Some Biochemical and Molecular Biology Studies on Bladder Cancer

Thesis
Submitted for the Fulfillment of
Ph.D. Degree in Biochemistry



By

Menha Malimoud Ahmed Swilam

B.Sc., M.Sc. Biochemistry Research Assistant National Research Center Cairo-Egypt

180 G 3

Under Supervision of

Prof. Dr. Mohamed M. Abdel Fattah
Professor of Biochemistry
Vice Dean for Postgraduate & Research
Faculty of Science
Ain Shams University

M. A. Faltal

Prof. Dr. Ali Khalifa Ali
Professor of Biochemistry
Head of Oncology
Diagnostic Unit
Faculty of Medicine
Ain Shams University

Porf. Dr. Yehia M. Shaker Professor of Biochemistry Head of Biochemistry Department National Research Center Dr. Sanaa Eissa Hamed Ass. Prof. of Biochemistry Faculty of Medicine Ain Shams University

Xelio chak Biochemistry Department
Faculty of Science
Ain Shams University
1999



Approval Sheet

Thesis entitled:

"Some biochemical and Molecular Biology Studies on Bladder Cancer"

Thesis supervisors

1-Prof.Dr. Mohamed M. Abdel Fattah M. A. Fattal Professor of Biochemistry Department - Vice Dean for Postgraduate and Research - Faculty of Science-Ain Shams University

2- Prof.Dr. Ali Khalifa Ali

Professor of Biochemistry Department - Head of Oncology Diagnostic Unit - Faculty of Medicine -Ain Shams University

3-Prof. Dr. Yehia Mohamed Shaker Line Clark Professor and Head of Biochemistry Department **National Research Center** 52 Cisso

4-Dr. Sanaa Eissa Hamed

Assistant Professor of Biochemistry Department-Faculty of Medicine – Ain Shams University

Head of Biochemistry Department

Prof.Dr. Nadia Abdelah

Ain Shams University Faculty of Science

Title Sheet

Student Name: Menha Mahmoud Ahmed Swilam

Degree: Doctor of Philosophy of Science in

Biochemistry

Department: Biochemistry Department

Faculty: Faculty of Science

University: Ain Shams University

Graduation year: 1989

Abstract

Flow cytometric analysis of DNA ploidy and SPF was performed on 136 bladder cancer patients and the proliferative rate was also emphasized using BrdU labeling technique. Moreover adaptation and evaluation of a new ELISA method for quantifying p21^{WAF-1/CIP1} in cell lysate was carried out in this study.

The results obtained indicated that DNA ploidy was only significant with the pathological type while SPF was significantly correlated with lymph node status, suggesting that FCM provide the urologist more prognostic information beyond that obtained from the clinicopathological data.

For BrdU labeling technique specimens with high SPF were high percentage of positive BrdU labeling

Considering the quantitation of p21^{WAF-1/CIP1} by ELISA it was confirmed by immunodot blotting and western blotting, the amount of p21^{WAF-1/CIP1} was significantly correlated with the pathological type, bilharziasis and age suggesting that detection of p21^{WAF-1/CIP1} in bladder tumors may be useful as prognostic value .

Acknowledgement

First and foremost thanks to God

I would like to extend great appreciation and gratitude to Prof.Dr. Mohamed Mohamed Abdel Fattah, Professor of Biochemistry Department and Vice Dean Research, Faculty of Science, Ain Shams University, for his kind supervision, continuous encouragement and generous attitude. His tremendous effort and intelligent remarks to finish up this work are highly appreciated. I owe my deepest thanks for his tutorial support and profound reading of the manuscript.

The words can never express my infinite gratitude to Prof.Dr. Ali Khalifa Ali, Professor of Biochemistry and Head of Oncology Diagnostic Unit, Ain Shams Faculty of Medicine. My deepest thanks for giving me the chance to work in this field and valuable advice throughout the work and ending by his prominent fingerprints during the finalization of his work. It is a great honour to work under his guidance and supervision.

I would like to express my thanks to Prof. Dr. Yehia Mohamed Shaker, Professor and Head of Biochemistry Department, National Research Center, for his sincere guidance, kind assistance and valuable advice to the accomplishment of this work. Many thanks for his great interest, encouragement in performing my work. His continuous advice, constant support are highly appreciated.

I have the greatest pleasure in acknowledgement Dr. Sanaa Eissa Hamed, Assistant Professor of Biochemistry, Faculty of Medicine, Ain Shams University, for her willing assistance, great guidance and continuous encouragement during the course of this study. I am indebted to her for teaching me the proper way of scientific thinking.

I am also grateful to Dr. Lila Saeda, lecturer of pathology, Faculty of Medicine, Banha University, for her kind assistance and for giving me the chance to learn the recent techniques applied in this work.

Finally, I would like to express my deepest gratitude and appreciation to my husband for his kind encouragement and constructive effort to accomplishment this research.

Contents

	Page
* List of abbreviations	I
* List of tables	V
* List of Figures	VII
* Introduction and aim of work	1
* Review of literature	4
Bladder cancer	4
-Epidemiology of bladder cancer	4
-Etiology of bladder cancer	5
-Anatomy of the urinary bladder	17
-Structure of the urinary bladder	18
-Blood supply of the urinary bladder	19
-Clinical presentation of bladder cancer	20
-Classification of the urinary bladder tumors	20
-Metastatic spread of bladder carcinoma	25
-Bladder cancer staging and grading	26
Flow cytometry	31
-History of FCM	31
-Principles of FCM	33
-Clinical FCM instrumentation	33
-Anatomy of FCM	35
-Determination of DNA content as a genetic marker of cancer cells using FCM	41
-DNA analysis by single parameter FCM and synthetic- phase fraction (FCM-SPF)	45
-Methodology of DNA analysis by FCM	49
-Applications of FCM	57
-Flow cytometry of bladder cancer	62

	Page
Cellular growth and differentiation, normal growth	67
and cancer development	
-Cell cycle and types of cells	67
-Regulation of cell cycle	69
-Regulatory genes and cancer development	73
Wild-type p53 activated fragment (WAF-1)	86
-Structure	86
-Mode of p21 ^{WAF-1/CIP1} association with cyclin-cdk complexes	89
-Role of p21 ^{WAF-1/CIP1} with p53-tumor suppressor gene	91
-Role of p21 WAF-1/CIP1 in oncology	94
* Material and Methods	100
-Tissue samples	100
-DNA analysis by FCM	102
-Preparation of cell lysate	107
-Estimation of protein concentration in the cell lysate	108
-Quantitative estimation of p21 WAF-1/CIP1 protein in cell	111
lysate fraction	
-Immunodot blot for p21 ^{WAF-1/CIP1} protein	115
-SDS polyacrylamide gel electrophoresis (SDS-PAGE)	117
-Western blotting for p21 ^{WAF-1/CIP1} protein	121
-Immunohistochemical localization of BrdU labeling	131
* Results	138
-Bilharziasis of bladder cancer in relation to clinicopathological parameters	138
-Pathological types of bladder cancer in relation to other clonicopathological data	138
-Lymph node status in relation to with stage, grade of the tumor and patient's age	138
-Correlation between stage and grade of the bladder tumor	138
-Flow cytometric analysis of bladder cancer	147

	Page
-Relation of DNA ploidy and clinicopathological parameters	147
-Relation of SPF and clinicopathological parameters	147
-Relation of DNA ploidy and synthetic phase fraction (SPF)	147
-BrdU labeling of bladder cancer	158
-ELISA performance characteristics for p21 ^{WAF-1/CIP1} expression	162
-Precision of the p21 ^{WAF-1/CIP1} ELISA assay	162
-Lower detection limit of p21 ^{WAF-1/CIPI} ELISA assay	162
-Analytical recovery of p21 WAF-1/CIP1 ELISA assay	162
-Cutoff point for p21 ^{WAF-1/CIPI} protein expression in bladder cancer	162
-Confirmation of p21 ^{WAF-1/CIP1} expression by ELISA,	165
•	165
immunodot blot and western blot	
-p21 ^{WAF-1/CIP1} expression in relation to clinicopathological data	169
-p21 ^{WAF-1/CIP1} expression in relation to DNA ploidy and SPF	169
-DNA ploidy and its relation to SPF and p21WAF-1/CIP1	169
* Discussion	175
* Summary and conclusion	188
* References	191
	226
* Appendix	220
* Arabic Summary	