**Mir-143 and Stemness Markers: Clinical and Pathological Implications In Bladder Cancer**

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**Abstract**

**Purpose:**

Sex determining region Y-box 2 (SOX2), and Nanog homebox (NANOG) are stem re­lated transcription factors, that confer self- renewal and pluripotency properties to embryonic stem cells (ESCs) and cancer stem cells (CSCs). So, the expression signature of the stemness state of primary tumors could be valuable for identifying patients who are most likely to suffer more aggressive tumor course or develop metastases and may also represent a promising molecular target for future therapeutic approaches. Evaluating the role of miRNAs as regulators of human malignancies represents an interesting research field. In this study we hypothesized that miR-143 may regulate bladder carcinogenesis, through regulating CSC markers (NANOG and SOX2). The expression of miR-143 was assessed in relation to the stemness markers Nanog and SOX2 in bladder cancer tissues and bladder carcinoma cell line. We also highlighted the possible association of our target genes expression with the clinicopathological features of BC patients.

**Material &Methods:** The present study included 27 tissue specimens from bladder cancer patients and 10 specimens from control subjects. In addition, human bladder cancer cell line (T24) and normal bladder epithelium cell line (HL14/12) were also studied. Reverse transcription quantitative Polymerase chain reaction (RT-qPCR) was used to assess gene expression of miR-143, SOX2 and NANOG. The expression of NANOG and SOX2 proteins was also detected by immunohistochemical staining in urinary bladder tissues from BC and control groups.

**Results:** miR-143 expression was significantly downregulated, while NANOG and SOX2 were significantly upregulated, in BC patients when compared to control group.miR-143 showed significant negative correlation with tumor grade, invasiveness, expression of NANOG and SOX2. Nanog and SOX2 mRNA were significantly elevated in tumor with higher grades, muscle invasive stages and showed significant positive correlation with each other. Downregulated miR-143, and upregulated Nanog and Sox2 could significantly predict muscle-invasive bladder cancer in crude and adjusted models.

**Conclusion:** miR-143 may have a role in regulating the NANOG and SOX2 stemness factors as shown by the significant negative correlation between miR-143 & both Nanog and Sox2 but the exact mechanisms of this role need to be further explored. Our study provides further proof of the pivotal role played by miRNAs in regulating the characteristics of CSCs in many tumors.

**Introduction:**

Bladder cancer (BC) is the most common malignancy affecting the urinary system and the ninth most common malignancy worldwide, with strong predominance in males. It is estimated that about half of bladder cancer deaths and more than 60% of all bladder cancer cases occur in less developed countries (1). In Egypt, BC is the second commonest malignancy in males (12.7%), and the third in both sexes. Estimated number of BC cases, in Egypt, is supposed to rise from 8234 cases in 2013 to 28337 cases in 2050 (2).

Currently, transitional cell carcinoma (TCC) is the predominant histologic type in Egypt, though it was squamous cell carcinoma in the past. This shift is due to the governmental strategy to eradicate Schistosomiasis and treat infected individuals, which was the underlying factor causing squamous cell carcinoma (SCC) predominance in the past (3).

The concept of cancer stem cells (CSCs) was introduced as an explanation for initiation and progression of tumors. These cells represent a small subset of cancer cells that act as analogues of adult stem cells in healthy tissues. They have unique properties as plasticity, quiescence, renewal, which confer their ability to generate differentiated cells’ progeny, that constitute the major tumor population (4). CSCs are responsible, as well, for drug resistance, recurrence and metastatic behavior of many tumors. (4, 5).

Homeobox protein (NANOG) and Sex determining region Y-box 2 (SOX2) are common stem related transcription factors. They are referred to as stemness markers due to their ability to repress differentiation and maintain self-renewal and pluripotency (stemness) of embryonic stem cells and CSCs. Hence, they are key mediators of tumor initiation, proliferation and dissemination. Several studies reported the expression of stemness markers in primary tumors including colon, prostate, and bladder cancers, suggesting the presence of CSCs (6, 7).

Dysregulation of microRNAs (miRNAs) has been implicated in initiation, progression, and metastasis of various cancers including bladder cancer. Emerging evidence suggests that miRNAs may be involved in regulation of CSCs properties (8). MiRNA 143 (miR-143) is regarded as a tumor suppressor miRNA, that have been previously suggested to be downregulated in many human malignancies, including bladder cancer (9, 10). However, its relationship with the pluripotency / stemness factors and tumor pathway, remains to be clarified (9, 10).

We hypothesized that miR-143 may control bladder cancer stem cell properties and may negatively affect the expression of genes associated with stemness and pluripotency, such as NANOG and SOX2.

To gain more insight into the pathogenesis of bladder cancer we studied the expression pattern of stemness genes (NANOG and SOX2) as well as miR-143 in bladder cancer tissues and a bladder cancer cell line. We focused on the association of our target genes with the clinicopathological data and highlighted their relationship with each other.