Clinical Utility of RASSF1A Gene Methylation Assayed By Methylation Specific Polymerase Chain Reaction In Ovarian Cancer Patients

Thesis

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بسم الله الرحمن الرحيم

﴿وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا﴾

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Al Shimaa Fahem Ali Omara

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List of Abbreviations

Abb.	Full term
AFP	*Alpha-fetoprotein*
ARID1A	*AT-rich interactive domain 1A*
BRCA1	*Breast cancer 1*
BRCA2	*Breast cancer 2*
CA 19-9	*Cancer antigen 19-9*
CA125	*Cancer antigen 125*
CDKN2A	*Cyclin-dependent kinase inhibitor 2A*
CEA	*Carcinoembryonic antigen*
CG	*Cytosine-guanine*
CICs	*Cortical inclusion cysts*
CLIA	*Chemiluminescent immunoassay*
COBRA	*Combined bisulfite restriction analysis*
CpG	*Cytosine phosphodinucleotides guanine*
CT	*Computerised topography*
DNA	*Deoxynucleic acid*
DNMTs	*DNA methyltransferases*
dNTP	*Deoxynucleoside triphosphates*
E2	*Estradiol*
ECLIA	*Electrochemiluminescence immunoassay*
ELISA	*Enzyme-linked immunosorbent assay*
EOC	*Epithelial ovarian cancer*
EORTC	*European Organization for Research and Treatment of Cancer*
ERBB2	*Erythroblastic leukemia viral oncogene homologue 2*
FDA	*Food and Drug Association*
FH.BC	*Family history of breast cancer.*
FH.OC	*Family history of ovarian cancer.*

List of Abbreviations Cont...

Abb.	Full term
FSH	*Follicle-stimulating hormone*
HBOC	*Hereditary breast and ovarian cancer*
HE4	*Human epididymis tissue protein E4*
HGSCs	*High-grade serous carcinomas*
HMTs	*Histone methyltransferases*
HNPCC	*Heritable non-polyposis colorectal cancer*
HS	*High significant*
ILs	*Interleukins*
IQR	*Inter-quartile range*
KRAS	*Kirsten rat sarcoma 2*
LH	*Luteinising hormone*
M11	*Murin11*
miRNAs	*Micro RNA*
MMR	*Mismatch repair*
MRI	*Magnetic resonance imaging*
MSP	*Methylation-specific polymerase*
NPC	*Nasopharyngeal carcinoma*
NPV	*Negative predictive value*
NS	*Non significant.*
OCPs	*Oral contraceptive pills*
OEC	*Ovarian epithelial carcinoma*
OR	*Odd's ratio*
OSE	*Ovarian surface epithelium*
P53	*Protein53*
PIK3CA	*Phosphatidylinositol 3-kinase catalytic alpha*
PTEN	*Phosphatase and tensin homologue*
RA	*Ras associated*
Ras	*Rat sarcoma*

List of Abbreviations Cont...

Abb.	Full term

RASSF..... Ras association domain family
ROMA..... Risk of Malignancy Algorithm
ssDNA..... Single stranded DNA
TSGs ..... Tumor suppressor genes
TSH..... Thyroid stimulating hormone
TVU ..... Transvaginal Ultrasound
WAP ..... Whey-acidic-protein
WHO ..... World Health Organization
β-HCG..... Beta-human chorionic gonadotropin

INTRODUCTION

Ovarian cancer is the leading cause of gynaecologic cancer death, although it constitutes only 3% of all female cancers worldwide ***(Hennessy et al., 2009*)**. Despite availability of screening measures, such as transvaginal ultrasound, cancer antigen 125 (CA125) or a combination of both modalities, mortality rates remain high due to the highly heterogeneous nature of ovarian cancer ***(Chu and Rubin, 2006 and American Cancer Society, 2012).***

Ovarian epithelial carcinoma (OEC) is the most common ovarian malignancy worldwide, with substantial histopathological heterogeneity. According to World Health Organization (WHO) classification scheme (2003), the most common histologic subtype is serous ovarian carcinoma (59%), while other subtypes include endometrioid (15%), clear cell (5%), transitional (8%), mucinous (9%), and undifferentiated (5%) subtypes ***(Leitzmann et al., 2009).***

The widely used "gold standard" tumor biomarker CA125, a high molecular weight glycoprotein, has limited sensitivity between 50% and 60% ***(Sreeja et al., 2012)***. Moreover, CA125 is elevated in some benign conditions, its levels exhibit fluctuations associated with menstrual cycle and pregnancy, all of which limit its specificity. As a result, CA125 assay has not been recommended in screening guidelines for the general population ***(Toss et al., 2015).***

It is recognized that both genetic and epigenetic events play a role in the development of ovarian cancer ***(Wei et al., 2006).*** Epigenetic changes are changes in gene expression, with no changes in DNA sequence, which are inheritable through mitosis or meiosis and lead to phenotypic changes ***(Chong et al., 2004).***

DNA methylation comprises the best-known epigenetic mechanism associated with gene expression. DNA methylation occurs on the cytosine residues of CG dinucleotides (also designated as CpG). Enzymes known as DNA methyltransferases (DNMTs) catalyse the addition of a methyl group to the cytosine ring to form methyl cytosine, employing S-adenosylmethionine as a methyl donor ***(Herman et al., 2003).***

The aberrant methylation of CpG islands in gene promoters has been correlated with a loss of gene expression, and it appears that DNA methylation provides an alternative pathway to gene deletion or mutation for the loss of tumor suppressor gene function ***(Toss et al., 2015).***

The RASSF family of tumor suppressor genes encode Ras superfamily effector proteins that, among their functions, mediate some of the growth inhibitory functions. Several members of this family are inactivated by promoter DNA hypermethylation; and, hence, inactivation of RASSF1 has been described in a growing number of tumor types ***(Baylin and Chen, 2005).***

Therefore, a study on the molecular mechanism underlying ovarian cancer progression, including a search for methylation status, is important for early diagnosis and effective therapy for ovarian cancer. There are limited data about the genetic cause in ovarian cancer ***(Tcherkassova et al., 2011).***

AIM OF THE WORK

The aim of this study is to investigate the association between methylation of RASSF1A and ovarian cancer and correlate results with the clinicopathological features of the disease, as well as with the tumor marker CA 125.

Chapter 1

OVARIAN CANCER

I) Epidemiology of Ovarian Cancer:

Although ovarian cancer has a life time risk of only 1.3% in the general population and accounts for only 1.3% of all new cancers and representing 3.8 % of all females' malignancies, it is the fifth-leading cause of cancer-related deaths in women ***(American Cancer Society, 2016).*** According to ***National Institutes of Health (2016)*** there are more than 22,200 new cases of ovarian cancer and more than 14,200 deaths from ovarian cancer in the United States ***(National Institutes of Health, 2016).*** According to the National Population-Based Cancer Registry Program in Egypt (2008 –2011); ovarian cancer is the fourth most common cancer among females with crude and age standardized incidence rates (4.6 and 6.3) per 100,000 population, respectively ***(Ibrahim et al., 2014).***

II) Classification of Ovarian Cancer:

The World Health Organization Histological Classification for ovarian tumors separates ovarian neoplasms according to the most probable tissue of origin: surface epithelial (65%), germ cell (15%), sex cord-stromal (10%), metastases (5%) and miscellaneous. Surface epithelial tumors are further classified by cell type (serous, mucinous,