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REVIEW ARTICLE

Epigenetic Modifiers Against Breast Cancer.

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ABSTRACT

Epigenetics is "the study of heritable change in gene expression and function without an alternation in DNA sequence itself. There are three main types of epigenetic mechanism named as DNA methylation, Histone modification and RNA interference (RNAi). DNA Methylation is generally considered as one of the most important epigenetic modifications occurring primarily at the cytosine residues of CpG dinucleotides. Histone Modifications are covalent post-translational modifications through mechanisms of acetylation, methylation, sumolyation, ADP-ribosylation, ubiquitylation and phosphorylation. The noncoding RNAs (ncRNAs) of epigenetics comprises of microRNAs (miRNAs), small interfering RNAs (siRNAs), Piwi-interacting RNA (piRNAs), and long noncoding RNAs (lncRNAs). Breast cancer is genetically highly heterogeneous disease. Epigenetics and genetic alterations causes to inactivation of cancer suppressors, deregulation of intracellular signaling cascades, metastasis, cancer micro environmental changes and deregulation of immune response, death which support cancer development. Recently, natural compounds or phytochemicals such as Epigallocatechin gallate (EGCG), Curcumin, Apigenin, Resveratrol etc., have been shown to alter epigenetic mechanisms and inhibit the synthesis of metabolic product i.e., leukotriene and prostaglandins. Quertcetin is well known for its antihypertensive, anti-obesity, anti-inflammatory and antihypertensive that block the cell cycle. Sulforaphane regulates tumor progression, apoptosis and tumor growth. Genistein regulates miRNA and inhibits DNMT and HDAC.

Keywords: Breast Cancer, DNA methylation, Histone Modification, RNA Interferon, Epigallocatechin gallate (EGCG), Curcumin.

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INTRODUCTION

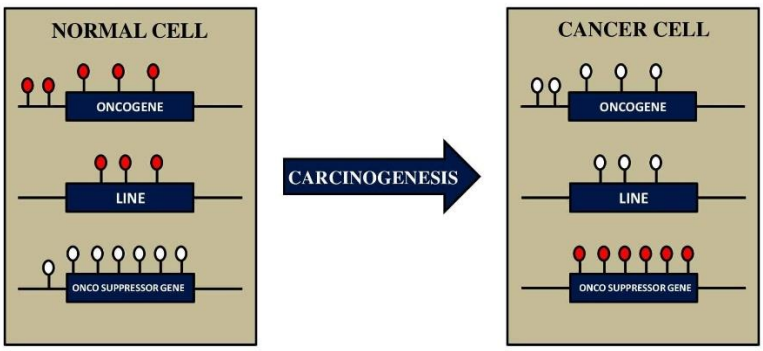
Epigenetics is the term that was first coined by Conrad H. Waddington in 1942. "The heritable change in gene expression and function without alternation in DNA due to changes based on sequence itself" [1]. There are three main type of epigenetic mechanism named as DNA methylation, histone modification and RNA interference (RNAi) [2]. The epigenetic modifications have its catalog called epigenome that occur in the genome [3]. The epigenetic modifications mediator enzymes have been found to be mutated in cancers. Cancer has a major significant role in global health. In 2018, around 18.1 million new individuals were diagnosed with cancer including 9.6 million mortalities. One of the leading cause of cancer death among women is Breast cancer, the most common diagnosed female cancers, accounts an estimate of about of 627,000 (6.6 %) deaths worldwide. Breast cancer progressions involve the accretion of aberrant changes both at genetic and epigenetic levels which ultimately results in tumorgenesis. Therefore, epigenetic regulations resulting due to DNA methylation, histone modification, nucleosome remodeling, and RNA-mediated gene targeting, are known to modulate a number of molecular, cellular and biological pathways associated with breast carcinogenesis [4].

EPIGENETIC MECHANISMS

DNA METHYLATION

DNA Methylation is generally considered as one of the most important epigenetic modifications occurring primarily at the cytosine residues of CpG dinucleotides [5]. DNA Methylation basically implies the mechanism of gene silencing via transcriptional repression. DNA methylation is basically one of the covalent modifications of DNA being moderated by DNMT. DNMT helps in removing methyl groups (-CH3) from S-adenosyl-L methionine (SAM) and add them to cytosine present in the so-called CpG island [6]. CpG regions are deposited inside large DNA sequences that are present in majority of gene promoters, intergenic regions and repeated elements in Human and Mouse genomes [7]. The alternations in DNA patterns are more common at the time of development and progressions of carcinogenesis [8]. The hypermethylation and hypomethylation in cancerous genes plays a critical role in the development of a variety of malignancies [9]. The hypomethylaion of LINE-1 gene is correlated with early carcinogenic characteristics in breast cancer; different clinical-pathological characteristics found in lung cancer and process of metastasis in colorectal adenocarcinoma [10]. DNA Methylation has also contributed to an increase in mortality rate in women having breast cancer and high risk of ovarian cancers. Please refer Figure A.

Figure A: DNA Methylation Changes In Carcinogenesis



HISTONE MODIFICATIONS

Histone Modifications are covalent post-translational modifications through mechanisms of acetylation, methylation, sumolyation, ADP-ribosylation, ubiquitylation and phosphorylation [11]. Histones are considered as an alkaline proteins found in the nucleus of eukaryotic cell [12]. Histones help in packaging of DNA into structural units naming nucleosomes. Nucleosome in turn has a core component called histone octamer. The two copies of each histone named H2A, H2B, H3 together forms an octamer along with histone H1 which always gets bind to the linker DNA between nucleosomes [13]. The histones

comprises of tails that are flexible in nature, arising out from nucleosomes lateral surfaces. These tails are more responsive for different chemical modifications taking place in different amino acids such as lysine (K), threonine (T), serine (S), and arginine(R) [14]. Histone proteins are structurally and functionally involved in the transitions happening between the active and inactive chromatin states. In case of highly packed chromatin (heterochromatin) the transcription of genes is blocked; whereas in case of less condensed chromatin (euchromatin) the transcription is activated [15]. Best studied mechanisms of histone modifications are acetylation and methylation. Aberrant histone modification can lead to development of cancers. In case of breast cancer global elevated histone levels of acetylation and methylation are often linked with favorable prognosis [16].

NON CODING RNA

The noncoding RNAs (ncRNAs) of epigenetics comprises of microRNAs (miRNAs), small interfering RNAs (siRNAs), Piwi-interacting RNA (piRNAs), and long noncoding RNAs (lncRNAs). MiRNAs are most studied ncRNAs which are small RNAs between 19 and 22 nucleotides in length and play important role in the regulation of gene expression by controlling mRNA translation. miRNAs usually target regions that are frequently associated with carcinogenesis [17]. The aberrant expression of miRNAs in cancer cells contribute to both the [18]. miRNA is of two types tumor promoting and tumor-suppressing. LncRNAs are another large group of noncoding RNAs that have an important role in tumorigenesis. Few lncRNAs are cancer type-specific, such as PCGEM1 in prostate cancer and HEIH in hepatocellular carcinoma. Dysregulation of HOTAIR has been noted in lung, pancreatic, and colorectal cancer [19].

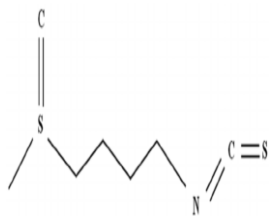
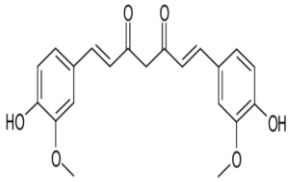
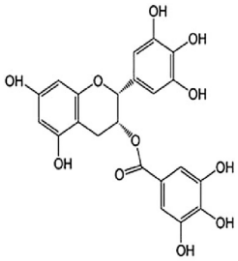
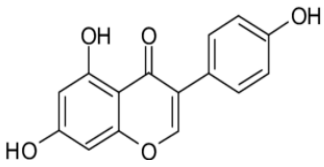
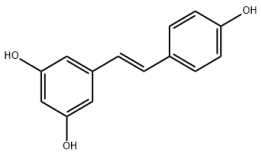
BREAST CANCER

A cancer develops from an uncontrolled cell growth acquired from the stepwise accumulation of heritable changes in gene function. Basically, cancer is caused due to changes in two different classes of genes: Tumor suppressor genes which inhibit cell growth and survival and oncogenes which promote cell growth and survival [20]. Epigenetic aberrations are key cause of carcinogenesis and its progression that lead to the activation or deactivation of specific genes that are typically regulated in normal cells [21]. Breast cancer is genetically highly heterogeneous disease. Breast cancer can be categorized into in situ carcinoma and invasive (infiltrating) carcinoma [22]. Breast cancer is the global community health crises. Breast cancer is the general carcinoma in women worldwide and is the leading cause of cancer-related mortality in females in developing and developed countries [23]. Number of factors are linked with the breast tumor growth, for example, gender, use of alcohol with high amount, diet (food), body movement, past of family history, lifestyle routine as well as endocrine aspect. Breast cancer characteristically shows no symptoms during its early stage that is why screening is vital for primary detect. Occasionally, breast cancer spreads to underarm lymph node which causes lump or swelling. Few common signs and symptoms like breast pain or heaviness; persistent alterations, like thickening swelling, or redness of the skin; and also, changes in nipple size, like spontaneous discharge, scaliness, or retraction, Slight tenacious alteration within the breast. However, its sensitivity and specificity is dissatisfactory [24]. In younger women, false-positive results are more common, who have previous breast biopsies, family history of Breast cancer and also who consume estrogen [25]. For better prognosis and therapeutic regimens specific biomarkers have been used which act as signatures for different subtypes of breast cancer [26].

EPIGENETICS AND BREAST CANCER

Epigenetic genomic alternations include promoter methylation of DNA and chromatin remodeling that plays an important role in tumorigenesis. Recent study states epigenetic alterations as one of the key factors in breast cancer [27]. These modifications are considered as targets for the therapeutics as they have the ability reversal. The breast cancer patient believes in understanding the role of epigenetic carcinogenesis which merge with targeted treatments, to overcome resistance and recover sensitivity to treatment. Please refer Table 1

Table 1 - Bioactive dietary compounds and their epigenetic functions

Bioactive dietary compounds and their epigenetic functions			
Dietary Compounds	**Structure**	**Food Source**	**Epigenetic Function(s)**
Sulforaphane (SFN)		Cruciferous vegetables (broccoli, kale, cabbage, Brussels sprouts)	HDAC inhibitor, DNMT inhibitor, Inhibits Htert (human telomerase reverse transcriptase), Increase the expression cyclin B1, Activates the poly(ADP-ribose) polymerase 1 and caspase family proteins.
Curcumin		Turmeric (Curry)	DNMT inhibitor, Upregulates the expression of some miRNAs to reduce the expression of Bcl-2, Enhances the efficacy of paclitaxel by deactivating NF-kB and MMP-9 expression in MDA-MB-231 cell.
Epigallocatechin-3-gallate (EGCG)		Green tea	DNMT inhibitor, HAT inhibitor, miRNA regulator, lowers the histone deacetylase activities, increases the acetylated lysine 5, 12 and 16 levels on histone H4 and acteylated lysine 9 and 14 levels on histone H3 (H3-Lys9 and 14), Decreases the levels of methylated H3-Lys 9, Increases the expression of epigenetically repressed TIMP-3 gene,
Genistein		Soy and fava beans	DNMT and HDAC inhibitor, miRNA regular, Regulates the p21 and p16 expression, Epigenetically restores Erα expression, Blocks the expression of DNMT1, Uprises the regulation of Brcal and Brca2 mRNA expressions in adult ovariectomised rats, Reduces the size of the tumours by 50%.
Resveratrol		Grapes, peanuts, mulberry, cocoa	DNMT and HDAC inhibitor, miRNA regulator, decreses RASSF-1α methylation, Activates SIRT1 and acetyl transferase p300, Decreases the expression of DNMT1, DNMT3a, and DNMT3b, HDAC1 and methyl CpG binding protein 2 (MeCP2) in MCF-7 cell line.
Lycopene		Tomato, carrot, watermelon, papaya, cherry	Upregulated the expression of GSTP1, demethylates GSTP1 promoter

EPIGENETIC MODIFICATIONS AGAINST BREAST CANCER DUE TO NATURAL COMPOUND

Epigenetics and genetic alterations cause the inactivation of cancer suppressors, deregulation of intracellular signaling cascades, overexpression of oncogenes, invasiveness, metastasis, cancer micro environmental changes and deregulation of immune response, failures causing cancer cell death which support cancer development [27]. Natural compound isolates natural sources i.e., fungi, marine life forms

and plant have overturned the field of anticancer therapeutics and rapid development in preclinical studies. An Epigenetics molecular mechanism that modulates DNA damage, gene expression and repair mechanisms could affect by natural compounds. Natural compounds depict positive health effects that are observable on specific molecular target by indirectly stabilizing conjugates which effect on metabolic pathways. The tumor suppressor genes (TSGs) were related to epigenetic silencing in carcinomas which abnormal has histone modifications like methylation of histone and acetylation [28]. Recently, natural compounds or phytochemicals such as epigallocatechin gallate (EGCG), curcumin, apigenin, resveratrol and also lycopene shown to alter epigenetic mechanisms and inhibit the synthesis of metabolic product i.e., leukotriene and prostaglandins are notion to be effective therapeutic agents against carcinoma.

EPIGALLOCATECHIN- GALLATE

The epigallocatechin is known as epigallocatechin-3-gallate, gallic acid and an ester of epigallocatechin. This component is extracted from green tea and if taken regularly then, the risk of breast and prostate cancer get reduced. Its mechanisms are unfavorable in cancer, bone regeneration, nervous system and vascularity. This review targets on effects of epigallocatechin, which includes antioxidant, anti-inflammatory, anti-cancerous, anticollagenase and antifibrosis effects to show the potential of EGCG. It has been detected that EGCG epigenetically reactivates Bax, PUMA and p21/wafl which further causes cell cycle arrest and programmed cell death moderated by proteasome degradation of class 1 HDACs [29] describes repression in the hormonal activity of androgen receptor (AR) by EGCG due to the lowering in acetylation of AR. It leads to decrease in promoting cell death and cell proliferation in LNCaP prostate cancer cell lines. It is considered as a potential epigenetic modifier of HDACs and DNMTs and it also restores epigenetically silenced gene in cervical and skin cancer. For example, in esophageal cancer EGCG leads to programmed cell death and also cell growth of ECa109 cells gets prohibited through p16 gene demethylation [30]. The protein levels of DNMT1, DNMT3a and DNMT3b get decreased in skin cancer cells due to ECGC effects.

CURCUMIN

It comprises of natural anti-inflammatory, antioxidant, anti-proliferative and anti-cancerous properties isolated from the roots of plant *Curcuma longa* (Turmeric), which is widely used in India and China for medicinal uses and has been used for treating medical conditions for many years. This natural compound has been proved to be a distinctive non-toxic hypomethylating agent for breast cancer therapies [31]. It is considered to be effective in treating chronic conditions like inflammatory bowel disease, rheumatoid arthritis, Alzheimer's and common malignancies like lung, breast, colon, stomach and skin cancers. Being principal component of turmeric, it is one of the curry spices which are used as an edible component through different parts of Asia, due to its flavor and color profile and so for its medicinal properties. Curcumin is used in ayurvedic medicine for the treatment of health conditions including liver disorders, respiratory illness, diabetic wounds and inflammatory disorders. It is also used in ancient Hindu medicines and traditional Chinese medicines. In ancient Hindu medicine; it is used to treat swellings and sprains and in traditional Chinese medicine; it is mainly used for treatment in condition related with abdominal pain. It inhibits DNMT1 expression and also restores the function of RASSFIA by initiating the promoter hypomethylation in estrogen positive MCF-7 breast cancer cell lines. Additionally, it also decreases the breast tumor growth rate and cell proliferation rate in situ [32].

RESVERATROL

It is a Stilbene–type bioactive molecule with a wide spectrum of reported biological effects. It is a sort of natural phenol produced by many plants. Resources of Resveratrol in diet food include blueberries, peanuts, raspberries and mulberries. It has been proved as a powerful anti–aging and anti-inflammatory. It also shows chemo preventive activities. It is phytoalexin which is common in many plants e.g., cranberries, grapes and blueberries. This is a polyphenolic agent who provides the therapeutics and chemo preventive effects within numerous types of cancer regulating biological functions mainly programmed cell death and metastasis, cell division and cell proliferation in mRNA expressions. These changes are found in specific oncogenes (RUNX2, MMP9, HK2, AURKA, CCNBI, DDIT4, DLGAP5, EYS, and FAM83D) [33]. In human MDA-MB-468 breast cancer cells and A2058 melanoma cells, it decreases the activity rate of demethylation of the ER-alpha promoter regions; it also reduces the

STAT3 acetylation activity. It is also sensitized to antiestrogen therapy and decrease the cell viability and cell death.

QUERCETIN

Quercetin comes from the latin word 'Quercetum', means oak forest, it belongs to the class known as flavonols which cannot be produced in the human body. It is one of the most important bioflavonoids found in fruits and vegetables such as green tea, onions, apple, red wine etc. It is present in more than twenty plants material, which is known for its antihypertensive, anti-obesity, anti – inflammatory, anti- atherosclerolemic and antihypertensive activity. Quercetin blocks the cell cycle and causes pro-apoptotic effect without damaging normal cells [34]. Furthermore, it is described that quercetin suppresses/restricts the binding activity of transactivators CREB2, C-jun and C/EBP beta, blocks the recruitment activity of the coactivator p300 to COX-2 promoter and also inhibits the p300 HAT activity [35] seen in colorectal cancer cells. It also inhibits the tumor growth by activating the p161NK4a effects via promoter demethylation. It promotes the cell death by fasL expression moderated by H3 acetylation in leukemic HL-60 cell line [36].

SULFORAPHANE

Sulforaphane is phytochemical which is derived from cruciferous and it regulates tumor progression, apoptosis and tumor growth [37]. It activates the anti-inflammatory and anti-aging responses by inducing Nrf2 pathway and interdicts NF-Kb. It also has an epigenetic effect by interdicting HDAC and DNA methyl transferase and changing mitochondrial dynamics [38] describing the hypermethylation in miR-9-3 promoter region in lung cancer cells. It also restores the miR-9-3 expressions via epigenetic regulation which declines the HDAC1, HDAC3, HDAC6 and CDH1 protein expression rates.

CONCLUSION

Breast cancer is based on malignancy with a substantial morbidity rate and rate of mortality among women. There are unusual kinds of treatment approaches. They have lower side effects attracting a huge deal of attention due to herbal treatments. Natural compounds play a major role for the treatments of breast cancer in the field of concretization which enhances the capability to eliminate all "pre-cancerous" tissues and reduce local recurrences. These natural compounds are significant factor of our diet unlike the demethylating chemicals. Natural compounds or phytochemicals provide positive health benefits due to their direct action on specific molecular targets such as genes, or by indirectly stabilizing conjugates that affect metabolic pathways. Compared to the chemicals, natural compounds are very much promising in breast cancer chemoprevention, due their less side effects and minimal toxicity in both *in vitro* and *in vivo* experiments. Studies shown that a number of phytochemicals such as curcumin, genistein, lycopene, and apigenin have been directly reported to inhibit biosynthesis of metabolic products such as prostaglandins and leukotriene's and therefore, considering them as the potent therapeutic agents in breast cancer chemoprevention.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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