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Association of BRCAI Epigenetic, with Breast Cancer

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Abstract

Breast cancer is the most frequent malignancy among Iraqi women cancer. Epigenetic alterations such as DNA methylation were involved in the earliest phases of tumorigenesis and help for early diagnoses of breast cancer.

In this study, the methylation and unmethylation BRACA1 gene have been detected of Iraqi women patients having breast cancer.

DNA extraction kit and DNA modification kits as well as polymerase chain reaction (PCR) have been used. The samples of 80 women patients' blood with breast cancer and 20 apparently healthy individual's blood sample are collected during the period of February2018- April2018 from Oncology Hospital in the Medical City - Baghdad, Iraq.

On using PCR(24) out of (80)patients havingmethylated BRACA1 and (56) having unmethylated BRACA1.

As discussed in this thesis, one can recommend by using BRACA1 gene as biomarker for prediction of early diagnoses.

Keywords: *methylation, unmethylation, BRACA1, modification, CPG*

Introduction

Epigenetic mechanisms in normal cells

Manytypes of human cancers and diseases are often induced by miss regulation of gene expression. Epigenetic modifications and genetic mutations are the mechanisms for this miss regulation (genetic instability) that disrupt the function of genes, including oncogenes and tumor suppressor genes, as well as other cancer-related genes [1],

Chromatin is made of repeating units of nucleosomes, which made of ~146 base pairs of DNA wrapped around an octamer of four core histone proteins (H4, H3, H2B and H2A) [2]. The main epigenetic mechanisms that modify chromatin structure are: histone modifications,DNA methylation. These modifications work together to regulate the functioning of the genome by changing the local structural dynamics of chromatin, primarily regulating its accessibility and compactness. The interplay of these modifications creates an 'epigenetic landscape' that regulates the way the mammalian genome manifests itself in different cell types, developmental stages and disease states, including cancer [3,4, 5].

DNA methylation

Vertebrate DNA is modified by addition of methyl residues at the 5' position of cytosine's residing mostly in CG (also known as CpG) dinucleotides [6] . Not all CGs are methylated in vertebrate genomes, and the distribution of unmethylated and methylated CGs in the genome is tissue-specific, resulting in a cell specific DNA methylation pattern [7].The DNA methylation reaction is catalysed by DNA methyltransferases (DNMTs) [8, 9].The C-terminal catalytic domain of DNMTs transfers methyl groups onto cytosine residues within the DNA, thus methyltransferases represent the critical enzyme class responsible for hypermethylation of tumor suppressor genes. In mammals, five members of the DNMT protein

family have been discovered (Dnmt1, Dnmt2, Dnmt3a, Dnmt3b, and Dnmt3L), of which only three were shown to possess catalytic methyltransferase activity (Dnmt1, Dnmt3a, and Dnmt3b) [10] .

Cancer was the first group of diseases to be related with DNA methylationallterations and to be considered for DNA methylation targeted therapeutics [15]. Aberrant expression of DNMT1 and other DNMTs,hypermethylation of tumor suppressor genes, and hypomethylation of repetitive sequences and unique genes are common epigenetic features of many types of cancers [16, 17, 18].

Silencing of tumor suppressor genes by DNA methylation provides a powerful molecular mechanism by which DNA methylation can provides a rationale for therapeutics aimed at inhibition of DNA methylationtrigger cancerandre-expression of silenced tumor suppressor genes [19] .

The original idea driving the study of DNA methylation changes in diseased states that limited sets of candidate genes are critical for disease progression and initiation. However, unbiased approaches could potentially detect new genes and new functional gene networks that are linked with a disease**,** whereas candidate approaches essentially allow proof of genes that are already known to be involved. Early studies attempting to take benefit of the emerging role of methylation of promoters of tumor suppressor genes in cancer examined whether methylation of specific CGs in tumor suppressor genes associates with different breast cancer clinical states[19]

BRCA1 is directly involved in preventing cells from growing and dividing in a controlled manner and repairing damaged DNA.BRCA1 (breast cancer) gene encodes a multifunctional protein involved in DNA repair, control of cell-cycle checkpoints, protein ubiquitination and chromatin remodelling [20]. It was basically detected as a gene responsible for familial breast cancer [21]

Material and Method

The study of this thesis include 20 healthy female and 80 female patients with breast cancer where their ages ranged between (30-60 years), who are suffering from breast cancer disease .there blood are collected from Medical city in Baghdad, Iraq during the period from February 2018 to April 2018.

DNA extraction kits and DNA modification kits have been used to extract and modify the DNA structural. Also nano drop techniques have been used to measure the DNA concentration before modify it. then Polymerase chain reaction have been used with special primers designed for BRACA1 gene Table (1)

Table (1) Primers Sequences and Their Size of Amplicon

BRCA1 -M-F	20	5'-GAGAGGTTGTTGTTTAGCGG-3
BRCA1 -M-R	20	5'-CGC GCAATCGCAATT TTA AT-3'
BRCA1-U-F	20	5'-TGG TAA TGG AAA AGT GTG GGA A-3
BRCA1- U-R	20	5'-CCC ATC CAA AAA ATC TCA ACA AA-3'

Result and Discussion

The result show that out of 80 patients with breast cancer disease 24 women's have methylation BRACA1 gene. And the remain 56 women's have un methylated BRACA1 gene Table (2).BRCA1 promoter methylation is associated with the early onset of breast cancer other Saudi Arabia studies also show that 13 women with breast cancer have methylation BRACA1 out of 47 , and 34 out of 47 have un methylated BRACA1 .

BRACA gene types	Women's with breast cancer disease
Methylation BRACA1 gene	24
Un methylation BRACA1 gene	56
Total	80

The association between *BRCA1* promoter methylation and the clinic- pathological features of breast tumours was assessed. A strong association($p<0.001$) was found between BRCA1 methylation and young age (≤40 years) at diagnosis (18)75%figure (1). Table(2) the proportion of methylated BRCA1 within the age of 40 and younger was 75% which confirm the important role for BRCA1 gene in the initiation and progression of breast cancer. While only 25% of the breast cancer patients aged 40 years and older have shown BRCA1 DNA methylation in from peripheral blood samples.-for more details one can see appendix (B.1) .

In Al-Moghrabi, studies show strong association between BRCA1 methylation and young age (≤40 years) [22]

Table (2) :BRCA1 * Age at diagnosis Cross tabulation

<= 40 **>= 40**			**Age at diagnosis**		**Total**
BRCA1	Methylated	Count	18	6	24
		% within BRCA1	75.0%	25.0%	100.0%
	Unmethylated	Count	14	42	56
		% within BRCA1	25.0%	75.0%	100.0%
Total % within BRCA1		Count	32	48	80
		40.0%	60.0%	100.0%	

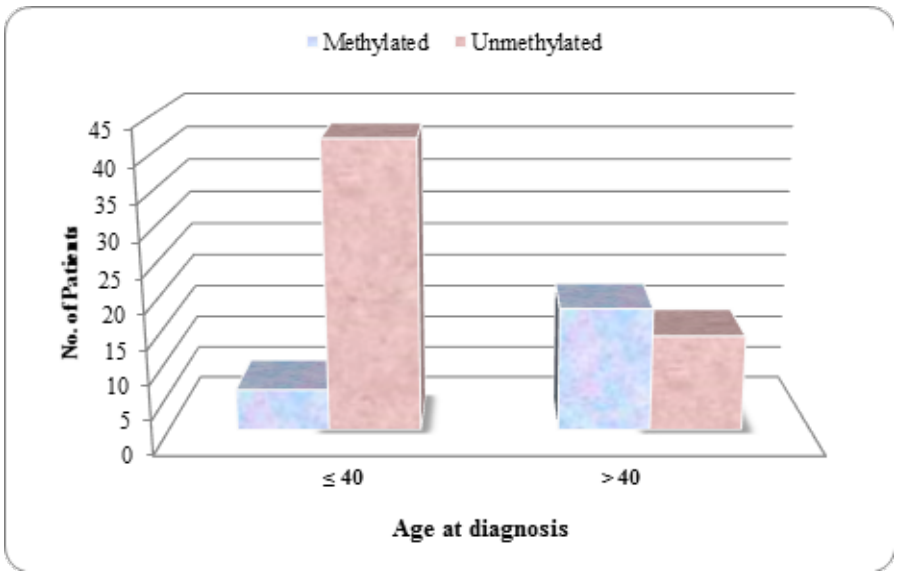

Figure (1) :Distribution of Patients According to Age at Diagnosis and the BRCAI

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under College of Science, Mustinisryah University, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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