Differential Protein Expression By Normal And Malignant Ovarian Epithelial Cells

رسالة

Thesis
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M.D. Degree in Obstetrics & Gynecology

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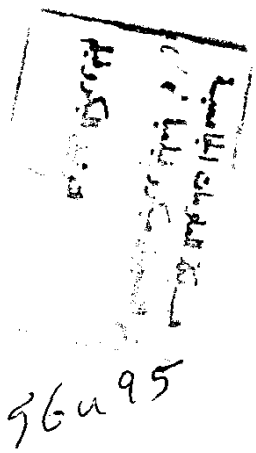

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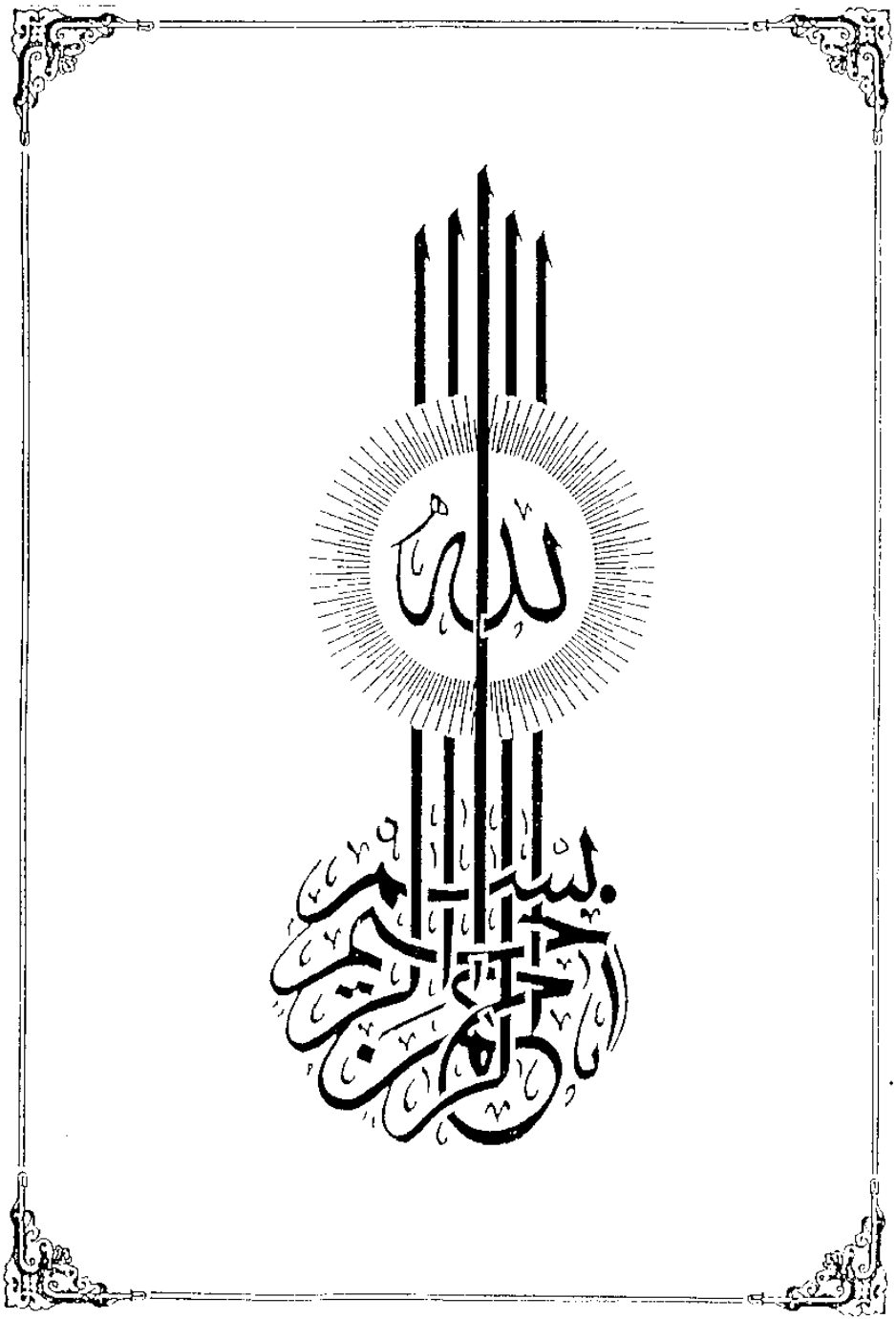

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LIST OF ABBREVIATIONS

(IL-1)	: Interleukin-1
(IL-6)	: Interleukin-6
2D	: two-dimensional
ATCC	: American Type Culture Collection
BSA	: Bovine serum albumin
CA	: Carbonic anhyderase
CASA	: Cancer- associated serum antigen
CEA	: Carcinoemberyonic antigen
CO2	: 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	: Mlli 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	: Tetramethylethylenediamine
TGF β	: Transforming growth factor β
TGF α	: Transforming growth factor α
TNF	: Tumor necrosis factor
U	: Unit

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

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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 mortailty is related to the advanced stage of the disease when it is first diagnosed. About 70% of cases are diagnosed at stage III or 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.

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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.

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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)***.

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

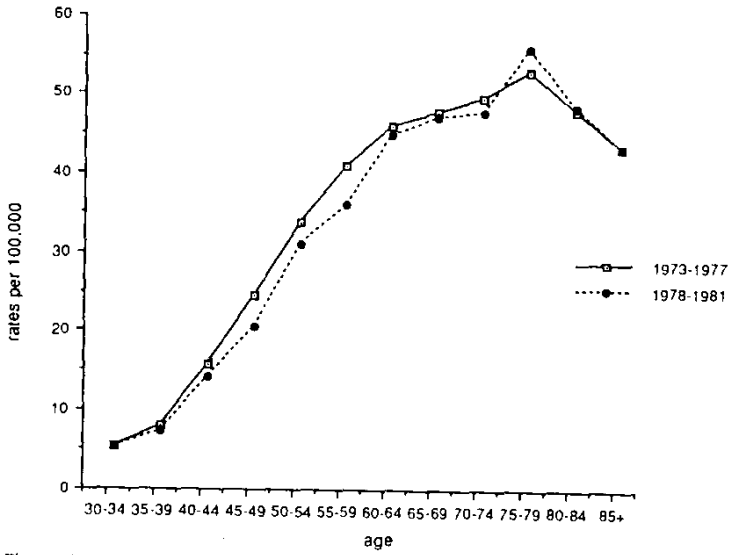

Figure 1. Ovarian cancer rates by age for 1973–1977 and 1978–1981. (*Data from* the SEER program: Cancer Incidence and Mortality in the United States, 1973–1981. US Department of Health and Human Services, 1984.)

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

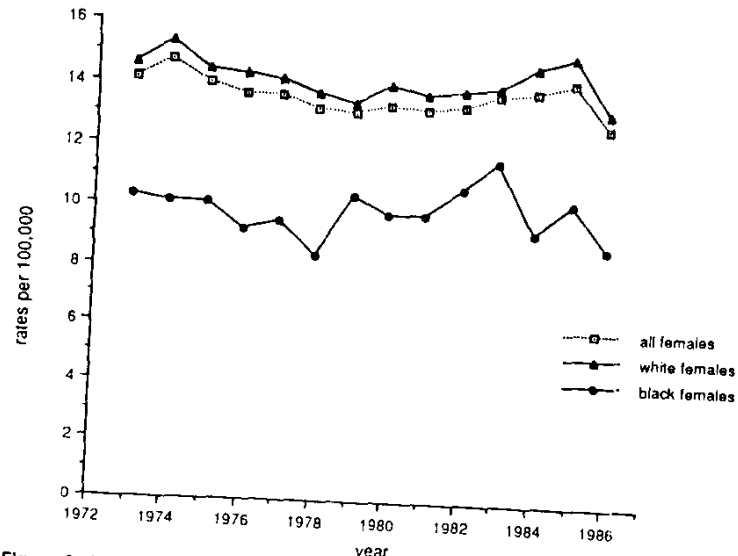

Figure 2. Age-adjusted ovarian cancer incidence by year and race for 1973–1986. (*Data from* the SEER program: Cancer Statistics Review, 1973–1986. US Department of Health and Human Services, 1989.)

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Significant geographic and ethnic variation in rates have been observed. Rates are highest for white women in the industerialized 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

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