Review

The most common genes involved in epigenetics modifications among Iranian patients with breast cancer: A systematic review

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Abstract: Breast cancer, with a lifelong risk of one in nine, is the most common cancer among women. In Iran, breast cancer is one of the growing and important women's health problems. Several environmental, genetic and epigenetics factors have been suggested to have a role in breast cancer development. Epigenetics alterations are heritable changes in gene expression that occur without causing any change in DNA sequence. DNA methylation as a main epigenetics modification in human cancer is found as a promising biomarker in early detection of breast cancer. Association between epigenetics changes of many gene promoters with the risk of breast cancer has been investigated worldwide. This aberrant methylation may be occur in specific genes related to cell cycle, cell adhesion, apoptosis and DNA repairing mechanisms and results in silencing of these important genes. In this review study, we have gathered all the data until December 2015 about epigenetics modifications among Iranian population with breast cancer. We searched international web databases such as: PubMed, Scopus, and Persian web databases; IranMedex and Magiran to investigate the association of epigenetics change and incidence of breast cancer among Iranian population. Using "methylation" or "epigenetics" key words and "Iran" as affiliation, all the published data were 31. After arbitrary limitation in search keywords the result have been 20 articles. Data analysis show that "*ER-α*" and "*E-Cadherin*" are most common studied genes in epigenetics modifications. Also, maximum studies were done in Tehran and Tabriz. We thought that more studies will be helpful to reveal the relation of methylation status in candidate genes with the breast cancer risk in Iranian populations.

Key words: Epigenetics, Methylation, Breast cancer, Iran.

Introduction

Breast cancer is the most common diagnosed type of cancer that affect women and it is associated with an great amount of morbidity and mortality worldwide (1). Breast cancer ranks first among cancers diagnosed in women in Iran (2) comprising 24.4% of all malignancies with a crude incidence rate and agestandardised incidence rates (ASR) of 17.4 and 23.1 per 100,000, respectively. Because of high incidence rate of breast cancer, it is generally regarded as one of the common health problems among different populations. Environmental, genetic and epigenetics factors have shown to play important roles in etiology of this disease. The epigenetics mechanisms are essential for normal development and maintenance of tissue-specific gene expression patterns in mammals. Change of epigenetics processes can lead to altered gene function and malignant cellular transformation (3). Epigenetics phenomena are mediated by several molecular mechanisms comprising histone modifications, polycomb/trithorax protein complexes, small non-coding or antisense RNAs and DNA methylation. Nowadays epigenetics is known as the collection of chemical modifications in chromatin that can influence the transcription and metabolism of the underlying DNA sequence. These different modifications are closely interconnected (4). Recent advances in the field of epigenetics have proven that human cancer cells are under the influence of global epigenetics abnormalities, in addition to numerous genetic alterations (3).

DNA methylation as a main epigenetics modification in human cancer is found as a hopeful biomarker in early detection of breast cancer (5). As mentioned above, epigenetics modification has a critical role in pathogenesis of breast cancer. Because of the importance of this mechanism we gathered all data about epigenetics studies among Iranian population with breast cancer. The aim of this study was to found what gene undergoes maximum epigenetics changes among Iranian patients. Also in order to show the neglect to these studies in Iran, we compared the number of articles about epigenetics in breast cancer patients in Iranian population with other country and was founded a gap in this field.
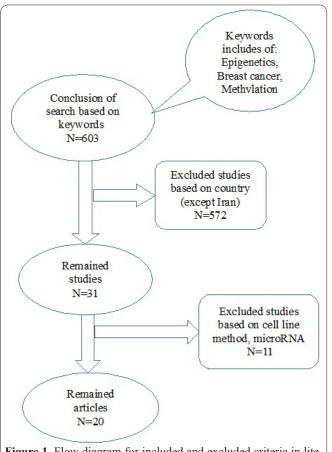
Research design and methods

Data sources and searches: We systematically searched international web databases: PubMed, Scopus, and Persian web databases: IranMedex and Magiran to investigate the association of epigenetics change and incidence of breast cancer among Iranian population up to December 2015. First, we searched "epigenetics" or

Figure 1. Flow diagram for included and excluded criteria in literature search process. In this diagram inclusion and exclusion criteria were shown.

"methylation" and "breast cancer" key words in title. With this search we founded about 600 results. Our results limited to 31 article when addition of "Iran" as affiliation. Also we use equivalents word similar mentioned words for Persian search in Persian web database and all of results matched with previous results from other databases. It should be noted that we excluded microRNA and cell line methods from epigenetics studies. As well as some results were excluded because of having patients with other nationalities. Finally, we analyzed 20 articles about epigenetics studies among Iranian population with breast cancer (Figure 1).

Results

Table 1 indicates the characteristics of included studies focusing on DNA methylation in breast cancer patients in Iran.

As shown in Figure 2, following genes had been mentioned in included studies.

RAR B2

Retinoic acid receptor beta 2 (RARB2) is the most functional form of retinoic acid receptor and is a candidate for a tumor suppressor gene (6). In several studies DNA hypermethylation was detected in 36.5% (50 out of 137) and 34.9 % (51 out of 146) for RARbeta2. The high expression of all of RARB convert to RARB2 was inversely proportional with the existence of RARB hypermethylation. Of the variables for B vitamins, there was notable downregulation of RARB with higher intakes of vitamin B2 and methionine. Dietary folate and

high cobalamin was correlated inversely with the hypermethylation status of RARB. Also High dietary vitamin B6 increased the chance of tumors having RARB hypermethylation about 2.15-fold over low intake (7, 8).

ER alpha

Estrogen receptor alpha (ERα) plays an important role in the etiology of breast cancer (9). Methylation analysis of ERα in several studies showed 51.1% methylation for this gene and ER protein expression decreased in 32.6% of cases. The plasma total homocysteine (tHcy) level was evaluated and determined markedly high among participants with hypermethylated genes. The hypermethylation status of ER-alpha was correlated negatively with folate and vitamin B12 plasma level status (7). Methylated ER3, ER4, and ER5 were found in 41.7, 11.3, and 43.3% of the samples, respectively (10). ERα methylation was detected in 52.9% (18 tumors out of 34) of luminal a subtype, 50% (7 tumors out of 14) of luminal B subtype, 87.5% (21 tumors out of 24) of Her2+ subtype and 89.3% (25 tumors out of 28) of basal subtype. There was a significant correlation between ERα methylation and poor prognosis, non luminal subtypes (basal and Her2+) in this study (11). This studies showed a significant association between the age of first full term pregnancy (FFTP) and ER-alpha methylation status. It seems that the ER-alpha methylation frequency tended to increase in breast tumors of nulliparous women or women with fewer children (12). Also A strong correlation was found between ERα methylation in ER3 region and ER negativity in tumors (13).
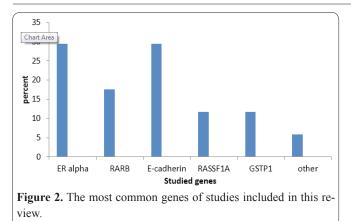
p16INK4a

The tumor suppressor gene p16INK4a (p16) regulates the cell cycle and antigenic switch. In human neoplastic tissues, cellular and molecular mechanisms that lead to loss of all of these type convert to p16INK4a gene activity are point mutations, homozygous deletions, and promoter hypermethylation in CpG islands (14). Analysis of p16INK4A promoter was performed using two different techniques including MSP and REP. Analyses of the samples using MSP showed that the methylation in p16INK4A promoter was homozygote (p16m+/u‹) in 15.7% (11/70), heterozygote (p16m+/u+) in 20% (14/70) and non-methylated in almost 64% (45/70) of samples. In REP analysis, hypermethylation of promoter in 40% (28/70) of samples was positive and in 60% (42/70) was negative. All samples with hypermethylation in the promoter of p16INK4A gene were found in patients with primary breast cancer tumors including DCIS (Ductular In situ carcinoma), LCIS (Lobular In situ carcinoma), and stages I and II tumor histology. Almost in 23% of patients with hypermethylation in p16INK4A gene, lymph node metastasis was detected (15).

DBC2

DBC2 gene (deleted in breast cancer 2), also known as RhoBTB2 participates in a variety of cellular functions such as cell cycle control, apoptosis, cytoskeleton regulation, and protein transport. DBC2 gene inacti-

Table 1. Characteristics of studies included focusing on DNA methylation in breast cancer patients in Iran.

Reference	Population	Sample size	Genes name	Method of DNA methylation study	Main result
Pirouzpanah et al, 2010	Tehran	Case :137 SampleType: tissue	*RARB2* *ER-α*	methylation sPesific PCR (MSP)	The methylation frequencies of RARbeta2 and ER alpha genes were observed in 36.5 and 51.1% of participants, respectively.
Vallian, et al 2009	Iranian patients mixed	Case: 70 Control: 70 Sample Type : blood	*p16INK4a*	MSP/restriction enzyme- related PCR(REP)	hypermethylation of p16 INK4a were 35.7% (25/70) and 40% (28/70) of samples, respectively.
Hajikhan mirzaei et al, 2011	Tehran	Case: 50 Control: 35 Sample type: blood and tissue	*DBC2*	Nested MSP	Frequency of the DBC2 promoter region methylation status in tumor and blood samples of the affected patients was significantly higher than that of the corresponding normal controls.
Ramezani et al,2012	Iranian patients mixed	Case: 60 Sample type: tissue	*ER-α*	MSP	Methylated ER3, ER4, and ER5 were found in 41.7, 11.3, and 43.3% of the samples, respectively.
Naghitorabi et al, 2013	Ahvaz	Case: 98 Control: 10 SampleType: tissue	*DNMT3B* promoter	Differential high resolution melting analysis(D-HRMA)	All the normal samples were found to be methylated at the DNMT3B promoter (the average methylation level 3.34%). Patients were identified as hypo-methylated (mean methylation level 0.8%), methylated (mean methylation level 2.48%) and hyper-methylated (mean methylation level 10.5%).
Rasti et al, 2009	Shiraz	Case: 81 Control: 100 SampleType: blood and tissue	*HIC1* *RASSF1A*	MSP	In this study ,HIC1 hypermethylation detected in 79% of invasive and metastasis tumors and RASSF1A gene hypermethylation in 51% of them.
Izadi et al,2014	Iran National Tumor Bank	Case: 99 Sample type: tissue	*ER-α*	MSP	Early FFTP(first full-term pregnancy) and increased number of Pregnancies were inversely correlated with epigenetics marks in the ER alpha gene in breast tumors.
Izadi et al , 2012	Iranian National Tumor Bank	Case:100 Sample type: tissue	*ER-α*	MSP	ERα methylation was detected in 98% of ER negative and 65% of ER positive breast tumors. ERα methylation not only associated to ER negativity in tumors but also associated to progesterone receptor negativity.
Pirouzpanah et al, 2015	Tehran	Case: 149 Sample type: tissue	*RARB* *BRCA1* *RASSF1A*	MSP	Folate and Cobalamin deficiencies are suggested to increase the frequency of tumors with methylation status at RARB and BRCA1 genes.
Gheibi et al, 2012	Isfahan	Case: 20 SampleType: tissue	*14−3−3 sigma*	MSP	Among breast cancer tissues 70% (14 of 20) were methylated and 30% (6 of 20) were unmethylated.
Vallian et al,2008	Isfahan	Case: 65 Control: 65 Sample type: tissue	*CKN2A*	MSP REP	An analysis of 65 patients by MPS and RPE showed hypermethylation of CKN2A in 29% (19.78) and 35% (23.78) of specimens, respectively.
Alizadeh Shargh et al,2011	Tabriz	Case: 50 Sample type: tissue	Glutathione S-transferase *P1(GSTP1)*	Bisulfite treated specific PCR	Out of 46 breast cancer samples, 28.3% (13 samples) were completely methylated, 13% (6 samples) were partially methylated and 58.7% (27 samples) were unmethylated.

Reference	Population	Sample size	Genes name	Method of DNA methylation study	Main result
Gharesouran et al,2015	Tabriz	Case: 50 Sample type: tissue	*E- cadherin*	Bisulfite specificpolymerase chain (PCR) sequencing	Among 50 breast cancer samples, the methylation frequency was as follows: a total of 94% was methylated (50% partial methylation and 44% full methylation), while only 6% was non-methylated. In normal samples, there were 76% nonmethylated samples and 24% methylated samples (22% was partially methylated and 2% was fully methylated).
A. Shargh et al,2011	Tabriz	Case: 50 Sample type: tissue	*E- cadherin*	Bisulfite conversion and BSP	Among breast cancer samples, 44% (22 of 50) were completely methylated, 50% (25 of 50) were partially methylated and 6% (3 of 50) were unmethylated. The overall hypermethylation rate in breast cancer tissues was 94% (47 of 50).
Vallian et al,2010	Isfahan	Case:15 Control: 5 Sample type: Blood and tissue	*PTEN*	MSP	PTEN promoter methylation was found to be present in 37 of 53(70%) tumor Tissues and none in 20 normal counterparts.
Yari et al,2016	Kermanshah and Ilam	Case: 103 Control: 50 Sample Type: blood	*PTEN* tumor suppressor	MSP	The frequency of PTEN-methylated (MM) genotype was 6 % in the healthy control group and 41.7 % in patients.
Sandeep Kumar Botla et al,2012	Tehran	Case: 117 Sample type: tissue	*GHSR* *SFRP2*	MSP	Normal-appearing breast tissues from cancer patients were also methylated at these loci but to a markedly lower extent.
Shakeri, et al,2016	Tabriz	Case : 80 Control:10 Sample type: tissue and blood	*RASSF1* *CDH13* *GSTP1*	MS-MLPA	The most frequently methylated genes were RASSF1 (48 %), CDH13 (44 %) and GSTP1 (36 %).
Alizadeh shargh et al, 2014	Tabriz	Case: 50 Control: 50 Sample type: tissue	*E-cadherin*	BSP	There were 95 % (19 of 20 samples) downregulation of E-cadherin expression in methylated samples comparing to unmethylated normal samples
Rasti et al,2009	Shiraz	Case :67 Sample type: tissue	*E-Cadherin* *ER-α*	MSP	Fifty percent of the samples showed aberrant methylation in at least one of the two tested loci. Hypermethylation of CDH1 was detected in 41% of invasive tumors and receptor-α gene hypermethylation in 18% of invasive tumor samples.

Figure 2. The most common genes of studies included in this review.

vation by methylation of CpG islands in the promoter region probably is a crucial step in the process of cell proliferation and susceptibility to different cancers, including breast cancer (16). Results of our survives showed 23 methylation-specific bands in whole blood samples of the 50 affected women and 5 methylation-specific bands in 30 normal controls. Of the 50 patients' tissue samples; 17 had only methylated bands; while 33 had both methylated and unmethylated bands (17).

DNM3B

DNA methylation is typically mediated by DNA methyl transferases (DNMT) and in mammalian genomes there are three DNMT genes. DNMT3a and DNMT3b are responsible for de novo methylation and modify unmethylated DNA. It seems that over-expression of DNMTs in cancer may be related to hypomethylation of the promoter region of their genes (18). Methylation status from 98 breast cancer FFPE tissues and 10 normal breast tissues were quantified with D-HRMA method. Statistical analyses showed that the mean methylation level of the normal samples was 3.34% and 48% of patients had the mean methylation level of 0.8 ±0.36%, therefore were classified as hypomethylated. 37.8% of patients were methylated with the average methylation level of 2.48 ± 0.92%. Also 14.3% of the patients had the mean methylation level of 10.5 ± 9.51% and were classified as hyper-methylated (19).

BRCA1

BRCA1 (breast cancer-1, early onset) is a TSG (Tumor suppressor gene). Mutation-independent transcriptional silencing of BRCA1 has been reported as a possible variation that is predisposed to sporadic breast cancer (20). In this survey, the hypermethylation status of BRCA1 promoters was 39.7 % (56 out of 141) of tumors. The relative frequency of BRCA1 hypermethylation was determined to be 35.7 % (5 of 14) in grade I, 27.8 % (10 of 36) in grade II, and 51.0 % (26 of 51) in grade III. The high folate and low methionine intake was significantly associated with a high level of BRCA1 expression (21).

HIC1

Hypermethylated in cancer 1 protein is a transcriptional regulator commonly methylated in a variety of human cancer. HIC1 is located at 17p13.3, a region

which is frequently hypermethylated Allelic (22). Loss of chromosomal arm 17p13 is found in approximately 40–60% of breast cancer. In this study from 81 malignant tumors, 63 tumors (79%) showed methylation of HIC1 promoter (23).

RASSF1A

RAS association domain family protein 1A (RASSF1A) gene hypermethylation has been a subject of interest in recent researches on cancer breast patients (24). RASSF1A identified at 3p21.3 was suggested as the major target tumor suppressor on the basis of its frequent epigenetics silencing (25). In our survey promoter methylation at RASS1A gene was reported 51%, 48% and 34.9% % in different studies (21, 23, 26). Dietary folate inversely contributed to the hypermethylation of RASSF1A. Cobalamin was associated with RASSF1A methylation (21).

GSTP1 (glutathione S-Transferase P1)

The glutathione S-transferase p1 (GSTP1) is thought to function in xenobiotic metabolism and play a role in susceptibility to cancer, and other diseases (27). The overall hypermethylation rate in breast cancer tissues was 41.3% and the majority of the normal samples (87.2%) were unmethylated. In another study Methylation of GSTP1 was associated with high histological grade (28).

E-cadherin

E-Cadherin (CDH1) is a tumor suppressor gene that mutations in this gene are correlated with gastric, breast, colorectal, thyroid, and ovarian cancers (29). Among 50 breast cancers, the methylation frequency was as follows: a total of 94% was methylated while only 6% was non-methylated. In normal samples, there were 76% nonmethylated samples and 24% methylated samples. In another study by Rasti et al; CDH1 hypermethylation was detected in 41% of invasive tumor and loss of CDH1 expression is associated with the acquisition of invasion, and metastatic potential (28). The result of this study showed that The CDH1 methylation was detected in 94% of breast tumors which was associated with higher tumor grade, tumor stage and tumor metastasis. Also, CDH1 methylation status in breast tumor specimen (ductal type) was observed in 94 % (47 of 50) comparing with normal samples (28).

14-3-3 sigma

14-3-3 proteins are a family of conserved regulatory molecules that are expressed in all eukaryotic cells (30). Promoter methylation analysis of 14-3-3 sigma gene was carried out in 20 breast cancer tissues and 20 normal breast tissues collected from breast cancer patients. Among breast cancer tissues 70% were methylated and 30% were unmethylated. Among normal breast tissues 20% were methylated and 80% were unmethylated (5).

CDKN2A

CDKN2A, also known as cyclin-dependent kinase Inhibitor 2A codes for two proteins, including p16 (or p16INK4a) and p14arf. Both act as tumor suppressors by regulating the cell cycle. Somatic mutations of CDKN2A are common in the majority of human cancers, with estimates that CDKN2A is the second most commonly inactivated gene in cancer after p53 (31). Methylation analysis of exon 1 CDKN2A gene showed 35% and 29% methylation with MSP and REP method respectively among 65 sporadic breast cancer patients (15).

GHSR

The growth hormone (GH)- secretagogue/ghrelin receptor (GHSR) is a member of the ghrelin receptor subfamily within class A of rhodopsin-like G protein-coupled receptors(GPCRs). Ghrelin, an octanoylated gastric peptide of 28 amino acids, was identified as the natural ligand of this receptor. There is now emerging evidence that ghrelin acts through GHSR to exert pleiotropic effects, which include not only the induction of GH secretion but also stimulation of appetite, regulation of energy metabolism, as well as having anti-inflammatory and immunomodulation properties (32). A human study demonstrated that the GHSR promoter region is frequently hypermethylated in primary breast tumors, and suggested that the methylation status of this region may be useful as a diagnostic biomarker. Analyses of this gene demonstrated a very high sensitivity and specificity of 89.3 and 100 %, respectively, for the GHSR methylation pattern (33).

CDH13

The cadherin's are a family of cell surface glycoproteins responsible for selective cell recognition and adhesion. Several family members, including CDH1 (E-cadherin) and CDH13 (H-cadherin), are located on the long arm of chromosome 16 [16q (2)], where loss of heterozygosity was reported in several human cancers including breast cancer. CDH13 expression is frequently silenced by aberrant methylation of the 5 region of the CDH13 gene in breast and lung cancers (34). Among our surveys only 1 study about CDH13 founded. In this study methylation of CDH13 was 44% and methylation status of CDH13 was associated with methylation of GSTP1(26).

PTEN

The PTEN (phosphatase and tensin homolog deleted from chromosome 10) tumor suppressor gene located at chromosome 10q23 is frequently mutated in a number of tumor lineages, including breast cancer (35). The PTEN protein is a negative regulator of the Akt pathway, leading to suppression of apoptosis and increased cell survival (36). In this investigation methylation of this gene was observed in 70% of tumor tissue (37) and in another study, The frequency of PTEN-methylated (MM) genotype was 6 % in the healthy control group, 23.3 % in relatives of patients, and 41.7 % in patients. Also, there was a 3.1-fold susceptibility to breast cancer in PTEN MM genotype compare to UU genotype. (38).

Conclusion

Breast cancer is the most common malignant tumor in women worldwide. According to estimations by the World Health Organization, Breast cancer leads to about 519,000 deaths per year in the world. Nowadays, the role of epigenetics change as a crucial mechanism to silence a variety of methylated tissue specific and imprinted genes has emerged in many cancer type such as breast cancer (5). Our investigation was revealed that USA and China had many epigenetics studies in breast cancer patients. The frequency of these studies was not comparable to the number of studies from other countries such as Iran. "BRCA1", "RASSFA" and "ERα" were most common genes that these studies focused on. In Iran, most studies attend to "*ERα*" and "*E-cadherin*". Among 20 studies, 5 articles were about "ERα" and 4 articles were about "*E-cadherin*". Also, maximum studies were done in Tehran, Tabriz, Isfahan and Shiraz provinces, respectively. The differences between these data may be representing different pattern of methylation around the world. As previously mentioned, the epigenetics role in silencing some specific genes, like tumor suppressor and cell cycle regulator genes, and therefore development of cancers such as breast cancer is clear. For this reason, further studies with a larger sample size to determine the role of epigenetics in the development of breast cancer in all part of Iran appear to be necessary.

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