

Serum MicroRNA-21(miR-21) as a Diagnostic Biomarker in human ovarian cancer and its relation with Programmed Cell Death 4(PDCD4) protein.

**Thesis submitted for partial fulfillment of
Master's degree in Clinical and Chemical Pathology by**

Marie-Claire Mounir Abdel-Messih

M.B.B.Ch., Cairo University

Under Supervision of

Prof. Dr. Ola Abdel Meneim El-Sisi

Professor of Clinical and Chemical Pathology

Faculty of Medicine

Cairo University

Dr. Enas Hamdy Mahmoud

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine

Cairo University

Dr. Amal Fawzy Mohammed

Assistant Professor of Clinical and Chemical Pathology

National Cancer Institute

Cairo University

استخدام مصل الحمض الريبونيوكلينك متناهي الصغر 21 كدلالة تشخيصية في سرطان المبيض وعلاقته ببروتين موت الخلية المبرمج 4.

رسالة توطئة للحصول على درجة الماجستير في الباثولوجيا الإكلينيكية و الكيميائية
مقدمة من

الطبيبة/ماريكليز منير عبدالمسيح

بكالوريوس الطب والجراحة
جامعة القاهرة

تحت إشراف

أ.د. علا عبد المنعم السيسى

أستاذ الباثولوجيا الإكلينيكية والكيميائية
كلية الطب
جامعة القاهرة

د. ايناس حمدي محمود

أستاذ مساعد الباثولوجيا الإكلينيكية والكيميائية
كلية الطب
جامعة القاهرة

د. أمل فوزي محمد

أستاذ مساعد الباثولوجيا الإكلينيكية والكيميائية
المعهد القومي للأورام
جامعة القاهرة

Acknowledgement

First of all, I would like to thank God for his grace, mercy, and for giving me the effort to complete this work.

I would like to express my great indebtedness and deep gratitude to **Prof. Dr. Ola Abdel-Meneim El-Sisi**, professor of clinical and chemical pathology, Faculty of Medicine, Cairo University for accepting the idea of this work, her kind assistance and great efforts, which helped me in accomplishing this thesis.

I also extend my profound thanks and appreciation to **Dr. Enas Hamdy**, Assist. Prof. of Clinical & Chemical Pathology, Faculty of Medicine, Cairo University for her invaluable guidance, great help and patience all through this work.

I would definitely like to thank **Dr. Amal Fawzy**, Assist. Prof. of Clinical & Chemical Pathology, NCI, Cairo University, for her support from the initial to the final level which enabled me to develop an understanding of the subject. She kindly offered me her valuable meticulous scientific help, precious time and effort.

I would also like to thank my colleagues of the clinical and chemical pathology department at national cancer institute, Cairo University for their great support.

To ovarian cancer patients, for whose benefit the present work was conducted, I wish it would help them a step forward.

I take this opportunity to express my profound gratitude to my beloved parents for their moral support and patience during completion of this work.

No words would ever be enough to express my feelings and gratitude to my lovely sincere husband for his great support, help and encouragement all through this work.

CONTENTS:

LIST OF FIGURES:	iv
LIST OF TABLES:	vi
LIST OF ABBREVIATION:	viii
ABSTRACT	xii
INTRODUCTION AND AIM OF WORK	1
REVIEW OF LITERATURE	4
CHAPTER I: THE OVARY	5
1-The Ovary:.....	5
1.1 Anatomy of the ovary:.....	5
1.2 Histology of the ovary:.....	6
1.3 The female (ovarian) cycle:.....	6
2- Ovarian Cancer:	8
2.1 Incidence and Epidemiology:.....	8
2.2 Risk factors:.....	10
3. Pathology of Ovarian Cancer:	14
3.1 Pathological types:	14
3.2 Staging of ovarian cancer:.....	17
3.3 Grading of ovarian cancer:	19

4. Diagnosis of Ovarian Cancer:	20
5. Treatment of Ovarian Cancer:	25
CHAPTER II: MICRORNAs	30
1. Discovery:.....	30
2. Biogenesis:.....	31
2.1 MiRNA Biogenesis	31
2.2 Regulation of MicroRNA Biogenesis	33
3. MicroRNA turnover:	35
4. MicroRNA Functions:	35
4.1 Gene Silencing:	35
4.2 Cellular development and differentiation:	36
4.3 MicroRNA and disease:	37
5. Clinical Implications of MiRNAs in Cancers:	41
5.1 Diagnostic Applications of MiRNAs in different cancers:	42
5.2 Therapeutic Application of MiRNAs:.....	45
CHAPTER III: MICRORNA-21	46
1. Introduction:.....	46
2. Regulation of miR-21:	47
3. Role of miR-21 in development:	51
4. Role of miR-21 in immune system:	52
5. Role of miR-21 in Cancer:.....	53

6- MicroRNA-21 and Ovarian Cancer	56
7- Identification of direct miR-21 targets.....	58
CHAPTER IV: PROGRAMMED CELL DEATH 4	64
1. Structure and subcellular distribution of PDCD4:.....	64
2. Regulation of PDCD4:.....	65
3. Function of PDCD4:	67
3.1 Role of PDCD4 in tumor development:.....	67
3.2 Potential Application of PDCD4 in Oncology:.....	68
4. Interaction between MiRNA 21 and PDCD4.	70
SUBJECTS AND METHODS	72
1. Samples collection:.....	74
2. Detection of serum miR-21 by Real-Time RT-PCR.....	74
3. Detection of serum protein PDCD4 by ELISA technique:	86
4. Statistical Methods:	91
RESULTS	92
DISCUSSION	109
SUMMARY AND CONCLUSION	119
RECOMMENDATIONS	122
REFERENCES	124

LIST OF FIGURES:

Figure 1: showing normal anatomy of the female reproductive system ...	6
Figure 2: Microscopic structure of the ovary and the ovarian cycle.....	7
Figure 3: Schematic drawing showing sites of origin of ovarian cancer	14
Figure 4: MiRNA biogenesis and mechanism of function	33
Figure 5: Workflow summarizing the progressive steps and the different issues considered to investigate the hypothesis of a concrete role of miRNAs in cancer biology and to support their possible clinical applications	40
Figure 6: Potential use of miRNAs in cancer diagnosis, prognosis and treatment	42
Figure 7: Conserved stem-loop structure of miR-21	46
Figure 8: Genomic location of pri-miR-21.....	47
Figure 9: miR-21 target genes and the next-generation hallmarks of cancer.	60
Figure 10: Structure of PDCD4.....	64
Figure 11: Overview of the known regulatory mechanisms for PDCD4 levels.	66
Figure 12: Example of amplification curve.....	85
Figure 13: preparation of 7 points of diluted standard	87
Figure 14: Standard Curve for PDCD4, Human ELISA.	90

Figure 15: Percentages of pathological grades of ovarian cancer.....	94
Figure 16: Percentages of FIGO stages in ovarian cancer patients.....	95
Figure 17: Comparison of miR-21 relative expression level between ovarian cancer patients and control group	98
Figure 18: comparison of PDCD4 level in ovarian cancer group and control group.	99
Figure 19: ROC curve of miR-21relative expression for diagnosis of ovarian cancer	105
Figure 20: ROC curve of PDCD4 (ng/ml) for diagnosis of ovarian cancer.	106
Figure 21: Correlation between relative expression of miR-21 and PDCD4 level in whole group.....	108

LIST OF TABLES:

Table1: Ranking of female malignant tumors- 4939 cases attending the Egyptian NCI (Statistics of gynecologic cancers, 2004).....	9
Table 2: Relative frequency of female genital tract malignancies – 462 cases attending NCI (Statistics of gynecologic cancers, 2004).....	9
Table 3: Histopathologic types of malignant ovarian tumors – 135 cases attending NCI (Statistics of gynecologic cancers, 2004).....	10
Table 4: Epithelial tumors: make up to 60–90% of all ovarian tumors; and make up 90% of all malignant ovarian tumors. (Hulse et al, 2004)	15
Table 5: TNM and FIGO Classifications for Ovarian Cancer (Edge et al., 2010).....	18
Table 6: Potential miRNAs biomarkers for types of cancers in blood and other body fluids. (Huiyin et al., 2015).	43
Table 7: shows factors Involved in miR-21 Gene Regulation (Jazbutyte V. and Thum, 2010).....	50
Table 8: primer sequence of miR-21 and U6	80
Table 9: shows Master Mix preparation	81
Table 10: Thermal cycling condition for the thermal cyclers.....	82
Table 11: Shows the amount of each component used in the PCR reaction.....	83
Table 12: Thermal cycling condition for probe optimization.	84

Table 13: Characteristics of the studied groups.....	94
Table 14: Frequency of the Clinicopathological Variable of the studied groups.....	96
Table 15: The pathological subtypes frequencies of malignant ovarian tumors.....	97
Table 16: level of miR-21 in ovarian cancer patients and control group.	98
Table 17: level of PDCD4 in ovarian cancer patients and healthy controls.	99
Table 18: showing relation between Serum miR-21 level, PDCD4 level and clinicopatological characteristics of ovarian cancer group	103
Table 19: showing Diagnostic accuracy of used markers.	104
Table 20: showing correlation between miR-21 and PDCD4	108

LIST OF ABBREVIATION:

3'UTR	3' –untranslated region
Ago	Argonaute
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AMO	Oligodeoxyribonucleotide
AP-1	activator protein 1
AR	androgen receptor
ara-C	arabinosyl cytosine
AST	Aspartate Transaminase
ATF2	Activating Transcription Factor 2
AUC	Area under the curve
Bcl-2	B-cell lymphoma 2
B-CLL	B-cell chronic lymphocytic leukemia
BMP4	Bone Morphogenetic Protein 4
BRCA1	Breast cancer type 1
BRCA2	Breast cancer type 2
Btg2	B-cell translocation gene 2
C/EBPα	CCAAT-enhancer-binding proteins
CA	serous cystadenoma
CA II	carbonic anhydrase type II
CA125	Cancer Antigen 125
CBC	Complete blood count
CDK12	Cyclin-dependant kinase 12
CEBPA	CCAAT/enhancer-binding protein alpha
COX-2	cyclo-oxygenase-2
CT	computed tomography
CT	Computed Tomography
DGCR8	DiGeorge syndrome critical region gene 8
DNA	Deoxyribonucleic acid
E2	Estrogen
EBV	Epstein–Barr virus
eIF	eukaryotic translation initiation factor
ELISA	enzyme-linked immunosorbent assay
EOC	Epithelial ovarian carcinoma
EREs	estrogen response elements

Era	estrogen receptor alpha
Erβ	estrogen receptor beta
ES	Embryonic stem
FDR	First degree relatives
FIGO	International Federation of Gynecology and Obstetrics
FNA	Fine-needle aspiration
FSH	Follicle-stimulating hormone
Gfi1	Growth factor independent-1
GI	Gastrointestinal
HE4	Human Epididymis Protein 4
HEK	human embryonic kidney
IL-12a or IL-12p35	Interleukin-12p35
LH	Luteinizing hormone
LLD	Lower Limit of Detection
LMP	Low Malignant Potential
LNA	locked nucleic acids
MCF7	Michigan Cancer Foundation-7
M-CSF	macrophage colony-stimulating factor
miR-21	MicroRNA-21
miRNA	MicroRNA
MMPs	Matrix metalloproteinases
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MyC	myelocytomatosis protein
NCI	National cancer Institute
NF1	Neurofibromin 1 gene
NFE2L1	nuclear factor erythroid-2-like 1
NFE2L2	Nuclear Factor, Erythroid 2 Like 2
NFI	nuclear factor I
NFIB	Nuclear Factor I B
NLS	nuclear localization sites
NOS	Not otherwise specified
NPM1	Nucleophosmin
NPV	negative predictive value

Nt	Nucleotides
O.D.	optical density
OSC	ovarian serous carcinomas
OVCAR3	ovarian papillary adenocarcinoma cell lines
OVX1	Ovarian Cancer Antigen X1
P53	protein 53
PAZ domain	named after the proteins Piwi, Argonaut and Zwillie
P-bodies	processing bodies
PDCD4	Programmed Cell Death 4
PET	Positron emission tomography
PI3K	phosphoinositide 3 kinase
PIWI domain	P-element induced wimpy testis
PMA	para-Methoxyamphetamine
PPARγ	peroxisome proliferator-activated receptor γ
PPV	positive predictive values
pre-miRNA	Precursor microRNA
pri-miRNAs	primary miRNA
PTEN	Phosphatase and tensin homologue
qPCR	Quantitative Polymerase chain reaction
RAR	retinoic acid receptor
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
RNase	Ribonuclease
ROC	The Receiver Operating Characteristic
RT	reverse transcription
SE	Standard error
Sp1	specificity protein 1
SPRY2	Sprouty2
Stat3	Signal transducer and activator of transcription 3
STAT4	Signal Transducer And Activator Of Transcription 4
TAG 72	tumor-associated glycoprotein 72

TGFBR2	TGF β receptor 2
TGF-β1	Transforming growth factor beta 1
TMB	Tetramethylbenzidine
TNM	Tumor Node Metastases
TPA	tetradecanoylphorbol-13-acetate
TPM1	Tropomyosin 1
TRBP	transactivating response RNA binding protein
UICC	The international Union against cancer
uPAR	urokinase receptor
UTR	untranslated region
YY1	Yin Yang 1
β-hCG	β -human chorionic gonadotropin