IMMUNOHISTOCHEMICAL STUDY OF THE EXPRESSION OF OCT-4 IN BLADDER UROTHELIAL CARCINOMA

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LIST OF ABBREVIATIONS

AJCC: American Joint Committee on Cancer

CIS: Carcinoma In-Situ

CSC: Cancer Stem Cell

DAB: Di-Amino Benzidine

H&E: Haematoxylin and Eosin

ISUP: International Society of Urological Pathology

TNM: Tumour, Nodal involvement, Metastasis

UBC: Urinary Bladder Cancer

WHO: World Health Organisation

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ABSTRACT

Bladder carcinoma is a significant problem in Egypt; being the 10th in the worldwide rank of incidence for both sexes. The disease is characterized by multifocality, multiple morphologies coexisting together and multiple recurrences after excision. A cancer stem cell model was proposed; that there is a subpopulation of tumour cells capable of resisting conventional therapies and surviving treatment to facilitate recurrence and metastasis. Oct-4 is a pluripotency marker of stem cells; which has been found to be associated with worse prognosis in multiple somatic tumours such as breast, brain, skin and thyroid.

We tested 84 urothelial tumour specimens including all grades, stages and some variants of urothelial carcinoma for the expression of Oct-4. Two sections were prepared per case for histologic evaluation and immunohistochemical staining by Oct-4 monoclonal antibody. The immunostaining was evaluated semiquantitavely through obtaining an H-score for each case.

All cases took up the Oct-4 stain except for low grade tumours and CIS cases. The highest percentage of positive cases was seen in variants of urothelial carcinoma (19.1%). Oct-4 expression was significantly associated with grade ($p= 0.03$), histopathologic type of urothelial tumour ($p< 0.001$), stage ($p < 0.001$), and tumour progression ($p = 0.001$). Conversely; the level of Oct-4 expression was not found to be associated with any of the studied parameters.

Our study proves that Oct-4 is expressed in bladder urothelial carcinoma, and its presence regardless of the level of expression seems to play a role in guiding tumour grade, stage, progression and histopathological type; which calls for considering Oct-4 as a novel prognostic marker that could be implemented in target therapies for urothelial carcinoma.

INTRODUCTION

Recent work in cancer research shows mounting evidence supporting the idea that tumors, similar to normal adult tissues, arise from a specific stem-like cell population, the cancer stem cells (CSCs),which are considered as the real driving force behind tumor growth, the ability to metastasize, as well as resistance to conventional antitumor therapy **(Dimov et al., 2010).**

Stem cells are unspecialized cells that undergo unlimited self-renewal and multilineage differentiation to become specialized cells. They usually constitute a small percentage of the total cell population in any tissue and are usually indolent; dividing very slowly (**Stricker and Kumar, 2010**). It is generally accepted that CSCs might arise from transformed stem cells or progenitor cells that have regained self-renewal activity; due to the autonomous cell-cycle control, alterations of cellular stress-control mechanisms, and interference of signaling pathways; as a result of accumulated genetic and epigenetic changes **(Dimov et al., 2010)**.

Extensive research in the field of cancer stem cells; in trying to isolate them and characterize their surface markers, showed that both normal stem cells and CSC share common markers and biological behavior **(Hatina and Schultz, 2012)**.

The stem cells of individual urothelial carcinomas appear to differ considerably and may contribute to the heterogeneity of this disease. The presence and quantity of urothelial carcinoma stem cells in each case may thus carry important clinical information that might allow a rationale stratification of urothelial cancer patients and probably pave the way for CSC targeted therapy. Many stem cell markers of normal urothelium have been the target of research in recent years, mainly CD44 and basal-type cytokeratins such as CK5 and CK 17, being usually expressed in the proposed normal urothelial stem cells (**Hatina and Schultz, 2012).**

However, in cancer biology, the relationship between embryogenesis and oncogenesis has long been a prevailing theme. Broadly speaking, cancer cells are similar to very early embryonic cells, which are immortal, undifferentiated, and invasive. It is important to study genes associated with embryogenesis and

tumorigenesis. The *POU* (Pit-Oct-Unc) family transcription factor member OCT-4 (POU5F1) acts as a key regulator of pluripotency in early stages of mammalian development. It has been shown that a critical amount of OCT-4 is required to sustain self-renewal of embryonic stem (ES) cells, and any up-regulation or down-regulation induces divergent cell fates **(Cheng et al., 2008)**.

AIM OF WORK

1. To understand whether OCT-4 is expressed in bladder urothelial carcinoma and its variants.
2. To predict the likely association of OCT-4 with the grade and stage of the tumour.
3. To delineate whether OCT-4 is a significant prognostic marker calling for special aggressive treatment postoperatively for patients with limited disease and/or target therapy.

ANATOMY OF THE URINARY BLADDER

The urinary bladder is an intrapelvic, extraperitoneal muscular structure designed to act as a reservoir of urine that allows controlled voiding whenever necessary.The adult empty bladder has the shape of an inverted pyramid and is enveloped by the vesical fascia. The superior surface faces superiorly and is covered by the pelvic peritoneum. The posterior surface, also known as the base of the bladder, faces posteriorly and inferiorly. Relations of this surface are of particular importance since it is this wall that harbours most of the bladder neoplasms. A growing neoplasm in this wall will directly infiltrate these posterior relations; which are the rectum separated by the seminal vesicles and the ampulla of the vasa differentia in males and the uterine cervix and the proximal portions of the vagina in females. The remainder of the pyramid is made up of two inferolateral surfaces, which together with the base of the bladder join to form the bladder neck. The bladder bed (structures on which the bladder neck rests) is formed posteriorly by the rectum in males and the vagina in females. Anteriorly and laterally it is formed by the internal obturator and the levator ani muscles as well as the pelvic bones **(Moore, 2013)**.

The “apex” or “dome” (the most anterior part of the bladder) is located at the point of contact of the superior surface and the two inferolateral surfaces. It marks the point of insertion of the median umbilical ligament (area where urachal carcinomas occur) **(Reuter, 1997)**. The ureters enter the bladder through a tunnel traversing the musculosa and mucosa. Each ureteric orifice is a small slit-like opening, and they are located about 2-3 cm apart and 2 cm above the internal urethral meatus in the adult bladder. The triangular region bound by these three openings in the posterior wall of bladder is called the trigone **(Engel, 1997)**.

HISTOLOGY OF THE URINARY BLADDER

The urinary bladder consists of a meshwork of thick muscle bundles that surround a highly vascular lamina propria lined by stratified epithelium (Reuter, 1992). The mucosa; or urothelium , is 5-7 cell layers thick in contracted bladder, 2-3 cells thick in distended bladder and is composed of 3 cell types: basal, intermediate, and superficial (umbrella) cells. The umbrella cells comprise a single layer of cells, and are relatively large and overlay multiple underlying cells in the intermediate cell layer. In the past their presence in histological preparations used to indicate a normal maturation process and thus lead to the exclusion of possible malignancy in a suspicious lesion, however , it is now known that they could be observed on the surface of low grade papillary urothelial neoplasms and often in carcinoma in situ (CIS). They appear elliptical with abundant eosinophilic cytoplasm and often binucleation or prominent nucleoli; and are p63 negative and stain positive for CK 18 and CK 20. Moreover, they contain trilaminar (asymmetric) unit membrane composed of two dense layers of unequal thickness and a central lucent layer, and apical plaques containing uroplakins **(Castello-Martin et al., 2010)**. Intermediate urothelial cells are cuboidal to low columnar with well defined borders and amphophilic cytoplasm rich in glycogen; nuclei are regularly arranged, ovoid with long axis at right angles to surface; chromatin is finely granular and has small nucleoli usually with no mitotic figures. Basal urothelial cells are more cylindrical and can be flat when bladder wall is stretched; some have longitudinal nuclear grooves. Both cell layers stain positive for p63, CK 5, CK 10 and CK 14 **(Koss, 1985)**;**(Castello-Martin et al., 2010)**.

Previously the bladder was thought to lack a muscularis mucosa and so the layer of fibrovascular connective tissue beneath the mucosa was termed lamina propria. However it is now well established that this layer contains wisps of incompletely differentiated smooth muscle fibres, that could occasionally be hyperplastic thus resembling the well developed muscularis propria. The importance of this observation is related to the staging of bladder carcinoma, and the significance attributed to tumour invasion of the muscle; where invasion of the muscularis propria carries a clinical significance far greater than invasion confined to muscularis mucosa, which calls for caution when interpreting biopsy specimens. A useful clue is that; in contrast to the well defined, rounded fascicles of the