Iraqi Journal of Biotechnology, 2017, Vol. 16, No.1, 101-105

Identification of E-cadehrin Methylation in Iraqi Breast Cancer Patients

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Received: October 10, 2016 / **Accepted:** November 22, 2016

Abstract: E-cadherin (CDH1), a Ca++ dependent transmembrane glycoprotein functioning in cell to cell adhesion placed in 16q22.1, is one of the cardinal regulators of morphogenesis. Decreased levels of CDH1 expression related with the advanced stage and poorly differentiated cancers. This study involved detection of CDH1 methylation in breast tissue after surgery for breast cancer patints and control cases that involved cases of fibeoadenoma, benign breast tissue and normal breast tissue. The study revealed presence of CDH1 methylation in 93.3% of patients while the unmethylated control samples were 80%.

Key words: breast cancer, E-cadherin, CDH1 methylation, promoter , CpG methylation, Methylation specific PCR.

Introduction

Breast cancer accounted for 23% (1.38 million) of total new cancer cases and 14% (458,400) of total cancer deaths in 2008 (1). The risk of developing breast cancer can be reduced by modifications to lifestyle and behaviours to minimize exposure to specific risk factors, such as obesity (2). Breast cancer forms from uncontrolled growth of abnormal breast cells, and its metastasis is one of the leading causes of death among women (3). Despite vast improvement in the overall survival rate of patients with noninvasive breast cancer, advanced metastatic breast cancer remains a life-threatening disease. One of the main challenges in mammary cancer research is now to identify key proteins modulating tumor invasion, which can serve as early markers for invasive tumors as well as new drug targets (4). The cadherins were first identified as a family of single-pass transmembrane glycoproteins mediating calcium-dependent cell-cell adhesion, and it is now well recognized that they play essential roles in development, cell polarity, and tissue morophology (5). Among the cadherin family membranes, reduced expression of E-cadherin (CDH1) and H-cadherin(CDH13) is common in a variety of human tumors(6). Decreased levels of CDH1 expression related with the advanced stage and poorly differentiated cancers (7). E-cadherin is expressed exclusively in all of the mammary epithelial cells, while P-cadherin is expressed in mammary epithelial cells of the alveoli and ducts, but also in myoepithelial cells. N-cadherin is expressed in mesenchymal cells of the mammary stroma. R-cadherin, which was first

identified in the retina, is expressed in the mammary epithelial cells (8). Aberrant methylation of the promoter regions of tumor suppressor genes seems to be the major mechanism of gene silencing in tumors (6).

Materials and Methods

Collection of samples: 70 tissue samples that were taken from surgical operations from Baghdad teaching hospital/medical city. The samples include 40 samples for control that include cases of fibroadenoma, normal breast tissues, accessory breast. The other 30 samples are from patients of breast cancer that include cases of mastectomy, recurrent lymph node post mastectomy, removal of the cancerous mass.DNA extraction: DNA was extracted from tissue samples using gSYNC^{TM} DNA Extracion kit, geneaid according to the protocol of the manufacturer with modifications followed by bisulfate modification of DNA using EZ DNA Methylation-Gold^{TM} kit, epigenetics according to the protocol of the manufacturer. Methylation specific PCR : the bisulfate modified DNA was amplified using AccuPower® PCR PreMix kit in a reaction volume of 20 µl according to the protocol of the manufacturer with modifications, the following primers were used (forward primer : 5′-GTTTAGTTTTGGGGAGGGGTT-3′) and (reverse primer:5′-ACTACTACTCCAAAA ACCCATAACT AA-3′) using the following program: 95ºC for 5 minutes, followed by 35 cycles of: (95ºC for 1minute, 50ºC for 30 seconds and 72ºC for 1 minute), and final extension72ºC for 5 minutes (9). Then 5µl of the PCR product was used in methylation specific nested PCR using AccuPower® PCR PreMix kit in a reaction volume of 20 µl using the following primers for methylated sequence (forward primer:5′-TGTAGTTACGTATTTATTTTTAGT GGC GTC-3′) and (reverse primer: 5′-CGAATACGTCGAATCGAACCG-3′) and using the following primers for unmethylated sequence (forward primer: 5′-TGGTTGTAGTTATGTATTTATTTT TA GTGGTGTT-3′) and (reverse primer: 5′-ACACCAATACAACAAATCAAACC A AA-3′) (9) using the same program. The product sizes of the methylated and unmethylated amplicons : 108 bp and 122 bp, respectively. The amplified products were visualized after electrophoresis in 3% agarose gel electrophoresis.

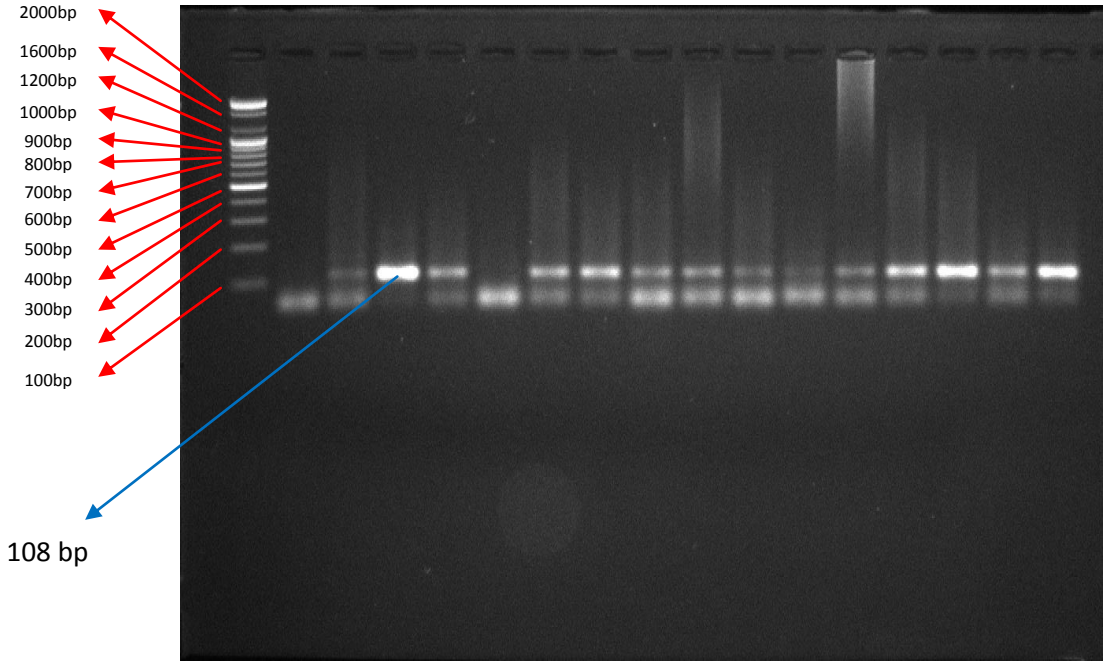

Figure (1):electrophoresis of Methylated samples, agarose concentration 3%, time 90 minutes, 100 volt.

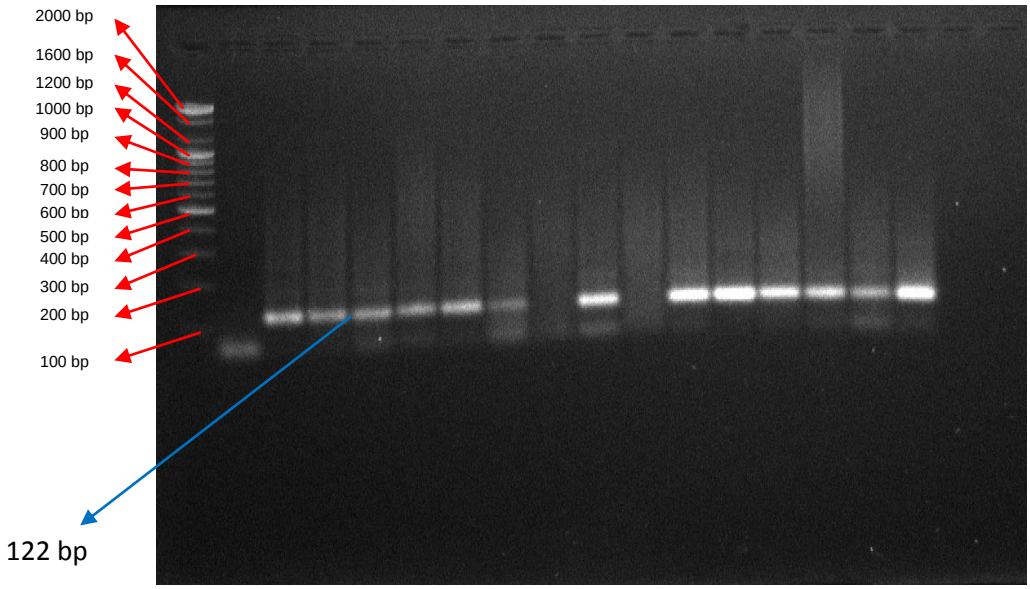

Figure (2): electrophoresis of unmethylated samples, agarose concentration 3%, time 90 minutes, 100 volt.

Results and Discussion

DNA methylation of CpG sites in the promoter regions of genes is a frequent acquired epigenetic event in the pathogenesis of many human cancers. This modification has important regulatory effects causing loss of gene expression (10). This study revealed that the methylated DNA samples from patients are 28 sample which represents 93.3% from all patients. While 2 samples did not give positive results for methylation and their percentage was 6.7%. The study also revealed that the control DNA samples that are methylated are 30 sample and their percnestage was 75% while the control DNA samples that are negative for methylation are 10 samples and their percentage was 25%.

The unmethylated DNA samples from patiens were 26 sample and their percentage was 86.67% , this unmethylation may be due to contamination with normal adjacent tissue. In this study the unmethylated DNA samples from controls were 32 sample and their percentage was 80% while DNA samples from controls that were negative for unmethylation were 8 samples and their percentage was 20%.

These results agreed with the results of other studies.

It was found that promoter hypermethylation occurs in CDH1 in both benign and malignant lesions of the breast tumors (11). Another study also found E-cadherin methylation in 31% (11/35) cases of ductal carcinoma in situ (DCIS) and in 52% (25/48) in invasive ductal carcinoma (IDC) cases and in samples harboring methylation, unmethylated alleles were invariably also evident (12). The results of the current study also agreed with the researches that had investigated the CDH1 methylation in different types of cancers other than the breast cancer.

A global study found methylation of CDH1 in 27% of prostate cancers and methylation of CDH13 in 31% of prostate cancer and 20% of benign diseases of prostate. Finally they found that CDH1, CDH13 were occasionally methylated in non malignant prostatic tissues (benign prostatic hypertrophy and prostitis) (10). Another study on human oral squamous cell carcinoma found 7 of 11 cell lines represent the unmethylated pattern, four of other cell lines exhibited bands for both methylation and unmethylation and three cell lines lacking the E-cadherin expression were markedly methylated (13). E-cadherin gene (*CDH1*) is considered a tumor suppressor gene, and its loss has also been demonstrated to promote tumor invasion and metastasis in various cancermodels (14). and methylation of its promoter is correlated with decreased gene expression (15). This can illustrates the presence of high percentage of methylated samples in breast cancer patients and unmethylated samples in the controls in the current study.

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