Exp Oncol 2013
35, 4, 246–249

Experimental ONCOLOGY

EPIGENETICS IN BREAST CANCER

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Epigenetic information has recently gained the attention of researchers and epigenetics in breast cancer is still an evolving area of research. Epigenetic modifications such as DNA methylation and histone acetylation have been thoroughly evaluated in breast cancer. New methods to detect epigenetic changes with higher specificity have been developed. These methods are utilized to find new markers for the diagnosis and prognosis of breast cancer. In addition, epigenetic modifications are assumed as new targets in the treatment of breast cancer and new drugs alone or in combination with conventional therapies such as chemotherapy or hormone treatment are being tested in clinical trials.
***Key Words*: breast, cancer, epigenetic, diagnosis, treatment.**

INTRODUCTION

Carcinogenesis is a multi-step process resulting mainly from direct changes in genome sequence. Large number of genetic alterations in cancers makes the detection of changes specific to individual cancers more difficult and time consuming. For this reason, new methods to study the relationship between changes in genome and cancer are developed. Epigenetic information has recently gained the attention of researchers and epigenetics is defined as "the inherited genome activity that does not depend on the naked DNA sequence" [1]. Epigenetic modifications are believed to be early events in carcinogenesis and these alterations could be a useful source for early detection, prognosis, and targeted therapy of cancer.

Breast cancer is a frequently diagnosed cancer among women with well-known hereditary and familial tendency. Tumor suppressor genes, *BRCA1* and *BRCA2*, play a role in breast cancer development. Besides, breast cancer has well-established precursor lesions indicating an increased risk of cancer such as moderate to severe atypical hyperplasia. Since epigenetic changes occur early in cancer development and even in normal tissues surrounding the tumor, detection of these changes may help early diagnosis of breast cancer as well as the determination of prognosis and response to treatment.

EPIGENETIC MODIFICATIONS

Epigenetic modifications are recently discovered issues and epigenetics is still an evolving area of genomic research. Epigenetic modifications encompass changes in DNA methylation, histone modifications, microRNA (miRNA) expression, nucleosome positioning and higher order chromatin structure on gene expression.

Received: May 24, 2013.
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Abbreviations used: CpG – cytosine-guanosine; DAPK – death-associated protein kinase; GSTP1 – glutathione S-transferase P1; miRNA – microRNA; PARP – poly(ADP-ribose) polymerase; RARβ – retinoic acid receptor beta; RASSF1A – Ras-associated domain family member 1A.

Methylation of DNA is the most widely studied epigenetic modification and can be detected as either hypo- or hypermethylation. Hypermethylation is described as covalent binding of a methyl group to cytosine-guanosine (CpG) dinucleotides located on genome by DNA methyl transferases. CpG dinucleotides form approximately 2–5% of the genome. CpG dinucleotides are found as either small clusters called CpG islands, usually located in or near the promoter regions of genes, or in clusters of large repetitive sequences like satellite sequences or centromeric repeats. Approximately 60% of protein coding genes harbor CpG islands in their promoter regions. CpG islands are normally unmethylated and the transcription factors can easily bind to the promoter regions of the related genes and activate these genes. In contrast, repetitive genomic sequences are heavily methylated and this property is thought to play an important role by silencing specific genes during the evolution as well as endoparasitic and retroviral transposons.

In cancer cells, hypermethylation occurs at the promoter regions of tumor suppressor genes inactivating these genes. As a result of deregulation of tumor suppressor genes, most of the important cellular networks such as cell cycle control, DNA repair, apoptosis, cell adhesion, and migration are negatively affected. On the other hand, global hypomethylation could also be detected in cancer genome and this phenomenon occurs in approximately 50% of breast cancer cases resulting in genome instability. In addition, hypomethylation may affect individual genes such as multidrug resistance gene in breast cancer. There are common hypermethylated genes in different cancers as well as genes specific to that cancer. Specific regions of hypermetyhlation can be useful in the diagnosis of various cancers.

Changes in methylation status of the genome are regulated by DNA methyl transferases. The role of DNA methyl transferases in cancer cells is not well defined. Studies in cancer cell lines suggest that certain subtypes of DNA methyl transferases may play a role in transformation to cancer. However, the frequency of overexpression of these enzymes is low in most cancers including breast cancer. Although

DNA methyl transferases are reported to correlate with worse prognostic factors such as higher grade and Ki-67 expression and estrogen receptor negativity in breast cancer, the relationship between DNA methyl transferases and hypermethylation in breast cancer still needs to be elucidated.

Histones are the protein backbone of the genome and play a critical role in the translation of genotype to phenotype. Post-translational modifications of histones determine the translational activity in genome. Histone modifications such as acetylation, methylation, phosphorylation, sumoylation, and ubiquitination determine the functioning of genome. Previously, acetylation and methylation of histones have been studied in detail. Type of the modification and the affected amino acid on histones determine DNA-histone interaction and the transcriptional activity of genome. Although histone modifications have been less well studied compared to DNA methylation, both of these epigenetic modifications interact with each other in the regulation of gene expression in cancer cells. Frequently, both of these epigenetic changes associate with gene silencing in tumor suppression genes and genomic instability. When the promoter regions of suppressor genes are methylated, these genes are repressed losing their functions. *BRCA1* gene is a tumor suppressor gene related to hereditary breast cancer and methylation of this gene has been reported in the previous studies. In case of hypermethylation, *BRCA1* gene loses its function like its mutant version.

The most recently discovered molecules in the epigenetic field are the small regulatory RNAs known as miRNAs. These are 20–30 nucleotides in length and are complementary to the untranslated regions of mRNAs. These molecules play a role in gene silencing by binding to mRNAs and resulting in either translational inhibition or mRNA degradation. Changes in miRNA number and distribution on genome lead to modulations in gene expression. Hypermethylation of promoter regions of genes coding for miRNAs downregulates the synthesis of miRNAs and as a result decreased number of miRNAs may activate the expression of oncogenes. Downregulation of miRNAs have been detected in various cancers including breast cancer and is frequently related to worse prognosis. On the other hand, deregulated miRNAs could be used as tumor markers or as anti-cancer drug targets making early diagnosis or better treatment options possible.

Gene silencing due to epigenetic modifications is a complex process. The molecular sequence of events is not well-established; however, there are suggestions on the mechanisms of gene silencing. Either histone modifications or DNA methylation could be the initiating event in gene silencing. Complex mechanisms take place in gene silencing and displaying the sequence of these mechanisms requires detailed experiments in a large number of breast cancer patients and individuals at higher risk for cancer.

METHODS FOR DETECTION OF EPIGENETIC CHANGES

Increased interest in epigenetics resulted in the development of high-throughput techniques to study epigenetic modifications in human genome. These techniques are principally based on common techniques utilized in molecular research. Genome-wide DNA methylation can be analyzed using four major approaches, namely, restriction endonuclease-based analysis, bisulfite-conversion of DNA, affinity and immunoprecipitation-based studies, and mass spectrometry-based analysis. These methods of analysis have been adapted to be used with array-based and sequence-based methods.

Evaluation of the epigenetic changes classically starts with bisulphite modification of genomic DNA. A frequently used method in bisulphite-modified genomic DNA is methylation specific polymerase chain reaction. This method enables the detection of methylated or unmethylated DNA utilizing specific primers. In addition, quantitative multiplex methylation specific polymerase chain reaction, which is a highly sensitive method, can be used for the quantitation of hypermethylated regions on genome. These methods have the advantage of detection using limited sample quantity and this advantage enables researchers to utilize samples from various body fluids such as nipple aspirate, ductal lavage fluid, or fine needle aspirate.

Methods specific for epigenetic modifications have changed the mode of studies conducted for breast cancer. Detection of genome-wide DNA methylation has several advantages over detection of specific gene mutations. Primarily, incidence of aberrant methylation is higher than those of mutations which enable easier and sensitive detection of aberrant methylation by methylation specific-polymerase chain reaction. In addition, detection of aberrant methylation is technically simple necessitating only one set of PCR primers. On the other hand, many primer sets are used to detect various mutations in the genome. Finally, aberrant methylation can be a useful marker in non-neoplastic and early stage cancer tissues [2].

EPIGENETICS IN DIAGNOSIS AND TREATMENT

Early diagnosis of breast cancer definitely prolongs survival. Specific markers are required for early diagnosis and the available markers are not specific enough for this purpose. Markers related to epigenetic changes in breast cancer may be more useful for early diagnosis since these changes occur even before cancer development. Researchers aim to discover a combination of epigenetic markers to develop an "epigenetic signature" specific to breast cancer. Another method to increase the specificity of any marker including epigenetic markers is to obtain samples as close as possible to the source of the tumor. Body fluids such as nipple aspirate or ductal lavage fluid may be better sources than serum for sample collection in breast cancer. In the previous studies, methylation

of various genes such as cyclin D2, retinoic acid receptor beta (RARβ), Twist, glutathione S-transferase P1 (GSTP1), p16, p14, Ras-associated domain family member 1A (RASSF1A), and death-associated protein kinase (DAPK) were reported in ductal lavage fluid and nipple aspirate of breast cancer patients [3, 4]. Besides, detection of methylated DNA in plasma/serum of breast cancer patients is more convenient compared to detection of specific mutations for breast cancer in whole genome. In addition, detection rate of breast cancer patients increased when a combination of different markers was utilized in the previous studies. Sensitivity for the detection of breast cancer cells increased from 43 to 71% using a panel of markers compared to cytology [5]. However, comparison between breast cancer patients and healthy subjects has not been evaluated thoroughly [6–10]. On the other hand, methylation of specific genes was reported to be related to prognosis in breast cancer. Methylation of RASSF1A and APC in serum samples correlated with poor prognosis in breast cancer patients [11].

In recent years, targeted therapies play an increased role in the treatment of breast cancer. Epigenetic modifications detected in cancer cells could be the new targets in the treatment of breast cancer. Especially process of DNA methylation could be reversed in the treatment. For the treatment of different malignancies, two groups of agents targeting epigenetic modifications have been studied previously, namely DNA methyl transferase inhibitors and histone deacetylase inhibitors. Demethylating agents were first used in the treatment of hematological malignancies with successful results. However, there is a definite need for more specific agents with higher affinity. Reversal of DNA methylation and histone acetylation in tumor suppressor genes is a logical method of targeting. Until now, only a few drugs have been utilized for this purpose. Decitabine and 5-azacytide are frequently utilized DNA methyl transferase inhibitors. On the other hand, there are four groups of histone deacetylase inhibitors such as hydroxamic acids, cyclic tetrapeptides, short-chain fatty acids, and benzamides. Histone deacetylase inhibitors are non-selective and the most potent ones exhibit their action by targeting the zinc cofactor at the active site of the enzyme. Enzyme inhibition causes re-expression of silenced genes. Among the histone deacetylase inhibitors, vorinostat and romidepsin have been approved for use in the treatment of lymphoma. In addition, vorinostat is currently evaluated in breast cancer in combination with chemotherapy, hormone treatment, and other targeted therapies. Estrogen and progesterone receptors are the oldest targets used in the treatment of breast cancer. However, hormone treatment is not suitable for the treatment of ER/PR negative tumors. Inhibition of histone deacetylases reactivates ERα and PR genes and re-establishes the expression of hormone receptors enabling the use of hormone treatment.

Whether drugs targeting epigenetic modifications should be used in combination with chemotherapeutic agents or alone in order to obtain optimal effect is unknown. However, combination of DNA methyl transferase inhibitors and histone deacetylase inhibitors showed a better effect in reversal of silenced gene expression. For this purpose, histone deacetylase inhibitor entinostat and DNA methyl transferase inhibitor 5-azacytidine are evaluated in patients with triple negative metastatic breast cancer.

Despite unwanted effects on cancer development and progression, epigenetic modifications result in better response to certain chemotherapy drugs. Hypermethylation of promoter regions of tumor suppressor genes inactivates these genes and *BRCA1* and *BRCA2* genes are similarly affected in breast cancer. Hypermethylation of *BRCA1* occurs in 10% of all sporadic breast cancers and similar genetic changes are observed as in *BRCA1* mutant cases. Basal-like subtype or triple negative breast cancer is highly prevalent among patients with germline *BRCA1* mutations. This gene is responsible for DNA repair in case of double-strand breaks. Hypermethylation of *BRCA1* gene makes the cancer cells sensitive to chemotherapeutic drugs such as platinum-based drugs and poly (ADP-ribose) polymerase (PARP) inhibitors [12]. These drugs principally cause DNA damage that needs to be repaired by *BRCA* genes. In sporadic breast cancer cases with hypermethylation of *BRCA* genes, response to these group of drugs are expected to increase. Breast cancer subtypes resistant to conventional therapies such as triple negative or multidrug resistant tumors can be treated with the addition of DNA methyl transferase inhibitors or histone deacetylase inhibitors.

Epigenetics in breast cancer is still an evolving area of research. In the future, more specific signatures will be developed for early diagnosis and to determine the prognosis of breast cancer patients. In addition, detailed understanding of epigenetic mechanisms underlying breast cancer will increase the rate of treatment. Either new targeted therapies will be utilized or the effectiveness of conventional therapies will increase. However, more research is definitely required to achieve all of these targets.

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