

# Differential Protein Expression By Normal And Malignant Ovarian Epithelial Cells

Thesis  
Submitted For Partial Fulfillment of  
M.D. Degree in Obstetrics & Gynecology

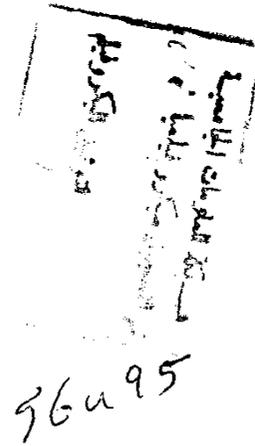


By

616.9946  
S.T.

**Salah Taha Ahmad Fayed**  
M.Sc. Obstetrics & Gynecology

Under Supervision of



**Prof. Dr. Khalil I. El Lamei**  
Chairman and Professor Of Dept.  
Of Obstetrics And Gynecology  
Faculty Of Medicine  
Ain Shams University

**Prof. Dr. Ali Khalifa Ali**  
Professor Of biochemistry  
Head Of Oncology Diagnostic Unit  
Faculty Of Medicine  
Ain Shams University

**Prof. Dr. Robert C. Bast Jr.**  
Chairman of Dept. Of medicine  
MD Anderson Cancer Center  
University Of Texas

**Dr. Maged R. Abu Seida**  
Assistant Professor Of  
Obstetrics And Gynecology  
Faculty Of Medicine  
Ain Shams University

**Ain Shams Faculty Of Medicine**

**1995**

## ***ACKNOWLEDGMENTS***

### ***First and foremost thanks to God***

Before presenting this thesis I would like to express my deep gratitude to ***Prof. Dr. Khalil El Lamei***, Chairman of Obstetrics & Gynecology department, Ain Shams Faculty Of Medicine, for his invaluable advice and his sincere help.

I really appreciate and feel indebted to ***Prof. Dr. Ali Khalifa*** Professor of Biochemistry and Head of Oncology Diagnostic Unit, Ain Shams Faculty Of Medicine for giving me the chance of doing this work and for his kind support and help throughout the duration of this thesis.

I wish to thank ***Dr. Maged Abu Seeda***, Assistant Professor of Obstetrics & Gynecology, Ain Shams Faculty of Medicine, for his valuable remarks and great help.

I am deeply indebted for ***Prof. Dr. Robert C. Bast***, Chairman of department of Medicine, MD Anderson Cancer Center, University of Texas, for his support, patience and his valuable guidance while working in his laboratory for ovarian and breast cancer biology at Duke Medical Center.

I do appreciate and thank ***Dr. Jon Wiener***, Associate Prof. in department of Medical Oncology, MD Anderson Cancer Center, for everything he has done throughout this work. He is the one I owe a lot of this work.





## Table Of Contents

• <b>Introduction &amp; Aim of the work</b> .....	1
• <b>Review</b>	
Ovarian Cancer, Epidemiology and Etiology .....	3
Ovarian Cancer, Biology .....	10
Ovarian Cancer, Pathology.....	15
Tumor Markers In Ovarian Cancer.....	18
Strategies For Tumor Marker Development .....	29
• <b>Materials and methods</b>	
<b>Cell culture</b> .....	37
(I) Labeling of cell proteins .....	38
(II) Sample preparation .....	39
<b>Two dimensional gel electrophoresis</b> .....	40
(I) First dimension isoelectric focusing (IEF) .....	40
(II) Second dimension SDS-PAGE .....	43
<b>Protein purification</b> .....	46
[I] Large scale cell culture supernatant preparation .....	46
[II] Concentration of supernatant .....	47
<b>Purification techniques</b> .....	48
Precipitation techniques: Introduction .....	48
(I) Ammonium sulfate precipitation .....	49
(II) PEG precipitation .....	52
Hydrophobic Interaction Chromatography (HIC) .....	53

Ion Exchange Chromatography (IEC) .....	55
Gel Filtration .....	58
• <b>RESULTS</b>	
Analysis of differential P30 protein expression by human ovarian epithelial cancer cell lines and normal ovarian epithelial cells.....	62
Purification of the p30 protein .....	88
(A) Expression of the p30 in the absence of serum .....	89
(B) Fractional precipitation of the p30 protein .....	94
(C) Hydrophobic Interaction Chromatography .....	102
(D) Ion Exchange Chromatography (IEC) .....	105
(E) Gel Filtration .....	111
(F) Ion Exchange Chromatography (IEC) .....	121
• <b>DISCUSSION</b> .....	127
• <b>SUMMARY &amp; CONCLUSION</b> .....	136
• <b>REFERENCES</b> .....	139
• <b>ARABIC SUMMARY</b>	



## LIST OF FIGURES

<b>Fig 1.</b> Ovarian cancer rates by age for 1973-1977 and 1978-1981.....	4
<b>Fig 2.</b> Age-adjusted ovarian cancer incidence by year and race for 1973-1986.....	4
<b>Fig 3.</b> Schematic representation of the stages of hybridization .....	32
<b>Fig 4.</b> Theoretical basis of ion exchange chromatography (IEC).....	57
<b>Fig 5.</b> Theoretical basis of gel filtration chromatography .....	59
<b>Fig 6.</b> 2-D gel electrophoresis of cell culture supernatant from the ovarian cancer cell line OVCA 429. [ <sup>35</sup> S]-methionine metabolic labeling.....	63
<b>Fig 7.</b> 2-D gel electrophoresis of cell culture supernatant from the ovarian cancer cell line OVCA 433.....	65
<b>Fig 8.</b> 2-D gel electrophoresis of cell culture supernatant from the ovarian cancer cell line OVCAR 3.....	67
<b>Fig 9.</b> 2-D gel electrophoresis of cell culture supernatant from the ovarian cancer cell line CAO V 3.....	69
<b>Fig 10.</b> 2-D gel electrophoresis of cell culture supernatant from the ovarian cancer cell line HEY.....	71
<b>Fig 11.</b> 2-D gel electrophoresis of cell culture supernatant from the ovarian cancer cell line OVCA 432.....	73
<b>Fig 12.</b> 2-D gel electrophoresis of cell culture supernatant from the normal ovarian surface epithelial cell culture N1.....	75
<b>Fig 13.</b> 2-D gel electrophoresis of cell culture supernatant from the normal ovarian surface epithelial cell culture N2.....	77
<b>Fig 14.</b> 2-D gel electrophoresis of cell culture supernatant from the normal ovarian surface epithelial cell culture N3.....	79
<b>Fig 15.</b> 2-D gel electrophoresis of cell culture supernatant from the normal ovarian surface epithelial cell culture N4.....	81
<b>Fig 16.</b> 2-D gel electrophoresis of cell culture supernatant from the normal ovarian surface epithelial cell culture N5.....	83

<b>Fig 17.</b> 2-D gel electrophoresis of cell culture supernatant from the normal ovarian surface epithelial cells N6.....	85
<b>Fig 18.</b> 2-D gel electrophoresis of cell culture supernatants from the ovarian cancer cell line OVCA 429 in the presence of 2% FBS and in absence of FBS .....	90
<b>Fig 19.</b> 2-D gel electrophoresis analysis of PEG precipitates of supernatant from the ovarian cancer cell line OVCA 429.....	92
<b>Fig 20.</b> 2-D gel electrophoretic analysis of fractional ammonium sulfate precipitation of OVCA 429 cell culture supernatant.....	99
<b>Fig 21.</b> 2-D gel electrophoresis of p30 proteins separated by hydrophobic interaction chromatography (HIC) .....	103
<b>Fig 22.</b> 2-D gel electrophoresis analysis of p30 protein separated by ion exchange chromatography (IEC) following the (HIC) step .....	107
<b>Fig 23.</b> Flowchart of a modified purification plan for the p30 protein.....	110
<b>Fig 24.</b> G75 sephadex gel filtration standard curve .....	112
<b>Fig 25.</b> 2-D gel electrophoresis analysis of the p30 protein purification by G75 sephadex gel filtration .....	115
<b>Fig 26.</b> Standard curve for the G 150 sephadex gel filtration column .....	118
<b>Fig 27.</b> 2-D gel electrophoresis analysis of the p30 protein purification using G 150 sephadex gel filtration column .....	121
<b>Fig 28.</b> 2-D gel electrophoresis analysis of the p30 protein purification by ion exchange chromatography (IEC) .....	124



## LIST OF TABLES

<b>Table 1.</b> Classification of primary common epithelial tumors of the ovary .....	15
<b>Table 2.</b> Standard precipitation table .....	51
<b>Table 3.</b> Detection of CA 125 and p30 in supernatants from cultures of normal human ovarian surface epithelium .....	87
<b>Table 4.</b> Detection of p30 in the supernatants of ovarian cancer cell lines.....	88
<b>Table 5.</b> Recovery of radiolabeled protein after precipitation with different concentrations of PEG.....	96
<b>Table 6.</b> Recovery of radiolabeled protein (cpm) after sequential fractionation with different concentrations of ammonium sulfate.....	98
<b>Table 7.</b> Recovery of p30 (cpm) after precipitation with different concentrations of ammonium sulfate.....	101
<b>Table 8.</b> Recovery of p30 (cpm) after separation by HIC using 0 M (no salt) eluent .....	105
<b>Table 9.</b> Comparison of purification of p30 by HIC and by IEC.....	106
<b>Table 10.</b> Calibration of a G 75 sephadex gel filtration column.....	111
<b>Table 11.</b> Calibration of G-150 sephadex gel filtration column.....	117
<b>Table 12.</b> Recovery of p30 protein (cpm) after G-150 sephadex gel filtration.....	120
<b>Table 13.</b> Recovery of p30 (cpm) after final step IEC.....	126
<b>Table 14.</b> Summary of the purification steps of p30 protein.....	126



## LIST OF ABBREVIATIONS

(IL-1)	: Interleukin-1
(IL-6)	: Interleukin-6
2D	: two-dimensional
ATCC	: American Type Culture Collection
BSA	: Bovine serum albumin
CA	: Carbonic anhydrase
CASA	: Cancer- associated serum antigen
CEA	: Carcinoembryonic antigen
CO <sub>2</sub>	: Carbon dioxide
cpm	: Count per minute
Cyto C	: Cytochrome C
DMEM	: Dulbecco's Minimal Essential Medium
EDTA	: Ethylenediaminetetraacetic acid
EGF	: Epidermal growth factor
EGFR	: Epidermal growth factor receptor
FBS	: Fetal bovine serum
g	: Gravity
h	: Hour
HIC	: Hydrophobic interaction chromatography
HMFG2	: Human milk fat globulin
IEF	: Isoelectric focusing
kD	: kiloDaltons
M-CSF	: Macrophage colony stimulating factor
MEM	: Minimum Essential Medium
ml	: Milli liter
mm	: Millimeter
mM	: Milli molar
MW	: Molecular weight
OCPs	: Oral contraceptive pills
PEG	: Polyethylene glycol
PEG	: Polyethylene glycol
pI	: Isoelectric point
PMSF	: Phenylmethylsulfonylfluoride
SDS-PAGE	: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TEMED	: Tetranethylethylenediamine
TGF $\beta$	: Transforming growth factor $\beta$
TGF $\alpha$	: Transforming growth factor $\alpha$
TNF	: Tumor necrosis factor
U	: Unit

ug	: Microgram
UGP	: Urinary gonadotropin peptide
ul	: Microliter
$V_o$	: Void volume
Vol	: Volume
$V_t$	: Total volume
w/v	: weight /volume



## **INTRODUCTION**

Ovarian cancer runs as the second common female genital malignancy though it is the leading cause of cancer deaths among all female genital cancers. This high mortality is related to the advanced stage of the disease when it is first diagnosed. About 70% of cases are diagnosed at stage III <sup>or</sup> stage IV when the chance of cure becomes extremely low. Patients with early stages of ovarian cancer stage I and II can achieve up to 90% cure rate with the currently available treatment modalities. This marked difference in prognosis makes the necessity of early detection and management of ovarian cancer extremely sore.

Different strategies have been tried for screening and early detection of ovarian cancer including the use of serum tumor markers and ultrasound specially vaginal ultrasound. Vaginal ultrasound is a highly sensitive tool but is not specific and its wide scale use is not cost effective. On the other hand, different tumor markers have been utilized either separately or in combination. The use of complementary tumor markers could improve sensitivity without sacrificing specificity, at the same time it is more feasible and cost effective before proceeding to vaginal ultrasound.

Most of the available tumor markers were developed by immunizing mice with whole cells of malignant surface epithelial ovarian tumor. Monoclonal antibodies were raised, selected for reactivity to malignant ovarian cells but not to normal or other malignant cells.

The availability of two dimensional gel electrophoresis which is a very powerful technique in analyzing complex mixtures of proteins has made it possible to compare the pattern of shed proteins from malignant and normal ovarian cell cultures. Proteins that are exclusively or differentially expressed by malignant cells could be identified and purified for further raising of monoclonal antibodies that will permit evaluation of these proteins' potential as tumor markers and will help their further purification if indicated.

### **Aim Of The Work**

- To test the hypothesis that malignant epithelial ovarian tumors express proteins that cannot be readily detected in normal ovarian epithelial cells.
- To identify cellular proteins that are differentially expressed by malignant ovarian cells but not by normal ovarian cells.
- To isolate (purify) the protein of interest for further evaluation this protein's potential as a novel tumor marker.



## **Ovarian Cancer**

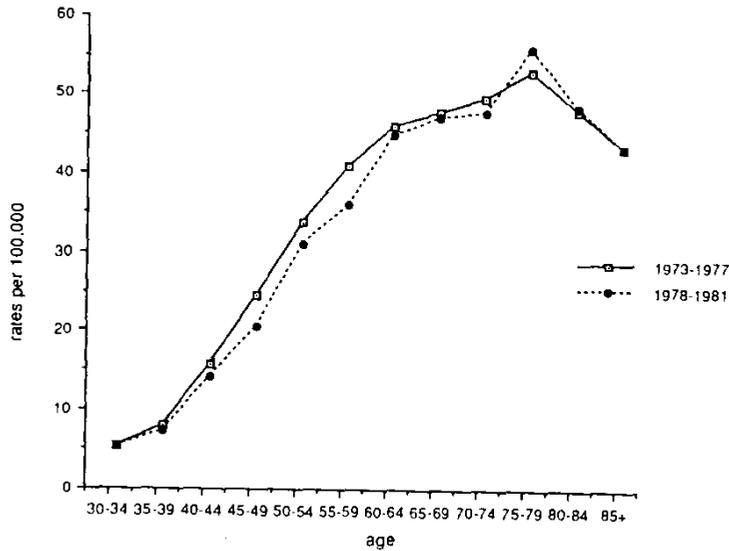
### **Ovarian Cancer, Epidemiology and Etiology**

Ovarian cancer is the leading cause of death from gynecologic malignancy among women in United States that accounts approximately for 13,000 deaths per year. Although it ranks second in incidence among gynecologic cancers, it causes more deaths than any other female genital tract malignancy. It is estimated that one of every 65 women will develop ovarian cancer by age 85. It accounts for 4% of all female cancers. An estimated 22,000 new cases of ovarian cancer in United States in 1993 (*American Cancer Society, 1993*).

Ovarian cancer has become the second most common gynecologic malignancy with an incidence of 13.7 per 100,000, after endometrial cancer that has an annual incidence of 21.2 per 100,000 women (*American Cancer Society, 1991*).

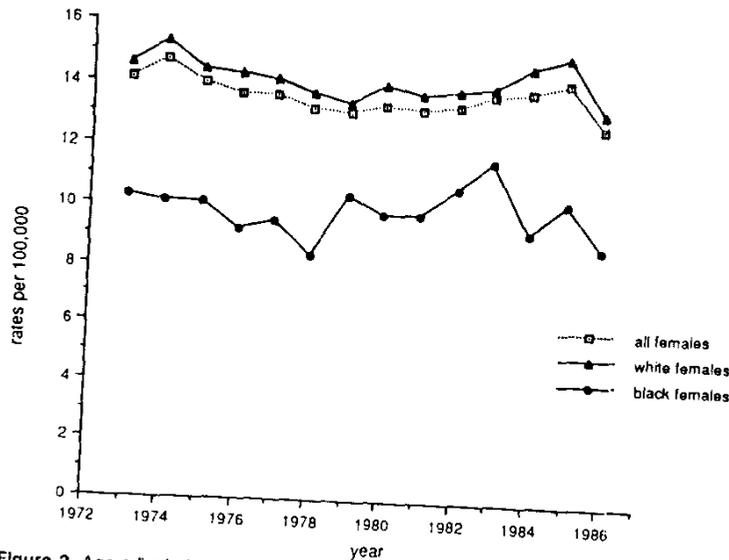
Malignant epithelial tumors comprise between 85 to 90% of all ovarian cancer and practically is a disease of perimenopausal and postmenopausal woman with a mean age of 52 years (*Wynder et al., 1969*). The disease incidence rates rises with each decade of life and peaks in the mid to late seventies (Fig 1) with acceleration of incidence coincident with the time of natural menopause (*Fathalla, 1972*). Age adjusted rates for ovarian cancer has remained relatively stable over the last two decades (Fig 2) although mortality rates have climbed as a result of the aging of the population and possibly due to the changing reproductive patterns (*La Vacchia et al., 1984*).

**Fig 1.** Ovarian cancer rates by age for 1973-1977 and 1978-1981.



**Figure 1.** Ovarian cancer rates by age for 1973-1977 and 1978-1981. (Data from the SEER program: Cancer Incidence and Mortality in the United States, 1973-1981. US Department of Health and Human Services, 1984.)

**Fig 2.** Age-adjusted ovarian cancer incidence by year and race for 1973-1986.



**Figure 2.** Age-adjusted ovarian cancer incidence by year and race for 1973-1986. (Data from the SEER program: Cancer Statistics Review, 1973-1986. US Department of Health and Human Services, 1989.)

Significant geographic and ethnic variation in rates have been observed. Rates are highest for white women in the industrialized countries of Europe and North America, and lowest in India and Asia. The wide geographic variations observed is roughly correlated with average family size (*Green et al., 1984*). Within the United States, the incidence rates for black, Hispanic, and American Indian women are approximately 40% lower than those for white women (*Soichet, 1978*), and among whites, rates are highest among Jewish women (*Fathalla, 1972*). These ethnic differences in rates could be partially explained by variation in reproductive patterns.

Gravidity is associated with a decreased ovarian cancer risk. Compared with nulligravid women, women with a single pregnancy have a relative risk of 0.6 to 0.8, with further lowering of the risk by 10% to 15% with each additional pregnancy (*Booth et al., 1989*). Some studies have reported an increased risk of ovarian cancer associated with late age at first pregnancy but in most studies that have adjusted the effects of age at first pregnancy for the number of pregnancies, no residual effects of age at first pregnancy persists (*Hartage et al., 1989*).

Booth and others have found a protective effect of breast feeding against ovarian cancer but this protective effect did not correlate with the duration of breast feeding possibly due to lack of correlation between breast feeding and suppression of ovulation (*Booth et al., 1989*).

Whittemore and colleagues found a high risk of ovarian cancer associated with nulliparity despite unprotected intercourse especially in patients with long ovulatory