

Detection of Aberrant Promoter Methylation of Retinoic Acid Receptor Gene (RAR) in the Urine of Bladder Cancer Patients

Thesis

Submitted for fulfillment of master degree
In biochemistry

By

Ahmed Ibrahim Abdel Fattah Nossier

B.Sc. Pharmaceutical sciences, 2001, Ain Shams University

Faculty of Pharmacy
Cairo University
2010

Detection of Aberrant Promoter Methylation of Retinoic Acid Receptor Gene (RAR) in the Urine of Bladder Cancer Patients

Thesis

Submitted for fulfillment of master degree
In biochemistry

By

Ahmed Ibrahim Abdel Fattah Nossier

B.Sc. Pharmaceutical sciences, 2001, Ain Shams University

Under supervision of

Prof.Dr. Mohamed Ahmed Hamdy

Professor of Biochemistry
Faculty of Pharmacy
Cairo University

Prof.Dr. Sanaa Eissa Mohamed

Professor of Medical Biochemistry
Faculty of Medicine
Ain Shams University

Prof.Dr. Ashraf Saad Zagloul

Professor of Surgical Oncology
National Cancer Institute
Cairo University

ACKNOWLEDGEMENT

First and foremost thanks to **ALLAH**

I wish to express my deepest gratitude and sincere appreciation to **Prof. Dr. Mohamed Hamdy**, professor of Biochemistry, Faculty of Pharmacy, Cairo University, for his guidance, kind supervision, constant support and encouragement throughout the work.

Words fail to express my deepest appreciation to **Prof. Dr. Sanaa Eissa**, professor of Biochemistry, Faculty of Medicine, Ain Shams University, for her great help starting from suggesting the point, her creative and instructive ideas in planning, discussing and revising this work.

I wish to express my deepest gratitude to **Prof. Dr. Ashraf Zagloul**, professor of Surgical Oncology, National Cancer Institute, Cairo University, for his ideal guidance and careful selection of the patients.

I wish to express my deepest gratitude to **DR. Inas el Kholy**, MD, Oncology Diagnostic Unit, Faculty of Medicine, Ain Shams University, for her help, constant support and continuous assistance throughout the course of this study.

I wish to express my deepest appreciation to **DR. Hanan Hussin**, lecturer of Biochemistry, Faculty of Medicine, Ain Shams University, for her kind supervision, constant support and her help in revising this work.

I will never forget the kind supervision and encouragement provided with *my parents*. I am also very grateful to *my wife*, for her assistance, constant support and encouragement throughout the work.

This work was supported by the Academy of Research and Technology. Technology Development and Scientific Sector, Science and Technology Center, Project 21, Egypt.

Ahmed Ibrahim Abdel Fattah Nossier

List of contents

Page No.

List of Abbreviations	I
List of Figures	VIII
List of Tables	XI
Introduction	1
Aim of Work	4
Review of Literature	4
Epidemiology of Bladder Cancer.....	5
Etiology and Risk factors.....	7
Bladder Cancer Staging.....	13
Grading of Bladder Cancer.....	17
Histopathological Types of Bladder Cancer.....	18
Diagnosis of Bladder Cancer.....	21
DNA Methylation.....	35
DNA Methylation and Gene Regulation.....	45
DNA Methylation and Cancer.....	49
DNA Methylation Analysis.....	62
Retinoids	65
Mechanism of Retinoid Receptors Action.....	73

Retinoic Acid and Cell Cycle Regulation.....	79
Loss of RAR- β_2 Expression as a Biomarker in Solid Tumors.....	80
Molecular Mechanisms Responsible for loss of RAR- β_2 Expression...	81
Molecular pathways involved in the mediation of tumor suppression by RAR- β_2	83
Materials and Methods	85
Subjects.....	85
Sample Collection and Lab Investigations.....	87
Detection of Bilharzial Antibodies in Sera	90
Molecular analysis.....	93
DNA Extraction from Urine Pellets	94
Bisulfite Conversion	97
PCR Amplification of the RAR β_2 Gene.....	101
Nested PCR Amplification of RAR β_2 Gene	105
Detection of PCR products by Agarose Gel Electrophoresis.....	109
Statistical Analysis	114
Results	117
Discussion	145
Summary and Conclusion	160
References	171
Arabic Summary	

List of abbreviations

5-ALA	5-aminolevulinic acid
ABP	4-aminobiphenyl
AF	Activation function domain
AJCC	American Joint Committee on Cancer
AP-1	Activating protein-1
APC	Adenomatous polyposis coli gene
bp	base pair
BPDE	Benzo(a)pyrene diol epoxide
BRCA1	Breast cancer 1 gene
BTA	Bard Tumor Antigen
CDKN2A	Cyclin-dependent kinase 2A
CDKs	Cyclin-dependent kinases
CFH	Complement factor H
CFH-rp	Complement factor H-related proteins
CIS	Carcinoma in Situ
CKs	Cytokeratins

COX-2	Cyclooxygenase-2
CpA	Cytosine phosphodiester Adenine
CpG	Cytosine phosphodiester Guanine
CpT	Cytosine phosphodiester Thymine
CRABP	Cytoplasmic retinoic acid binding protein
CRBP	Cytoplasmic retinol binding protein
CYP1A2	Cytochrome p450 1A2

DAPK	Death-associated protein kinase gene
DBD	DNA-Binding Domain
DMH	Differential methylation hybridization
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
DR	Direct repeat

E-cad	Epithelial cadherin gene
EDNRB	Endothelin receptor B gene
EDTA	Ethylene Diamine Tetra Acetic acid
EGFR	Epidermal growth factor receptor
ELISA	Enzyme Linked Immunosorbent Assay

Erk1/2	Extracellular signal-regulated protein kinases 1 and 2
FDP	Fibrinogen degradation products
FHIT	Fragile histidine triad gene
FISH	Fluorescent in situ hybridization
GSTM1	Glutathione S-transferase M1
GSTP1	Glutathione S-transferase π 1 gene
GTFs	General transcription factors
HAase	Hyalourindase
HAT	Histone acetyltransferases
HDAC	Histone deacetylases
HMT	Histone methyltransferase
HPLC	High performance liquid chromatography
HPV	Human papilloma virus
IHA	Indirect Haemagglutination Assay
ISUP	International Society of Urological Pathology

JNK	Jun amino-terminal kinase
LBD	Ligand-Binding Domain
LOH	Loss of heterozygosity
Lys	Lysine
M	Distant metastasis
MAPK	Mitogen-activated protein kinase
MBDs	Methyl-binding domain proteins
MGMT	O(6)-methylguanine-DNA methyltransferase gene
MKK	Mitogen-activated protein kinase kinase
MLH1	MutL homologue 1 gene
MMP	Matrix metalloproteinase
Ms-AP-PCR	Methylation-sensitive arbitrarily primed polymerase chain reaction
MSP	Methylation-specific polymerase chain reaction
MTA2	Metastasis-associated protein 2
MUMA	Nuclear mitotic apparatus

N	Regional lymph node metastasis
NAT2	N-acetyltransferase 2
NCI	National Cancer Institute
NCoR	Nuclear receptor corepressor
NMP22	Nuclear matrix protein22
NNCs	N-nitroso compounds
NPV	Negative predictive value
NSCLC	Non-small cell lung cancer
p53	Tumor suppressor gene
PAX6	Paired box transcription factor 6 gene
PBS	Phosphate buffer saline
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PI-3K	Phosphoinositide-3 kinase
PPV	Positive predictive value
RA	Retinoic acid
RARs	Retinoic acid receptors
RARβ	Retinoic acid receptor β gene
RAREs	Retinoic acid response elements

RASSF1a	Ras association domain family 1A gene
Rb	Retinoblastoma protein
RbAp46/48	Retinoblastoma-associated protein 46/48
RBCs	Red Blood Cells
RLGS	Restriction landmark genomic scanning
RNA	Ribonucleic acid
RNA pol II	RNA polymerase II
RRIG1	Retinoid receptor-induced gene-1
RT-PCR	Reverse transcriptase polymerase chain reaction
RXR_s	Retinoid X receptors
SAP18/30	Sin3-associated polypeptides 18/30
SCC	Squamous Cell Carcinoma
SMCC	Srb and Mediator protein containing complex
SMRT	Silencing mediator for retinoid and thyroid hormone receptor
SPSS	Statistical Package for the Social Sciences
T	Size of primary tumors
T.B.E	Trizma. Boric acid .EDTA

TCC	Transitional Cell Carcinoma
TF	Transcription factors
TGS	Transcriptional gene silencing
TPA	Tissue polypeptide antigen
UBC	Urinary bladder cancer
VHL	Von Hippel–Lindau gene
WBCs	White Blood Cells
WHO	World Health Organization
ZF	Zinc-finger domain

List of Figures

Fig. No.	Page No.
Figure (1):	Diagram showing the T stages of bladder cancer15
Figure (2):	DNA methylation reaction catalyzed by DNA methyltransferase39
Figure (3):	The DNA methyltransferases of mammals43
Figure (4):	A family of methyl-CpG-binding proteins45
Figure (5):	Mechanism of gene inactivation by DNA methylation48
Figure (6):	Possible roles of increased CpG islands and decreased global DNA methylation in tumour development50
Figure (7):	DNA methylation changes in cancer cells.....51
Figure (8):	Naturally occurring retinoids66
Figure (9):	Functional domains and the major phosphorylation sites of nuclear retinoid receptors70
Figure (10):	Retinoids response elements73

Figure (11):	Three-step mechanism of retinoid receptor action	78
Figure (12):	Positivity rate of Bilharziasis among different groups of study	120
Figure (13):	Positivity rate of cytology in urine samples of different groups of study	123
Figure (14):	Positivity Rate of RAR β_2 gene methylation among different groups of study	126
Figure (15):	MS-PCR product analysis of urinary RAR β_2 gene by agarose gel electrophoresis in the normal group.....	128
Figure (16):	MS-PCR product analysis of urinary RAR β_2 gene by agarose gel electrophoresis in the benign group.....	129
Figure (17):	MS-PCR product analysis of urinary RAR β_2 gene by agarose gel electrophoresis in the malignant group.....	130
Figure (18):	Roc curve analysis for the PMA of RAR β_2 gene in malignant group versus benign and normal control groups	135