OCT4 and miRNA145 Expression in Bladder Cancer

Thesis
Submitted for fulfillment of Master Degree in Medical Biochemistry

By
Lina Abd El-Hady Mohamed
(M.B.B.Ch)

Demonstrator of Medical Biochemistry
Benha University

Supervised by

PROF. DR. THANAA HAMED BELAL
Professor of Medical Biochemistry
Faculty of medicine
Benha university

ASSIST. PROF. DR. MAMDOH ZAKI ABADIR
Assistant Professor of Medical Biochemistry
Faculty of medicine
Benha university

ASSIST. PROF. DR. HAMMOUDA WAHEEB SHERIF
Assistant Professor of Urology
Faculty of medicine
Benha university

DR. INAS ABDULMONEM EL-SAYED
Lecturer of Medical Biochemistry
Faculty of medicine
Benha university

2015
ACKNOWLEDGEMENT

I express my deep thanks and gratitude to Prof. Dr. Thanaa Hamed Belal professor of Medical Biochemistry, Faculty of Medicine, Benha University, for giving me the privilege of working under her supervision, for her encouragement, continuous support, patience, fruitful comments and constructive supervision throughout this work.

I offer my sincere thanks to Prof. Dr. Mamdoh Zaki Abadir Assistant professor of Medical Biochemistry, Faculty of Medicine, Benha University, for his guidance, generous time, great support and generous help.

I express my thanks to Prof. Dr. Hammouda Waheeb Sherif Assistant professor of Urology, Faculty of Medicine, Benha University, for his great help, generous time and fruitful comments.

I also offer my deepest thanks and gratitude to Dr. Inas Abdulmonem El-Sayed Lecturer of Medical Biochemistry, Faculty of Medicine, Benha University, for her generous support, great efforts, patience and constructive comments.

Many thanks to Molecular Biology Unit team especially to Prof. Dr. Amal Idris, the head of the unit, for helping me in performing the molecular analysis of Oct4 and miR-145.
<table>
<thead>
<tr>
<th>Item</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>AIM OF THE WORK</td>
<td>3</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td></td>
</tr>
<tr>
<td>Chapter (I): Bladder Cancer</td>
<td>4</td>
</tr>
<tr>
<td>i. Introduction</td>
<td>4</td>
</tr>
<tr>
<td>ii. Incidence and Epidemiology</td>
<td>4</td>
</tr>
<tr>
<td>iii. Molecular Basis of Bladder cancer</td>
<td>5</td>
</tr>
<tr>
<td>iv. Risk Factors</td>
<td>6</td>
</tr>
<tr>
<td>v. Pathology</td>
<td>8</td>
</tr>
<tr>
<td>vi. Diagnosis of Urinary bladder cancer</td>
<td>11</td>
</tr>
<tr>
<td>vii. Treatment</td>
<td>20</td>
</tr>
<tr>
<td>Chapter (II): MicroRNA and Oct4</td>
<td>21</td>
</tr>
<tr>
<td>MicroRNA</td>
<td></td>
</tr>
<tr>
<td>i. Definition of microRNA(miRNA)</td>
<td>21</td>
</tr>
<tr>
<td>ii. Discovery of miRNAs</td>
<td>21</td>
</tr>
<tr>
<td>iii. Nomenclature</td>
<td>21</td>
</tr>
<tr>
<td>iv. miRNA genes</td>
<td>23</td>
</tr>
<tr>
<td>v. Biogenesis of miRNAs</td>
<td>24</td>
</tr>
<tr>
<td>vi. Mode of silencing</td>
<td>28</td>
</tr>
<tr>
<td>vii. miRNA turnover</td>
<td>30</td>
</tr>
<tr>
<td>viii. Biological functions of miRNA</td>
<td>32</td>
</tr>
<tr>
<td>ix. miRNA and diseases</td>
<td>36</td>
</tr>
<tr>
<td>miR-145</td>
<td>42</td>
</tr>
<tr>
<td>i. miR-145 identification</td>
<td>42</td>
</tr>
<tr>
<td>ii. miR-145 gene</td>
<td>42</td>
</tr>
<tr>
<td>iii. miR-145 regulation</td>
<td>42</td>
</tr>
<tr>
<td>iv. Role of miR-145</td>
<td>43</td>
</tr>
<tr>
<td>v. miR-145 as a biomarker</td>
<td>47</td>
</tr>
<tr>
<td>vi. miR-145 in cancer therapy</td>
<td>48</td>
</tr>
<tr>
<td>OCT4</td>
<td>50</td>
</tr>
<tr>
<td>i. Introduction</td>
<td>50</td>
</tr>
<tr>
<td>ii. Oct4 definition</td>
<td>50</td>
</tr>
<tr>
<td>iii. Oct4 gene</td>
<td>51</td>
</tr>
<tr>
<td>iv. Oct4 structure</td>
<td>51</td>
</tr>
<tr>
<td>Item</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>v. Oct4 function</td>
<td>52</td>
</tr>
<tr>
<td>vi. Regulation of Oct4 expression</td>
<td>53</td>
</tr>
<tr>
<td>vii. Oct4 target genes</td>
<td>56</td>
</tr>
<tr>
<td>viii. Oct4 and cancer</td>
<td>57</td>
</tr>
<tr>
<td><strong>Chapter(III):Relationship between miR-145, Oct4 and bladder cancer</strong></td>
<td>59</td>
</tr>
<tr>
<td>i. miR-145 and bladder cancer</td>
<td>60</td>
</tr>
<tr>
<td>ii. Oct4 and bladder cancer</td>
<td>62</td>
</tr>
<tr>
<td>iii. miR-145 and Oct4</td>
<td>64</td>
</tr>
<tr>
<td><strong>SUBJECTS AND METHODS</strong></td>
<td>66</td>
</tr>
<tr>
<td><strong>RESULTS</strong></td>
<td>77</td>
</tr>
<tr>
<td><strong>DISCUSSION</strong></td>
<td>107</td>
</tr>
<tr>
<td><strong>SUMMARY AND CONCLUSION</strong></td>
<td>144</td>
</tr>
<tr>
<td><strong>RECOMMENDATIONS</strong></td>
<td>118</td>
</tr>
<tr>
<td><strong>REFERENCES</strong></td>
<td>119</td>
</tr>
<tr>
<td><strong>ARABIC SUMMARY</strong></td>
<td></td>
</tr>
<tr>
<td>Table (N)</td>
<td>List of Tables</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(1)</td>
<td>2009 TNM classification of urinary bladder cancer</td>
</tr>
<tr>
<td>(2)</td>
<td>World Health Organization (WHO) grading of urinary tumours in 1973 and 2004</td>
</tr>
<tr>
<td>(3)</td>
<td>Sensitivity and specificity with advantages and disadvantages of urinary markers</td>
</tr>
<tr>
<td>(4)</td>
<td>Volumes of total RNA, miRNA and primers used for cDNA synthesis.</td>
</tr>
<tr>
<td>(5)</td>
<td>Reaction setup for reverse transcription master mix preparation.</td>
</tr>
<tr>
<td>(6)</td>
<td>Reaction setup for real-time PCR.</td>
</tr>
<tr>
<td>(7)</td>
<td>Primer sequence of target and endogenous control genes.</td>
</tr>
<tr>
<td>(8)</td>
<td>The mean ± SD and range of the age/years of the studied groups.</td>
</tr>
<tr>
<td>(9)</td>
<td>Sex distribution among the studied groups.</td>
</tr>
<tr>
<td>(10)</td>
<td>Number, percentage and test of significance of smoking habit distribution in bladder cancer group as compared to that in control group.</td>
</tr>
<tr>
<td>(11)</td>
<td>The mean ±SD and test of significance of miR-145 fold change of bladder cancer group as compared to that of control group.</td>
</tr>
<tr>
<td>(12)</td>
<td>The mean ± SD and test of significance of Oct4 fold change of bladder cancer group as compared to that of control group.</td>
</tr>
<tr>
<td>(13)</td>
<td>Spearman’s correlation coefficient(ρ) between miR-145 fold change and the age in the studied groups.</td>
</tr>
<tr>
<td>(14)</td>
<td>Spearman’s correlation coefficient(ρ) between Oct4 fold change and the age in the studied groups.</td>
</tr>
<tr>
<td>(15)</td>
<td>The mean ±SD and test of significance of miR-145 fold change of female as compared to male subjects of the studied groups.</td>
</tr>
<tr>
<td>(16)</td>
<td>The mean ±SD and test of significance of Oct4 fold change of female as compared to male subjects in the studied groups.</td>
</tr>
<tr>
<td>(17)</td>
<td>The mean ±SD and test of significance of miR-145 fold change of smoker as compared to non smoker subjects in the studied groups.</td>
</tr>
<tr>
<td>(18)</td>
<td>The mean ±SD and test of significance of Oct4 fold change of smoker as compared to non smoker subjects in the studied groups.</td>
</tr>
<tr>
<td>(19)</td>
<td>Pathological data of bladder cancer group.</td>
</tr>
<tr>
<td>(20)</td>
<td>The mean ±SD and test of significance of miR-145 fold change of patients with small cell carcinoma as compared to those with transitional cell carcinoma.</td>
</tr>
<tr>
<td>Table (N)</td>
<td>List of Tables</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(21)</td>
<td>The mean ±SD and test of significance of Oct4 fold change of patients with small cell carcinoma as compared to those with transitional cell carcinoma.</td>
</tr>
<tr>
<td>(22)</td>
<td>The mean ±SD and test of significance of miR-145 fold change of patients with moderately differentiated tumors as compared to those with well differentiated tumors.</td>
</tr>
<tr>
<td>(23)</td>
<td>The mean ±SD and test of significance of miR-145 fold change of patients with poorly differentiated tumors as compared to those with well differentiated tumors.</td>
</tr>
<tr>
<td>(24)</td>
<td>The mean ±SD and test of significance of miR-145 fold change of patients with poorly differentiated tumors as compared to those with moderately differentiated tumors.</td>
</tr>
<tr>
<td>(25)</td>
<td>Differences in mean values of miR-145 fold change of bladder cancer patients regarding tumor grades.</td>
</tr>
<tr>
<td>(26)</td>
<td>The mean ±SD and test of significance of Oct4 fold change of patients with moderately differentiated tumors as compared to those with well differentiated tumors.</td>
</tr>
<tr>
<td>(27)</td>
<td>The mean ±SD and test of significance of Oct4 fold change of patients with poorly differentiated tumors as compared to those with well differentiated tumors.</td>
</tr>
<tr>
<td>(28)</td>
<td>The mean ±SD and test of significance of Oct4 fold change of patients with poorly differentiated tumors as compared to those with moderately differentiated tumors.</td>
</tr>
<tr>
<td>(29)</td>
<td>Differences in mean values of Oct4 fold change of bladder cancer patients regarding tumor grades.</td>
</tr>
<tr>
<td>(30)</td>
<td>The mean ±SD and test of significance of miR-145 fold change of patients with muscle invasive tumors as compared to those with non muscle invasive tumors.</td>
</tr>
<tr>
<td>(31)</td>
<td>The mean ±SD and test of significance of Oct4 fold change of patients with muscle invasive tumors as compared to those with non muscle invasive tumors.</td>
</tr>
<tr>
<td>(32)</td>
<td>Spearman’s correlation coefficient($\rho$) between miR-145 fold change and Oct4 fold change in the studied samples</td>
</tr>
<tr>
<td>Figures (N)</td>
<td>List of Figures</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(1)</td>
<td>Diagram showing the T stages of bladder</td>
</tr>
<tr>
<td>(2)</td>
<td>Field of pTa G2 bladder tumor with photodynamic diagnosis versus white light cystoscopy</td>
</tr>
<tr>
<td>(3)</td>
<td>Field of pTa G2 bladder tumor with narrow-band imaging versus white light cystoscopy</td>
</tr>
<tr>
<td>(4)</td>
<td>Nomenclature of miRNA</td>
</tr>
<tr>
<td>(5)</td>
<td>MicroRNA genomic organization</td>
</tr>
<tr>
<td>(6)</td>
<td>MicroRNA maturation and function</td>
</tr>
<tr>
<td>(7)</td>
<td>Primary microRNA structure and site of Drosha cleavage</td>
</tr>
<tr>
<td>(8)</td>
<td>Biogenesis of miRNA</td>
</tr>
<tr>
<td>(9)</td>
<td>Biogenesis and mode of silencing of miRNA</td>
</tr>
<tr>
<td>(10)</td>
<td>miRNA degradation by highly complementary mRNA targets and viral transcripts</td>
</tr>
<tr>
<td>(11)</td>
<td>MicroRNAs as oncogenes or tumour suppressor genes</td>
</tr>
<tr>
<td>(12)</td>
<td>Oct4 domains</td>
</tr>
<tr>
<td>(13)</td>
<td>Embryonic stem cells and Oct4 expression</td>
</tr>
<tr>
<td>(14)</td>
<td>MicroRNA-Regulated Stem Cell</td>
</tr>
<tr>
<td>(15)</td>
<td>Model of a regulatory loop between Oct4 and miR-145</td>
</tr>
<tr>
<td>(16)</td>
<td>Age distribution among the studied groups</td>
</tr>
<tr>
<td>(17)</td>
<td>Sex distribution among the studied groups</td>
</tr>
<tr>
<td>(18)</td>
<td>Non-significant difference in smoking habit distribution between the studied groups</td>
</tr>
<tr>
<td>(19)</td>
<td>Significant decrease in mean value of miR-145 fold change of bladder cancer group as compared to that of control group</td>
</tr>
<tr>
<td>(20)</td>
<td>Significant increase in mean value of Oct4 fold change of bladder cancer group as compared to that of control group</td>
</tr>
<tr>
<td>(21)</td>
<td>Non-significant correlation between miR-145 fold change and the age of control group</td>
</tr>
<tr>
<td>(22)</td>
<td>Non-significant correlation between miR-145 fold change and the age of bladder cancer group</td>
</tr>
<tr>
<td>(23)</td>
<td>Non-significant correlation between Oct4 fold change and the age of control group</td>
</tr>
<tr>
<td>(24)</td>
<td>Non-significant correlation between Oct4 fold change and the age of bladder cancer group</td>
</tr>
<tr>
<td>(25)</td>
<td>Non-significant difference in mean value of miR-145 fold change regarding sex in control group</td>
</tr>
<tr>
<td>(26)</td>
<td>Non-significant difference in mean value of miR-145 fold change regarding sex in bladder cancer group</td>
</tr>
<tr>
<td>(27)</td>
<td>Non-significant difference in mean value of Oct4 fold change regarding sex in control group</td>
</tr>
<tr>
<td>Figures (N)</td>
<td>List of Figures</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(28)</td>
<td>Non-significant difference in mean value of Oct4 fold change regarding sex in bladder cancer group</td>
</tr>
<tr>
<td>(29)</td>
<td>Non-significant difference in mean value of miR-145 fold change regarding smoking habit in control group</td>
</tr>
<tr>
<td>(30)</td>
<td>Non-significant difference in mean value of miR-145 fold change regarding smoking habit in bladder cancer group</td>
</tr>
<tr>
<td>(31)</td>
<td>Non-significant difference in mean value of Oct4 fold change regarding smoking habit in control group</td>
</tr>
<tr>
<td>(32)</td>
<td>Non-significant difference in mean value of Oct4 fold change regarding smoking habit in bladder cancer group</td>
</tr>
<tr>
<td>(33)</td>
<td>Distribution of bladder cancer patients regarding tumor type</td>
</tr>
<tr>
<td>(34)</td>
<td>Distribution of bladder cancer patients regarding tumor grades</td>
</tr>
<tr>
<td>(35)</td>
<td>Distribution of bladder cancer patients regarding tumor stages</td>
</tr>
<tr>
<td>(36)</td>
<td>Non-significant difference in mean value of miR-145 fold change regarding tumor types</td>
</tr>
<tr>
<td>(37)</td>
<td>Non-significant difference in mean value of Oct4 fold change regarding tumor types</td>
</tr>
<tr>
<td>(38)</td>
<td>Non-significant differences in mean values of miR-145 fold change of bladder cancer patients regarding tumor grades</td>
</tr>
<tr>
<td>(39)</td>
<td>Differences in mean values of Oct4 fold change of bladder cancer patients regarding tumor grades</td>
</tr>
<tr>
<td>(40)</td>
<td>Non-significant difference in mean value of miR-145 fold change regarding tumor stages</td>
</tr>
<tr>
<td>(41)</td>
<td>Significant increase in mean value of Oct4 fold change of patients with muscle invasive tumors as compared to that of patients with non muscle invasive tumors</td>
</tr>
<tr>
<td>(42)</td>
<td>Significant negative correlation between fold change of miR-145 and Oct4 in the studied samples</td>
</tr>
</tbody>
</table>
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ADAM</td>
<td>A disintegrin and metalloproteinase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>BACE 1</td>
<td>Beta-site Amyloid precursor protein-Cleaving Enzyme 1</td>
</tr>
<tr>
<td>BC</td>
<td>Bladder cancer</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacille Calmette-Guérin</td>
</tr>
<tr>
<td>Bel-2</td>
<td>B-cell lymphoma 2</td>
</tr>
<tr>
<td>B-CLL</td>
<td>B-cell chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>bp</td>
<td>Base-pair</td>
</tr>
<tr>
<td>BTA</td>
<td>Bladder tumor antigen</td>
</tr>
<tr>
<td>ºC</td>
<td>Celsius</td>
</tr>
<tr>
<td>C. elegans</td>
<td>Caenorhabditis elegans</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete Blood Count</td>
</tr>
<tr>
<td>CBFB</td>
<td>Core-binding factor b subunit</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>Cdx2</td>
<td>Caudal type homeobox 2</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>CIS</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>CLINT1</td>
<td>Clathrin Interactor 1</td>
</tr>
<tr>
<td>c-Myc</td>
<td>Avian myelocytomatosis viral oncogene homolog</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CSC</td>
<td>Cancer Stem Cell</td>
</tr>
<tr>
<td>CT</td>
<td>Computerised tomography</td>
</tr>
<tr>
<td>Ct</td>
<td>Cycle threshold</td>
</tr>
<tr>
<td>CYFRA 21-1</td>
<td>Cytokeratin 19 fragment</td>
</tr>
<tr>
<td>DGCR 8</td>
<td>DiGeorge Syndrome Critical Region 8</td>
</tr>
<tr>
<td>DN</td>
<td>Dopaminergic neuron</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>Drice</td>
<td>Drosophila interleukin 1 beta -converting enzyme</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>Eri-1</td>
<td>Enhanced RNA interference -1</td>
</tr>
<tr>
<td>ESC</td>
<td>Embryonic stem cell</td>
</tr>
<tr>
<td>Esrrb</td>
<td>Estrogen-related receptor b</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ETS</td>
<td>E26 transformation-specific</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast growth factor</td>
</tr>
<tr>
<td>FGFR 3</td>
<td>Fibroblast growth factor receptor 3</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescence in situ hybridization</td>
</tr>
<tr>
<td>Fli-1</td>
<td>Friend leukemia integration 1</td>
</tr>
<tr>
<td>FSCN1</td>
<td>Fascin actin-bundling protein 1</td>
</tr>
<tr>
<td>5-FU</td>
<td>5-fluorouracil</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GCNF</td>
<td>Germ cell nuclear factor</td>
</tr>
<tr>
<td>G phase</td>
<td>Gap phase</td>
</tr>
<tr>
<td>GSTM 1</td>
<td>Glutathione S-transferase mu 1</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>HA</td>
<td>Hyaluronic acid</td>
</tr>
<tr>
<td>HA-ase</td>
<td>Hyaluronidase</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>Hid</td>
<td>Head involution defective</td>
</tr>
<tr>
<td>HIF-1 α</td>
<td>Hypoxia-inducible factor 1α</td>
</tr>
<tr>
<td>HIV-1</td>
<td>Human immunodeficiency virus type 1</td>
</tr>
<tr>
<td>hsa</td>
<td>Homo sapien</td>
</tr>
<tr>
<td>ICM</td>
<td>Inner cell mass</td>
</tr>
<tr>
<td>IGF-IR</td>
<td>Insulin like growth factor I receptor</td>
</tr>
<tr>
<td>INF</td>
<td>Interferon</td>
</tr>
<tr>
<td>INR</td>
<td>International normalized ratio</td>
</tr>
<tr>
<td>IPF</td>
<td>Idiopathic pulmonary fibrosis</td>
</tr>
<tr>
<td>IRE 1 α</td>
<td>Inositol-requiring enzyme1α</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Insulin receptor substrate-1</td>
</tr>
<tr>
<td>ISH</td>
<td>In situ hybridisation</td>
</tr>
<tr>
<td>ISUP</td>
<td>International Society of Urological Pathology</td>
</tr>
<tr>
<td>IVP</td>
<td>Intravenous pyelogram</td>
</tr>
<tr>
<td>KLF 4</td>
<td>Kruppel-like factor 4</td>
</tr>
<tr>
<td>let-7</td>
<td>Lethal-7</td>
</tr>
<tr>
<td>Limk-1</td>
<td>Lim domain containing protein kinase-1</td>
</tr>
<tr>
<td>LOH</td>
<td>Loss of heterozygosity</td>
</tr>
<tr>
<td>LRC</td>
<td>Laparoscopic radical cystectomy</td>
</tr>
<tr>
<td>MCMV</td>
<td>Murine cytomegalovirus</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>MIBC</td>
<td>Muscle invasive bladder cancer</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>miR-LAT</strong></td>
<td>Latency-associated miRNA</td>
</tr>
<tr>
<td><strong>miRNA</strong></td>
<td>MicroRNA</td>
</tr>
<tr>
<td><strong>MMP</strong></td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td><strong>MRI</strong></td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td><strong>mRNA</strong></td>
<td>Messenger RNA</td>
</tr>
<tr>
<td><strong>MS</strong></td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td><strong>MSA</strong></td>
<td>Microsatellite analysis</td>
</tr>
<tr>
<td><strong>MSH</strong></td>
<td>Mismatch repair</td>
</tr>
<tr>
<td><strong>MUC1</strong></td>
<td>Mucin 1</td>
</tr>
<tr>
<td><strong>NAFLD</strong></td>
<td>Non-alcoholic fatty liver disease</td>
</tr>
<tr>
<td><strong>NAT 2</strong></td>
<td>N-acetyltransferase 2</td>
</tr>
<tr>
<td><strong>NBI</strong></td>
<td>Narrow-band imaging</td>
</tr>
<tr>
<td><strong>NMIBC</strong></td>
<td>Non-muscle invasive bladder cancer</td>
</tr>
<tr>
<td><strong>NMP 22</strong></td>
<td>Nuclear matrix protein 22</td>
</tr>
<tr>
<td><strong>nt</strong></td>
<td>Nucleotide</td>
</tr>
<tr>
<td><strong>oar</strong></td>
<td>Ovis aries</td>
</tr>
<tr>
<td><strong>Oct 4</strong></td>
<td>Octamer-binding transcription factor 4</td>
</tr>
<tr>
<td><strong>ORC</strong></td>
<td>Open radical cystectomy</td>
</tr>
<tr>
<td><strong>PAK1</strong></td>
<td>P21 activated protein</td>
</tr>
<tr>
<td><strong>p70S6K1</strong></td>
<td>p70 ribosomal protein S6 kinase 1</td>
</tr>
<tr>
<td><strong>PC</strong></td>
<td>Prothrombin concentration</td>
</tr>
<tr>
<td><strong>PDD</strong></td>
<td>Photodynamic diagnosis</td>
</tr>
<tr>
<td><strong>PFV-1</strong></td>
<td>Primate foamy virus type 1</td>
</tr>
<tr>
<td><strong>PGC</strong></td>
<td>Primordial germ cell</td>
</tr>
<tr>
<td><strong>PIK3CD</strong></td>
<td>Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta isoform</td>
</tr>
<tr>
<td><strong>Pitx 3</strong></td>
<td>Pituitary homeobox 3</td>
</tr>
<tr>
<td><strong>PK</strong></td>
<td>Parkinson's disease</td>
</tr>
<tr>
<td><strong>POU5f1</strong></td>
<td>POU domain, class 5, transcription factor 1</td>
</tr>
<tr>
<td><strong>PPP3CA</strong></td>
<td>Protein phosphatase 3 catalytic subunit α isoform</td>
</tr>
<tr>
<td><strong>Pre-miRNA</strong></td>
<td>Precursor-miRNA</td>
</tr>
<tr>
<td><strong>Pri-miRNA</strong></td>
<td>Primary-miRNA</td>
</tr>
<tr>
<td><strong>PT</strong></td>
<td>Prothrombin time</td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>Phosphatase and tensin homolog</td>
</tr>
<tr>
<td><strong>PTT</strong></td>
<td>Partial thromboplastin time</td>
</tr>
<tr>
<td><strong>PUNLMP</strong></td>
<td>Papillary urothelial neoplasms of low malignant potential</td>
</tr>
<tr>
<td><strong>qRT-PCR</strong></td>
<td>Quantitative reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td><strong>Ran</strong></td>
<td>Ras-related nuclear protein</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Ras</td>
<td>Rat sarcoma</td>
</tr>
<tr>
<td>RB</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td>Rex1</td>
<td>Reduced expression 1</td>
</tr>
<tr>
<td>RISC</td>
<td>RNA-induced silencing complex</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acids</td>
</tr>
<tr>
<td>RU</td>
<td>Relative unit</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SDN</td>
<td>Small RNA degrading nuclease</td>
</tr>
<tr>
<td>SF-1</td>
<td>Steroidogenic factor-1</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematosis</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
</tr>
<tr>
<td>SOCS 7</td>
<td>Suppressor of cytokine signalling 7</td>
</tr>
<tr>
<td>Sox2</td>
<td>Sex determining region Y-box 2</td>
</tr>
<tr>
<td>S phase</td>
<td>Synthesis phase</td>
</tr>
<tr>
<td>SUMO-1</td>
<td>Small ubiquitin-related modifier-1</td>
</tr>
<tr>
<td>TCC</td>
<td>Transitional cell carcinoma</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumour-node-metastases</td>
</tr>
<tr>
<td>Tr 2</td>
<td>Testicular receptor 2</td>
</tr>
<tr>
<td>TRBP</td>
<td>Transactivating response RNA-binding protein</td>
</tr>
<tr>
<td>TU</td>
<td>Transcription unit</td>
</tr>
<tr>
<td>TURBT</td>
<td>Transurethral resection of bladder tumor</td>
</tr>
<tr>
<td>UC</td>
<td>Urothelial carcinoma</td>
</tr>
<tr>
<td>UICC</td>
<td>Union International Contre le Cancer</td>
</tr>
<tr>
<td>UTR</td>
<td>Untranslated region</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VSMC</td>
<td>Vascular smooth muscle cell</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>Wwp2</td>
<td>Ww domain-containing protein 2</td>
</tr>
<tr>
<td>XRN2</td>
<td>Exoribonuclease 2</td>
</tr>
<tr>
<td>y</td>
<td>Year</td>
</tr>
</tbody>
</table>
INTRODUCTION

Bladder cancer (BC) represents a global health problem (Zarzour et al., 2008). It ranks ninth in worldwide cancer incidence. It is the 7th most common cancer in men and the 17th most common cancer in women (Ploeg et al., 2009). Approximately 386,300 new BC cases and 150,200 deaths caused by the BC worldwide were registered in 2008 (Ferlay et al., 2010). In Egypt, carcinoma of the bladder is the most prevalent cancer, accounting for as many as 31% of all cancer cases. The estimated incidence in males in rural areas in Egypt is about 32 per 100,000 (Zarzour et al., 2008). There have been lots of efforts in the field to find non-invasive, sensitive, and specific molecular markers for bladder cancer (Ratert et al., 2013).

MicroRNAs (miRNAs) are endogenous, non-coding RNA molecules of about 22 nucleotides in length that regulate gene expression. They regulate their targeted messenger RNA (mRNA) by repressing mRNA translation and/or directing mRNA cleavage (Bartel, 2004).

A large number of studies have demonstrated that miRNAs are key regulators of a variety of fundamental biological processes such as development, cell proliferation, apoptosis, haematopoiesis and, importantly, tumourigenesis (Huang et al., 2011). Many miRNAs were significantly upregulated or downregulated in bladder urothelial carcinoma compared to matched histologically normal urothelium. Most importantly, different cancer types, stages or differentiation states have unique miRNA expression profiles, suggesting that miRNAs play important roles in the initiation and progression of cancer (Han et al., 2011).
miR-145 was identified as a tumor-suppressive miRNA that is downregulated in several cancer types (Dip et al., 2013), including prostate cancer (Porkka et al., 2007), bladder cancer (Ichimi et al., 2009), colon cancer (Akao et al., 2007) and ovarian cancer (Nam et al., 2008), suggesting that miR-145 over-expression has a growth inhibitory effect (Sachdeva et al., 2009).

Tumor recurrence and multifocality are two common features of bladder tumors. Several previous reports suggested that these tumors are derived from a primary transformed progenitor cell (Hatefi et al., 2012). Based on Cancer Stem Cell (CSC) model, CSCs are characterized by self-renewal, heterogeneity (potential for multidirectional differentiation), resistance to apoptosis, and resistance to conventional therapies. CSCs have been isolated from a variety of solid tumors such as breast cancer, lung, prostate, colon tumors (Al-Hajj and Clarke, 2004) and bladder cancer (Tran et al., 2010). The most important members of CSCs' regulatory core are transcription factors such as Octamer-binding transcription factor 4 (Oct4), Sex determining region Y-box 2 (Sox2), and Nanog, which are defined as key players in the regulatory network for maintaining the “stemness” state of stem cells (Hatefi et al., 2012).

Oct4 (octamer-binding transcription factor 4) is a transcription factor that is required for pluripotency during early embryogenesis and the maintenance of embryonic stem cell (ESC) and pluripotent cell identity. Misexpression of Oct4 is correlated with tumorigenesis and can affect the behavior of tumors such as recurrence or resistance to therapy (Hatefi et al., 2012).
AIM OF THE WORK

This study was designed to clarify the role of miR-145 and Oct4 in bladder cancer by determining their expression in relation to various clinico-pathological parameters. Moreover, to determine the relationship between the expression of miR-145 and Oct4.
i. Introduction:

Bladder cancer (BC) is a heterogeneous disease with a variable natural history. At one end of the spectrum, low-grade tumors have a low progression rate and require initial endoscopic treatment and surveillance but rarely present a threat to the patient. At the other extreme, high-grade tumors have a high malignant potential associated with significant progression and cancer death rates (Kirkali et al., 2005).

ii. Incidence and Epidemiology:

Bladder cancer ranks ninth in worldwide cancer incidence. It is the 7th most common cancer in men and the 17th most common cancer in women (Ploeg et al., 2009). Approximately 386,300 new BC cases and 150,200 deaths caused by the BC worldwide were registered in 2008 (Ferlay et al., 2010).

Globally, the incidence of bladder cancer varies significantly, with Egypt, Western Europe, and North America having the highest incidence rates, but Asian countries having the lowest rates (Colombel et al., 2008).

The risk of developing bladder cancer at ˂75 years (y) of age is 2% to 4% for men and 0.5% to 1% for women. The median age at diagnosis is 65 to 70 y (Kirkali et al., 2005).

Ninety percent of BC are urothelial carcinomas (UC), previously known as transitional cell carcinomas, and the majority are papillary low-grade, non-muscle invasive cancers that recur in up to 80% of cases but
rarely progress to muscle invasion \cite{McConkey2010}. In contrast, 10 to 20% of tumors are muscle invasive at diagnosis, and 50% of patients die from metastatic disease \cite{Borden2005}.

iii. Molecular Basis of Bladder cancer:

The molecular pathways underlying the two main distinct types of UC, low-grade non-muscle invasive UC and high-grade muscle invasive UC \cite{Jebbar2005} have been investigated to identify new potential markers for diagnosis, disease monitoring, prognosis and the development of new targeted therapies \cite{Castillo-Martin2010}.

The most common genetic alteration of BC associated with low-grade and low-stage is an activating mutation of the Fibroblast growth factor receptor 3 (FGFR3) gene \cite{Castillo-Martin2010}, whereas mutations in p53, Retinoblastoma (RB1) and Phosphatase and tensin homolog (PTEN) genes have been identified as being characteristic of the carcinogenesis pathway for high-grade invasive BC \cite{Pandith2013}.

Other events playing roles in the development and progression of BC are related to alterations in protein expression, including Deoxyribonucleic acid (DNA) methylation, histone acetylation and abnormalities in the expression of microRNAs (miRNAs) \cite{Dip2013}.

miRNAs are members of small single-stranded regulatory Ribonucleic acids (RNAs) 21-25 nucleotides (nt) that can suppress translation or promote degradation of mRNAs, thereby regulating the expression of target genes, including transcription factors, oncogenes and
tumor suppressor genes. Moreover, miRNAs have been reported to be differentially expressed in several types of cancers (Dip et al., 2013).

iv. Risk Factors:

Several factors have different impacts on the incidence and pathophysiology of BC. This phenomenon is called etiologic fraction or attributable risk (Burger et al., 2013). These factors include:

1. Smoking:

Smoking is recognized as the most important risk factor for BC and is estimated to account for 50% of tumors (Burger et al., 2013). In fact, cigarette smokers have a 2- to 4-fold increased risk of bladder cancer compared to non-smokers (Kirkali et al., 2005), and the risk increases with increasing intensity and/or duration of smoking. Upon cessation of cigarette smoking, the risk of bladder cancer falls >30% after 1–4 y and >60% after 25 y but never returns to the level of risk of non-smokers (Colombel et al., 2008).

Tobacco smoke contains aromatic amines, such as-naphthylamine, and polycyclic aromatic hydrocarbons known to cause BC. These are renally excreted and exert a carcinogenic effect on the entire urinary system (Burger et al., 2013).

2. Occupational risk:

Occupational exposure to urothelial carcinogens is the second most important risk factor, accounting for 5–20% of all bladder cancers (Kogevinas et al., 2003).

Current or historical exposure to aromatic amines(e.g. benzidine, 2-naphthylamine and 4-aminobiphenyl) used in rubber and dye industries; and polycyclic aromatic hydrocarbons used in the aluminum
and coal industries have all been associated with the development of bladder cancer. An increased risk of bladder cancer has also been reported in painters, varnishers, and hairdressers (Colombel et al., 2008).

3. Medical conditions:

Medical conditions may predispose individuals to bladder tumorigenesis through direct causation or as a side effect of treatment. Examples of direct causative roles include (a) chronic urinary retention and upper tract dilation increasing urothelial exposure to carcinogens and (b) carcinogenesis associated with chronic inflammation or schistosomiasis (Burger et al., 2013). Squamous cell carcinoma of the urinary bladder has been known for many years to be associated with Schistosoma haematobium infection (Kirkali et al., 2005).

With regard to treatment, BC may arise as a consequence of exposure to ionizing radiation and pharmaceutical agents (Burger et al., 2013). Abern et al. (2013) found an increased age-standardized incidence rate of BC following external-beam radiotherapy for prostate cancer.

Two pharmacologic agents have also been related to BC; cyclophosphamide is an alkylating agent mainly applied in lymphoma and leukemia, and a long-term use increases BC incidence (Burger et al., 2013). Pioglitazone, an antidiabetic drug of the thiazolidinedione class, has been found to have a weak relation to BC incidence with longer-term use (MacKenzie et al., 2011).

For diabetes mellitus, an increased BC incidence has been reported, which was greater with longer duration and use of oral hypoglycemic medication (Lewis et al., 2011).
4. Dietary factors:

Inadequate consumption of fruits, vegetables, and certain vitamins may also play a role in the development of bladder cancer *([Colombel et al., 2008](#))*. Although it has been suggested that coffee consumption and artificial sweeteners may be associated with an increased risk of bladder cancer, results from epidemiologic studies investigating these agents have been inconclusive *([Colombel et al., 2008](#))*. A major problem in evaluating the independent effect of coffee consumption on the development of bladder cancer is its relationship to cigarette smoking *([Murta-Nascimento et al., 2007](#))*. 

5. Genetic susceptibility:

The risk of BC is two-fold higher in first-degree relatives of BC patients. Inherited genetic factors, such as the genetic slow acetylator N-acetyltransferase 2 (NAT2) variants and glutathione S-transferase mu 1 (GSTM1)–null genotypes, have been established as risk factors for BC. However, factors such as slow acetylation may not intrinsically lead to BC but may confer additional risk to exposure of carcinogens such as tobacco products *([Burger et al., 2013](#))*. 

v. Pathology:

1. Major pathologic subtypes:

Transitional cell carcinoma (TCC) is the most common primary pathologic subtype of bladder cancer and is observed in >90% of tumours. Squamous cell carcinoma and adenocarcinoma are less common and occur in approximately 5% and 1% of bladder cancers, respectively *([Colombel et al., 2008](#))*. 

In certain regions of the world where schistosomiasis (also known as bilharziasis) infection is endemic, squamous cell carcinoma can account for up to 75% of bladder cancers (Colombel et al., 2008).

2. Staging:

The most widely used and universally accepted staging system is the tumour-node-metastases (TNM) system approved by the Union International Contre le Cancer (UICC), which was updated in 2009, shown in (table 1) (fig.1) (Sobin et al., 2009).

**Table (1):** 2009 TNM classification of urinary bladder cancer (Sobin et al., 2009).

<table>
<thead>
<tr>
<th>T: Primary tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX Primary tumour cannot be assessed</td>
</tr>
<tr>
<td>T0 No evidence of primary tumour</td>
</tr>
<tr>
<td>Ta Non invasive papillary carcinoma</td>
</tr>
<tr>
<td>Tis Carcinoma in situ(CIS): “flat tumour”</td>
</tr>
<tr>
<td>T1 Tumour invades subepithelial connective tissue</td>
</tr>
<tr>
<td>T2 Tumour invades muscle</td>
</tr>
<tr>
<td>T2a Tumour invades superficial muscle (inner half)</td>
</tr>
<tr>
<td>T2b Tumour invades deep muscle (outer half)</td>
</tr>
<tr>
<td>T3 Tumour invades perivesical tissue</td>
</tr>
<tr>
<td>T3a Microscopically</td>
</tr>
<tr>
<td>T3b Macroscopically (extravesical mass)</td>
</tr>
<tr>
<td>T4 Tumour invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall</td>
</tr>
<tr>
<td>T4a Tumour invades prostate, uterus, or vagina</td>
</tr>
<tr>
<td>T4b Tumour invades pelvic wall or abdominal wall</td>
</tr>
</tbody>
</table>
### N: Lymph nodes

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)</td>
</tr>
<tr>
<td>N2</td>
<td>Metastasis in multiple lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)</td>
</tr>
<tr>
<td>N3</td>
<td>Metastasis in a common iliac lymph node(s)</td>
</tr>
</tbody>
</table>

### M: Distant metastasis

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MX</td>
<td>Distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

**Fig. (1): Diagram showing the T stages of bladder cancer.**
Grading:

Traditionally, bladder carcinomas have been graded according to the World Health Organization (WHO) 1973 grading of urothelial papilloma: well differentiated (G1), moderately differentiated (G2), or poorly differentiated (G3). In 2004, the WHO and the International Society of Urological Pathology (ISUP) published a new grading system that employs specific cytologic and architectural criteria (*Sauter et al.*, 2004).

Comparisons of the 1973 and 2004 classification systems are shown in (table 2) (*Colombel et al.*, 2008).


<table>
<thead>
<tr>
<th>1973 WHO grading</th>
<th>2004 WHO grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urothelial papilloma</td>
<td>Urothelial papilloma</td>
</tr>
<tr>
<td>Grade 1: (G1) Well differentiated</td>
<td>PUNLMP= papillary urothelial neoplasms of low malignant potential</td>
</tr>
<tr>
<td>Grade 2: (G2) Moderately differentiated</td>
<td>Low-grade papillary urothelial carcinoma</td>
</tr>
<tr>
<td>Grade 3: (G3) Poorly differentiated</td>
<td>High-grade papillary urothelial carcinoma</td>
</tr>
</tbody>
</table>

vi. Diagnosis of Urinary bladder cancer

1. Clinical picture

Bladder cancer commonly presents with intermittent or persistent microscopic or macroscopic haematuria; rates may be as high as 25%
in patients with macroscopic haematuria and 9.4% in patients with microscopic haematuria (Khadra et al., 2000).

The symptom complex of bladder irritability and urinary frequency, urgency and dysuria is the second most common presentation of bladder cancer and is usually associated with diffuse carcinoma in situ (CIS) or invasive bladder cancer (Kirkali et al., 2005).

Other symptoms and signs of bladder cancer include flank pain caused by ureteral obstruction, lower extremity edema and a palpable pelvic mass. Very rarely, patients present with symptoms of advanced disease, such as weight loss and abdominal or bone pain from distant metastases. However, these symptoms almost never occur without microscopic or macroscopic hematuria (Kirkali et al., 2005).

2. Investigations:

A. Cytology:

Urine cytology is an essential modality for the detection of urothelial neoplasia. It has long been known that urine cytology is accurate in the diagnosis of high-grade urothelial carcinoma. However, it carries a much lower diagnostic yield for low-grade urothelial neoplastic lesions (Brimo et al., 2009).

Many studies have evaluated the accuracy of urine cytology in the detection of bladder cancer. Overall, the reported sensitivity ranges from 20% to 97.3%; specificity ranges from 74% to 99.5% (Brimo et al., 2009).

B. Cystoscopy and biopsy:

Conventional or white-light cystoscopy is the “gold standard” for the detection of bladder cancer. A disadvantage of conventional
cystoscopy is the difficulty in detecting flat lesions such as carcinoma in situ (CIS) (Jacobs et al., 2010). This has led to the development of newer technologies such as photodynamic diagnosis (PDD) and narrow-band imaging (NBI) cystoscopy (Cheung et al., 2013).

**Photodynamic diagnosis (PDD):**

Photodynamic diagnosis or fluorescence cystoscopy aims to improve the visualization of bladder cancer based on cystoscopic detection of fluorescent signals from neoplastic tissue. This fluorescence is accomplished by the intravesical administration of photosensitizing agents 5-aminolevulenic acid or its derivative hexaminolevulinate which cause selective accumulation of photoactive porphyrins in rapidly proliferating cells (e.g., tumor cells) (fig.2) (Cauberg Evelyne et al., 2011).

![Field of pTa G2 bladder tumor with photodynamic diagnosis (right) versus white light cystoscopy (left)](Cauberg Evelyne et al., 2011)

**Narrow-band imaging (NBI):**

Narrow-band imaging is a straightforward optical technique designed for endoscopy to enhance the visualization of (sub) mucosal vessels. The working mechanism is based on the filtering of white light
into two narrow bandwidths of light that are centered around 415 nm (blue light) and 540 nm (green light), which penetrate tissue only superficially and are specifically absorbed by hemoglobin (Song et al., 2008). Because bladder tumors tend to be well vascularised, narrow-band imaging will increase the contrast between these lesions and normal bladder mucosa (fig.3) (Cauberg Evelyne et al., 2011).

**Fig.(3):** Field of pTa G2 bladder tumor with narrow-band imaging (right) versus white light cystoscopy (left) (Cauberg Evelyne et al., 2011).

**C. Imaging:**

Ultrasonography is being used more frequently and is advantageous because it does not require contrast agents (Jacobs et al., 2010).

Imaging with computerised tomography (CT) scan has essentially replaced intravenous pyelograms (IVP) in many centers. Magnetic resonance imaging (MRI) had been a reliable modality for patients with renal failure. CT scan and MRI may be used to determine the stage of bladder cancer; however, they are unable to accurately detect early metastatic disease particularly in normal-sized lymph nodes (Jacobs et al., 2010).
D. Urinary markers:

Numerous urinary markers have been investigated (table 3), with the aim of reducing frequency of cystoscopy (Yutkin et al., 2010). Several are commercially available, but none has been adopted into routine standard of care, owing to poor sensitivity and/or expense. These markers may serve as an adjunctive diagnostic test in cases where urine cytology is equivocal (Cheung et al., 2013).

a) Fluorescence in situ hybridization (FISH):

FISH can be used to detect urinary cells that have chromosomal abnormalities consistent with a diagnosis of bladder cancer. For example, The UroVysion Bladder Cancer Kit (UroVysion Kit) uses fluorescently labeled DNA probes to detect aneuploidy in chromosomes 3, 7, and 17 and loss of the 9 p21 locus of the P16 tumor suppressor gene (Cheung et al., 2013).

b) Microsatellite analysis (MSA)

Microsatellites are highly polymorphic, short, tandem DNA repeats found in the human genome. Two types of microsatellite alterations can be found in many cancers: loss of heterozygosity (LOH), an allelic deletion, and somatic alteration of microsatellite repeat length (Vrooman and Witjes, 2008). In bladder cancer, most mutations are in the form of LOH (Turyn et al., 2006). Microsatellite alterations in exfoliated urine are detected by a polymerase chain reaction (PCR) using DNA primers for a panel of known microsatellite markers (Vrooman and Witjes, 2008).

c) ImmunoCyt™

Immunocytology is based on the visualisation of tumour associated antigens in urothelial carcinoma cells using monoclonal antibodies. Three fluorescently marked antibodies label two mucin like proteins and a high
molecular weight form of carcinoembryonic antigen. After this process the cells are examined under a fluorescent microscope (Vrooman and Witjes, 2008).

d) Telomerase:
Telomeres are repetitious sequences at the end of chromosomes that protect genetic stability during DNA replication. There is loss of telomeres during each cell division, which causes chromosomal instability and cellular senescence. Bladder cancer cells express telomerase, an enzyme that regenerates telomeres at the end of each DNA replication and therefore sets the cellular clock to immortality. Determination of telomerase activity is a PCR-based technology and must be performed in specialized laboratories (Vrooman and Witjes, 2008).

e) Bladder tumor antigen (BTA) BTA-TRAK™ and BTA-stat™:
BTA-TRAK and BTA-stat (Alidex Inc, Redmond, WA, USA) are both versions of the bladder tumour antigen assay that measures complement factor H–related protein in urine. BTA-stat is an immunoassay that can be performed “on bench” within several minutes. BTA-TRAK is a quantitative test that is performed in a laboratory (Vrooman and Witjes, 2008).

f) Hyaluronic acid and hyaluronidase
Hyaluronic acid (HA) is a glycosaminoglycan and a normal component of tissue matrices and body fluids. In tumour tissues, elevated HA is mostly localised to tumour stroma. In bladder carcinoma HA is found in tumour cells, and elevated HA levels have been shown in urinary samples of bladder cancer patients (Lokeshwar et al., 2005). The concentration of HA is also associated with tumour metastases (Lokeshwar et al., 2001). Hyaluronidase (HA-ase) is an enzyme that
cleaves HA into fragments. HA-ase levels are elevated in bladder tumour tissue, and an increase is correlated with tumour grade (*Vrooman and Witjes, 2008*).

g) **Nuclear matrix protein 22 (NMP22):**

NMP22 is a nuclear matrix protein and is an important regulator of mitosis. In tumour cells the nuclear mitotic apparatus is elevated and NMP22 is released from cells in detectable levels (*Vrooman and Witjes, 2008*).

h) **Cytokeratins:**

Cytokeratins are intermediate filaments; their main function is to enable cells to withstand mechanical stress. In humans 20 different cytokeratin isotypes have been identified. Cytokeratins 8, 18, 19, and 20 have been associated with bladder cancer. The Urinary Bladder Cancer test detects cytokeratin 8 and 18 fragments in urine (*Vrooman and Witjes, 2008*).

Cytokeratin 19 fragment (CYFRA 21-1) is a soluble fragment of cytokeratin 19, is analysed with Enzyme-linked immunosorbent assay (ELISA), and is measurable in serum and urine (*Pariente et al., 2000*).

i) **Survivin:**

Survivin is a member of the family of proteins that regulate cell death, the so-called inhibitor of apoptosis family. Its overexpression inhibits extrinsic and intrinsic pathways of apoptosis (*Moussa et al., 2006*). Survivin is expressed during foetal development but not in terminally differentiated adult tissues (*Dabrowski et al., 2004*). However, it is one of the most commonly overexpressed genes in cancer (*Black et al., 2006*). In bladder cancer, survivin is expressed in urine, and its expression is associated with disease recurrence, stage, progression
and mortality (Shariat et al., 2007). Reverse transcription polymerase chain reaction (RT-PCR) provides a diagnostic tool to detect survivin messenger RNA (mRNA) in urine (Vrooman and Witjes, 2008).

**Table (3):** Sensitivity and specificity with advantages and disadvantages of urinary markers (Vrooman and Witjes, 2008).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH</td>
<td>69–87</td>
<td>89–96</td>
<td>Unaffected by BCG</td>
<td>Labour intensive and expensive</td>
</tr>
<tr>
<td>MSA</td>
<td>72–97</td>
<td>80–100</td>
<td>Also detection of low grade tumour</td>
<td>Complex and expensive</td>
</tr>
<tr>
<td>Immunocy</td>
<td>38.5–100</td>
<td>73–84.2</td>
<td>High interobservervariability</td>
<td></td>
</tr>
<tr>
<td>Telomerase</td>
<td>70–100</td>
<td>60–70</td>
<td>Sensitivity</td>
<td>Influenced by inflammation and age</td>
</tr>
<tr>
<td>BTA-TRAK</td>
<td>24–89</td>
<td>52–93</td>
<td>Influenced by benign genitourinary conditions</td>
<td></td>
</tr>
<tr>
<td>BTA-stat</td>
<td>57–79</td>
<td>48–95</td>
<td>On bench test</td>
<td>Influenced by benign genitourinary conditions</td>
</tr>
<tr>
<td>HA-HA-ase</td>
<td>83–94</td>
<td>77–93.4</td>
<td>Also detection of low grade tumour</td>
<td>Needs further study</td>
</tr>
<tr>
<td>NMP22</td>
<td>49.5–65</td>
<td>40–87.3</td>
<td>Unaffected by BCG and detection of low grade tumour</td>
<td>No clearly defined cut-off value</td>
</tr>
<tr>
<td>CYFRA 21-1</td>
<td>43–79.3</td>
<td>68–84</td>
<td>Influenced by benign genitourinary conditions and instillations</td>
<td></td>
</tr>
<tr>
<td>Survivin</td>
<td>64–94</td>
<td>93–100</td>
<td>Sensitivity and specificity</td>
<td>Needs further study</td>
</tr>
</tbody>
</table>

E. microRNA:

microRNAs are receiving growing attention because of numerous reports on their dysregulation in human diseases and their potential as diagnostic and therapeutic targets. Because of their stability and presence in almost all body fluids, miRNAs constitute a novel class of non-invasive biomarkers (Brase et al., 2010). Some miRNAs have been reported to be up-regulated in bladder cancer tissues. For example, miR-129 was the most commonly upregulated and its up-regulation was associated with poor outcome (Dyrskjøt et al., 2009); the expression of miR-96 and miR-183 in urine were significantly correlated with tumor stage and grade, and their expressions were significantly decreased after radical surgery (Yamada et al., 2011); miR-133b and miR-518c were also strongly up-regulated in bladder cancer tissues (Dyrskjøt et al., 2009).

Meanwhile, some miRNAs were reported to be downregulated in cancer tissues and might function as tumor suppressors. miR-200 family members were lower in urine sediment of bladder cancer patients and increased significantly following surgery which suggested this microRNA family could be used as diagnostic and prognostic markers of bladder cancer (Wang et al., 2012). These aberrant expression miRNAs in bladder cancer are attractive as potential biomarkers and new targets for bladder cancer therapy (Feng et al., 2014).

F. Metastatic work-up

For invasive bladder tumors, metastatic evaluation should include chest radiography, liver function tests, and alkaline phosphatase (Kirkali et al., 2005).

Abdominal and pelvic imaging (MRI or CT) is not accurate for staging of the primary bladder tumor but may be useful when metastatic disease is suspected (Kirkali et al., 2005).
A bone scan is unnecessary in all cases, but it should be performed in the presence of bone pain or elevated alkaline phosphatase (Kirkali et al., 2005).

vii. Treatment:

1. Non-muscle invasive bladder cancer (NMIBC)

   Transurethral resection of bladder tumor (TURBT) is the first-line treatment for patients with NMIBC. Unfortunately, the high rate of recurrence and progression after TURBT necessitates the use of adjuvant treatments (Lerner and Au, 2008). This entails instillation of chemotherapeutic, usually mitomycin-C, or immunotherapeutics agents such as Bacille Calmette – Guérin (BCG), either alone or in various combinations (Cheung et al., 2013).


   Open radical cystectomy (ORC) is the current gold-standard treatment for MIBC and for high-risk recurrent NMIBC. Ideally, all patients with MIBC should receive platinum-based neo-adjuvant chemotherapy (Grossman et al., 2003).

   ORC has a peri-operative complication rate of 25 to 62% (Novotny et al., 2007). Therefore, minimally invasive techniques such as laparoscopic radical cystectomy (LRC) have been explored (Cheung et al., 2013). The advantages of LRC include decreased blood loss, reduced postoperative pain, early return of bowel function and shorter hospital stay (Haber et al., 2008). Furthermore, LRC has good early oncologic outcomes with low morbidity in large cohorts with up to 5 years follow-up (Haber and Gill, 2007).
CHAPTER (II)

MICRORNAS AND OCT4

i. Definition of microRNA(miRNA):

miRNAs are endogenous, non-coding short RNA molecules ~22 nucleotides (nt) long that are considered to posttranscriptionally regulate the cleavage of target mRNAs or just repress their translation (Bartel, 2004).

ii. Discovery of miRNAs:

Lee et al. (1993) discovered the first miRNA lin-4 during a study of developmental timing in Caenorhabditis elegans (C. elegans). About few years later, a second miRNA lethal-7 (let-7) was characterized (Reinhart et al., 2000). These two discoveries of miRNA encouraged further studies on discovery of new miRNAs, and consequently a large class of small non-coding RNAs emerged with a diverse range of biological functions, such as temporal regulation of development, cell death and proliferation, hematopoiesis and tumourigenesis (Kim et al., 2009).

Over 2500 miRNAs have been identified in the human genome, which are thought to regulate more than 30% of the protein-coding genes (Cui et al., 2014).

iii. Nomenclature:

The name for a miRNA consists of the prefix “mir” followed by a dash and a number. The number indicates order of naming. For example, mir-123 was named and discovered prior to mir-456. The uncapitalized (r) in "mir-" refers to immature miRNA, while a capitalized (R) in "miR"
Chapter (II) : MicroRNAs and OCT4

refers to the mature form. miRNAs with nearly identical sequences except for one or two nucleotides are annotated with an additional lower case letter. For example, miR-123a is closely related to miR-123b. Precursor-miRNAs (pre-miRNA) that lead to 100% identical mature miRNAs but that are located at different places in the genome are indicated with an additional dash-number suffix. For example, the pre-miRNAs mir-194-1 and mir-194-2 lead to an identical mature miRNA (miR-194) but are located in different regions of the genome (fig. 4) (Ambros et al., 2003).

Species of origin is designated with a three-letter prefix, e.g., Homo sapien-miR-123 is a human miRNA (hsa-miR-123) and Ovis aries-miR-123 is a sheep miRNA (oar-miR-123). Other common prefixes include 'v' for viral miRNA encoded by a viral genome and 'd' for Drosophila miRNA (for example d-mir-281 in Drosophila). When two mature microRNAs originate from opposite arms of the same pre-miRNA, they are denoted with a -3p (3′ arm) or -5p (5′ arm) suffix. In the past, this distinction was also made with 's' (sense) and 'as' (antisense). When relative expression levels are known, an asterisk following the name indicates a miRNA expressed at low levels relative to the miRNA in the opposite arm of a hairpin, for example, miR-123 and miR-123* would share a pre-miRNA hairpin, but more miR-123 would be found in the cell (Griffiths-Jones et al., 2006).

Fig. (4): Nomenclature of miRNA
(http://blog-biosyn.com/2013/04/11/how-can-microRNAs-be-described/).
iv. miRNA genes:

Initially, it was thought that miRNA genes were located in the intergenic regions, but later studies have shown that most of the mammalian miRNA genes are present in the defined transcription units (TUs) (Rodriguez et al., 2004). On the basis of their location in the annotated TUs, a majority of the miRNA genes can be categorized into three groups: 1) intronic miRNA genes in protein-coding TUs, 2) intronic miRNA genes in noncoding TUs and 3) exonic miRNA genes in noncoding TUs (fig.5) (Kim and Nam, 2006).

The miRNA genes located in the TUs are thought to be cotranscribed with the host genes, whereas the miRNA genes located in intergenic regions have their own promoters (Aravin et al., 2003).

Fig. (5): MicroRNA genomic organization (Wahid et al., 2010).
v. Biogenesis of miRNAs

The biogenesis of miRNAs occurs in a stepwise fashion in the nucleus and the cytoplasm. Mature microRNAs are shortened RNAs that are the result of a series of cleavage processes that begins with a primary microRNA (pri-miRNA) (fig. 6) (Friedman et al., 2009).

**Fig.(6):** MicroRNA maturation and function (Macfarlane and Murphy, 2010).
1. Transcription of primary microRNAs

Most miRNA genes are transcribed by RNA polymerase II to generate a pri-miRNA which can range in size from hundreds of nucleotides to tens of kilobases (Cai et al., 2004).

Pri-miRNA has a hairpin stem of approximately 33 base-pairs (bp), a terminal loop and single-stranded RNA segments at both the 5′ and 3′ sides (fig.7) (Ha and Kim, 2014). Following transcription, the pri-miRNA undergoes several steps of maturation (Yang et al., 2011).

2. Nuclear Processing of primary microRNAs:

Inside the nucleus, pri-miRNA is first processed into a pre-miRNA of about 65 nucleotides long. Pri-miRNA is cleaved by a miRNA microprocessor composed of Drosha and its essential cofactor DiGeorge Syndrome Critical Region 8 (DGCR8). Drosha is a RNase III endonuclease that cleaves the pri-miRNA 11 base-pairs away from the bottom of the hairpin stem where the 5′ and 3′ flanking regions separate (fig.7) (Ha and Kim, 2014).

While Drosha acts as the catalytic subunit of the microprocessor, DGCR8 recognizes the pri-miRNA and also stabilizes its interaction with Drosha (Yeom et al., 2006). In addition, DGCR8 functions as a molecular ruler that allows Drosha to cut at an accurate position forming pre-miRNA (Yang et al., 2011).
Fig. (7): Primary microRNA structure and site of Drosha cleavage

(Ha and Kim, 2014).
3. Export of precursor microRNAs to the cytoplasm:

Following Drosha processing, pre-miRNA transported from the nucleus to the cytoplasm, a process mediated by Exportin-5 protein (Lynam-Lennon et al., 2009). Exportin-5-mediated transport is energy-dependent, using guanisine triphosphate (GTP) bound to Ras-related nuclear protein (Ran)(fig.8) (Murchison and Hannon, 2004).

4. Cytoplasmic processing of precursor microRNA and formation of RNA induced silencing complex (RISC):

The final processing step is facilitated by the RNA III enzyme Dicer and Transactivating response RNA-binding protein (TRBP) which cuts both strands of the pre-miRNA at the base of the stem-loop. This produces a duplex molecule approximately 22 nucleotides long (fig.8) (Lynam-Lennon et al., 2009).

The duplex molecule contains the single-stranded mature miRNA and a fragment termed miRNA*, which is derived from the opposite complementary arm of the pre-miRNA (Schwarz et al., 2003). The miRNA : miRNA* molecule is then incorporated into a large protein effector complex called the RNA induced silencing complex (RISC). The RISC is activated upon unwinding of the miRNA : miRNA* duplex. The miRNA* strand is subsequently degraded, whilst the miRNA molecule guides the RISC complex to the target mRNA. It is the interaction of the miRNA/RISC and its target mRNA that results in gene regulation (Lynam-Lennon et al., 2009).
vi. Mode of silencing:

The interaction between a miRNA and its target mRNA is restricted to the 5′-end of the miRNA. Sequence complementarity between nucleotides 2-8 is vital for target sequence recognition (Lewis et al., 2003). The degree of complementarity between the 3′ - untranslated region (UTR) of the target mRNA and the 5′- end of the miRNA determines the mechanism by which the miRNA regulates the target (fig.9). If the miRNA bears sufficient sequence complementarity (near perfect) to the target mRNA, then regulation is carried out by a process
called RNA interference, in which the RISC complex is directed to cleave the target mRNA (*Hutvagner and Zamore, 2002*).

If there is insufficient complementarity, which is generally the case in mammals (*Lewis et al., 2003*), regulation is achieved by repression of translation (*Lynam-Lennon et al., 2009*). Several studies have provided evidence that translational repression occurs preinitiation of translation (*Wang et al., 2006*). However, other studies suggest that repression occurs post-initiation of translation (*Maroney et al., 2006*).

![Biogenesis and mode of silencing of miRNA](http://www.the-scientist.com/?articles.view/articleNo/14848/title/MicroRNA-Shows-Macro-Potential/)
vii. miRNA turnover:

1. miRNA nucleases:

As compared to miRNA transcription and processing, relatively little is known about the regulation of mature miRNA degradation. In *Arabidopsis thaliana*, miRNAs are protected from degradation by 2′-O-methyl modification of their 3′-terminal ribose mediated by the Hen1 methyl transferase (*Yu et al., 2005*). Unmethylated *Arabidopsis* miRNAs and are subjected to 3′ polyuridylation (*Li et al., 2005*) and degradation by 3′-to-5′ exonucleases of the Small RNA Degrading Nuclease (SDN) family (*Ramachandran and Chen, 2008*). However, in *C. elegans*, the 5′-3′ exoribonuclease 2 (XRN2) degrades mature miRNAs (*Bronevetsky and Ansel, 2013*).

The identity of the enzymes that degrade mature miRNAs in mammals remains unknown. However, at least two ribonucleases have been shown to negatively regulate the expression of mature miRNAs (*Bronevetsky and Ansel, 2013*). Inositol-requiring enzyme1α (IRE1α), an endoplasmic reticulum transmembrane RNase, activated in response to ER stress and subsequently cleaves precursors corresponding to miR-17, miR-34a, miR-96, and miR-125b and mediates rapid decay of their expression in response to sustained cellular stress (*Upton et al., 2012*).

Enhanced RNA interference-1 (Eri-1) is a 3′-to-5′ exoribonuclease with a double-stranded RNA binding domain (*Gabel and Ruvkun, 2008*). In mammals Eri-1 was found to limit miRNA abundance in CD4+ T cells and natural killer cells (*Thomas et al., 2012*).
2. **Target mediated degradation:**

It has been suggested that the interaction between miRNAs and their targets can result in degradation of the miRNA. This is often accompanied by 3′ oligo(U) or (A) tailing and trimming of the miRNA (fig.10) \(\text{(Ameres et al., 2010)}\). In *Drosophila*, extensive complementarity between a target RNA and miRNA triggers miRNA tailing and 3′-to-5′ trimming. Sequence-specific degradation of miRNAs following addition of RNA targets has also been observed in mammalian cells \(\text{(Bronevetsky and Ansel, 2013)}\).

3. **Virus mediated degradation:**

Previous studies have also identified mechanisms by which viruses direct miRNA decay in infected cells. For example, during infection of Jurkat T cells with herpes virus saimiri, miR-27 abundance is dramatically decreased. This decrease is dependent on two virally encoded transcripts that contain several miR-27 binding sites \(\text{(Cazalla et al., 2010)}\). Another example demonstrated that miR-27 is similarly regulated by a transcript encoded by murine cytomegalovirus (MCMV) in infected fibroblasts \(\text{(Libri et al., 2012)}\). Finally, during poxvirus infection of mouse fibroblasts, a virally encoded poly(A) polymerase mediates 3′ polyadenylation of host miRNAs, leading to their degradation (fig.10) \(\text{(Backes et al., 2012)}\).

![Fig. (10): miRNA degradation by highly complementary mRNA targets and viral transcripts \(\text{(Bronevetsky and Ansel, 2013)}\).](image-url)
viii. Biological functions of miRNA:

Several studies have indicated that miRNAs play a pivotal role in most critical biological events, including development, proliferation, differentiation, cell fate determination, apoptosis, signal transduction, organ development, hematopoietic lineage differentiation and host-viral interactions (Bartel, 2009).

1. Cell proliferation and differentiation:

miRNAs have been established as potent controllers of cell proliferation and differentiation (Wang et al., 2007).

A neuron-specific miRNA, miR-132, regulates neuronal growth by decreasing the levels of a GTPase-activating protein (Vo et al., 2005). Another brain specific miRNA, miR-134, is expressed in the synaptodendritic compartment of rat hippocampal neurons, where it is capable of down-regulating Lim domain containing protein kinase-1 (Limk-1), a protein responsible for spine development (Schratt et al., 2006).

Adipose cell differentiation has been shown to be partially controlled by the expression of miR-143 (Esau et al., 2004). Also, miR-1 and miR-133 are important regulators of skeletal muscle proliferation and differentiation (Chen et al., 2006).

2. Apoptosis:

Apoptosis, programmed cell death, is an integral part of animal tissue development. Once apoptosis is activated, caspase proteins cleave both the structural and functional elements of the cell. Therefore, cell death and survival depend largely on the control of active caspases in the cell (Wang et al., 2007). Because caspases are ubiquitous, it makes sense
that miRNAs would play a role in their regulation. Indeed, in the *Drosophila* eye, the absence of miR-14 leads to an increase in the cell death effector, Drosophila interleukin 1 beta-converting enzyme (Drice), suggesting that miR-14 is an inhibitor of apoptosis (*Xu et al.*, 2003). Likewise, the *bantam* gene encodes a miRNA that when over-expressed, suppressed apoptosis in the *Drosophila* retina. One of the targets for *bantam* was identified as the pro-apoptotic gene, *head involution defective* (*hid*), which possesses sequences of complementarity to *bantam* (*Brennecke et al.*, 2003).

It has been known that viruses must prevent apoptosis in order to survive in the host cell. It has been discovered that the herpes simplex virus-1 inhibits apoptosis through a latency-associated miRNA (miR-LAT) that modulates transforming growth factor beta (TGF-β) signaling (*Gupta et al.*, 2006).

3. **MiRNAs and nervous system regulation:**

Previous studies have shown that miRNA is not only required for the development of early embryonic stem cell survival and differentiation, but also plays an important role in maintaining the survival of mature neurons and their function (*Huang et al.*, 2011).

Studies suggest that growth and synaptic plasticity may be regulated by miR-134, which contributes to synaptic development, maturation and plasticity (*Sempere et al.*, 2004).

miR-133b is expressed specifically in midbrain dopaminergic neurons (DNs) and is deficient in midbrain tissue from patients with Parkinson's disease (PD). miR-133b regulates the maturation and function of midbrain DNs within a negative feedback circuit that includes the transcription factor pituitary homeobox 3 (Pitx3) (*Kim et al.*, 2007).
4. miRNAs and immunity:

miRNA control has emerged as a critical regulatory principle in the mammalian immune system. Genetic ablation of the miRNA machinery, as well as loss or deregulation of certain individual miRNAs, severely compromise immune development and can lead to immune disorders like autoimmunity and cancer (Huang et al., 2011).

miR-223 controls the generation and activation of granulocytes; the loss-of miR-223 in mice results in an expanded granulocytic compartment resulting from a cell autonomous increase in the number of granulocyte progenitors. In addition, granulocytes lacking miR-223 are hypermature, hypersensitive to activating stimuli and display increased fungicidal activity. Thus, miR-223 acts as a fine-tuner of granulocyte production and the inflammatory response (Johnnidis et al., 2008).

5. miRNAs and viral infection:

Viruses use miRNAs in their effort to control their host cell; reciprocally, host cells use miRNAs to target essential viral functions. Experimental results have shown that miRNAs are involved in innate immunity and function as gene regulators and as a host cell defense against both RNA and DNA viruses (Williams, 2008).

An anti-viral miRNA, which effectively restricts the accumulation of the retrovirus primate foamy virus type 1 (PFV-1) in human cells, was the first to be reported (Lecellier et al., 2005). In addition, the miRNA-silencing machinery plays a physiological role in controlling human immunodeficiency virus type 1 (HIV-1) replication (Triboulet et al., 2007). Moreover, it was also found that the expression of host cell miR-122 can inhibit the replication of hepatitis C virus (HCV) (Pedersen et al., 2007).
6. miRNAs and angiogenesis:

Wang et al. (2008) found that an endothelial cell-restricted miR-126 mediates developmental angiogenesis in vivo. They also showed that targeted deletion of miR-126 in mice causes leaky vessels, hemorrhage and partial embryonic lethality, due to a loss of vascular integrity and defects in endothelial cell proliferation, migration and angiogenesis. Moreover, the subset of mutant animals that survives displays defective cardiac neovascularization following myocardial infarction. They suggested that the vascular abnormalities of miR-126 mutant mice resemble the consequences of diminished signaling by angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). Accordingly, miR-126 enhances the proangiogenic actions of VEGF and FGF and promotes blood vessel formation by repressing the expression of Spred-1, an intracellular inhibitor of angiogenic signaling. Their results showed that miR-126 mediated integration of hemodynamics and VEGF signal during angiogenesis.

7. miRNAs and mammalian reproduction:

miRNAs regulate physiological processes such as oocyte maturation, luteum development and early embryo development (Huang et al., 2011). A previous study carried out by Tesfaye et al. (2009), revealed the differential expression of 59 miRNAs, of which 31 and 28 miRNAs were found to be expressed preferentially in immature and mature bovine oocytes, respectively.
8. Signal transmission:

Exosomes are small (50–90 nm) vesicles of endocytic origin that are released into the extracellular environment (*Van Niel et al., 2006*). These vesicles can mediate communication between cells. Studies in animals have confirmed that genetic exchange between cells can occur in exosome mediated transfer of miRNAs (*Huang et al., 2011*). Previous study showed that exosomes from a mouse and a human mast cell line (MC/9 and HMC-1, respectively), contain miRNAs that can be delivered to another cell, and can be functional in this new location (*Valadi et al., 2007*).

ix. miRNA and diseases:

1. MicroRNAs and liver diseases:

miRNAs are involved in different kinds of liver diseases, including viral hepatitis, drug-induced injury, non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, primary biliary cirrhosis, liver fibrosis, and hepatocellular carcinoma (HCC) (*Bala et al., 2009*). For example, miR-122, a liver-specific microRNA, has been shown to enhance the replication of hepatitis C virus (*Jopling et al., 2005*). miR-122 is also up-regulated in NAFLD. It may also play a critical role in NAFLD since miR-122 is known to regulate the genes involved in fatty acid biosynthesis and the antagonist of miR-122 decreases the synthesis of hepatic fatty acid and cholesterol (*Jin et al., 2009*).

2. MicroRNAs and cardiovascular diseases:

Some miRNAs have been shown to play roles in ventricular hypertrophy, fibrosis, heart failure, and arrhythmogenesis (*Yang et al., 2007*). For example, the heart-specific microRNAs, miR-1 and miR-133,
have been reported to have critical regulatory roles in cardiac hypertrophy. As the expression level of these two miRNAs are down-regulated in both mouse and human models of cardiac hypertrophy and suppression of miR-133 can induce hypertrophy (Carè et al., 2007).

3. MicroRNAs and lung diseases:

microRNAs also play important roles in many chronic lung diseases, such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), asthma, and idiopathic pulmonary fibrosis (IPF) (Oglesby et al., 2010). For example, miR-21 is up-regulated in human IPF. An enhanced expression of miR-21 promotes Transforming growth factor beta (TGF-β)-induced fibrosis, while the inhibition of miR-21 reduces the severity of fibrosis (Liu et al., 2010).

miR-155 is another microRNA involved in IPF pathogenesis. Expression of miR-155 is up-regulated in the lungs of IPF patients and is correlated with the degree of lung fibrosis in a bleomycin-treated mouse model (Pottier et al., 2009). Expression of miR-29 is reduced in IPF and the bleomycin-treated mouse model. It has been shown that knockdown of miR-29 leads to an increase in genes associated with pulmonary fibrosis, including Integrin, alpha 11, etc. (Cushing et al., 2011).

4. Neurodegenerative diseases:

Notably, previous studies elucidating miRNA functions in neurodegenerative diseases (NDs) have shed new light on disease pathogenesis and may lead to novel treatment strategies. For example, a systemic miRNA profiling in peripheral blood mononuclear cells from Parkinson's disease patients revealed miR-30b, miR-30c, and miR-26a to be associated with the susceptibility of the disease (Martins et al., 2011).
An analysis of miRNA and mRNA expression in brain cortex from Alzheimer’s disease (AD) and age-matched control subjects demonstrated strong correlations between the expression levels of miRNAs and predicted mRNA targets, implying functional relevance of microRNA-mediated regulations in AD pathogenesis (Nunez-Iglesias et al., 2010). More specifically, the expression of miR-29a, miR-29b-1 and miR-9 was significantly decreased in AD patients (Hebert et al., 2008), resulting in abnormally high expression of their target beta-site Amyloid precursor protein-Cleaving Enzyme 1 (BACE1), a protein playing an important role in AD pathogenesis (Willem et al., 2006).

5. Immune-related diseases:

Many common immune-related diseases, including multiple sclerosis (MS), systemic lupus erythematosus (SLE), type I/II diabetes, have shown established correlations with cellular miRNAs. Dozens of miRNA signatures were identified by comparing the miRNA expression profiles of relapsing remitting MS and healthy controls (Keller et al., 2009). Specifically, the expression of miR-145 alone was found to distinguish affected patients from healthy controls with high specificity and sensitivity (Li and Kowdley, 2012). Increased expression of miR-34a, miR-155 and miR-326 was observed in MS lesions (Junker et al., 2009), with additional evidence indicating that high levels of miR-326 had a strong correlation with increased severity of MS (Du et al., 2009).

Decreased expression of miR-146a demonstrated a strong correlation with increased risk for SLE among European populations (Löfgren et al., 2012).

miRNA expression profiling has also identified type 2 diabetes-related miRNAs including miR-144, miR-146a, miR-150 and miR-182.
In addition, miR-103 and miR-107 were shown to negatively regulate glucose homeostasis and insulin sensitivity in type 2 diabetes by targeting caveolin-1, a critical regulator of insulin receptor (Trajkovski et al., 2011).

6. Cancers:

Since the early stages of miRNA research, cancer has been the most prominent of human diseases with a clear role for miRNA regulation. The first evidence came from a study by Calin et al. (2002) in which they demonstrated a frequent deletion of miRNA genes miR-15 and miR-16 among 65% of B-cell chronic lymphocytic leukemia (B-CLL) patients.

Subsequent expression profiling studies further demonstrated the correlation between aberrant miRNA expression patterns and increased occurrence of different types of cancers. Notably, the deregulation of miR-125b, miR-145, miR-21, and miR-155 expression was associated with the increased risk of breast cancer (Iorio et al., 2005). In addition, up-regulation of miR-155 and down-regulation of let-7a were correlated with poor survival of lung cancer patients (Yanaihara et al., 2006). Interestingly, miRNA expression patterns were also able to stage cancer progression (Lu et al., 2005), indicating that miRNA levels were not only useful in diagnosis but also potentially in prognosis of diseases (Li and Kowdley, 2012).

These cancer-related miRNAs were categorized into tumor suppressors and oncogenes due to their associations with opposite clinical outcomes with altered expressions (fig.11). For example, miR-15, miR-16 and let-7are known tumor suppressors while miR-21 and miR-155 serve as oncogenes (Li and Kowdley, 2012). miR-15 and miR-
16 were found to repress the expression of anti-apoptotic gene *B-cell lymphoma 2* (*Bcl-2*) thereby promoting cell death in cancerous cells (*Cimmino et al., 2005*). Likewise, let-7 family members demonstrate anti-cancer properties due to their ability to repress the expression of the oncogene, *rat sarcoma* (*ras*) (*Johnson et al., 2005*). In contrast, miR-21 directly serves as an anti-apoptotic factor in glioblastomas (*Chan et al., 2005*). Similarly, miR-155 interferes with the process of mismatch repair by repressing the expression of the *mismatch repair* (*MSH*) gene family members in colorectal cancer (*Valeri et al., 2010*).

miRNAs also play key roles in tumor invasion and metastasis. miRNA expression profiling revealed the stepwise down-regulation of miR-145 levels with progression of primary gastric cancers and secondary metastases (*Gao et al., 2013*), as well as metastatic prostate cancer (*Peng et al., 2011*). Similarly, increased expression of miR-210 was observed during the invasive transition of breast cancer (*Volinia et al., 2012*).

While profiling studies establish disease correlations, mechanistic studies characterize the role of miRNAs in greater detail. For example, through the use of synthetic miRNA mimics, miR-7 and miR-29b were shown to suppress the metastasis of liver cancer by targeting phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta isoform (*PIK3CD*) (*Fang et al., 2012*) and matrix metalloproteinase-2 (*MMP-2*) (*Fang et al., 2011*), respectively. These cancer-related miRNAs are potentially useful for developing not only early diagnosis, but also novel anti-cancer strategies (*Li and Kowdley, 2012*).
Fig. (11): MicroRNAs as oncogenes or tumour suppressor genes (Ahmad et al., 2013).
miR-145

i. miR-145 identification:

miR-145 was first identified in mice from heart tissue using small RNA cloning techniques (Lagos-Quintana et al., 2001), and then later reported in human (Michael et al., 2003). Human miR-145 (hsa- miR-145) is enriched in germline and mesoderm-derived tissues, such as uterus, ovary, testis, prostate, spleen, and heart (Lakshmipathy et al., 2007).

ii. miR-145 gene:

Slightly over half (52.5%) of miRNA genes are located in cancer associated regions or fragile sites in the genome. Consistently, miR-145 is located on chromosome 5 (5q32-33), a well-known fragile site in the human genome, and is suggested to be co-transcribed with miR-143 (Cui et al., 2014).

iii. miR-145 regulation

Transcriptional and posttranscriptional regulation could play an important role in miR-145 expression (Sachdeva and Mo, 2010). Previous reports suggest that miR-145 can be induced transcriptionally by p53 (Sachdeva et al., 2009). Interestingly, it has been suggested that there is positive feedback regulation between miR-145 and p53 (Cui et al., 2014).

Another report showed a novel mechanism of posttranscriptional regulation of miR-145 via p53-mediated RNA processing. p53 interacts with Drosha processing complex through association with p68 and
facilitates the processing of primary transcript of miR-145 (Suzuki et al., 2009).

iv. Role of miR-145:

1. miR-145 in stem cells and differentiation:

   It has been reported that miR-145 is induced during stem cell differentiation, and it directly silences the stem cell self renewal and pluripotency program. Ectopic expression of miR-145 in human embryonic stem cells blocks self-renewal while blockade of miR-145 with antisense oligonucleotides increases the self-renewal (Xu et al., 2009).

2. miR-145 in the vascular smooth muscle cells (VSMCs)

   miR-145 is the most enriched miRNA in arteries and its expression is significantly downregulated in vascular walls with neointimal lesions formation (Cheng et al., 2009). Moreover, miR-145 is cardiac-specific and smooth-muscle specific miRNA (Cordes et al., 2009).

   The role of miR-145 in differentiation of VSMC has been investigated. Cordes et al. (2009), found that miR-145 is necessary and sufficient to induce differentiation of multipotent neural crest stem cells into VSMCs. They demonstrated that miR-145 can direct smooth muscle fate by regulating multiple transcription factors including Kruppel-like factor 4 (KLF4) and myocardin, thereby repressing the proliferation of smooth muscle cells and promoting its differentiation. Based on this observation, they suggested the possible role of miR-145 in suppressing smooth muscle hyperplasia observed in vascular injuries and atherosclerosis.
Another line of evidence regarding the expression of miR-145 in smooth muscle cells comes from a study carried out by Elia et al. (2009). The study suggested that miR-143/145 cluster is expressed mostly in the smooth muscle cell (SMC) compartment in vessels and SMC-containing organs during development and postnatally and the loss of this cluster induces structural modifications of the aorta, because of an incomplete differentiation of VSMCs.

3. miR-145 in tumors:

Down-regulation of miR-145 has been observed in many types of cancers, suggesting that it may serve as a tumor suppressor. So far, miR-145 has been shown to be involved in regulating various cellular processes, such as the cell cycle, proliferation, apoptosis and invasion, by targeting multiple oncogenes. Moreover, reduced expression of miR-145 is associated with a worse prognosis for many cancers, indicating that it may serve as a potential cancer biomarker and an attractive target for cancer therapy (Cui et al., 2014).

A. miR-145 as a tumor growth inhibitor:

The tumor-suppressive function of miR-145 is related to its regulation of cell proliferation (Cui et al., 2014). The first report describing tumor growth inhibition by miR-145 was from a study in colon cancer cells, where miR-145 suppressed tumor growth by targeting insulin receptor substrate-1 (IRS-1) (Shi et al., 2007). IRS-1 is also involved in miR-145 ability to suppress cell proliferation, and cell motility in gastric cancer and hepatocellular carcinoma (Xing et al., 2013). Interestingly, the insulin like growth factor I receptor (IGF-IR) is also a predominant target of miR-145, by which miR-145 suppresses the tumor growth of colorectal cancer (La Rocca et al., 2009) and
hepatocellular carcinoma (Law et al., 2012). These findings suggested that the IGR/IRS1 pathway plays an important role in miR-145-mediated anti-proliferative function (Cui et al., 2014).

Sachdeva et al. (2009) revealed that miR-145 causes cell cycle arrest at Gap phase (G0-G1 phase) and a decrease in synthesis phase (S phase), partially through silencing the expression of Avian myelocytomatosis viral oncogene homolog (c-Myc). Consistent with these results, the miR-145-induced G1/S-phase arrest is mediated by targeting c-Myc in non-small cell lung cancer cell (Chen et al., 2010).

It has been suggested that miR-145 regulates certain oncogenic transcription factors to suppress cell growth. For example, E26 transformation-specific (ETS) transcriptional factors are well-known proto-oncogenes with mitogenic and transforming activity (Seth and Watson, 2005). Some members of the ETS family were identified as targets of miR-145, and are involved in miR-145-mediated apoptosis and repression of cell proliferation (Cui et al., 2014).

B. Role of miR-145 in cell invasion and metastasis:

Like many other miRNAs, miR-145 can also impact cell migration and invasion (Sachdeva and Mo, 2010). miR-145 was observed to be differentially expressed in node-negative patients or node-positive gastric cancer patients (Tchernitsa et al., 2010).

Mucin 1 (MUC1) is considered as an important metastasis gene. miR-145 suppresses cell invasion and lung metastasis of breast cancer by silencing MUC1 (Sachdeva and Mo, 2010).
Several researches also described the relationship between miR-145 and some adhesion or cytoskeleton molecules. For example, the oncogenic *fascin actin-bundling protein 1 (FSCN1)* is a candidate target gene of miR-145. miR-145 participates in the modulation of proliferation and invasion by targeting FSCN1 in various cancers, including oesophageal squamous cell carcinoma (*Kano et al., 2010*), prostate cancer (*Fuse et al., 2011*) and breast cancer cells (*Götte et al., 2010*).

miR-145 targets N-cadherin, a calcium-dependent cell adhesion molecule associated with an increased invasive potential, suppressing invasion and metastasis, rather than inhibiting cell proliferation in gastric cancer (*Gao et al., 2013*).

miRNA-145 also targets Metalloproteinase 17 (ADAM 17), an important member of the 'A disintegrin and metalloprotease' (ADAM) family involved in cancer invasion (*Mochizuki and Okada, 2007*).

miR-145 is specifically expressed in pericytes in microvasculature and it inhibits migration of microvascular cells in response to growth factor gradients by directly targeting friend leukemia integration 1 (Fli-1) transcription factor (*Larsson et al., 2009*).

**C. Role of miR-145 in angiogenesis:**

Angiogenesis is a process by which new blood vessels sprout from existing vasculature. It is vital for tumor growth and metastasis, because cancer cells cannot grow beyond 2 mm³ without vascular support (*Holmgren et al., 1995*). miR-145 exhibits tumor suppressive functions by modulating angiogenesis. Vascular endothelial growth factor (VEGF), one of the strongest angiogenic factors, is a direct target of miR-145, regulating the invasion and metastasis of osteosarcoma cells, which
implies a role of miR-145 in modulating tumor angiogenesis (Fan et al., 2012). In addition, miR-145 decreases hypoxia-inducible factor 1 α (HIF-1 α) expression, a major transcriptional regulator of VEGF in response to hypoxia, as well as decreasing VEGF expression by targeting p70 ribosomal protein S6 kinase 1 (p70S6K1) in colorectal cancer, leading to the inhibition of tumor growth and angiogenesis (Xu et al., 2012).

v. miR-145 as a biomarker

1. miR-145 as a biomarker in diseases

Of considerable interest, miR-145 may serve a biomarker for several disorders. For example, in blood samples miR-145 level was found highly deregulated in polycythemia Vera (Bruchova et al., 2008) and multiple sclerosis (MS). Moreover, miR-145 is the best single microRNA marker that allows discriminating MS from controls with a specificity of 89.5%, a sensitivity of 90.0%, and an accuracy of 89.7% from blood (Keller et al., 2009).

2. miR-145 as a biomarker in tumors:

Specific miRNAs have been found to be differentially expressed in the majority of tumor cases; therefore, miRNA expression patterns are capable of distinguishing between malignant and non-malignant tissue (Cui et al., 2014). miR-145 is differentially expressed in breast cancers and normal breast tissues, and its down-regulation is closely associated with invasive breast cancer pathobiological features (Iorio et al., 2005).

Furthermore, as a non-invasive, blood-based diagnostic tool, cell free miRNAs have received much interest in recent years (Cui et al., 2014).
Serum miR-145 has a distinct level in cancer patients compared with healthy ones, suggesting that detection of serum miR-145 has potential as a novel method for early cancer diagnosis (Chung et al., 2013). Moreover, recent evidence has revealed that a combination of circulating miRNAs biomarkers show better sensitivity and specificity for cancer diagnosis (Cui et al., 2014). For example, in two independent studies, a combination of plasma markers miR-145 and miR-451 (Ng et al., 2013), or a combination of miR-145, miR-155 and miR-382, were suggested to increase the sensitivity and specificity for discriminating breast cancer from healthy controls (Mar-Aguilar et al., 2013). Likewise, circulating miR-145 combined with three other circulating miRNAs (miR-20a, miR-21 and miR-221) significantly identified aggressive prostate cancer patients (Shen et al., 2012).

vi. miR-145 in cancer therapy

The observation of decreased levels of tumor-suppressive miRNAs in cancers has led to the concept of miRNA replacement therapy (Cui et al., 2014). Encouraging results have been achieved in several animal models (Takeshita et al., 2010). A systemic or local application of a polyethylenimine-mediated delivery of unmodified miR-145 in a mouse model of colon carcinoma showed a decrease in the tumor growth (Ibrahim et al., 2011). Moreover, delivery of miR-145 mimics by mesenchymal stem cells significantly decreased the migration of glioma cells and the self-renewal of germline stem cells (Lee et al., 2013).

On the other hand, miR-145 can influence the sensitivity of tumors to chemo- or radiation therapy, and a combination of miR-145 and chemo- or radiation therapy represents a novel antitumor strategy.
Treatment with miR-145 inhibited gastric cancer cell growth and increased its sensitivity to 5-fluorouracil (5-FU) \cite{Takagi et al., 2009}. Similarly, an adenoviral constructed miR-145 was injected into breast cancer orthotopic mouse models intratumorally, resulting in significant suppression of tumor growth. Furthermore, a treatment combining adenoviral miR-145 and 5-FU produced enhanced retardation of tumor growth, compared with treatment with either drug alone \cite{Kim et al., 2011}.
OCT4

i. Introduction

Tumor recurrence and multifocality are two common features of bladder tumors. Several previous reports suggest that these tumors are derived from a primary transformed progenitor cell (Hatefi et al., 2012). Based on Cancer Stem Cell (CSC) model, CSCs are characterized by self-renewal, heterogeneity (potential for multidirectional differentiation), resistance to apoptosis, and resistance to conventional therapies. CSCs have been isolated from a variety of solid tumors such as breast cancer, lung, prostate, colon tumors (Al-Hajj and Clarke, 2004) and bladder cancer (Tran et al., 2010). The most important members of CSCs' regulatory core are transcription factors such as Octamer-binding transcription factor4 (Oct4), Sex determining region Y-box 2(Sox2), and Nanog, which are defined as key players in the regulatory network for maintaining the “stemness” state of stem cells (Hatefi et al., 2012).

ii. Oct4 definition:

Octamer-binding transcription factor4 (Oct4) also known as Oct3, Oct3/4 and POU5f1 (POUdomain, class5, transcription factor1) (Atlasi et al., 2007). Oct4 belongs to POU (Pit-Oct-Unc) family of DNA binding proteins. These proteins control the expression of their target genes by binding to the octamer motif ATGCAAAT within their promoter or enhancer regions (Zeineddine et al., 2014).

Oct4 is a transcription factor that is required for pluripotency during early embryogenesis and the maintenance of embryonic stem cell (ESC) and pluripotent cell identity. Misexpression of Oct4 is
correlated with tumorigenesis and can affect the behavior of tumors such as recurrence or resistance to therapy (Hatefi et al., 2012).

**iii. Oct4 gene:**

Oct4 encoded by *Pou5f1* gene which maps to chromosome number 6 in humans and chromosome 17 in mouse. It is separated into 5 exons. Oct4 upstream region consists of a proximal promoter upstream of transcription start site, proximal and distal enhancers. All three regions are important regulatory elements during embryonic development (Ashok and Reddy, 2009).

**iv. Oct4 structure:**

The Oct4 protein comprises three domains: a central POU (Pit-Oct-Unc) domain, an N-terminal domain (N domain) and a C-terminal domain (C domain) (fig.12) (Shi and Jin, 2010).

POU domain consists of two structurally independent subdomains: a 75 amino acid amino-terminal POU specific (POUs) region and a 60 amino-acid carboxyl-terminal homeodomain (POUh). Both domains make specific contact with DNA through a helix-turn-helix structure and are connected by a variable linker of 15 to 56 amino-acids. Regions outside the POU domain are not critical for DNA binding and exhibit little sequence conservation (Pan et al., 2002).

The N-terminal domain is rich in proline and acidic residues, while the C-terminal domain is rich in proline, serine and threonine residues. Both domains play a role in transactivation. It was subsequently demonstrated that the activity of C domain is cell type specific and is regulated through phosphorylation, whereas the N domain is not (Pan et al., 2002).
In humans, *Pou5f1* encodes 2 isoforms that are generated by alternative splicing of Oct4 mRNA. These isoforms, Oct4-IA and Oct4-IB (360 and 265 amino acids, respectively), of which 225 amino acids at the C-termini are identical, differ in sequence at their N termini. Critical amounts of human Oct4-IA are required to sustain stem cell self-renewal and it has been shown that Oct4-IB is not related to stemness (Zeineddine et al., 2014).

v. Oct4 function:

Oct4 is expressed in both mouse and human ESCs and primordial germ cells (PGCs) and its expression rapidly decreases upon differentiation (fig. 13) Oct4 expression is necessary for the maintenance of pluripotentiality in ESC and PGC (Cheng et al., 2007).

1. Embryonic development:

Oct4 is essential for early embryonic development. The fundamental role of Oct4 expression is the preservation of pluripotent cells in the inner cell mass (ICM) of blastocysts (fig. 13) (Cheng et al., 2007). In the absence of Oct4, embryos die at the time of implantation because of a lack of pluripotent ICM cells (Shi and Jin, 2010). Interestingly, the precise expression level of Oct4 is a critical determinant of ESC fates, and their pluripotent potential can be sustained only when the Oct4 expression level is maintained within a normal range (Zafarana et al., 2009). Reducing Oct4 expression by one-half induces
ESCs to differentiate into trophoblasts, while less than two fold over-expression of Oct4 triggers ESC differentiation into primitive endoderm and mesoderm (*Niwa et al., 2000*).

2. Germ cell viability

In addition to its role in the maintenance of pluripotentiality, Oct4 may also play a role in maintenance of viability of the mammalian germline, functioning as a ‘stem cell survival’ factor (*Cheng et al., 2007*). Primordial germ cells lacking Oct4 expression have been shown to undergo apoptosis rather than differentiation (*Kehler et al., 2004*).

![Embryonic stem cells and Oct4 expression](Pan et al., 2002)

**vi. Regulation of Oct4 expression**

Precise control of Oct4 expression is essential for the maintenance and the re-establishment of pluripotency. Oct4 expression is controlled at multiple levels (*Shi and Jin, 2010*).
1. Epigenetic regulation of Oct4:

Epigenetics is a phenomenon in which modification of genes takes place without changing gene sequence. It includes modifications such as addition of methyl (− CH3) groups. DNA methylation and histone modification (acetylation, methylation and ubiquitination) help to either activate or repress genes depending on the stage of development (Ashok and Reddy, 2009).

In order to maintain pluripotential state of ESCs, Oct4 gene must remain in an unmethylated state. This is because methylation of Oct4 gene compromises its ability to carry out efficient transcription. Oct4 gene is unmethylated in the blastula stage but undergoes de novo methylation and remains modified in all adult somatic tissues (Ashok and Reddy, 2009).

2. Oct4 regulation at transcriptional level:

At the transcriptional level, direct binding of transacting regulators to the Oct4 locus also plays a critical role in the modulation of Oct4 expression. Several members of the orphan nuclear receptor family have been found to participate in the control of Oct4 expression (Shi and Jin, 2010).

Orphan nuclear receptor Steroidogenic factor-1 (SF-1) and orphan nuclear receptor estrogen-related receptor b (Esrrb) were shown to directly activate the transcription of human Oct4 and mouse Oct4, respectively, to sustain pluripotency in ESCs (Zhang et al., 2008).

 Additionally, germ cell nuclear factor (GCNF), an orphan nuclear receptor, has been found to repress Oct4 gene activity by specifically binding within the proximal promoter. GCNF is critical for repressing
Oct4 gene activity as pluripotent stem cells differentiate (Fuhrmann et al., 2001).

Interestingly, the orphan nuclear receptor testicular receptor 2 (Tr2) alternates between being an activator and a repressor of Oct4 expression depending on its phosphorylation, sumoylation and associated coregulators (Gupta et al., 2008).

3. Oct4 regulation at post-transcriptional level:

miRNAs play important roles in the post-transcriptional regulation of Oct4 expression. Xu et al. (2009), reported that miR-145 directly repressed the 3’ untranslated region of Oct4 mRNA during human ESC differentiation.

Although miRNAs usually target the 3’ untranslated regions of mRNAs, miR-296, miR-470 and miR-134 were found to be upregulated in differentiated mouse ESCs and to target the coding sequences of Oct4, Nanog and Sox2 in various combinations (Tay et al., 2008).

In addition to these negative regulators of Oct4 during ESC differentiation, Qiu et al. (2010), found that Lin28, a repressor of miRNA processing, bound Oct4 mRNA directly within its coding region and recruited RNA helicase A to promote the translation of Oct4 in human ESCs.

4. Oct4 regulation at post-translational level:

The stability and activity of Oct4 proteins are subject to modifications at the post-translational level (Shi and Jin, 2010).
A. Phosphorylation:

An early study suggested that the differential phosphorylation of Oct4 might affect the transactivation ability of the Oct4 carboxyl-terminal transactivation domain (Brehm et al., 1997).

B. Ubiquitination:

Moreover, ubiquitination is a common modification of proteins where Ubiquitin (a small regulatory protein) is attached to substrate proteins and act as a signal for their degradation (Schnell and Hicke, 2003). A previous study indicated that Ww domain-containing protein 2 (WwP2) plays an important role in Oct4 ubiquitination and degradation during the differentiation of embryonic carcinoma cells (Liao and Jin, 2010).

C. Sumoylation:

In addition to ubiquitination, Oct4 is a target of small ubiquitin-related modifier-1 (SUMO-1) modification (Wei et al., 2007). SUMO is an ubiquitin-like protein that is covalently attached to a variety of target proteins. Unlike ubiquitination, sumoylation does not target proteins for proteolytic breakdown, but is instead involved in regulating protein functional properties including protein stability, protein activity or protein-protein interactions (Park-Sarge and Sarge, 2009).

Wei et al. (2007), found that the sumoylation resulted in increased stability, DNA binding and transactivation of Oct4.

vii. Oct4 target genes:

Oct4 mainly functions through the activation of pluripotency associated and self-renewal-associated genes, while simultaneously repressing genes that promote differentiation (Shi and Jin, 2010).
Oct4 maintains the self-renewal and pluripotency of ESCs in coordination with other pluripotency factors and coregulators. Among the partners of Oct4, Sox2 is the best characterized. Oct4 and Sox2 synergistically activate the expression of target genes which play important roles in maintaining pluripotency (Shi and Jin, 2010).

Reduced expression 1 (Rex1) gene is one of Oct4 targets. Rex1 encodes a protein that is functionally important in the reacquisition and maintenance of pluripotency and is widely used pluripotency marker (Son et al., 2013). Interestingly, Oct4 activates or represses Rex-1 promoter depending on the Oct4 expression level. At both low and high levels Oct4 represses Rex1 expression but at intermediate levels it activates Rex1 expression (Shi and Jin, 2010).

In addition to activating pluripotency factors, Oct4 also prevents the differentiation of pluripotent cells by acting as a repressor of lineage-specific transcription factors (Shi and Jin, 2010). Oct4 forms a repressive complex with and inhibits the transcription of Caudal type homeobox 2 (Cdx2), a transcription factor essential for trophectoderm specification (Niwa et al., 2005).

In addition to protein coding genes, noncoding RNA targets of Oct4 have been found. For example, Oct4/Sox2 positively regulates miR-302, a cluster of eight miRNAs expressed specifically in ESCs (Card et al., 2008).

**viii. Oct4 and cancer:**

Phenotypically, human preimplantation embryonic cells resemble cancer cells in many ways, especially in their ability to grow indefinitely. Both types of cells undergo deprogramming to a proliferating state and
become immortal, self-renewing, and invasive. These similarities suggest that some embryonic genes may be re-expressed or re-activated in cancer cells (Pan et al., 2002). An early study reported that Oct4 and three other novel embryonic genes are expressed in human tumors but not in normal somatic tissues, in agreement with the hypothesis that embryonic genes are re-activated in tumor cells (Monk and Holding, 2001).

Indeed, Gidekel et al. (2003) found that the level of Oct4 expression dictated the oncogenic potential of ESCs in a dose-dependent manner. Moreover, the expression of Oct4 has already been reported in germ cell tumors, a small number human kidney and lung cancer samples (Looijenga et al., 2003), human breast cancer (Ezeh et al., 2005), osteosarcoma biopsies (Gibbs et al., 2005) and bladder cancer (Atlasi et al. 2007).

Thus, it has been believed that over-expression of Oct4, Sox2 and Nanog, together or separately, led to tumorigenicity, tumor metastasis, and even distant recurrence after chemoradiotherapy in different types of cancer (Zeineddine et al., 2014).
microRNAs are receiving growing attention because of numerous reports on their dysregulation in human diseases and their potential as diagnostic and therapeutic targets. Because of their stability and presence in almost all body fluids, miRNAs constitute a novel class of non-invasive biomarkers (Brase et al., 2010).

miRNA have emerged as integral components of nearly every biological process, including cell proliferation, migration, differentiation, apoptosis and angiogenesis (Cui et al., 2014). Moreover, miRNAs have been related to carcinogenesis through regulating the expression of target genes, including transcription factors, oncogenes and tumor suppressor genes (Dip et al., 2013).

The association between miRNAs and human cancers was first recognized when miRNA genes were found to be specifically deleted in patients with leukaemia (Calin et al., 2002). Following this initial discovery, many studies have demonstrated that overexpressed miRNAs can act as oncogenes by repressing tumour suppressor genes, and underexpressed miRNAs can function as tumour suppressors by negatively regulating oncogenes (Mavrakis et al., 2011).

Furthermore, studies have shown that some miRNAs control the activity of major cancer-related signalling molecules such as p53 family proteins (Ory and Ellisen, 2011), retinoblastoma protein (Noonan et al., 2010), and epidermal growth factor receptor (EGFR) (Erkan et al., 2011). Interestingly, expression levels of some miRNAs have been found to be
associated with recurrence or metastasis and prognosis of cancers (Nair et al., 2012).

i. miR-145 and bladder cancer:

The first report of altered miRNA expression profiles in bladder cancer was reported by Gottardo et al. (2007). They identified 10 miRNAs that were significantly upregulated in bladder cancer compared with normal bladder tissue. Dyrskjøt et al. (2009) found that miR-133b and miR-518c were strongly up-regulated in bladder cancer tissues. They also reported that miR-129 was the most commonly upregulated and its up-regulation was associated with poor outcome.

Meanwhile, some miRNAs were reported to be down-regulated in cancer tissues and might function as tumor suppressors. Down-regulated miRNAs in bladder cancer was first reported by Lin et al. (2009) who found 38 down-regulated miRNAs using hybridization-based miRNA array, four of which (miR-143, miR-145, miR-125b, and miR-199b) were found to be significantly down-regulated on subsequent quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of the same samples.

The increasing number of studies investigating miRNA expression profiles specific to bladder cancer indicate the growing interest in searching for specific miRNAs to function as diagnostic and/or prognostic biomarkers (Yoshino et al., 2011).

1. Down-regulation of miR-145 in bladder cancer:

Song et al. (2010) reported that the expression profile of miRNAs was significantly different between bladder urothelial carcinoma tissue and adjacent normal bladder tissue using microarray technology. The
microarray data were validated by real-time RT-PCR analysis for 10 miRNAs, mir-1, mir-145, mir-143, mir-100, mir-200b, mir-708, mir-133a, mir-133b, mir-125b, and mir-99. Their study showed that miR-1, miR-133a mir-133b, miR-143 and miR-145 were down-regulated in bladder urothelial carcinoma suggesting that these miRNAs may contribute to tumorigenesis and might be serve as biomarkers for tumor.

Among the miRNA expression profiles of bladder cancer that have been published, miR-145 is the miRNA most frequently reported to be down-regulated (Yoshino et al., 2013). miR-145 expression was analysed by real-time RT-PCR in bladder cancer tissues and noncancerous bladder tissue specimens and was found to be significantly lower in bladder cancer tissue (Ichimi et al., 2009).

Furthermore, Ostenfeld et al. (2010) performed in situ hybridisation (ISH) analysis and showed staining of miR-145 in the normal urothelium but weak expression in tumor samples, suggesting actual differences in miR-145 expression between normal and transformed urothelial cells.

Moreover, the miR-145 level in urine was able to distinguish bladder cancer patients from non-cancer controls (77.8% and 61.1% specificity) for non-muscle invasive bladder cancer, and (84.1% sensitivity and 61.1% specificity for muscle invasive bladder cancer (Yun et al., 2012).
2. Tumor suppressive role of miR-145 in bladder cancer:

Previous studies showed that miR-145 was commonly down-regulated in several human cancers and that their transfection could suppress cancer cell tumorigenicity. miR-145 has been demonstrated to inhibit cell proliferation and migration in miR-145-transfected bladder cancer cell lines through direct regulation of fascin actin-bundling protein 1 (FSCN1) gene (Chiyomaru et al., 2010). The protein product of this gene is required to form protrusions of the cellular membrane and cytoplasmic movements related to migration. In malignant neoplasms, FSCN1 activity has been correlated to high-grade disease, extensive invasion, metastasis and poor prognosis (Vignjevic et al., 2006). Chiyomaru et al. (2010) concluded that there is an association between FSCN1 oncogene over-expression due to miR-145 under-expression in BC, which leads to a more aggressive phenotype.

Another study demonstrated that, ectopic expression of miR-145 in bladder cancer cells induced interferon (IFN)-beta mediated apoptosis by targeting the suppressor of cytokine signalling 7 (Socs7) (Noguchi et al., 2013).

Ostenfeld et al. (2010) also reported that miR-145 induced caspase-dependent and - independent cell death in human urothelial cancer cells by targeting Clathrin Interactor 1 (CLINT1), core-binding factor b subunit (CBFB) and protein phosphatase 3 catalytic subunit α isoform (PPP3CA).

ii. Oct4 and bladder cancer:

According to the new Cancer Stem Cell (CSC) concept, only a small subset of cancer cells are thought to be tumorigenic and capable of
self-renewing to generate additional CSCs. Based on this school of thought, CSCs have high self-renewal capacity and can be generated via dysregulation of self-renewal process in normal stem or early progenitor cells (Atlasi et al., 2007).

It has been proposed that Oct4 acts as a multifunctional factor in cancer and stem cell biology (Atlasi et al., 2007). Based on the reports that Oct4 increases the malignant potential of ESC in a dose dependent manner (Gidekel et al., 2003), a possible oncogenic role was also attributed to Oct4. On the other hand, ectopic expression of Oct4 in epithelial tissues could lead to a dysplastic induction through inhibition of epithelial stem or progenitor cell differentiation (Hochedlinger et al., 2005).

The expression of Oct4 has already been reported in germ cell tumors, a small number human kidney and lung cancer samples (Looijenga et al., 2003), human breast cancer (Ezeh et al., 2005) and osteosarcoma biopsies (Gibbs et al., 2005).

Atlasi et al. (2007) demonstrated, for the first time, that Oct4 is highly expressed in bladder tumors. They found that compared with adjacent apparently non tumor samples, bladder cancer samples had higher expression of the transcription factor Oct4 at the mRNA and protein levels.

Furthermore, Chang et al. (2008) showed that Oct4 is highly expressed in bladder cancer. Most importantly, they found that higher Oct4 expression correlates with tumor progression and metastasis, as well as contributes to patient mortality.
Moreover, a previous study carried out by Zhu et al. (2009) detected the expression of Oct4 mRNA and protein in bladder cancer cell line T24. Zhu et al. (2009) also explored the role of Oct4 in bladder cancer showing that Oct4 gene might play a role in the proliferation of T24 bladder cancer cells.

iii. miR-145 and Oct4:

Indeed, numerous lines of evidence implicate the miRNA family of posttranscriptional regulators as central players in the maintenance of normal ESC function (Chivukula and Mendell, 2009). miRNAs play important roles in the post-transcriptional regulation of Oct4 expression (Shi and Jin, 2010). Xu et al. (2009) reported that miR-145 directly repressed the 3′ UTR of Oct4 mRNA during human ESC differentiation. They demonstrated that increasing miR-145 expression represses expression of pluripotency genes including Oct4, SOX2, and KLF4. This leads to induction of lineage-restricted differentiation and inhibition of human ESC self renewal. Furthermore, they found a reciprocal negative regulation between miR-145 and Oct4 (fig.14,15).

Given the role of miR-145 in silencing pluripotency during embryonic stem cell differentiation, it is not surprising that miR-145 also inhibits the proliferation of CSCs and induces differentiation, contributing to suppression of tumor growth, migration (Cui et al., 2014). Huang et al. (2012) showed that forced expression of miR-145 suppressed tumour formation and expression of CSC markers and ‘stemness’ factors, including CD133, CD44, Oct4, c-Myc and Klf4 in prostate cancer cells, and inhibited tumourigenesis of prostate cancer.
Chapter (III): Relationship between miR-145, Oct4 and bladder cancer

Fig. (14): MicroRNA-Regulated Stem Cell (Chivukula and Mendell, 2009).

Fig. (15): Model of a regulatory loop between Oct4 and miR-145 (Stuwe et al., 2014).
SUBJECTS AND METHODS

This study was carried out between July 2013 and July 2014 after approval of the study scheme by the research ethical committee of Benha Faculty of Medicine and obtaining informed consent from included patients.

The study included 50 subjects of both sexes selected from Urology Department, Faculty of Medicine, Benha University Hospital. Their ages ranged from 49-83 years (y) with mean value 65.3 ± 8.4 y.

The subjects were categorized into 2 groups:

A. Malignant lesion group: included 35 patients, they were diagnosed as bladder cancer patients by clinical, radiological, cystoscopic and histopathological examinations.

B. Control group: Comprised 15 persons, age and sex matched, with histopathologically normal urothelium. Tissue biopsies were obtained during endoscopic removal of renal or ureteric stones with diagnostic cystoscopy.

Exclusion criteria:

Patient with previous history of receiving chemotherapy or radiotherapy.

All patients were subjected to:

1. Full history taking with attention to:
   a) Special habits including, tobacco smoking.
   b) Urological symptoms including, dysuria, frequency and hematuria.
2. General and local urological examinations specially P/R.
3. Radiological investigations including:
   - Abdominopelvic plain X-ray.
   - Abdominopelvic ultrasonography to detect stone or mass in the urinary bladder.
   - Abdominopelvic CT for detection and staging of urinary bladder cancer.
4. Routine preoperative laboratory investigations including:
   - Urine analysis.
   - Complete Blood Count (CBC).
   - Fasting blood sugar (Trinder, 1969).
   - Liver function tests: serum albumin, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Reitman and Frankel, 1957).
   - Kidney function tests: serum urea (Tobacco et al., 1979) and serum creatinine (Bowers and Wong, 1980).
   - Coagulation profile: plasma prothrombin concentration (PC) prothrombin time (PT), partial thromboplastin time (PTT) and international normalized ratio (INR) (Hirsh et al., 1995).
5. Electrocardiogram (ECG) (if indicated for preoperative assessment).
6. Diagnostic cystoscopy and biopsy for histopathology.
7. Molecular biology investigations: Quantitative reverse transcription polymerase chain reaction (qRT-PCR) for detection of miR-145 and Oct4 gene expression levels.

**Methods:**

**Sampling:**
Subjects and Methods

Bladder biopsies were obtained during endoscopic removal of renal or ureteric stones from subjects of control group while bladder mass biopsies were obtained during surgical excision by cystoscopy from malignant group. Each biopsy was divided into two parts: the first part was used for histopathological examination and the second part was immediately kept frozen at -80 °C (celsius) to be used for detection of gene expression levels of miR-145 and Oct4 by qRT-PCR.

Estimation of miR-145 and Oct4 gene expression levels according to the following steps:

i. Extraction of miRNA and mRNA from tissue samples.

ii. Complementary deoxyribonucleic acid (cDNA) synthesis.

iii. qRT-PCR for detection of miR-145 and Oct4 gene expression levels.

i.a. Extraction of miRNA:

Using the RNeasy Mini Kit (QIAGEN, Germany) (Eldh et al., 2012) and RNeasy MinElute Cleanup Kit (QIAGEN, Germany) (Naccache et al., 2013) according to manufacturer's instructions.

Procedure:

1. The tissue sample was excised (30mg).

2. The tissue sample was placed directly into 700μl QIAzol Lysis Reagent in a suitably sized vessel for disruption and homogenization.

3. The tissue sample was immediately homogenized using the Tissue Ruptor Homogenizer (QIAGEN, Switzerland) until the sample is uniformly homogeneous usually 30s (seconds).
4. The tube containing the homogenate was placed on the benchtop at room temperature for 5 min (minutes).

5. Chloroform (140 μl) was added to the tube containing the homogenate then the tube was capped securely and shaken vigorously for 15 s.

6. The tube containing the homogenate was incubated at room temperature for 3 min.

7. Centrifugation was done for 15 min at 12,000 x g at 4 °C. After centrifugation, the sample was separated into 3 phases: an upper colorless aqueous phase containing RNA, a white interphase and a lower red organic phase.

8. The upper aqueous phase was transferred to a new reaction tube. Ethanol 70% was added (350 μl) and mixed thoroughly by vortexing.

9. The sample was pipetted (700 μl) into an RNeasy Mini spin column placed in a 2 ml collection tube. The lid was gently closed and centrifugation was done at 8000 x g for 15 s (seconds) at room temperature. The flow-through containing miRNA was pipetted into a 2 ml reaction tube.

10. Ethanol 100% was added (450 μl) to the flow-through and mixed thoroughly by vortexing.

11. The sample was pipetted (700 μl) into an RNeasy MinElute spin column placed in a 2 ml collection tube. The lid was closed gently and centrifugation was done for 15 s at 8000 x g at room temperature. The flow-through was discarded.

12. Buffer RPE was pipetted (500 μl) into the RNeasy MinElute spin column. The lid was closed gently and centrifugation was done for 15 s at 8000 x g at room temperature. The flow-through was discarded.
13. Ethanol 80% was added (500μl) to the RNeasy MinElute spin column. The lid was closed gently and centrifugation was done for 2 min at 8000 x g at room temperature to dry the spin column membrane. The flow-through and the collection tube were discarded.

14. The RNeasy MinElute spin column was placed into a new 2 ml collection tube. Centrifugation was done for 5 min at ≥8000 x g at room temperature.

15. The RNeasy MinElute spin column was placed into a 1.5 ml collection tube and RNase-free water was pipetted (14μl) onto the spin column membrane. The lid was closed gently and centrifugation was done for 1 min at ≥8000 x g at room temperature to elute the miRNA-enriched fraction.

i.b. Extraction of mRNA:

Using the RNeasy Mini Kit (QIAGEN, Germany) (Eldh et al., 2012) according to manufacturer's instructions.

Procedure:

1. The tissue sample was excised (30mg).
2. The tissue sample was placed directly into 700μl QIAzol Lysis Reagent in a suitably sized vessel for disruption and homogenization.
3. The tissue sample was immediately homogenized using the Tissue Ruptor Homogenizer(QIAGEN, Switzerland) until the sample is uniformly homogeneous 30s.
4. The tube containing the homogenate was incubated at room temperature for 5 min.
5. Chloroform (140μl) was added to the tube containing the homogenate then the tube was capped securely and shaked vigorously for 15s.
6. The tube containing the homogenate was incubated at room temperature for 3 min.

7. Centrifugation was done for 15 min at 12,000 x g at 4 °C. After centrifugation, the sample separates into 3 phases: an upper colorless aqueous phase containing RNA, a white interphase and a lower red organic phase.

8. The upper aqueous phase was transferred to a new reaction tube. Ethanol 70% was added (350 μl) and mixed thoroughly by vortexing.

9. The sample was pipetted (700 μl) into an RNeasy Mini spin column placed in a 2 ml collection tube. The lid was gently closed and centrifugation was done at 8000 x g for 15 s at room temperature.

10. Buffer RWT was pipetted (700 μl) into the RNeasy Mini spin column. The lid was gently closed and centrifugation was done for 15 s at 8000 x g at room temperature to wash the spin column membrane. The flow-through was discarded.

11. Buffer RPE was added (500 μl) to the RNeasy Mini spin column. The lid was gently closed and centrifugation was done for 15 s at 8000 x g at room temperature to wash the spin column membrane. The flow-through was discarded.

12. Another Buffer RPE was pipetted (500 μl) into the RNeasy Mini spin column. The lid was gently closed and centrifugation was done for 15 s at 8000 x g to wash the spin column membrane. The flow-through and the collection tube were discarded.

13. The RNeasy Mini spin column was placed in a new 2 ml collection tube. Centrifugation was done at 14000 x g for 1 min at room temperature.
14. The RNeasy Mini spin column was placed into a new 1.5ml collection tube. RNase-free water was pipetted (30μl) directly onto the spin column membrane. The lid was gently closed and centrifugation was done for 1min at 8000 x g at room temperature to elute the total RNA.

**ii. cDNA synthesis:**

Using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) (*Wiame et al., 2000*) according to manufacturer's instructions.

**Procedure:**

1. Template RNA was left to thaw on ice and mixed by pipetting.
2. RNA quantity(μg/1μl) was measured by NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA).
3. In different tubes the following were added(table 4):

**Table (4):** Volumes of total RNA, miRNA and primers used for cDNA synthesis.

<table>
<thead>
<tr>
<th>Oct4, GAPDH</th>
<th>MiR-145</th>
<th>U6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RNA equivalent to 1μg</td>
<td>Total miRNA equivalent to 1μg</td>
<td>Total miRNA equivalent to 1μg</td>
</tr>
<tr>
<td>Oligo dT primer 1μl</td>
<td>miR-145 RT primer* 1μl</td>
<td>U6 RT primer* 1μl</td>
</tr>
<tr>
<td>Different volumes of water were added to complete total volume 12μl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sequences of these primers are mentioned in (table 7).

4. Gentle mixing and brief centrifugation were done.
5. The tubes were incubated at 65 °C for 5min then returned in ice.
6. Brief centrifugation was done, then the tubes were returned in ice again.

7. The following components were added to the previous tubes in the indicated order as in (table 5) to complete final volume 20µl:
Table (5): Reaction setup for reverse transcription master mix preparation.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume/reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X Reaction Buffer</td>
<td>4µl</td>
</tr>
<tr>
<td>RiboLock RNase Inhibitor</td>
<td>1µl</td>
</tr>
<tr>
<td>10 mM dNTP Mix</td>
<td>2µl</td>
</tr>
<tr>
<td>RevertAid M-MuLV Reverse Transcriptase</td>
<td>1µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>8µl</td>
</tr>
</tbody>
</table>

8. Centrifugation was done briefly.

9. The tubes were placed in G-Storm Thermal Cycler (Gene Technologies, UK) for cDNA synthesis according to the following program: Incubation at 42 °C for 60min followed by incubation at 70 °C for 5min.

10. The cDNA was immediately used for qRT-PCR.

iii. qRT-PCR for detection of mRNAs and mature miRNAs:

Using Maxima SYBR Green qPCR Master Mix (2X) (Longo et al., 1990) (Thermo Scientific, USA) according to manufacturer's instructions.

Procedure:

1. The PCR reaction mix was prepared in a total volume of 25µl / well (used in 96-well plate) as in (table 6):
Table (6): Reaction setup for real-time PCR.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume/reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxima SYBR Green qPCR Master Mix (2X)</td>
<td>12.5µl</td>
</tr>
<tr>
<td>Forward Primer*</td>
<td>0.4µl</td>
</tr>
<tr>
<td>Reverse Primer*</td>
<td>0.4µl</td>
</tr>
<tr>
<td>ROX Solution</td>
<td>0.1µl</td>
</tr>
<tr>
<td>Template DNA</td>
<td>2.5µl</td>
</tr>
<tr>
<td>Water, nuclease-free</td>
<td>9.1µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>25µl</td>
</tr>
</tbody>
</table>

*Sequences of these primers are mentioned in (table 7).

2. The plate was placed in the 7900HT Fast Real-Time PCR System, (Applied Biosystem, Singapore) and the run was started according to the following program:
   a) Initial activation step: for 10 min at 95°C.
   b) Cycling step (40 cycles): denaturation at 95 °C for 15 seconds followed by annealing at 54 °C for 30 seconds then extension at 72 °C for 45 seconds.
   c) Final extensions step at 72 °C for 2 min.

After the end of the program the expression of miRNA and mRNA expression levels in each sample were finally determined after correction with U6 and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression levels respectively. Controls were chosen as the reference samples, and fold change of target genes (miR-145 and Oct4) were
determined by $\Delta\Delta C_t$ (cycle threshold) method and expressed as relative units (RU) using StepOne software (Applied Biosystems, USA).

**Table (7):** Primer sequence of target and endogenous control genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>RT Primer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiR-145</td>
<td>5 CGTGGAGT-3</td>
<td>5 CCCAGG-3</td>
<td>5 AGTGCCTGTCTGGAGGACAGGATT-3</td>
<td>(Shao et al., 2013)</td>
</tr>
<tr>
<td>U6</td>
<td>5 CACATATACTAAAAAT-3</td>
<td>5 TTGCCTGTCAT-3</td>
<td>CGCTTCACGAATTGCTGTCAT-3</td>
<td>(Shao et al., 2013)</td>
</tr>
<tr>
<td>Oct4</td>
<td>5 TCCTGCAGTGTC-3</td>
<td>5 TTCCTTAGT-3</td>
<td></td>
<td>(Atlasi et al., 2007)</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5 CAGGCGCAATTTCTTCTTGT-3</td>
<td>5 GACAAATC-3</td>
<td></td>
<td>(Villadsen et al., 2012)</td>
</tr>
</tbody>
</table>
STATISTICAL ANALYSIS

The collected data were summarized in terms of mean ± Standard Deviation (SD) and range for quantitative data; and frequency and percentage for qualitative data. Comparisons between cases and control were carried out using the Mann-Whitney test (z), to compare quantitative data between two groups and Chi- squared ($\chi^2$) test, to compare proportions of two or more groups. Spearman correlation coefficient (RHO, $\rho$) was used to estimate the correlation between miRNA-145, Oct4 and age of the studied group. The corresponding test statistics were calculated and the corresponding P-values were obtained. P-value $\leq 0.05$ was considered statistically significant, while P-value $> 0.05$ was considered statistically non-significant. The statistical analysis was conducted using STATA version 11 (StataCorp, 2009).
RESULTS

Table (8) and Fig. (16): Show mean ± SD and range of the age/years of control group as well as bladder cancer group.

Table (8): The mean ± SD and range of the age/years of the studied groups.

<table>
<thead>
<tr>
<th>Age(years)</th>
<th>Group</th>
<th>Control group</th>
<th>Bladder cancer group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>65.1</td>
<td>65.4</td>
</tr>
<tr>
<td></td>
<td>±SD</td>
<td>±7.2</td>
<td>±8.9</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>52-80</td>
<td>49 - 83</td>
</tr>
</tbody>
</table>

Fig.(16): Age distribution among the studied groups.
**Table (9) and Fig.(17):** Show sex distribution in control group as well as bladder cancer group.

**Table(9):** Sex distribution among the studied groups.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control group</th>
<th>Bladder cancer group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number(n)</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Percent%</td>
<td>53.3</td>
<td>65.7</td>
</tr>
<tr>
<td>Female:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number(n)</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Percent%</td>
<td>46.7</td>
<td>34.3</td>
</tr>
</tbody>
</table>

**Fig. (17):** Sex distribution among the studied groups.
Table (10) and fig.(18): Show that there is statistically non-significant difference in smoking habit distribution in bladder cancer group as compared to that in control group.

Table (10): Number, percentage and test of significance of smoking habit distribution in bladder cancer group as compared to that in control group.

<table>
<thead>
<tr>
<th>Smoking habit</th>
<th>Control group</th>
<th>Bladder cancer group</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non smokers:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number(n)</td>
<td>10</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent%</td>
<td>66.7</td>
<td>65.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number(n)</td>
<td>5</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent%</td>
<td>33.3</td>
<td>34.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2 = 0.004$, 0.95

$P \leq 0.05$ significant

$P > 0.05$ non-significant

Fig.(18): Non-significant difference in smoking habit distribution between the studied groups.
Results

Table (11) and Fig.(19): Show that there is statistically significant decrease in mean value of miR-145 fold change of bladder cancer group as compared to that of control group.

Table (11): The mean ±SD and test of significance of miR-145 fold change of bladder cancer group as compared to that of control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control group</th>
<th>Bladder cancer group</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>39.3 ±8.9</td>
<td>9.2 ±2.3</td>
<td>Z=5.56</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 significant

*P > 0.05 non-significant

Fig. (19): Significant decrease in mean value of miR-145 fold change of bladder cancer group as compared to that of control group.
Table (12) and Fig.(20): Show that there is statistically significant increase in mean value of Oct4 fold change of bladder cancer group as compared to that of control group.

Table (12): The mean ±SD and test of significance of Oct4 fold change of bladder cancer group as compared to that of control group.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Control group</th>
<th>Bladder cancer group</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>fold change of Oct4(RU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>5 ± 1</td>
<td>46.4 ± 9.8</td>
<td>z=5.6</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 significant

*P > 0.05 non-significant

Fig. (20): Significant increase in mean value of Oct4 fold change of bladder cancer group as compared to that of control group.
Table (13) and Fig. (21): Show that there is non-significant correlation between miR-145 fold change and the age of control group.

Table (13) and Fig. (22): Show that there is non-significant correlation between miR-145 fold change and the age of bladder cancer group.

Table (13): Spearman’s correlation coefficient(\(\rho\)) between miR-145 fold change and the age in the studied groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age /years</th>
<th>Control group (n=15)</th>
<th>Bladder cancer group (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rho((\rho))</td>
<td>P</td>
<td>rho((\rho))</td>
</tr>
<tr>
<td>miR-145</td>
<td>-0.004</td>
<td>0.99</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Fig.(21): Non-significant correlation between miR-145 fold change and the age of control group.
Fig.(22): Non-significant correlation between miR-145 fold change and the age of bladder cancer group.
Table (14) and Fig.(23): Show that there is non-significant correlation between Oct4 fold change and the age of control group.

Table (14) and fig.(24): Show that there is non-significant correlation between Oct4 fold change and the age of bladder cancer group.

Table (14): Spearman’s correlation coefficient(ρ) between Oct4 fold change and the age in the studied groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n=15)</th>
<th>Bladder cancer group (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rho(ρ)</td>
<td>P</td>
</tr>
<tr>
<td>Oct4</td>
<td>-0.16</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Fig.(23): Non-significant correlation between Oct4 fold change and the age of control group.
Fig.(24) : Non-significant correlation between Oct4 fold change and the age of bladder cancer group.
Results

Table (15) and Fig.(25): Show that there is statistically non-significant difference in mean value of miR-145 fold change of female as compared to that of male subjects in control group.

Table (15) and Fig.(26): Show that there is statistically non-significant difference in mean value of miR-145 fold change of female as compared to that of male patients in bladder cancer group.

Table (15): The mean ±SD and test of significance of miR-145 fold change of female as compared to male subjects of the studied groups.

<table>
<thead>
<tr>
<th>Studied group</th>
<th>Male</th>
<th>Female</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>37.4</td>
<td>41.5</td>
<td>z=0.81</td>
<td>0.42</td>
</tr>
<tr>
<td>± SD</td>
<td>±9.2</td>
<td>±8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bladder cancer group:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.2</td>
<td>9.4</td>
<td>z=0.31</td>
<td>0.75</td>
</tr>
<tr>
<td>± SD</td>
<td>±2.3</td>
<td>±2.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P* ≤ 0.05 significant

*P* > 0.05 non-significant

Fig.(25): Non-significant difference in mean value of miR-145 fold change regarding sex in control group.
Fig.(26): Non-significant difference in mean value of miR-145 fold change regarding sex in bladder cancer group.
**Results**

**Table (16) and Fig.(27):** Show that there is statistically non-significant difference in mean value of Oct4 fold change of female as compared to that of male subjects in control group.

**Table (16) and Fig. (28):** Show that there is statistically non-significant difference in mean value of Oct4 fold change of female as compared to that of male patients in bladder cancer group.

**Table (16):** The mean ±SD and test of significance of Oct4 fold change of female as compared to male subjects in the studied groups.

<table>
<thead>
<tr>
<th>Sex Studied group</th>
<th>Male</th>
<th>Female</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.2 ±0.7</td>
<td>4.7 ±1.3</td>
<td>z=1.04</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Bladder cancer group:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>46.9 ±9.2</td>
<td>45.4 ±11.2</td>
<td>z=0.29</td>
<td>0.77</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 significant  
P > 0.05 non-significant*

**Fig.(27):** Non-significant difference in mean value of Oct4 fold change regarding sex in control group.
**Fig.(28):** Non-significant difference in mean value of Oct4 fold change regarding sex in bladder cancer group.
Table (17) and fig.(29): Show that there is statistically non-significant difference in mean value of miR-145 fold change of smoker as compared to that of non-smoker subjects in control group.

Table (17) and fig.(30): Show that there is statistically non-significant difference in mean value of miR-145 fold change of smoker as compared to that of non-smoker patients in bladder cancer group.

Table (17): The mean ±SD and test of significance of miR-145 fold change of smoker as compared to non-smoker subjects in the studied groups.

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Studied group</th>
<th>Non smoker</th>
<th>Smoker</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group:</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non smoker</td>
<td>38.9 ± 8.8</td>
<td>40 ± 10.1</td>
<td>z=0.61</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>40 ± 10.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder cancer group:</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non smoker</td>
<td>8.9 ± 2.1</td>
<td>9.8 ± 2.7</td>
<td>z=0.87</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>9.8 ± 2.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P ≤ 0.05 significant
P > 0.05 non-significant

Fig.(29): Non-significant difference in mean value of miR-145 fold change regarding smoking habit in control group.
Fig. (30): Non-significant difference in mean value of miR-145 fold change regarding smoking habit in bladder cancer group.
Table (18) and Fig.(31): Show that there is statistically non-significant difference in mean value of Oct4 fold change of smoker as compared to that of non smoker subjects in control group.

Table (18) and Fig.(32): Show that there is statistically non-significant difference in mean value of Oct4 fold change of smoker as compared to that of non smoker patients in bladder cancer group.

Table (18): The mean ±SD and test of significance of Oct4 fold change of smoker as compared to non smoker subjects in the studied groups.

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Studied group</th>
<th>Non smoker</th>
<th>Smoker</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group:</td>
<td>Mean ± SD</td>
<td>5 ±1.1</td>
<td>4.9 ±0.9</td>
<td>z=0.12</td>
<td>0.90</td>
</tr>
<tr>
<td>Bladder cancer group:</td>
<td>Mean ± SD</td>
<td>45.5 ±10.3</td>
<td>48 ±9</td>
<td>z=0.68</td>
<td>0.50</td>
</tr>
</tbody>
</table>

\( P \leq 0.05 \) significant
\( P > 0.05 \) non-significant

Fig.(31): Non-significant difference in mean value of Oct4 fold change regarding smoking habit in control group.
**Fig.(32):** Non-significant difference in mean value of Oct4 fold change regarding smoking habit in bladder cancer group.
**Table (19):** Shows pathological data in bladder cancer group regarding tumor type, tumor grade and tumor stage.

**Table (19):** Pathological data of bladder cancer group.

<table>
<thead>
<tr>
<th>Pathological data Cases</th>
<th>Tumor type n=35</th>
<th>Grade(G) n=35</th>
<th>Stage n=35</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transitional cell carcinoma</td>
<td>Small cell carcinoma</td>
<td>Grade (1)</td>
</tr>
<tr>
<td>Number (n)</td>
<td>34</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Percent %</td>
<td>97.1</td>
<td>2.9</td>
<td>20</td>
</tr>
</tbody>
</table>

*NMIBC: Non-muscle invasive bladder cancer
*MIBC: Muscle invasive bladder cancer

**Fig.(33):** Distribution of bladder cancer patients regarding tumor types.
**Results**

*Fig.(34)*: Distribution of bladder cancer patients regarding tumor grades.

*Fig.(35)*: Distribution of bladder cancer patients regarding tumor stages.
**Results**

**Table (20) and fig.(36):** Show that there is statistically non-significant difference in mean value of miR-145 fold change of patients with small cell carcinoma as compared to that of patients with transitional cell carcinoma.

**Table (20):** The mean ±SD and test of significance of miR-145 fold change of patients with small cell carcinoma as compared to those with transitional cell carcinoma.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Transitional cell carcinoma</th>
<th>Small cell carcinoma</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold change of miR-145 (RU)</td>
<td>9.3 ±2.3</td>
<td>6.3</td>
<td>z=1.68</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*P* < 0.05 significant

*P* > 0.05 non-significant

**Fig.(36):** Non-significant difference in mean value of miR-145 fold change regarding tumor types.
**Table (21) and Fig.(37):** Show that there is statistically non-significant difference in mean value of Oct4 fold change of patients with small cell carcinoma as compared to that of patients with transitional cell carcinoma.

**Table (21):** The mean ±SD and test of significance of Oct4 fold change of patients with small cell carcinoma as compared to those with transitional cell carcinoma.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Fold change of Oct4(RU)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transitional cell carcinoma</td>
<td>45.8 ±9.4</td>
<td>z=1.68</td>
<td>0.09</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>64.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P ≤ 0.05 significant*

*P > 0.05 non-significant*

**Fig.(37):** Non-significant difference in mean value of Oct4 fold change regarding tumor types.
**Results**

Table (22): Shows that there is statistically non-significant difference in mean value of miR-145 fold change of patients with moderately differentiated tumors as compared to that of patients with well differentiated tumors.

### Table (22): The mean ±SD and test of significance of miR-145 fold change of patients with moderately differentiated tumors as compared to those with well differentiated tumors.

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Well differentiated Grade (1)</th>
<th>Moderately Differentiated Grade (2)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>8.3 ±2</td>
<td>9.8 ±2</td>
<td>z=1.62</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 significant*

*P > 0.05 non-significant*

Table (23): Shows that there is statistically non-significant difference in mean value of miR-145 fold change of patients with poorly differentiated tumors as compared to that of patients with well differentiated tumors.

### Table (23): The mean ±SD and test of significance of miR-145 fold change of patients with poorly differentiated tumors as compared to those with well differentiated tumors.

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Well differentiated Grade (1)</th>
<th>Poorly Differentiated Grade (3)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>8.3 ±2</td>
<td>9.4 ±2.5</td>
<td>z=1.05</td>
<td>0.29</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 significant*

*P > 0.05 non-significant*
Table (24): Shows that there is statistically non-significant difference in mean value of miR-145 fold change of patients with poorly differentiated tumors as compared to that of patients with moderately differentiated tumors.

Table (24): The mean ± SD and test of significance of miR-145 fold change of patients with poorly differentiated tumors as compared to those with moderately differentiated tumors.

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Moderately differentiated Grade (2)</th>
<th>Poorly Differentiated Grade (3)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold change of miR-145 (RU)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>z</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>9.8 ±2</td>
<td>9.4 ±2.5</td>
<td>0.61</td>
<td>0.54</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 significant

*P > 0.05 non-significant*
Table (25) and Fig.(38): Show that there is statistically non-significant difference in mean value of miR-145 fold change of patients with moderately differentiated tumors as compared to those with well differentiated tumors. In addition, they show that there is statistically non-significant difference in mean value of miR-145 fold change of patients with poorly differentiated tumors as compared to those with well differentiated tumors. Moreover, they show that there was statistically non-significant difference in mean value of miR-145 fold change of patients with poorly differentiated tumors as compared to those with moderately differentiated tumors.

Table (25): Differences in mean values of miR-145 fold change of bladder cancer patients regarding tumor grades.

<table>
<thead>
<tr>
<th></th>
<th>Well differentiated (n=7)</th>
<th>Moderately differentiated (n=8)</th>
<th>Poorly differentiated (n=20)</th>
<th>Z1</th>
<th>P1*</th>
<th>Z2</th>
<th>P2*</th>
<th>Z3</th>
<th>P3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-145</td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Z1</td>
<td>P-value obtained using the Mann-Whitney test; ; after Bonferroni correction, P-value is considered significant if &lt;0.017.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.31±2</td>
<td>6.6-12.2</td>
<td>9.8±2</td>
<td>7-12.9</td>
<td>9.4±2.5</td>
<td>6.3-13.3</td>
<td>1.62</td>
<td>0.10</td>
<td>1.05</td>
</tr>
</tbody>
</table>

* P-value obtained using the Mann-Whitney test; ; after Bonferroni correction, P-value is considered significant if <0.017.  

P1 for comparison between well differentiated and moderately differentiated groups.  
P2 for comparison between well differentiated and poorly differentiated groups.  
P3 for comparison between moderately differentiated and poorly differentiated groups.

Fig.(38): Non-significant differences in mean values of miR-145 fold change of bladder cancer patients regarding tumor grades.
Table (26): Shows that there is statistically significant increase in mean value of Oct4 fold change of patients with moderately differentiated tumors as compared to that of patients with well differentiated tumors.

Table (26): The mean ± SD and test of significance of Oct4 fold change of patients with moderately differentiated tumors as compared to those with well differentiated tumors.

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Well differentiated Grade (1)</th>
<th>Moderately Differentiated Grade (2)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>33 ±5.7</td>
<td>44 ±6.8</td>
<td>z=2.66</td>
<td>0.008</td>
</tr>
</tbody>
</table>

$P \leq 0.05$ significant

$P > 0.05$ non-significant

Table (27): Shows that there is statistically significant increase in mean value of Oct4 fold change of patients with poorly differentiated tumors as compared to that of patients with well differentiated tumors.

Table (27): The mean ±SD and test of significance of Oct4 fold change of patients with poorly differentiated tumors as compared to those with well differentiated tumors.

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Well differentiated Grade (1)</th>
<th>Poorly Differentiated Grade (3)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>33 ±5.7</td>
<td>52 ±6.6</td>
<td>z=3.82</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

$P \leq 0.05$ significant

$P > 0.05$ non-significant
**Table (28):** Shows that there is statistically non-significant difference in mean value of Oct4 fold change of patients with poorly differentiated tumors as compared to that of patients with moderately differentiated tumors.

**Table (28):** The mean ±SD and test of significance of Oct4 fold change of patients with poorly differentiated tumors as compared to those with moderately differentiated tumors.

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Moderately Differentiated Grade (2)</th>
<th>Poorly Differentiated Grade (3)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold change of Oct4 (RU)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>z=2.24</td>
<td>0.02</td>
</tr>
<tr>
<td>Moderately Differentiated Grade (2)</td>
<td>44 ±6.8</td>
<td>52 ±6.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P ≤0.05 significant

*P > 0.05 non-significant
Table (29) and Fig. (39): Show that there is statistically significant increase in mean value of Oct4 fold change of patients with moderately differentiated tumors as compared to those with well differentiated tumors. Moreover, they show that there is statistically significant increase in mean value of Oct4 fold change of patients with poorly differentiated tumors as compared to those with well differentiated tumors. However, they show that there is statistically non-significant difference in mean value of Oct4 fold change of patients with poorly differentiated tumors as compared to those with moderately differentiated tumors.

Table (29): Differences in mean values of Oct4 fold change of bladder cancer patients regarding tumor grades.

<table>
<thead>
<tr>
<th></th>
<th>Well differentiated (n=7)</th>
<th>Moderately differentiated (n=8)</th>
<th>Poorly differentiated (n=20)</th>
<th>Z 1</th>
<th>P1*</th>
<th>Z 2</th>
<th>P2*</th>
<th>Z 3</th>
<th>P3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>24.7-39.3</td>
<td>35.1-53.5</td>
<td>39-64.9</td>
<td>2.66</td>
<td>0.008</td>
<td>3.82</td>
<td>0.0001</td>
<td>2.24</td>
<td>0.02</td>
</tr>
<tr>
<td>Oct4</td>
<td>33±5.7</td>
<td>44±6.8</td>
<td>52±6.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P-value obtained using the Mann-Whitney test; ; after Bonferroni correction, P-value is considered significant if <0.017.

P 1 for comparison between well differentiated and moderately differentiated groups.
P 2 for comparison between well differentiated and poorly differentiated groups.
P 3 for comparison between moderately differentiated and poorly differentiated groups.

Fig.(39): Differences in mean values of Oct4 fold change of bladder cancer patients regarding tumor grades.
Table (30) and Fig. (40): Show that there is statistically non-significant difference in mean value of miR-145 fold change of patients with muscle invasive tumors as compared to that of patients with non muscle invasive tumors.

Table (30): The mean ±SD and test of significance of miR-145 fold change of patients with muscle invasive tumors as compared to those with non muscle invasive tumors.

<table>
<thead>
<tr>
<th>Tumor stage fold change of miR-145 (RU)</th>
<th>Non muscle invasive (Ta,CIS,T1)</th>
<th>Muscle invasive (T2,T3,T4)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>9.2 ±2.2</td>
<td>9.2 ±2.4</td>
<td>z=0.03</td>
<td>0.97</td>
</tr>
</tbody>
</table>

P ≤ 0.05 significant
P > 0.05 non-significant

Fig.(40): Non-significant difference in mean value of miR-145 fold change regarding tumor stages.
Table (31) and Fig.(41): Show that there is statistically significant increase in mean value of Oct4 fold change of patients with muscle invasive tumors as compared to that of patients with non muscle invasive tumors.

Table (31): The mean ±SD and test of significance of Oct4 fold change of patients with muscle invasive tumors as compared to those with non muscle invasive tumors.

<table>
<thead>
<tr>
<th>Tumor stage</th>
<th>Non muscle invasive (Ta,CIS,T1)</th>
<th>Muscle invasive (T2,T3,T4)</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>36.6 ±7.1</td>
<td>51.5 ±6.6</td>
<td>z=4.17</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 significant

Fig.(41): Significant increase in mean value of Oct4 fold change of patients with muscle invasive tumors as compared to that of patients with non muscle invasive tumors.
Table (32) and Fig. (42): Show that there is a significant negative correlation between fold change of miR-145 and Oct4 in the studied samples.

Table (32): Spearman’s correlation coefficient (ρ) between miR-145 fold change and Oct4 fold change in the studied samples (n=50).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oct4 Samples (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rho(ρ)</td>
</tr>
<tr>
<td>miR-145</td>
<td>-0.70</td>
</tr>
</tbody>
</table>

Fig. (42): Significant negative correlation between fold change of miR-145 and Oct4 in the studied samples (n=50).
DISCUSSION

Bladder cancer is still the most common malignant tumor among males in Egypt, some African and Middle East countries (Khaled, 2013). According to the National Cancer Institute in Cairo, Egypt, it constitutes 30.3% of all cancers (Eissa et al., 2010). The annual death rate from this disease is significant and every year there is an increase in its incidence globally (Khochikar, 2011).

The prognosis of bladder cancer is stage and grade dependent; the lower the stage (T2 or less) the better is the survival. Delay in the diagnosis and treatment does alter the overall outcome. Therefore, there is a clear need for early detection of bladder cancer and screening program (Khochikar, 2011).

The current methods of bladder cancer diagnosis are urine cytology and cystoscopy. Urine cytology is a procedure with 95% specificity but low sensitivity, especially in low-grade tumors. Cystoscopy is the current gold-standard method for bladder cancer detection, but it is an invasive and expensive procedure with low specificity and sensitivity in detecting flat CIS tumors (Hatefi et al., 2012). Therefore, there have been lots of efforts in the field to find sensitive, and specific molecular markers for bladder cancer (Ratert et al., 2013). It has been suggested that miRNAs are useful in this respect (Dip et al., 2012).

The involvement of miRNAs in gene regulatory processes and their implication in several diseases, including cancer (Gottardo et al., 2007), makes them very attractive for diagnosis, prognosis, and treatment in clinical application (Calin and Croce, 2006). The increasing number of studies investigating miRNA expression profiles specific to bladder
cancer indicate the growing interest in searching for specific miRNAs to function as diagnostic biomarkers (Ratert et al., 2013).

Several studies support the existence of a highly tumorigenic subpopulation of bladder cancer cells that possesses most characteristics of cancer stem cells (CSCs), including high tumorigenicity and involvement in bladder cancer progression (Tran et al., 2010). Oct4 has been proposed as a biomarker for CSC-like cells (Kumar et al., 2012). It has been detected in a variety of cancer types including human breast cancer (Ezeh et al., 2005), prostate (Sotomayor et al., 2009), and bladder cancer (Atlasi et al., 2007).

The current work aimed to study the role of miR-145 and Oct4 in bladder cancer by determining their expression in relation to various clinico-pathological parameters. Also to determine the relationship between the expression of miR-145 and Oct4.

We used quantitative reverse transcription polymerase chain reaction (qRT-PCR) to analyze the expression of miR-145 and Oct4 in tissue biopsies of control subjects (n=15) and bladder cancer patients (n=35).

The age of bladder cancer patients ranged from 49-83 years (y) with mean value 65.4 ± 8.9 y (table 8, fig. 16).

This was in agreement with many other studies and reviews which demonstrated that bladder cancer incidence increases with age, arising most commonly in the seventh decade of life (Shariat et al., 2009) and the median ages at diagnosis are 69 years for men and 71 for women (Volanis et al., 2010). Proposed mechanisms for the increased incidence of cancer in the aging population include an accumulation of
genetic and cellular damage, prolonged exposure to carcinogens, and fundamental changes in the host environment (Taylor and Kuchel, 2009).

As regard sex in our BC studied patients, males represent 65.7% (n=23) while females represent 34.3% (n=12) (table 9, fig.17).

This result was in agreement with Horstmann et al. (2008) who previously suggested that bladder cancer appeared to have a higher incidence in men than in women. They found that the male-to-female bladder cancer incidence ratio was 2.2:1.

Also, Fajkovic et al. (2011), demonstrated that men are nearly 3-4 times more likely to develop bladder cancer than women. They proposed that the disparity between genders might be attributed to the result of differences exposure to carcinogens (i.e., tobacco and chemicals) as well as environmental factors.

The results of the current study showed that non-significant difference regarding smoking habit distribution between bladder cancer group and control group (table 10, fig.18).

This result was in contrast to a study carried out by Jiang et al. (2012). Their study demonstrated that the risk of bladder cancer was significantly higher among smokers and increased with increasing number of cigarettes smoked per day and number of years of regular smoking.

The results of the present study showed that genetic expression of miR-145 was significantly decreased in bladder cancer patients as compared to controls (Table 11, Fig.19).
In agreement with our results, several studies have shown miR-145 under-expression in BC when compared to normal bladder epithelium (Ichimi et al., 2009) and (Dip et al., 2013). Moreover, Song et al. (2010) used real-time RT-PCR and showed that miR-1, miR-133a mir-133b, miR-143 and miR-145 were down-regulated in bladder urothelial carcinoma as compared to adjacent normal bladder tissue suggesting that these miRNAs may contribute to tumorigenesis and might serve as biomarkers for tumor.

Through performing quantitative real-time PCR analysis, Kou et al. (2014) found that the expression of miR-145 was significantly lower in superficial and invasive bladder cancer tissue than that in normal bladder epithelium. Moreover, their study showed that miR-145 transfection in bladder cancer cell lines inhibited bladder cancer cell invasion, at least partly through targeting p21 activated protein (PAK1) which is responsible of cell growth and invasion. Thus, they suggested that restoration or replacement of miR-145 could be an efficient approach to inhibit PAK1 and bladder cancer development in the tumor therapy.

The mechanism of miR-145 down-regulation in cancer is unknown, p53 response elements identified in the putative promoter region of miR-145 suggested that upon p53 inactivation, the transcription of miR-145 may be reduced (Sachdeva et al., 2009). But Mitra et al. (2007) showed that p53 is seldomly inactivated in early stage of bladder tumors (Ta) and thus cannot account for the early loss of miR-145, implying other mechanisms involved.

Ostenfeld et al. (2010) showed significant overall increase in DNA methylation in a CpG-rich region 250 base pair (bp) upstream of miR145 in bladder tumors (Ta) tumors compared with normal bladder biopsies.
They suggested that DNA hypermethylation could contribute to the reduced expression, although other regulatory mechanisms are most likely also involved.

Furthermore, a study carried out by Suh et al. (2011), showed that DNA methylation in the upstream sequence of miR-145 contributed to the down-regulation of miR-145 in prostate cancer, and, importantly, interfered with the binding of p53 to the p53 response element in the upstream region of miR-145.

The current results also showed non-significant difference in miR-145 expression regarding tumor grades (table 25, fig. 38) and stages (table 30, fig. 40).

These results agreed with a study carried out by Dip et al. (2013), showing non-significant difference upon comparing miR-145 expression in papillary(p)Ta and pT2/pT3 when considering stage and grade.

Also, another study conducted by Kou et al. (2014), found that miR-145 level in Ta-T1 was not significantly higher than that in T2-T4 bladder cancers.

In contrast to these results Ostenfeld et al. (2010), showed that miR-145 expression exhibited decrease in low stage tumors and carcinoma in situ (CIS) lesions than in T1 and T2–T4 tumors. They suggested that reduction of miR-145 is necessary to achieve a survival advantage for cancerous cells during early oncogenesis, but not for later steps of carcinogenesis. The difference between our results and their results could be attributed to difference in the measuring technique. Ostenfeld and His colleagues performed their study using in situ
hybridisation (ISH) technique to demonstrate difference in miR-145 expression regarding tumor stage but we used qRT-PCR in this regard.

Furthermore, the results of the present study showed significant increase in Oct4 expression in bladder cancer tissues compared with normal bladder tissues (Table 12, Fig.20).

This result was consistent with Atlasi et al. (2007), who previously explained the mechanism of Oct4 up-regulation in bladder cancer tissues. They suggested that high expression of Oct4 may be due to expansion of bladder cells that intrinsically express Oct4 or through acquisition of self-renewal capacity by other cancer cells. Accordingly, they concluded that Oct4 can potentially be regarded as a new molecular marker of bladder tumors, in which its expression might indicate the existence of stem-like cancer cells in these tumors.

Also, Chang et al. (2008), confirmed a previous finding by Atlasi et al. (2007), showing high expression of Oct4 in bladder cancer but not in normal human bladder tissues. They also suggested that Oct4 might also correlate with stem-like cell properties of cancer cells apart from being a marker for ESCs. Moreover, their study indicated that the expression of Oct4 in bladder cancer promoted tumor progression and metastasis through the activation of matrix metalloproteinase-13 (MMP-13), MMP-2, and MMP-9 expressions which are associated with cancer promotion and invasion. Based on this observation, Chang et al. (2008) suggested that the expression of Oct4 may contribute to the group of bladder cancer patients with poorer survival, and Oct4 may act as a novel target for cancer therapy especially in cancers with high tendency for metastasis.
Moreover, the results of the current study showed that Oct4 expression was significantly increased with advanced tumor grades (Table 29, Fig. 39) and stages (Table 31, Fig. 41).

These results were in agreement with Hatefi et al. (2012). They reported a significant correlation between the expression level of Oct4 and the grade and stage of the samples suggesting the suitability of Oct4 expression as a molecular marker for the diagnosis of bladder tumors and as a prognostic molecular marker to predict the malignant nature of bladder cancers.

The results of this study were in contrast with the results reported by Atlasi et al. (2007). They showed no significant correlation between the expression level of Oct4 and tumor grade or stage. The difference between both results may be due to difference in the technique used to detect Oct4 expression as Atlasi et al. (2007) used semiquantitative RT-PCR.

The current study showed a significant negative correlation between miR-145 and Oct4 expression in bladder cancer (Table 32, Fig. 42).

In agreement with the results of the current study, Xu et al. (2009) reported that Oct4 down-regulated miR-145 expression in human ESC through repressive binding to its promoter. Interestingly, they found that miR-145 directly repressed the 3’ UTR of Oct4 mRNA during human ESC differentiation, uncovering a double-negative feedback loop for Oct4 and miRNA expression.

The inhibitory role of Oct4 on miR-145 was further confirmed by a study conducted by Jia et al. (2012). They showed that the tumor suppressive role of miR-145 on cancer stem cell like cells derived from hepatocarcinoma was partly reversed by over-expression of Oct4.
CONCLUSION

It could be concluded that the significant decrease in miR-145 expression level in bladder cancer patients compared to the controls suggests its role as a tumor suppressor. Such decrease may be attributed to significant over expression of Oct4, a well known stem cell marker, in bladder cancer group compared to control group supporting the theory of cancer stem cell.
RECOMMENDATIONS

The followings are recommended:

- Further studies including larger number of both sexes are needed.
- Investigation of role of miR-145 as a therapeutic agent in bladder cancer cell lines.
- Study the expression level of miR-145 and Oct4 in urine as an easier and non invasive molecular marker in the diagnosis of bladder cancer.
- Study of the expression level of miR-145 and Oct4 as biomarkers in the follow up of bladder cancer.
SUMMARY

Bladder cancer represents a global health problem. It ranks ninth in worldwide cancer incidence. In Egypt, carcinoma of the bladder is the most prevalent cancer, accounting for as many as 31% of all cancer cases. The global burden of bladder cancer is predicted to increase significantly in the foreseeable future as a result of the progression of the tobacco epidemic and increasing exposure to occupational carcinogens in developing countries.

Therefore, there have been lots of efforts to find non-invasive, sensitive, and specific molecular markers for bladder cancer. It has been suggested that miRNAs are useful in this respect. Many miRNAs were significantly upregulated or downregulated in different tumors. miR-145 was identified as a tumor-suppressive miRNA that is downregulated in several cancer types, including prostate cancer, ovarian cancer, colon cancer and bladder cancer.

Tumor recurrence and multifocality are two common features of bladder tumors. Moreover, several previous reports suggested that these tumors are derived from a primary transformed progenitor cell. CSCs have been isolated from a variety of solid tumors such as breast cancer, lung, prostate, colon tumors and bladder cancer. The most important members of CSCs' regulatory core are transcription factors such as Oct4, Sox2, and Nanog, which are defined as key players in the regulatory network for maintaining the “stemness” state of stem cells. Oct4 is a transcription factor that is required for pluripotency during early embryogenesis and the maintenance of embryonic stem cell (ESC) and pluripotent cell identity. Misexpression of Oct4 is correlated with tumorigenesis and can affect the behavior of tumors such as recurrence or
resistance to therapy. Oct4 is largely expressed in human germ cell
tumors and its expression has also been identified in several somatic
cancers including prostate, breast and bladder carcinomas.

The current work aimed to study the role of Oct4 and miR-145 in
bladder cancer by determining their expression in relation to various
clinico-pathological parameters. Also to determine the relationship
between the expression of Oct4 and miR-145.

50 subjects of both sexes were selected from Urology Department,
Faculty of Medicine, Benha University Hospital. Their ages ranged from
49-83 years (y) with mean value 65.3±8.4 y.

The subjects were categorized into 2 groups:
A. **Malignant lesion group**: Included 35 patients, they were diagnosed
   as bladder cancer patients by clinical, radiological, cystoscopic and
   histopathological examinations.
B. **Control group**: Comprised 15 persons, age and sex matched, with
   histopathologically normal urothelium.

All patients were subjected to:
1. Full history taking.
2. General and local urological examinations.
3. Routine preoperative laboratory investigations including: urine
   analysis, complete blood count (CBC), fasting blood sugar, liver
   function tests, kidney function tests and coagulation profile.
4. Radiological investigations including: abdominopelvic plain X-ray,
   ultrasonography and CT.
5. ECG (if indicated for preoperative assessment).
6. Diagnostic cystoscopy and biopsy for histopathology.
7. Molecular biology investigations: Quantitative reverse transcription polymerase chain reaction (qRT-PCR) for detection of miR-145 and Oct4 gene expression levels.

Bladder biopsies were obtained during endoscopic removal of renal or ureteric stones from subjects of control group while bladder mass biopsies were obtained during surgical excision by cystoscopy from malignant group. Each biopsy was divided into two parts: the first part was used for histopathological examination and the second part was immediately kept frozen at -80 °C to be used for estimation of miR-145 and Oct4 gene expression levels by qRT-PCR.

The results of the current study showed that miR-145 expression level significantly decreased in bladder cancer group compared to the control group. Whereas Oct4 showed significant increase in bladder cancer group compared to control group. Moreover, Oct4 showed significant correlation with the tumor stages and grades. In addition, our results revealed significant negative correlation between miR-145 and Oct4 expression levels in bladder cancer group as well as control group.
REFERENCES


References


Bronevetsky, Y. and Ansel, K.M. (2013):


Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. Rna, 10:1957-1966.


Cauberg Evelyne, C.C.; de la Rosette, J.J. and de Reijke, T.M. (2011):


Cheung, G.; Sahai, A.; Billlia, M.; Dasgupta, P. and Khan, M.S.(2013):


The exonuclease ER1-1 has a conserved dual role in 5. 8S rRNA processing and RNAi. Nat. Struct. Mol. Biol., 15:531–533.


Haber, G.P. and Gill, I.S. (2007):  
Laparoscopic radical cystectomy for cancer: oncological outcomes at up to 5 years. BJU Int., 100:137-42.


http://blog-biosyn.com/2013/04/11/how-can-micrornas-be-described/


Bladder cancer in 2010: how far have we come?. CA- Cancer J. Clin., 60 : 244 - 272.

FGFR3 and Ras gene mutations are mutually exclusive genetic events in urothelial cell carcinoma. Oncogene, 24: 5218-25.


RAS is regulated by the let-7 microRNA family. Cell, 120(5):635–47.


MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in Type 2 Diabetes Mellitus. PLoS One, 6:e22839.


Li, Y. and Kowdley, K.V. (2012):


Liu, G.; Friggeri, A.; Yang, Y.; Milosevic, J.; Ding, Q.; Thannickal, V.J.; Kaminski, N. and Abraham, E. (2010):


Nair, V.S.; Maeda, L.S. and Ioannidis, J.P.(2012):


Oglesby, I.K.; McElvaney, N.G. and Greene, C.M. (2010):  


Park-Sarge, O.K. and Sarge, K.D.(2009):


Identification of miRs-143 and -145 that is associated with bone metastasis of prostate cancer and involved in the regulation of EMT. PLoS One,6:e20341.


Reitman, S. and Frankel, S. (1957):


ETS transcription factors and their emerging roles in human cancer.  

Shao, Y.; Qu, Y.; Dang, S.; Yao, B. and Ji, M. (2013):  


Micro RNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. J. Biol. Chem., 282: 32582-32590.


Son, M.Y.; Choi, H.; Han, Y.M. and Cho, Y.S. (2013):  


StataCorp. (2009): Statistical Software: Release 11. College Station, TX: StataCorp LP.


Tran, M.N.; Goodwin Jinesh, G.; McConkey, D.J. and Kamat, A.M.(2010):

Triboulet, R.; Mari, B.; Lin, Y.L.; Chable-Bessia, C.; Bennasser, Y.;
Lebrigand, K.; Cardinaud, B.; Maurin ,T.; Barbray ,P.; Baillat, V.;
Suppression of microRNA-silencing pathway by HIV-1 during virus

Trinder, P.(1969):
Determination of blood glucose using an oxidase-peroxidase system
158–161.

Genomic instability analysis of urine sediment versus tumor tissue in
transitional cell carcinoma of the urinary bladder. Oncol. Rep.,

Upton, J.P.; Wang, L.; Han, D.; Wang, E.S.; Huskey, N.E.; Lim, L.;
Truitt, M.; McManus ,M.T.; Ruggero, D.; Goga, A.; Papa, F.R.
IRE1α cleaves select microRNAs during ER stress to derepress

Valadi, H.; Ekstrom, K.; Bossios, A.; Sjostrand, M.; Lee, J.J. and
Exosome-mediated transfer of mRNAs and microRNAs is a novel

Valeri, N.; Gasparini, P.; Fabbri, M.; Braconi, C.; Veronese, A.;
Lovat, F.; Adair, B.; Vannini, I.; Fanini, F.; Bottoni, A.; Costinean,
Ferracin, M.; Lanza, G.; Volinia, S.; Negrini, M.; McIlhatton,
Modulation of mismatch repair and genomic stability by miR-155.


Wang, G.; Chan, E.S.; Kwan, B.C.; Li, P.K.; Yip, S.K.; Szeto, C.C. and Ng, C.F. (2012):


Williams, A.E. (2008):


الملخص العربي

مقدمة البحث:

تمثل سرطان المثانة مشكلة صحية عالمية، فهو يحتل المركز الثالث في من حيث مختلف أنواع السرطانات على مستوى العالم. ويعتبر سرطان المثانة في مصر من أكثر أنواع السرطانات انتشاراً وهو يمثل ما يصل إلى 31% من جميع حالات السرطان. ومن المتوقع أن العدد العالمي لسرطان المثانة سي榛ر لتزايد كبيرة في المستقبل المنظور، وذلك نتيجة لزيادة استهلاك التبغ وال تعرض للمواد السامة خاصة في البلدان النامية. وذلك كان من الضروري البحث عن دلالات جزيئية دقيقة وحساسية والتشخيص المبكر ومتابعة سرطان المثانة. وتعتبر الأمراض النووية الريبوزية المتناهية الصغر مفيدة في هذا الصدد.

فقد أثبتت الدراسات السابقة أن العديد من الأمراض النووية الريبوزية المتناهية الصغر تتأثر عملية نسخها من الجينات المنتجة لها بشكل ملحوظ في مختلف أنواع السرطانات. ومؤخرًا اكتشف أن الحمض النووي الريبوزي المتناهي الصغر يملأ كمثبط للأورم، فقد وجد أن عملية نسخه تقل في مختلف أنواع السرطانات، والتي من ضمنها سرطان البروستاتا، سرطان المبيض، سرطان القولون، وكذلك سرطان المثانة.

ومن الجدير بالذكر أن العديد من الأبحاث السابقة أشارت إلى أن الأورام تنشأ منخلايا جذعية سرطانية، والتي تنمي بالتجديد الذاتي، والمقاومة للموت المبرمج، ومقاومة للعوامل التحسينية للسرطان. وقد تم عزل الخلايا الجذعية السرطانية من مجموعة متنوعة من الأورام، ومن ضمنها سرطان المثانة.

فقد وجد أن عامل النسخ أوكت 4 والمطلوب بدوره في أوائل مرحلة التطور الجنيني وصيانة الخلية الجذعية الجنينية. يلعب دورًا هاماً في تنظيم تطور الخلايا الجذعية السرطانية.

فقد وجد أن التمثيل الجنيني لعامل النسخ أوكت 4 يزداد بصورة واضحة في العديد من الأورام والتي من ضمنها سرطان البروستاتا والمبيض والمثانة.

الهدف من البحث:

تم إجراء هذا البحث لدراسة الحمض النووي الريبوزي المتناهي الصغر 145 وعامل النسخ أوكت 4 في سرطان المثانة من خلال تحديد التمثيل الجنيني لهما وعلاقتهما بمختلف المعايير الأكليتيكية والباثولوجية، بالإضافة إلى تحديد العلاقة بين التمثيل الجنيني لعامل النسخ أوكت 4 والحمض النووي الريبوزي المتناهي الصغر 145.
طرق البحث:

تم إجراء هذا البحث في الفترة من 25/7/2014 حتى 20/7/2015 بعد الموافقة على خطة الدراسة من قبل اللجان الأخلاقية للبحث بكلية طب بنها والحصول على الموافقة من المرضى المدرجة في البحث. وشملت الدراسة 50 مريضاً من كلا الجنسين تم اختيارهم من قسم المسالك البولية بمستشفى بنها الجامعي. وتراوحت الأعمار ما بين 44-83 عاماً. تم تقسيم المرضى إلى مجموعتين كالتالي:

المجموعة المصابة بالسرطان (أ) وتشمل (35) مريضاً ويبدأ تحليل الأنسجة والخلايا للنسيج المبطن لغشاء المثانة على الإصابة بالسرطان.

المجموعة الضابطة (ب) وتشمل (15) شخصاً ويبدأ تحليل الأنسجة والخلايا للنسيج المبطن لغشاء المثانة على خلوي من الأمراض.

تم استعداد الحالات الآتية:

الحالات التي سبق لها التعرض للعلاج الكيميائي أو العلاج بالأشعة.

طريقة الدراسة:

قد خضع جميع المرضى للفحوصات والتحليلات الآتية:

1- الحصول على التاريخ المرضي بالكامل.
2- الفحص الكامل.
3- التحاليل المعملية الروتينية (قبل إجراء العملية) وتشمل:
   1. تحليل بول.
   2. قياس الهيموجلوبين.
   3. قياس مستوى السكر في مصل الدم أثناء الصيام.
   4. وظائف الكبد وتشمل: قياس مستوى إنزيمات الكبد.
   5. اختبارات عوامل تجلط الدم وتشمل زمن البروثرومبيين، تركيز البروثرومبيين وزمن الثرومبوبلاستين الجزيئي.
   6. وظائف الكلي وتشمل: قياس مستوى اليوبريا والكرياتينين.
4- رسم كهربي القلب (قبل إجراء العملية).
5- إجراء الفحوصات بالأشعة وتشمل:
   • أشعة عادية على الورك والحوض.
   • الموجات الفوق صوتية التشخيصية على الورك والحوض.
   • أشعة مقطعية على الورك والحوض.
6- منظور المثانة وأخذ عينة لإجراء تحليل الأنسجة والخلايا.
7- تفاعل البلامرة المتسلسل الكمي لكل من الحمض النووي الريبوزي المتناهي الصغر و أوكت.

أخذ العينات:

بعد الحصول على موافقة طبية من المرضى تم الحصول على عينة من المثانة بالمناظر أثناء إزالة حصائى الكلي أو الحالب من المجموعة الضابطة وآثار أخذ العينة من الورم من المجموعة المصابة بالسرطان.

كل العينات تم تقسيمها إلى جزئين: الجزء الأول تم استخدامه في تحليل الأنسجة والخلايا.

والجزء الثاني تم حفظه مجمعا عند - 80 درجة سيلزية وقد خضع للاختبار الآتي:

- الكشف عن التمثيل الجيني للحمض النووي الريبوزي المتناهي الصغر وعامل النسخ أوكت 4 بواسطة تفاعل البلامرة المتسلسل الكمي.
- وقد تم تحديد مستوى التمثيل الجيني لكل من الحمض النووي الريبوزي المتناهي الصغر و أوكت 4.

1- استخلاص الأحماض النووية الريبوزية المتناهية الصغر و الحمض النووي الريبوزي الرسول من العينات.

2- تصنيع الحمض النووي الديوكسي ريبوزي المتمم من الأحماض النووية الريبوزية المتناهية الصغر و الحمض النووي الريبوزي الرسول.

3- تفاعل البلامرة المتسلسل الكمي لتحديد التمثيل الجيني لكل من الحمض النووي الريبوزي المتناهي الصغر وعامل النسخ أوكت 4.

نتائج البحث:

أظهر التعبير الجيني للحمض النووي الريبوزي المتناهي الصغر 145 نقصاً ذو دلالة إحصائية، في حين أن التعبير الجيني لعامل النسخ أوكت 4 أظهر زيادة ذو دلالة إحصائية في مرضى سرطان المثانة مقارنة بالمجموعة الضابطة. كما أنه وجد ارتباط ذو دلالة إحصائية بين مستوى التعبير الجيني للأوكت 4 ومراحل ودرجات تطور المرض. بينما أظهرت النتائج وجود علاقة عكسية ذو دلالة إحصائية بين التعبير الجيني للحمض النووي الريبوزي المتناهي الصغر 45 وعامل النسخ أوكت 4.
الخلاصة:

نستخلص من هذه الدراسة أن التعبير الجيني للحمض النووي الريبوزي المتئاخي الصغر 45 يمثل بصورة ملحوظة في مرضى سرطان المثانة، مما يشير إلى دوره كمثبط للورم. ومن المحتمل أن هذا التقصير يرجع إلى الزيادة الواضحة في التعبير الجيني لعامل النسخ أوكت 4، والذي يلعب دوراً هاماً في تنظيم تطور الخلايا الجذعية السرطانية، مؤكداً على أن سرطان المثانة ينشأ من خلايا جذعية سرطانية.

توصيات البحث:

بعد إجراء البحث نوصى بالآتي:

• أن تشمل الدراسات المستقبلية على عدد أكبر من الحالات من كلا الجنسين.
• دراسة دور الحمض النووي الريبوزي المتئاخي الصغر 45 كمثبط لورم استخدام في علاج سرطان المثانة.
• دراسة التمثيل الجيني للحمض النووي الريبوزي المتئاخي الصغر 45 أو عامل النسخ أوكت 4 في البول كوسيلة أسهل للكشف عن سرطان المثانة.
• استخدام مستوى التعبير الجيني لكل من الحمض النووي الريبوزي المتئاخي الصغر 45 أو أوكت 4 كدلائل لتشخيص و متابعة المرض.
التعبير الجيني لكلاً من أوكت 4 والحمض النووي الريبوزي المتناهي الصغر 145 في سرطان المثانة
رسالة
توطئة للحصول على درجة الماجستير في الكيمياء الحيوية الطبية

مقدمة من الطبيبة/ ليلى عبد الهادي محمد
(بكالوريوس الطب والجراحة)
المعيدة بقسم الكيمياء الحيوية الطبية
جامعة بنها

تحت إشراف
أ.د./ ثناء حامد بلال
أ.د./ ممدوح زكي أبادير
أ.د./ إيناس عبد المنعم السيد
أ.د./ حموده وهيب شريف

أ.د./ ممدوح زكي أبادير
أ.د./ حموده وهيب شريف

2015