Validation of Breast Cancer Microarray Analysis Using Molecular Biology Techniques

Thesis

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LIST OF ABBREVIATIONS

Abbrev.	Full Term

Ajcc.....American joint committee on cancer

Arg.....Argenine

BPS .....Between PH and SH2 domains

BRCA-1 ..... Breast Related Cancer Antigen- 1

BRCA-2 ..... Breast Related Cancer Antigen- 2

CDKIs ..... Cyclin dependant kinase inhibitors

CDKs..... Cyclin dependant kinases

cDNA..... Complementary Deoxyribonucleic Acid

CIS ..... Non-invasive carcinomas

CK : ..... Cytokeratins

cRNA..... complementaryribonucleic Acid

DCIS ..... Ductal carcinoma in situ

DNA..... Deoxyribonucleic Acid

EDTA.....Ethylene diamine tetraacetic acid

EGRF ..... Epidermal growth factor receptor

EphB1..... Ephrin type-B receptor

ER..... Estrogen Receptors

ErbB1..... Avian erythroblastosis oncogene B-1

ErbB2..... Avian erythroblastosis oncogene B-2

ErbB3..... Avian erythroblastosis oncogene B-3

ErbB4..... Avian erythroblastosis oncogene B-4

ERK1/2 ..... Extracellular signal-regulated kinase

FAK..... Focal adhesion kinase

FN ..... Fibronectin

LIST OF ABBREVIATIONS (CONT.)

Abbrev.	Full Term
G0 phase	Gap zero phase of the cell cycle
GAPDH	Glyceraldehyde 3 phosphate dehydrogenase
GM	Region for Grb and Mig
Grb10	Human growth factor receptor bound protein 10
Grb14	Human growth factor receptor bound protein 14
Grb7	Human growth factor receptor bound protein 7
HER-2	Human Epidermal growth factor Receptor 2
IHC	Iimmunohistochemistry
JNK	c-Jun N-terminal kinase
kDa	kilodalton
KOR	Kappa opioid receptor
LCIS	Lobular carcinoma in situ
Lys	Lysine
M phase	Mitosis phase of the cell cycle
MAPKs	Mitogen-activated protein kinase
P16	Gene codes for a 16 kilodalton protein
P27	Gene codes for a 27 kilodalton protein
p38	Gene codes for a 38 kilodalton protein
P53	Gene codes for a 53 kilodalton protein
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PH	Pleckstrin homology domain

LIST OF ABBREVIATIONS (CONT.)

Abbrev.	Full Term
Phe	Phenylalanine
PI3-K	Phosphatidylinositol 3-kinase
PR	Progesterone Receptors
pRb	Retinoblastoma protein
QC	Quality control
RA	Ras-associating domain
Ras	Rat sarcoma
RT-PCR	Reverse transcriptase - polymerase chain reaction
Rb	Retinoblastoma gene
RNA	Ribonucleic Acid
TBE	Trisma, Boric and EDTA
S phase	DNAsynsesis phase of the cell cycle
SH2	Src homology 2
SNP	Single nucleutide polymorphism
Src family	Sarcoma virus
STAT3	Signal transducer and activator of transcription-3
TTYH1	Tweety homologue 1
TTYH2	Tweety homologue 2
TTYH3	Tweety homologue 3
Tyr	Tyrosine

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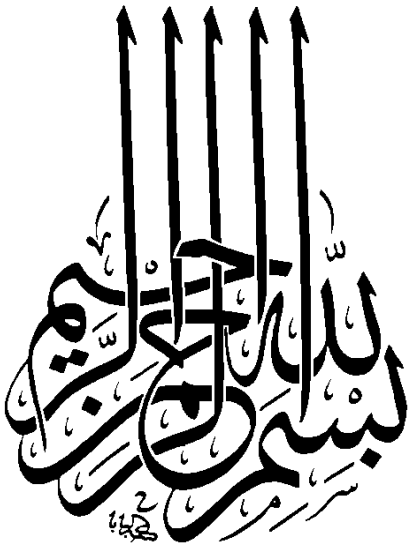

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا

إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

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Introduction

Breast cancer is a major health burden worldwide. It is the most common cause of cancer among females in both developed and developing countries. It is responsible for over 1 million of the estimated 10 million neoplasms diagnosed worldwide each year in both sexes ***(Bray et al., 2004).***

Breast cancer is the second leading cause of cancer deaths in general after lung cancer and it is the most common cancer among women worldwide ***(Laurance and Jeremy, 2006).***

In Egypt, breast cancer is the most common cancer among women, representing 18.9% of total cancer cases among the Egyptian National Cancer Institute (NCI) and represents 37.5% of all reported tumors in Egyptian females ***(Salem et al., 2010).***

In the past several years, a new technology, called microarray, has attracted great interests among biologists. This is because traditional methods generally work on a gene in one experimental basis, which means that the throughput is very limited. This technology promises to monitor the whole genome on a single chip so that the researchers can have a better picture of the interactions among thousands of genes simultaneously. They are useful when one wants to survey a large number of genes quickly or when the sample to be studied is small.

A previously done microarray data on breast cancer by ***Hana et al. (2009)*** showed that members of the growth factor receptor family overexpression have been of considerable interest in tumorigenesis (e.g. Grb7). While, it also highlighted some genes to be related to breast cancer for the first time. Among these was the gene called TTYH1 which was found to be significantly under expressed in Breast cancer.

Growth factor receptor-bound protein-7 (Grb-7) is a member of the Grb-7/-10/-14 family. It has been implicated in important cellular and physiological functions such as signal transduction, cell motility and tumor progression ***(Cariou et al., 2004).***

Tweety, belongs to a family which includes three members, designated as TTYH1 (Tweety homologue 1), TTYH2 and TTYH3. It recently identified Cl^- channels predicted to be modified by N-glycosylation ***(Yaowu et al., 2008).***

Aim of the Work

The aim of this study is to validate the results obtained from the previous microarray data analysis with respect to the above mentioned genes (Grb-7 & TTYH1). Also, we will study the relation between their expression and the development of the disease. Moreover, their relation with the different bad prognostic indicators will also be addressed.

Breast Cancer

Breast cancer represents a serious health problem and is currently the most frequent malignancy in female population.

It is the most common cause of cancer related mortalities among women worldwide. The basic understanding of breast cancer initiation and progression is still incomplete.

In addition, there is a need to develop improved methods to stratify breast cancer patients into different risk groups more accurately than can be achieved with current clinicopathologic classification methods. Hence, low-risk patients can be spared unnecessary treatment, avoiding side effects and reducing the cost of treatment ***(Li and Brattain, 2006).***

Epidemiology

According to the American Cancer Society, every three minutes a woman in the United States is diagnosed with breast cancer. This cancer incidence in women has increased from one in 20 in 1960 to one in eight nowadays. About 1.3 million women are expected to be diagnosed with this cancer annually worldwide and about 465,000 will die from the disease. Breast cancer death rates have been dropping steadily since 1990 because of earlier detection and better treatment ***(Mehmet et al., 2012).***

According to the National Cancer Institute, Cairo, Egypt, breast cancer is the most common cancer among women,

representing 18.9% of total cancer cases. Among the Egypt National Cancer Institute (NCI) series of 10,556 patients during the year 2001 ***(Salem et al., 2010).***

There is an international geographical variation in the incidence of Breast Cancer. Incidence rates are higher in the developed countries than in the developing countries. Incidence rates are also higher in urban areas than in the rural areas ***(Vorobiof et al., 2001).***

The mortality rates of breast cancer are declining in the developed world (Americas, Australia and Western Europe) as a result of early diagnosis, screening, and improved cancer treatment programs, the converse is true in the developing world ***(Adesunkanmi et al., 2006).***

The hallmarks of the disease in Africa are patients presenting at advanced stage, lack of adequate mammography screening programs, preponderance of younger pre-menopausal patients, and a high morbidity and mortality ***(Parkin et al., 2005).***