates of 9.6 and 3.2 per 100,000 in men, about four times those of women globally. Thus, the disease ranks higher among men, in whom it is the sixth most common cancer and ninth leading cause of cancer death (Bray et al., 2018).

In Egypt, it is the second most common malignancy after liver cancer among Egyptian males (Ibrahim et al., 2014). Egyptian men with bladder cancer have the greatest mortality rates (16.3%), twice as high as the greatest rate in Europe (8.3% in Spain and 8.0% in Poland) and 4 times greater than in the USA (3.7%) (Salem and Mahfouz, 2012). The main risk factor for bladder cancer in Egypt was urinary schistosomiasis which was more frequent in Upper (South) Egypt, and its prevalence decreased when going north. Despite control of schistosomiasis, its effect on bladder cancer needs time to disappear (Ibrahim et al., 2014).

Cancer stem cell (CSC) theory can help in understanding the cellular and molecular events during cancer progression contributing to therapy resistance, recurrence, and metastasis. The CSC theory of cancer progression presents tumors as hierarchically organized tissue with CSC population at the top rank that then generate the more differentiated bulk of the tumor cells with lower or limited proliferative potentials.

✉ Suzan M. Talaat
Suzabella0000@gmail.com

Sabrine A Ahmad
sabrine.ali80@gmail.com

Nour El Hoda S. Ismael
nourelhodaismael@hotmail.com

Lubna O. Abdel-Salam
lobystar2004@yahoo.com

[1] Pathology Department, Ahmed Maher Teaching Hospital, Cairo, Egypt

[2] Pathology Department, El Sahel Teaching Hospital, Cairo, Egypt

[3] Pathology Department, Faculty of Medicine, Cairo university, Cairo, Egypt

CSCs share similar properties with normal stem cells, including the ability of self-renewal and differentiation that give rise to heterogeneous, differentiated cancer cells making up the bulk of the tumor. Due to this similarity, CSCs are commonly characterized by the expression of surface markers associated with stem cells, such as CD133, CD44, and CD90, although no single marker can be used to define the CSC populations (Beck and Blanpain, 2013).

The existence of bladder CSCs has been suggested as a possible reason for the failure of adjuvant treatment and poor oncological control outcomes with tumor recurrence (Hofner et al., 2014).

Cancer stem cell markers including basal urothelial cell cytokeratins (CK5, CK14, CK17) (Ho et al., 2012) or adhesion molecules (CD44) (Immervoll et al., 2011 and Ho et al., 2012), aldehyde dehydrogenase 1 family, member A1 (ALDH1A1) (Ho et al., 2012), and tumor protein 63 (p63) (Ho et al., 2012) have been previously used as prognostic factors and applied to identify urothelial carcinoma stem cells (Hatina and Schulz, 2012 and Ho et al., 2012).

Aldehyde dehydrogenase 1(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-renewal ability of either normal or cancer stem cells (Senol et al., 2015).

CD44 is a transmembrane glycoprotein that mediates cell adhesion to the extracellular matrix through its interaction with hyaluronate (Custódio et al., 2018). Also, CD44 presents cytokines and chemokines to their complimentary receptors on the cellular membrane (Naor et al., 2008). CD44 interacts with osteopontin and regulates its cellular functions leading to tumor progression (Rangaswami et al., 2006). It even interacts with collagen, laminin, and fibronectin where their physiological function is unclear (Jaggupilli and Elkord, 2012). CD44 functions involve ligand-binding receptor, coreceptor, and organizer in cortical actin skeleton (Naor et al., 2008).

CD44 is expressed on cancer cell surface and assists hematogenous spread (Napier et al., 2007). It is also involved in numerous complex signaling cascades enhancing tumor initiations by interacting with neighboring receptors like tyrosine kinase (Jaggupilli and Elkord, 2012).

Material and method

Sixty cases of urothelial carcinoma covering different age groups were retrieved from the pathology department, Ahmed Maher teaching hospital, Egypt, during the period from January 2016 to December 2018. Demographic and clinical data of the patients were collected from the hospital files.

Five-micrometer-thick sections were cut from Formalin-fixed paraffin-embedded tissue blocks and stained with hematoxylin and eosin for histopathological examination and determination of tumor type, grade, variant, the extent of invasion, presence or absence of associated carcinoma in situ, bilharziasis, vascular and perineural invasion, and status of dissected lymph nodes in the radical cystectomy specimens.

Immunohistochemical staining was performed using immunostainer (Shandon Sequenza) using the labeled streptavidin biotin method with the following reagents: Citrate buffer: 10X concentrate, pretreatment antigen–retrieval (Spring Bioscience, Catalog number: DCB-125), Hydrogen peroxide block (Lab vision, USA, Catalog number: TA-060-HP), Ultravision large volume detection system (Lab vision, USA, Catalog number: TP-060- HL) including Ultra V block, Biotinylated goat anti-polyvalent plus (link) & Streptavidin peroxidase plus (label), and DAB plus substrate system (Lab vision, USA, Catalog number: TA-060-HDX) including DAB plus chromogen & DAB plus substrate. The primary antibodies were ALDH1A1: rabbit polyclonal antibody (Gene Tex Catalog number: GTX123973), supplied as a vial (0.1 ml), concentrated to be diluted with a diluent (phosphate buffered saline) at a concentration of 1:700 and CD44: Mouse monoclonal antibody (Thermo Scientific Catalog number: MS-668-R7), supplied as a vial (7 ml), ready to use.

Sections of normal liver and breast carcinoma were used as positive control for ALDH1A1 and for CD44, respectively (Keymoosi et al., 2014; Schlossman et al., 1995). Sections of the same tissue were used following the same procedure, but the PBS was used instead of the primary antibody used as internal negative controls.

ALDH1A1 positivity was defined as cytoplasmic staining of tumor cells, while CD44 positivity was defined as membranous staining of tumor cells.

The staining intensity of antibodies were evaluated applying a semi-quantitative system, ranging from negative to strong: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong staining). The percentage of positive cells for each antibody was graded as follows: 1 (< 25% positive cells), 2 (25–50% positive cells), 3 (50–75% positive cells), and 4 (> 75% positive cells). The overall score was calculated by H-score (histochemical score) for each case by multiplying the intensity of staining by the percentage of positive cells, and a final score of 0 to 300 was given. The mean of H-scores was chosen as cut-off value to classify the samples as high or low expression (Keymoosi et al., 2014).

Data collected was revised, coded, tabulated, and introduced to a PC using statistical package for social sciences (IBM SPSS 20.0). Chi-square, Fisher's exact tests, and Pearson correlation coefficient (r) were used. P value is significant when ≤ 0.05.

Results

Fifty-four out of our sixty cases were derived from transurethral resection of bladder tumors (TURBT) and six from radical cystectomies.

The mean age of patients was 58.53, and the age ranged between 24 and 83 years with a standard deviation of 12.62 years. Fifty patients were males (83.3%), and ten patients were females (16.7%).

Regarding immunohistochemical results, there was only a statistically significant relationship between the presence of bilharziasis and expression of ALDH1 ($P = 0.020$). Nine cases (81.8%) out of 11 bilharziasis-associated urothelial carcinomas showed ALDH1 high expression (Fig. 1a). There was no statistically significant relationship between CD44 expression and presence of bilharziasis.

There was no statistically significant relationship between ALDH1 or CD44 expression and age, gender, tumor grade, histological variants of urothelial carcinoma, depth of invasion (T), presence of carcinoma in situ in adjacent mucosa, lymphovascular invasion, lymph node involvement, and tumor recurrence.

Also, there was no statistically significant relationship between ALDH1 and CD44 expression or between combined ALDH1 and CD44 expression and all clinicopathological parameters (Table 1).

Tumor recurrence was detected in 14 cases (23.3%). Four of them had the same grade and stage of the primary tumor, while the remaining ten cases showed progression in grade and/or depth of invasion (T).

Among recurrent cases, eight cases showed high ALDH1 expression, and six showed low ALDH1 expression. Seven recurrences showed high CD44 expression, and the other seven showed low CD44 expression.

Five of seven of primary tumors with low ALDH1 expression showed high ALDH1 expression within the recurrent tumor, and the other two cases remained of low expression. Four out of these five cases (80%) had progression in grade and/or depth of invasion in the recurrent tumor. Regarding CD44, four of eight of primary tumors with low CD44 expression showed high CD44 expression within the recurrent tumor, and the other four cases remained of low expression. Three out of these four cases (75%) had progression in grade and/or depth of invasion in the recurrent tumor.

Discussion

Bladder cancer is the 10th most common form of cancer worldwide (Bray et al., 2018). In Egypt, it is the second most common malignancy after liver cancer among Egyptian males (Ibrahim et al., 2014).

Cancer stem cells (CSCs) are subpopulation of cancer cells that are distinct from normal stem cells and bulk cancer cells in terms of tumorigenic and self-renewal capacities and are responsible for tumor initiation, differentiation, recurrence, metastasis, and drug resistance (Jinesh et al., 2014).

The target of conventional anticancer treatment is mature cancer cells, which account for most cancer cells. Although CSCs only constitute a small proportion of cancer cells, they have strong vitality and natural resistance to conventional therapies, which often leads to treatment failure. The discovery of therapies targeting CSCs will achieve better treatment effects at a lower cost (Liu et al., 2015).

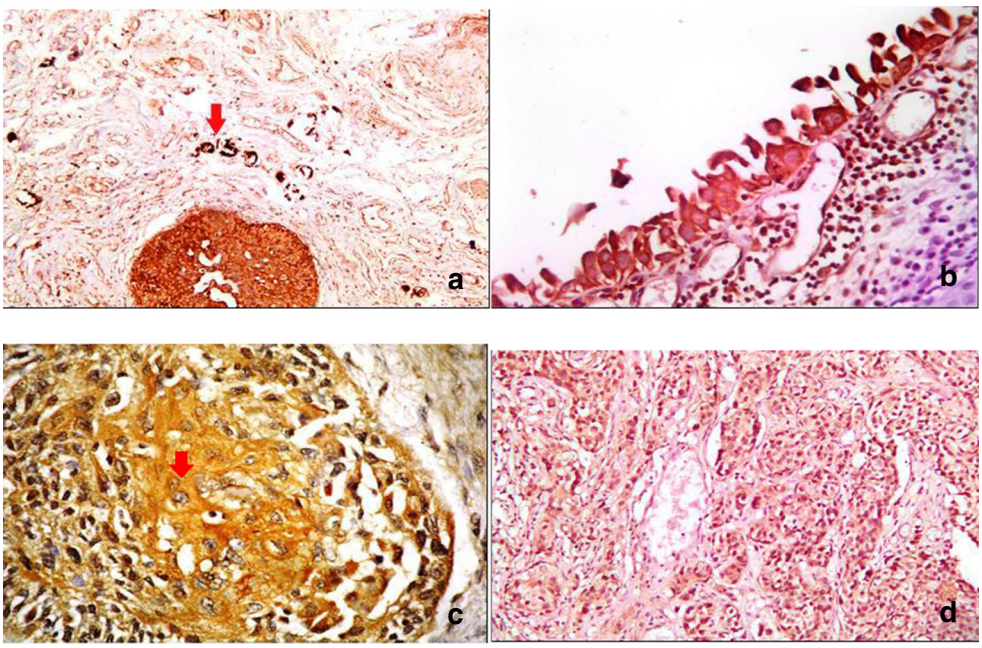

Fig. 1 (**a**) High ALDH1expression in a case of infiltrating urothelial carcinoma associated with bilharzias ova (arrow) (ALDH IHC, × 200). (**b**) A focus of carcinoma in situ in a case of infiltrating urothelial carcinoma showing high ALDH1 expression (ALDH IHC, × 400). (**c**) High ALDH1expression in a case of infiltrating urothelial carcinoma with focal squamous differentiation (arrow) (ALDH IHC, × 400). (**d**) Low ALDH1 expression in a case of nested variant of urothelial carcinoma (ALDH IHC, × 200)

Table 1 Clinicopathological parameter among cases

Parameter	Number	%
Histologic variant		
Conventional UC	48	80
UC with squamous differentiation	6	10
Nested variant of UC	2	3.3
UC with glandular differentiation	1	1.7
UC with squamous and glandular differentiation	1	1.7
Sarcomatoid variant of UC	2	3.3
Grade		
Low	44	73.3
High	16	26.7
Depth of invasion		
Ta	15	25
T1	30	50
T2	12	20
T3	2	3.3
T4	1	1.7
Lymph node involvement		
Non-applicable	54	90
N0	4	6.7
N1	2	3.3
Vascular invasion		
No	55	91.7
Yes	5	8.3
CIS		
No	59	98.3
Yes	1	1.7
Bilharziasis		
No	49	81.7
Yes	11	18.3
ALDH1 expression		
Low expression	30	50
High expression	30	50
CD44 expression		
Low expression	32	53.3
High expression	28	46.7

The expression of ALDH genes, especially ALDH1, has been reported to be associated with UCSCs with a worse prognosis in a large number of previous studies (Kitamura et al., 2013 and Shen et al., 2015).

CD44 has been applied individually or in combination with other putative CSC markers to isolate CSCs in various solid tumors (Jaggupilli and Elkord, 2012).

In the current study, the expression of ALDH1 among UC cases was low in 30 cases (50%) and high in 30 cases (50%). These findings are in contrary to those of Su et al. (2010), of Kitamura et al. (2013), and of Liu et al. (2015) who revealed higher number of low than high ALDH1 expression cases, but all these studies had larger sample size and used monoclonal antibody, while we used polyclonal one. Moreover, all these studies used different scoring systems; both Kitamura et al. and Liu et al. considered at least one ALDH1-positive cell as being positive. Su et al. used a cutoff value of 10% to determine whether a tumor had low or high ALDH1A1 expression.

There was only a statistically significant relationship between the presence of bilharziasis and expression of ALDH1 ($P = 0.020$). This suggests that ALDH1 may play a role in carcinogenesis of urinary bladder urothelial carcinoma as it is related to the presence of bilharziasis which is a known risk factor. Bilharziasis or schistosomiasis is a snail-borne poverty-related disease caused by blood-dwelling trematode worms. It is Schistosoma haematobium, causing urogenital schistosomiasis that with continuous infection and reinfection through many years can lead to a state of chronic inflammation which results in nitric-oxide-mediated DNA alterations which may lead to genetic instabilities and potential malignant transformation. Inflammation and passage of parasite eggs through the tissue result in repeated tissue damage which lead to restorative hyperplasia of the damaged tissue. This may promote the propagation of cells in which genotoxic DNA damage has been completed.

Although there was no relationship between ALDH1 expression and other clinicopathological parameters, it is worth to be mentioned that 75% of urothelial carcinoma with differentiation or special variant show low ALDH1 expression (Fig. 1 c and d); this suggests relationship between urothelial carcinoma with differentiation or special variant and low ALDH1 expression but not at significant level, perhaps due to small number of cases.

Su et al. (2010) found that ALDH1 expression was significantly associated with tumor recurrence and high-grade carcinoma, while Keymoosi et al. (2014) found significant correlation between ALDH1expression and high-grade carcinoma, depth of tumor invasion, lymph node metastasis, and lymphovascular invasion. Liu et al. (2015) also found significant correlation between ALDH1expression and high-grade carcinoma and depth of tumor invasion. El-Dien et al. (2017) detected significant relation with depth of invasion.

Senol et al. (2015) detected that ALDH1 expression was significantly associated with presence of concomitant carcinoma in situ (Fig. 1b) and tumor recurrence.

Contrasting results may be explained by several factors: (1) different antibodies used for the analysis, (2) different criteria used to classify positive staining and different cutoff used to discriminate high and low expression tumors, (3) different treatment modalities done before sample collection, and (4) non-homogeneity of patient selection criteria.

Keymoosi et al. (2014), Su et al. (2010), Kim et al. (2013), and Senol et al. (2015) reported that overexpression of ALDH1 was positively associated with prognostic factors of

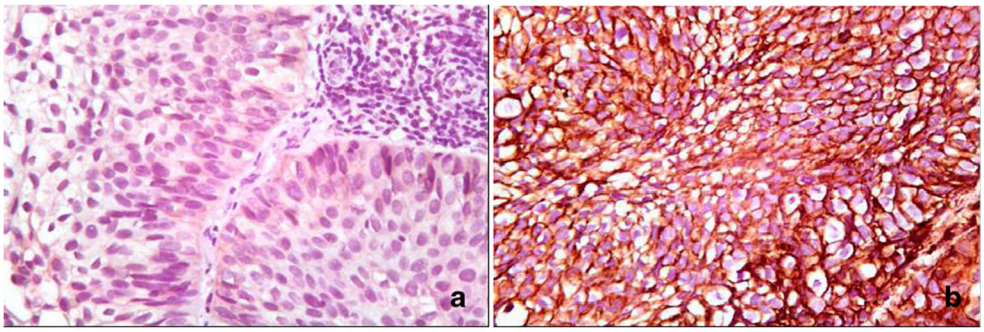

Fig. 2 (**a**) Low CD44 expression in a case of low-grade urothelial carcinoma, T1 (CD44 IHC, × 400). (**b**) High CD44 expression in a case of infiltrating high-grade urothelial carcinoma, T2 (CD44 IHC, × 400)

bladder cancers indicating that ALDH1 could be applied as a prognostic marker in urothelial carcinomas.

Regarding the expression of CD44 in UC cases, the present study revealed low expression in 32 cases (53.3%) and high expression in 28 cases (46.7%) (Fig. 2). These findings are similar to that of Keymoosi et al. (2014) but in contrary to that of El-Dien et al. (2017).

There was no relationship between CD44 expression and any of the clinicopathological parameters.

Omran and Ata (2012) found that CD44 expression is significantly associated with depth of invasion. Omar et al. (2015) reported significant relationship with tumor grade, depth of invasion, and vascular invasion.

El-Dien et al. (2017) reported that higher CD44 expression was associated with bilharziasis, while Wasfy and El-Guindy (2017) reported significant relationship with both tumor grade and depth of invasion.

It is to be noted that the antibody used in this study detects the standard isoform of CD44 (CD44s). Many isoforms are generated out of alternative splicing of CD44 during oncogenic signaling. The presence of many isoforms of CD44 undoubtedly contributes to some of the variability in research (Basakran, 2015).

In the current study, the pattern of CD44s staining in normal urothelium was membranous and slightly cytoplasmic, with the maximum intensity in the basal cell layers. The extent of staining decreased in a step-wise manner towards the surface, leaving the upper part of the intermediate and the most superficial layers of urothelium with highly differentiated umbrella cells entirely negative for CD44; this agrees with Kuncova et al. (2007) and Salih et al. (2016).

Gadalla et al. (2004) reported that there was markedly less expression of CD44 in invasive UC than in normal and pre-invasive carcinomas. Erdogan et al. (2008) reported that higher CD44 expression was revealed in low-grade and non-invasive tumors. And Salih et al. (2016) documented that higher CD44 expression was revealed in low-grade and non-invasive tumors. All of these findings are supporting evidence indicating involvement of CD44 in the tumorogenesis of UC.

There was no significant relationship between combined expression of ALDH1 and CD44 proteins among studied cases ($P = 0.796$). This is in contrary to the significant correlation found by Keymoosi et al. (2014) from combined analysis of both ALDH1 and CD44 that showed 30% of urothelial carcinomas displayed the ALDH1+/CD44+ phenotype, whereas 58% of cases were ALDH1−/CD44+ phenotype, and only 3% tumors expressed ALDH1+/CD44− phenotype. This finding suggests that the ALDH1+ population is a subset of the CD44+ bladder cancer cells. Liu et al. (2015) concluded that only a proportion of the CD44-positive cells express ALDH1, while most ALDH1-positive cells express CD44, thereby indicating that ALDH1 has higher specificity as a CSC marker as compared to CD44.

There was no correlation between combined expression of ALDH1 and CD44 and the clinicopathological parameters of UC cases. No data was detected in literature discussing this point to compare with.

It is worth to be mentioned that the findings among the fourteen recurrent cases suggest that increased expression of either ALDH1 or CD44 within the recurrent tumor may be associated with progression in grade or pathological T stage of tumor or both.

Referring to the current study and the related previous researches, diverse conclusions have been achieved, but many related results were detected. Most conclusions documented that in a way or another, ALDH1 and CD44 stem cell marker expression is related to at least one clinicopathological parameter of urinary bladder urothelial carcinoma, and so they could be considered as prognostic markers. This topic is worthy to be further evaluated and studied on a larger number of patients as it has a very important role in introducing therapies targeting CSCs and that will achieve better treatment effects at a lower cost and may prevent morbidities and mortalities caused by invasive treatment.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human participants or animals performed by any of the authors so no informed consent needed.

References

Basakran NS (2015) CD44 as a potential diagnostic tumor marker. Saudi Med J 36:273–279

Beck B, Blanpain C (2013) Unravelling cancer stem cell potential. Nat Rev Cancer 13:727–738

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424

Custódio M, Pelissari C, Santana T, Trierveiler M (2018) Expression of cancer stem cell markers CD44, ALDH1 and p75NTR in actinic cheilitis and lip cancer. Eur Arch Otorhinolaryngol 275:1877–1883

El-Dien MM, Abdallah RA, Zanaty F (2017) Role of ALDH1a and CD44 in transitional and squamous cell carcinomas of urinary bladder. Egyptian Journal of Pathology 37(1):137–144

Erdogan G, Kucukosmanoglu I, Akkaya B et al (2008) CD44 and MMP-2 expression in urothelial carcinoma. Turkish Journal of Pathology 24(3):147–152

Gadalla HA, Kamel NA, Badary FA et al (2004) Expression of CD44 protein in bilharzial and non-bilharzial bladder cancers. B.J.U. International 93(1):151–155

Hatina J, Schulz WA (2012) Stem cells in the biology of normal urothelium and urothelial carcinoma. Neoplasma 59(6):728–736

Ho PL, Kurtova A, Chan KS (2012) Normal and neoplastic urothelial stem cells: getting to the root of the problem. Nat Rev Urol 9(10): 583–594

Hofner T, Macher-Goeppinger S, Klein C, Schillert A, Eisen C, Wagner S, Rigo-Watermeier T, Baccelli I, Vogel V, Trumpp A, Sprick MR (2014) Expression and prognostic significance of cancer stem cell markers CD24 and CD44 in urothelial bladder cancer xenografts and patients undergoing radical cystectomy. Urol Oncol 32:678–686

Ibrahim AS, Khaled HM, Mikhail NN et al (2014) Cancer incidence in Egypt: results of the National Population-Based Cancer Registry Program. J Cancer Epidemiol 2014:437971

Immervoll H, Hoem D, Steffensen OJ, Miletic H, Molven A (2011) Visualization of CD44 and CD133 in normal pancreas and pancreatic ductal adenocarcinomas: non-overlapping membrane expression in cell populations positive for both markers. J Histochem Cytochem 59:441–455

Jaggupilli A, Elkord E (2012) Significance of CD44 and CD24 as cancer stem cell markers: an enduring ambiguity. Clin Dev Immunol 2012: 1–11

Jinesh GG, Willis DL, Kamat AM (2014) Bladder cancer stem cells: biological and therapeutic perspectives. Current Stem Cell Research & Therapy 9:1–13

Keymoosi H, Gheytanchi E, Asgari M, Shariftabrizi A, Madjd Z (2014) 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 15(5):2013–2020

Kim YJ, Yoon HY, Kim JS, Kang HW, Min BD, Kim SK, Ha YS, Kim IY, Ryu KH, Lee SC, Kim WJ (2013) HOXA9, ISL1 and ALDH1A3 methylation patterns as prognostic markers for non-muscle invasive bladder cancer: array-based DNA methylation and expression profiling. Int J Cancer 133:1135–1143

Kitamura H, Torigoe T, Hirohashi Y, Asanuma H, Inoue R, Nishida S, Tanaka T, Fukuta F, Masumori N, Sato N, Tsukamoto T (2013) Prognostic impact of the expression of ALDH1 and SOX2 in urothelial cancer of the upper urinary tract. Mod Pathol 26:117–124

Kuncova J, Urban M, Mandys V (2007) Expression of CD44s and CD44v6 in transitional cell carcinomas of the urinary bladder: comparison with tumour grade, proliferative activity andp53 immunoreactivity of tumour cells. APMIS 115:1194–1205

Liu J, Jin J, Yang Y, Han W, Yu W, Shen C, Zhang N, du P, Zhao Q, Wang S, Tang X (2015) Prognostic impact of ALDH1 expression in transitional cell carcinoma of the renal pelvis. Anticancer Res 35: 4829–4836

Naor D, Wallach-Dayan SB, Zahalka MA, Sionov RV (2008) Involvement of CD44, a molecule with a thousand faces, in cancer dissemination. Semin Cancer Biol 18(4):260–267

Napier SL, Healy ZR, Schnaar RL, Konstantopoulos K (2007) Selectin ligand expression regulates the initial vascular interactions of colon carcinoma cells: the roles of CD44V and alternative sialofucosylated selectin ligands. J Biol Chem 282(6):3433–3441

Omar ES, GM EL-o, Abd El-Aziz TA et al (2015) Prognostic role of CD44, smoothelin and MUC1 in urinary bladder carcinoma. Med J Cairo Univ 83(1):449–458

Omran OM, Ata HS (2012) CD44s and CD44v6 in diagnosis and prognosis of human bladder cancer. Ultrastruct Pathol 36(3):145–152

Rangaswami H, Bulbule A, Kundu GC (2006) Osteopontin: role in cell signaling and cancer progression. Trends Cell Biol 16(2):79–87

Salem HK, Mahfouz S (2012) Changing patterns (age, incidence, and pathologic types) of Schistosoma-associated bladder cancer in Egypt in the past decade. Urology 79(2):379–383

Salih SM, Chalabi RA, Saad S, Jawad H (2016) Macrophage migration inhibitory factor level and its receptor CD74and CD44 expression in urinary bladder carcinoma. Int J Curr Microbiol App Sci 5(1):667–678

Schlossman SF et al (1995) Leucocyte Typing V. Oxford Univ. Press, Oxford, pp 1713–1719

Senol S, Yildırım A, Akalin I, Uruç F, Çobanoğlu B, Yilmaz S, Ceyran B, Kösemetin D, Ece D, Aydın A (2015) Relation of stem cell markers ALDH1 and CD44 with clinicopathological factors in urothelial carcinomas of urinary bladder. Int J Clin Exp Med 8(3):4195–4203

Shen H, Blijlevens M, Yang N, Frangou C, Wilson KE, Xu B, Zhang Y, Zhang L, Morrison CD, Shepherd L, Hu Q, Zhu Q, Wang J, Liu S, Zhang J (2015) Sox4 expression confers bladder cancer stem cell properties and predicts for poor patient outcome. Int J Biol Sci 11: 1363–1375

Su Y, Qiu Q, Zhang X, Jiang Z, Leng Q, Liu Z, Stass SA, Jiang F (2010) Aldehyde dehydrogenase 1 A1–positive cell population is enriched in tumor-initiating cells and associated with progression of bladder Cancer. Cancer Epidemiol Biomark Prev 19(2):327–337

Wasfy RES, El-Guindy DM (2017) CD133 and CD44 as cancer stem cell markers in bladder carcinoma: an immunohistochemical study. Egyptian Journal of Pathology 37(1):204–208