EXPRESSION OF CANCER STEM CELL MARKER OCTAMER-BINDING TRANSCRIPTION FACTOR 4 IN HIGH-GRADE TRANSITIONAL CELL CARCINOMA

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ABSTRACT

Objectives: The objectives of this study were to provide an outlook of urothelial carcinoma through the immunohistochemical expression patterns of octamer-binding transcription factor 4 (OCT4) in high-grade transitional cell carcinoma (TCC) of the urinary bladder.

Methods: A total number of 60 tissue samples were collected for the study. Patients were divided into two groups according to the pathological diagnosis of the bladder tissue, Group A: 30 cases with high-grade TCC of the bladder and Group B: 30 cases with apparently normal bladder tissue. Tissue immunohistochemical analysis was applied to investigate the expression patterns of cancer stem cell (CSC) markers OCT4 in bladder samples.

Results: OCT4 was positive in 80% of specimens of Group A and 3.3% in specimens of Group B. The association between OCT4 marker result and certain histopathological features in high-grade group: Positive OCT4 result was found in patients with inflammation and necrosis (90.9%) with a significant association (p=0.013). Regarding muscular invasion, we noticed that 87.5% of patients with muscular invasion showed positive OCT4 marker result with a significant association (p=0.039) between OCT4 marker result and muscular invasion. As well, OCT4 marker was highly sensitive and specific (sensitivity=66.7%, specificity=96.7%, and accuracy=76.7%).

Conclusion: There was a significant expression of CSC OCT4 in high-grade TCC, OCT4 can be considered as a key regulator of tumor progression, aggressive behavior, and metastasis.

Keywords: Octamer-binding transcription factor 4, Transitional cell carcinoma, Bladder.

INTRODUCTION

Urothelial carcinoma (transitional cell carcinoma [TCC]) of the urinary bladder represents 90% of all primary tumors of this organ, and one of the most common 10 malignancies in Iraq and worldwide. These tumors can range from low-grade papillary neoplasm to less frequent more aggressive and invasive high-grade tumors [1]. More than 70% of superficial tumors recur, and about one-third of the patients have tumor progression, which can affect the quality of their life [2]. Treatment failure, recurrence, and metastasis in bladder cancer are attributed to a subset of tumor cells expressing cancer stem cell (CSC) markers. CSCs have been isolated from leukemia and from a wide spectrum of solid tumors, including breast cancer, using putative CSC markers such as octamer-binding transcription factor 4 (OCT4), CD133, aldehyde dehydrogenase 1, and CD44 [3]. Some unique characteristics of CSCs include an increased expression of telomerase and adenosine triphosphate-binding cascade transporters and evasion of apoptosis. CSCs have become the target in treating various cancers. It has to be tested what connections are between CSCs and tumor-initiating cells [4]. Characteristic markers and proteins may help to identify bladder CSCs and thus early stages of bladder cancer. OCT-4 (the gatekeeper of self-renewal), also known as POU domain, class 5, transcription factor 1 (POU5F1), is a protein that in humans is encoded by the POU5F1 gene [5]. OCT-4 is a homeodomain transcription factor of the POU family. It is critically involved in the self-renewal of undifferentiated embryonic stem cells. As such, it is frequently used as a marker for undifferentiated cells. OCT-4 is a member of the octamer transcription factor family, so named because they bind the octameric (8-unit) DNA nucleotide sequence ATTTGCAT [6]. The OCT-4 transcription factor is initially active as a maternal factor in the oocyte and remains active in embryos throughout the pre-implantation period. OCT-4 can form a heterodimer with SOX2 so that these two proteins bind DNA together [7]. Mouse embryos that are OCT-4 deficient or have low expression levels of OCT-4 fail to form the inner cell mass, lose pluripotency, and differentiate into trophectoderm [8]. Therefore, the level of OCT-4 expression in mice is vital for regulating pluripotency and early cell differentiation since one of its main functions is to keep the embryo from differentiating [9]. In a mature organism, OCT4 is not present in mature and differentiated cells and is found only in germ cells. OCT4 gene encodes three transcripts and four protein isoforms that are generated by alternative splicing, OCT4A, and OCT4B, and OCT4B1. It is suggested that OCT4A and OCT4B can be distinguished by their distinct subcellular localization [10]. Only the OCT4A form, which is present in cell nuclei, exhibits transcription factor functions and is responsible for maintaining cells at an undifferentiated stage, stem cell properties, and the ability for self-renewal. OCT4 regulates the expression of several target genes, including NANOG, SOX-2, REX-1, and CDX-2, involved in the regulation of pluripotency. OCT4 is generally considered a universal marker of pluripotent stem cells [11]. OCT4 expression in cancer cells: The presence of OCT4 protein is associated with, for example, poor prognosis in non-small-cell lung cancer, hepatic cancer, and esophageal squamous cell carcinoma. One possible mechanism responsible for the more aggressive behavior of cancers and worse clinical outcomes with cells expressing OCT4 is the presence of the stem cell phenotype in cancers related to OCT4-mediated dedifferentiation and related chemoresistance [12].

METHODS

A total number of 60 tissue samples were collected for the study, 30 samples of Group A retrospective obtained from archives of histopathology unit with 30 prospective samples were obtained from the Directorate of Forensic Medicine at Baghdad Medical City. The patients' medical reports, with full histopathological parameters, were

collected and reviewed. After appropriate trimming, a serial of four micrometer-thick tissue sections was obtained using the automated microtome. For each case, two sections were taken; the first was placed on an ordinary slide and stained with hematoxylin and eosin to confirm the diagnosis and to determine the histological type and grade for the tumor and the second section was put on the positively charged slides for immunohistochemical staining with anti-OCT4 antibody.

Immunohistochemical staining

Slides preparation was placed in semi-vertical position in the oven at 65°C overnight. The slides were covered by water until ready to perform antigen retrieval; they should be kept wet because it will yield a non-specific antibody binding.

- Heat-induced epitope: Slides were put in a vertical position then put in 250 ml (10 mmol sodium citrate buffer complete with wash buffer, ph 6) in a plastic container then cover and heated at 95 for 5 min allow the slides to cool in the buffer for approximately 20 min. Wash in deionized H_2O 3 times for 2 min each, aspirate excess liquid from slides.
- Peroxidase block: Incubate for 7–10 min in 50 ul hydrogen peroxide in a humid chamber to quench endogenous peroxidase activity. Wash in phosphate-buffered saline (PBS) twice for 5–7 min each then drained.
- Protein block: The slides were incubated with protein block UltraCruz® blocking reagent in a humid chamber for 1 h to eliminate non-specific background staining then drained for a few seconds without a rinse and wipe around with a piece of tissue paper.
- Primary antibody: 50 ul of prediluted primary antibody was placed into sections (dilution 1:70 for OCT4) incubated in a wet chamber at 4°C overnight for OCT4.
- The slides were washed with fresh PBS twice for few minutes each. Then, the slides were drained.
- Conjugated secondary antibody enough drops of secondary antibody were applied to cover the specimen and incubated in a humid chamber at room temperature for 60 min. Then, the slides rinse with PBS 2 × 5 min then drained and blotted.
- Substrate chromogen solution: 50 drops of diaminobenzidine (DAB) substrate with one drop of chromogen were mixed, few drops were added and incubated for 10 min in the humid chamber or until desired stain intensity develops, then washed with tap water for few minutes each.
- Counterstain with Mayer's hematoxylin was used for 1 min, then washed with tap water, followed by distal water for few minutes then slides were drained and blotted.
- Mounting: One to two drops of mounting media are applied onto the sections, then covered with coverslips and left to dry overnight.

Evaluation of immunostaining scores

The cells were scored as positive or negative staining depending on the presence of distinct brown nuclear staining. The accuracy of the positive and strongly positive categories was further tested and confirmed by ranking each slide from the lowest to highest intensity and extent of staining and location was also revealed for each marker. The slides were examined with low-power microscopy ×10 to determine the regions of highest staining, if they show no staining at low power, reexamination was done by high power ×400 to determine area of weak staining, five fields of each slide were examined and scored semi-quantitatively by calculating the proportion of positively stained cells over the total number of tumor cells examined (%) and samples were graded according to the extent of staining and intensity.

Statistical analysis

The data analyzed using the Statistical Package for the Social Sciences version 25. The data presented as mean, standard deviation, and ranges. Categorical data presented by frequencies and percentages. Pearson's Chi-square test was used to assess the statistical association between different associated variables. A level of $p<0.05$ was considered statistically significant.

RESULTS

In this study, OCT4 was positive in 80% of specimens of Group A and 3.3% of specimens of Group B. We noticed that the highest proportion of patients with positive OCT4 result was diagnosed with TCC (97.6%) with a significant association ($p=0.001$) between OCT4 marker result and prevalence of TCC (Table 1).

The association between the OCT4 marker result and certain clinicopathological features is shown in Table 2. The highest prevalence of positive OCT4 result was found in patients with inflammation and necrosis (90.9%) with a significant association ($p=0.013$) between OCT4 result and inflammation with necrosis. Regarding muscular invasion, we noticed that 87.5% of patients with muscular invasion showed positive OCT4 marker result with a significant association ($p=0.039$) between OCT4 marker result and muscular invasion. There was no significant association ($p≥0.05$) between OCT4 marker result and other histopathological features of patients in Group A (Fig. 1).

Regarding OCT4 marker, it was highy sensitive and specific (sensitivity=80%, specificity=96.6%), (Table 3).

DISCUSSION

OCT4 was positive in 80% of Group A and 3.3% in Group B, in concomitant to Chinese study (2007) conducted on 49 bladder cancer biopsy samples and found that 40 of 49 bladder cancer samples (81.6%) showed the expression of OCT-4 of cancer cells [13]. In comparison to

Table 1: Immunohistochemical staining result of OCT4

CSC marker result	Group A n=30 (%)	Group B n=30 (%)	Total n=60 (%)
Positive	24 (80.0)	1 (3.3)	25 (41.6)
Negative	6 (20.0)	29 (96.7)	35 (58.3)

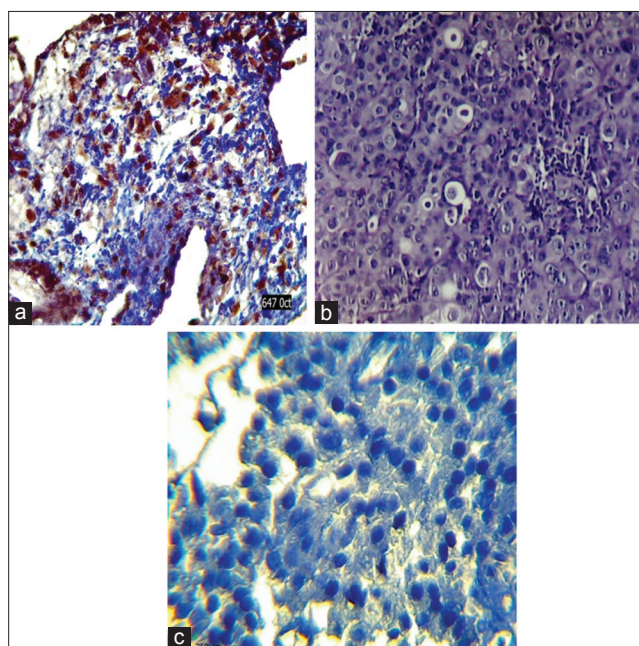OCT4: Octamer-binding transcription factor 4, CSC: Cancer stem cell

Figure 1: Histopathological sections of bladder. (a) High-grade transitional cell carcinoma (TCC), magnification ×10, (Hematoxylin-eosin). (b) +ve IHC expression of octamer-binding transcription factor 4 (OCT4) marker in high-grade TCC of the bladder, brownish discoloration of the nuclear stain, strong intensity, × 40. (c) –ve IHC expression of OCT4 marker in high-grade TCC of the bladder, bluish discoloration of the nuclear stain, × 40

Table 2: Association between OCT4 marker result and certain histopathological features of Group A

Variable	OCT4 result		Total n=30	p value
	Positive (%) n=24	Negative (%) n=6		
Age (years)				
51–60	5 (71.4)	2 (28.6)	7 (23.3)	0.809
61–70	14 (82.4)	3 (17.6)	17 (56.7)	
71–80	5 (83.3)	1 (16.7)	6 (20.0)	
Gender				
Male	21 (84.0)	4 (16.0)	25 (83.3)	0.22
Female	3 (60.0)	2 (40.0)	5 (16.7)	
Inflammation with necrosis				
Yes	20 (90.9)	2 (9.1)	22 (73.3)	0.013
No	4 (50.0)	4 (50.0)	8 (26.7)	
Vascular invasion				
Yes	10 (71.4)	4 (28.6)	14 (46.7)	0.272
No	14 (87.5)	2 (12.5)	16 (53.3)	
Muscular invasion				
Yes	21 (87.5)	3 (12.5)	24 (80.0)	0.039
No	3 (50.0)	3 (50.0)	6 (20.0)	
Glandular differentiation and squamous metaplasia				
Yes	9 (69.2)	4 (30.8)	13 (43.3)	0.197
No	15 (88.2)	2 (11.8)	17 (56.7)	
Prostate involvement				
Yes	6 (100)	0 (0)	6 (20.0)	0.17
No	18 (75)	6 (25)	24 (80.0)	

OCT4: Octamer-binding transcription factor 4, CSC: Cancer stem cell

Table 3: Percentage of CSC marker

CSC marker	TCC		Total
	Yes	No	
OCT4			
Positive	24	1	25
Negative	6	29	35
Total	30	30	60

TCC: Transitional cell carcinoma, OCT4: Octamer-binding transcription factor 4, CSC: Cancer stem cell

another study conducted in Iran (2007), a higher results observed as they detected the expression of OCT4 in almost all 96% of the examined tumor samples of bladder (31/32) 33% of Group C samples (3/9 of those without carcinoma) (Atlasi *et al.*, 2007), and in Iran 2009, on 140 patients with bladder carcinoma, as noticed that among 140 cases, 9 (6%) cases showed no immunoreactivity for the OCT4 protein, whereas 131 (94%) cases showed positive immunoreactivity with a variety of intensities [14]. Lower results conducted in Egypt at 2017 on 84 urothelial tumor specimens, and found, of all the tumor specimens, 30 (35.8%) cases showed tumor positivity, whereas 54 (64.2%) cases were negative for OCT4 staining, in which all positive results found in high-grade urothelial tumor specimens [15]. The most reasonable explanation for this discrepancy in results was probably the different representation of different grade specimens in each study.

Our results also showed that there was a significant association between the OCT4 positive result and inflammation with necrosis (p=0.013) in 90.9% of patients in Group A. Furthermore, with muscular invasion, it significantly related (p=0.039, 87.5%) of patients and no significant association (p≥0.05) with other clinicopathological features. Similarly, a study conducted in Japan (2011) showed that the immunohistochemical analysis demonstrated that the positive rate of OCT4 expression was significantly associated with higher grade cancer (G2 and G3) in comparison with that of the lower grade (G1) [16]. Furthermore, in Iran (2017), researchers found that there was a significant correlation between the expression of OCT4 and the tumor stage (p<0.001), indicating a higher level of expression in higher stage tumors and a significant correlation was also observed between the OCT4 intensity with muscular invasion (p=0.02) but differ in that no correlation with grade of tumor (Sedaghat *et al.*, 2017). In Egypt (2016), researchers noticed that a significant association between OCT4 expression and tumor grade (p=0.003), tumor morphology (p<0.001), tumor stage (p<0.001), and progression of the tumor from non-muscle invasive to invasive urothelial carcinoma (p=0.001) (Asar *et al.*, 2017).

Differently, Iranian researchers notice that the sensitivity and specificity of OCT4 expression as a molecular marker in the detection of bladder tumors were determined as 96 and 66%, respectively, in a study conducted in 2007 (Atlasi *et al.*, 2007).

CONCLUSION

There was a significant expression of OCT4 in high-grade TCC; OCT4 can be considered as a key regulator of tumor progression, aggressive behavior, and metastasis. Furthermore, it is a reliable marker for the early diagnosis and the designed chemotherapy of bladder cancer.

CONFLICTS OF INTEREST

None.

SOURCE OF SUPPORT

None.

AUTHORS' CONTRIBUTIONS

All authors contribute equally in data collection, experimental design, interpretation, statistical analysis, literature review, manuscript preparation, and review.

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