

Immunohistochemical Expression of CD 133 and CD 24 for Detection of Cancer Stem Cells in Urinary Bladder Carcinoma (Pilot Study)

Thesis

Submitted for the Fulfillment of M.D Degree in Pathology

By Tag Ibrahim Omran

Supervised by

Prof. Sanaa Abd El-Maged Sammour

Professor of Pathology Faculty of Medicine - Ain Shams University

Prof. Zeinab Abd El-kader Shehab El-Din

Professor of Pathology Faculty of Medicine - Ain Shams University

Prof. Manal Ibrahim Salman

Professor of Pathology
Faculty of Medicine - Ain Shams University

Dr. Rola Mohammed Farid

Assistant Professor of Pathology Faculty of Medicine - Ain Shams University

> Faculty of Medicine Ain Shams University 2014



First thanks to Allah, the most merciful, the compassionate, all praise is to you.

Thanks to **Prof. Sanaa AbdEl-Maged Sammour,** Prof. of pathology, Faculty of Medicine, Ain—Shams University for constant guidance, Constructive supervision and following the performance and progress of this thesis.

To**Prof**. Zeinab AbdEl-kader ShehabEl-Din, Prof. of pathology, Faculty of Medicine, Ain – ShamsUniversity, for her valuable support, guidance and great concern about this work.

To **Prof.** Manalibrahim Salman, Prof. of pathology, Ain-Shams University for her effective supervision and great help during the histopathological and immunohistochemical results in this thesis, support, constructive and greatly appreciated advice, great concern about the completion of this studyand for her continuous encouragement throughout the course of this work.

To **Dr. RolaMohammedFarid**, Assistant Professor of pathology, Ain-Shams University who was abundantly helpful and offered great support and guidance, advice, directions and revision of study.

To all the staff members of Pathology Department, for their sincere encouragement and support throughout the study.

I'd likethank **Professor.** Nahed Khamis, the Head of the Department and all the respectful Professors as well as my beloved colleagues.

Special thanks for technicians of Pathology Department, for their help and cooperation.

LIST OF CONTENTS

Title	Page No.	
List of tables	i	
List of figures	iv	
List of histograms	viii	
List of abbreviations	ix	
Introduction	1	
Aim of the work	5	
Review of literature		
-Bladder Carcinoma	6	
-Stem Cells	34	
-Bladder cancer and cancer stem cells	49	
-Stem cell markers	56	
Material and methods	61	
Results	71	
Cases	100	
Discussion	119	
Summary	128	
Conclusion	133	
Recommendations	134	
References	136	
Arabic Summary	-	

List of Tables

No. Title	Page
Table (1):The WHO (2004) TNM classification for staging of	27
bladder cancer	
Table (2): The American Joint Committee on Cancer for	28
staging of bladder cancer.	
Table (3): The American Joint Committee on Cancer for	30
(Anatomic Stage/Prognostic Groups)	
Table (4):Clinicopathologic parameters of the urinary	71
bladder carcinoma	
Table (5):Transitional cell carcinoma variants	73
Table (6):Tumor stage and type	75
Table (7): CD 133 and CD 24 expression in bladdercarcinoma	77
Table(8): Mode of expression of CD 133 and CD 24 in bladde carcinoma	
Table (9): Relation between expression of CD133 and age	80
Table (10): Relation between expression of CD 24 and age	80
Table (11):Relation between expression of CD133 and gender	81
Table (12): Relation between expression of CD24 and gender	81
Table (13):Relation between CD 133 expression and	82
tumortype	
Table (14): Relation between CD 24 expression and tumor	
type. Table (15): Relation between CD 133 expression and pattern	83
Table (13): Kelation between CD 133 expression and pattern	03

of growth(TCC).	
Table (16):Relation between CD 24 expression and pattern	
of growth(TCC)	
Table(17): Relation between CD 133 expression and tumor	85
grade(TCC)	
Table (18)Relation between CD 24 expression and tumor	86
grade (TCC)	
Table (19):Relation between CD 133 expression and tumor	88
stage	
Table (20):Relation between CD 24 expression and tumor	89
stage	
Table (21): Relation between CD 133 expression and the	90
bilharzial associated tumors	
Table (22):Relation between CD 24 expression and the	90
bilharzial associated tumors	
Table(23): Combined CD 133 and CD 24 expression.	91
Table(24): Combined CD 133 and CD 24 expression.	92
Table(25): The intensity of combined CD 133 and CD 24	93
expression	
Table (26): Relation between combined CD 133 and CD 24 expression and tumortype	94
Table (27):Relation between combined CD 133 and CD 24	95
expression and pattern of growth (TCC).	
Table (28):Relation between combined CD 133 and CD 24	
expression and tumor grade(TCC).	
Table (29):Relation between combined CD 133 and CD 24	

expression and tumor stage.	
Table (30):Relation between CD 133 and CD 24 expression	99
and clinicopathologic features of bladdercarcinoma	

List of Figures

Fig.NoTitle	Page
Fig . (1) :Molecular Oncogenesis model ofbladder carcinomaand their precursors lesions	9
Fig.(2):Urinary bladder carcinoma, Gross picture	19
Fig.(3):Precursor lesions of invasive transitionalcellcarcinoma	21
Fig.(4): Non-invasive low-grade papillary transitional cell carcinoma.	21
Fig.(5): The American Joint Committee on Cancer (AJCC) (TNMclassification for bladdercarcinoma, 7 th edition	31
Fig.(6): Role of staging in determining the line of treatmentin transitional cell carcinoma of the urinary bladder	32
Fig.(7):Self-renewalof stem cells.	36
Fig.(8):Properties and differentiation of stemcells.	37
Fig.(9):Stem cell division and differentiation.	38
Fig.(10): Pluripotent embryonic stem cells originate as inner mass cells within a blastocyst.	39
Fig.(11): Comparison of self-renewal during hematopoietic stem cell development and leukemic transformation	42
Fig.(12): The cardinal features of CSCs	43
Fig.(13):Origin of cancer stem cells	44
Fig.(14): How a cancer stem cell may arise	
Fig.(15):Stem cell specific and conventional cancer therapies	
Fig.(16): Conventional therapies may shrink tumors by killing mainly cells with limited proliferative potential	47
Fig.(17): A proposed model for the development of bladderUCCSCs.	52

Fig.(18): High grade papillary transitional cellshowing lamina	100	
propria invasion		
Fig.(19): Transitional cellcarcinoma with clear cellfeatures		
Fig.(20) :Micro-papillary variant of transitional cellcarcinoma showing perineural invasion	101	
Fig.(21): Micro-cystic variant of transitional cell carcinoma	101	
Fig.(22) : High grade non-papillary muscle invasive transitional cell carcinoma showing tumor nests	102	
	100	
Fig.(23): Nested variant of transitional cell carcinoma.	102	
Fig.(24): Keratinizing moderately differentiated squamous cel carcinoma with bilharzial ova.	103	
Fig.(25): Moderatelydifferentiated adenocarcinoma.	103	
Fig.(26): Positive Control : Normal kidney tissue (CD 133).	104	
Fig.(27): Positive Control : Normal kidney tissue(CD 133).	104	
Fig.(28): Positive Control: Invasive Ductal Carcinoma ofbreast (CD 24).	105	
Fig.(29): Low grade papillary lamina propria invasive transitional	105	
carcinoma showing negativeCD 133expression		
Fig.(30): High gradenested variant of transitional cellcarcinoma showing negative CD133expression.	106	
Fig.(31): Squamous cell carcinoma showingnegative expression of CD 24.	106	
Fig.(32): High gradetransitional cell carcinomashowingperinuclear single cell expression showing low expression of CD 133.	107	
Fig.(33): High gradetransitional cell carcinomashowing lowexpression of CD 133.	107	
Fig.(34): High gradetransitional cell carcinomashowing lowexpression of CD 133	108	
Fig.(35): High gradetransitional cell carcinomashowinglowexpression of CD 133	108	
Fig.(36):High gradetransitional cell carcinoma showinglowexpression of CD 133	109	

Fig.(37):High gradetransitional	109
cellcarcinomashowinglowexpression of CD 24	
Fig.(38):High gradetransitional	110
cellcarcinomashowinglowexpression of CD 24protein	
expression in tumor cells	
Fig.(39):Moderately differentiatedadenocarcinoma	110
showinglowexpression of CD 24	
Fig.(40): High grade transitional cell	111
carcinomashowinghighexpressionof CD 133.	
Fig.(41):High gradetransitional cell	111
carcinomashowinghighexpression of CD 133	
Fig.(42):Poorlydifferentiated squamous cell	112
carcinomashowinghighexpression of CD 133	
Fig.(43):High gradetransitional cell carcinoma (micro-cystic	112
variant.)showinghighexpression of CD 133	
Fig.(44):High gradetransitional cell	113
carcinomashowinghighexpression of CD 133	
Fig.(45):High gradetransitional cell	113
carcinomashowinghighexpression of CD 133	
Fig.(46):High grade transitional cell carcinoma with clear cell	114
features showingmembranous staining (high CD133	
expression)	
Fig.(47):High gradetransitional cell	114
carcinomashowinghighexpression of CD 133	
Fig.(48):High gradetransitional cell	115
carcinomashowinghighexpression of CD 133	
Fig.(49): High gradetransitional cell carcinomashowinghighCD 24expression	115
Fig.(50) :High gradetransitional cell carcinoma showinghigh CD 24	116
expression	110
Fig.(51):High gradetransitional cell carcinoma showing	116
perineuralinvasion with highexpression of CD 24	
Fig.(52):High gradetransitional cell carcinoma	117

showinghighexpression of CD 24	
Fig.(53):Moderately differentiated squamous cell carcinoma	117
showinghighexpression of CD 24	
Fig.(54): Moderately differentiated adenocarcinoma showinghigh	118
expression of CD 24	

List of Histograms

Graph (1):Gender distribution.	72		
Graph (2):Histological types of tumor.			
Graph (3):Grade of the tumor.	74		
Graph (4):Tumor stage.	76		
Graph (5): Expression of CD 133in bladder carcinoma cases.	77		
Graph (6): Expression of CD 24 in bladder carcinoma cases.	78		
Graph (7):Relation between CD 133 expression and the pattern of growth (TCC)			
Graph (8):Relation between CD 24expression and the pattern of growth(TCC).	84		
Graph (9): Relation betweenCD 133 expression and tumor grade.	85		
Graph (10):Relation betweenofCD 24expression and tumor grade	87		
Graph (11):Relation between CD 133 expression and tumor stage.	88		
Graph (12): Relation betweenCD 24expressionandtumorstage.			
Graph (13):Combined CD 133 and CD 24 expression			
Graph (14):Combined CD 133 and CD 24 expression.	93		
Graph (15):Combined CD 133 and CD 24 expression and tumor type.	94		
Graph (16):Relation between combined CD 133 and CD 24 expression and tumor type	95		
Graph (17):Relation between combined CD 133 and CD 24 expression and the pattern of growth (TCC).	96		
Graph (18):Relation between combined CD 133 and CD 24 expression and tumor grade.	97		
Graph (19):Relation between combined CD 133 and CD 24 expression and tumorstage.	98		

List of Abbreviations

TCC:Transitional Cell Carcinoma

SCC: Squamous Cell Carcinoma

CIS:Carcinoma In Situ

BC:Bladder Carcinoma

IHC: Immunohistochemistry

TUR: Trans-Urothelial Resection

UCs: UrothelialCarcinomas

CSCs: Cancer Stem Cells

NCI:National Cancer Institute

EBRT: External Beam Radiation Therapy

HNPCC:Hereditary Non-Polyposis Colorectal Cancer

NOS: NotOtherwise Specified

AJCC: American Joint Committee on Cancer

ICM: Inner Cell Mass

ESC: Embryonic stem cells

HSCs:Hematopoietic Stem Cells

UCC:UrothelialCarcinoma Cells

TICs: Tumor Initiating cells

CIS: Carcinoma In Situ

CT: Computed Tomography

IARC: International Agency for Research on Cancer

HGPUC:High-grade Papillary Urothelial Carcinoma

LGPUC: Low-grade Papillary Urothelial Carcinoma

HTCs: Highly Tumorigenic Cells

TIC: Tumorigenic Initiating Cancer

EMT:Epithelial Mesenchymal Transition

MET: Mesenchymalto-Epithelial Transition

PSGL-1:P-selectin Glycoprotein Ligand-1

PUNLMP:Papillary Urothelial Neoplasm of Low Malignant Potential

PAHs: polycyclic Aromatic Hydrocarbons

INTRODUCTION

Bladder cancer (BC) is the ninth most commonly diagnosed cancer worldwide, with more than 380,000 new cases each year and more than 150,000 deaths per year, and an estimated male-female ratio of 3.8:1.0 (**Siegel et al., 2013**).

It is the sixth most common cancer in the United States after lung cancer, prostate cancer, breast cancer, colon cancer, and lymphoma. It is the third most common cancer in men but only the eleventh most common cancer in women. Of the roughly 70,000 new cases annually, about 53,000 are in men and about 18,000 are in women. Of the roughly 15,000 annual deaths, over 10,000 are in men and fewer than 5,000 are in women. The reasons for this disparity between the sexes are not well understood. Estimated new cases and deaths from bladder cancer in the United States in 2014: New cases: (74,690)and Deaths:(15,580) (American Cancer Society, 2014).

Over 90% of these bladder cancers are transitional cell carcinomas (TCC) of urothelial origin (urothelial carcinomas or UCs). At presentation, over 70% will be non–muscle-invasive or stage Ta/T1 tumours, with the remainder being muscle-invasive or stages T2-4 (Van Rhijn et al., 2009).