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Epigenetic Pathways in Breast Cancer: Diagnostic Markers, Transgenerational Impacts, and Emerging Therapies

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ABSTRACT: Breast cancer affects the lives of millions of women each year, and there is still no cure for this disease. There are known links between breast cancer and epigenetics, which is the mechanism of altering gene expression and affecting biological activities without changing the gene sequence. Given this, researchers are investigating breast cancer development, diagnosis, and treatment. This paper will investigate how transgenerational epigenetic changes affect the growth of breast cancer, diagnosis, and what are therapeutic approaches to mitigate these effects. Understanding the epigenetic links to breast cancer is key to improving diagnosis and treatment. Through a thorough examination of the existing literature, this paper analyzes the transgenerational epigenetic development of breast cancer, the diagnosis of epigenetic markers of breast cancer, and therapeutic approaches for treating epigenetic markers of breast cancer. Several therapeutic approaches are developed targeting epigenetic markers, including the development of epidrugs, which inhibit key parts of the epigenetic pathways leading to breast cancer; these are comprehensively studied in this paper. Overall, understanding and targeting epigenetic mechanisms underlying breast cancer is essential for advancing diagnosis, developing effective treatments, and potentially reducing the transgenerational impact of the disease.

KEYWORDS: Biomedical and Health Sciences; Genetics and Molecular Biology of Disease; Epigenetics; Breast Cancer; Therapeutics.

Introduction

Breast cancer killed 670,000 people in 2022 worldwide, and 2.3 million women were diagnosed in the same year.[1] However, this lethal and troublesome disease cannot be cured but only alleviated.[2] Traditional treatments like surgery, radiotherapy, and chemotherapy can be indiscriminately applied to patients. Additionally, some more personalized and advanced treatments, which will be discussed later, are available depending on the subtype of breast cancer. Breast cancer is categorized by receptors on the tumor cells, which are proteins that can receive and transduce signals.[3] For patients who are positive for estrogen (ER) or progesterone receptors (PR), cancer growth can be caused by the presence of hormones.[4,5] Accordingly, endocrine therapy using drugs that degrade or inhibit the hormone receptors can help mitigate cancer progression, though the risk of recurrence and resistance remains a problem.[3] Noteworthily, these therapeutics can have adverse effects when paired with traditional treatment approaches. On the other hand, hormone-negative patients mainly rely on chemotherapy and radiotherapy as there are limited receptors to target, which makes those diagnoses more lethal.[3] The most progressive subtype with the worst prognostic is triple-negative breast cancer (TNBC). There aren't specific treatment options for this subtype due to the absence of receptors. Thus, a standardized treatment for TNBC is a prospective future direction for research.[3] As demonstrated, there is hardly an almighty cure for this disease.[6]

Epigenetics can be responsible for cancer's occurrence. Epigenetics alters the phenotype of the organisms in response to an environmental stimulus without changing the DNA sequences.[7] Epigenetic regulation influences how the DNA folds (chromatin structure) so that the amount of the protein that is produced by the regulated segment of the gene is increased (an open chromatin) or decreased (a closed chromatin).[8] Proteins act out a specific function, resulting in phenotypic change. There are several types of epigenetic regulation. DNA methylation was the first discovered epigenetic alteration and is, therefore, the best studied.[9] A methyl group is added to the cytosines (usually in the regulatory area near the promoter) to close the chromatin structure, decreasing the transcription rate and silencing the gene.[8,10] Another mode of epigenetic modification is histone modification, which can take various forms (de-/acetylation, methylation, phosphorylation, sumoylation). In acetylation, the acetyl groups of two carbon, three hydrogens, and one oxygen atom are added to the amino acid lysine. Thereby, DNA becomes less compacted around the histone core and more open to make more protein, increasing the transcription rate.[11] Another component modifying chromatin structure is noncoding RNA (ncRNA). Unlike transfer RNA and messenger RNA that transcribe/translate DNA to make protein, ncRNA performs the regulatory work after the transcription. It affects gene expression states, especially in cell development and differentiation, and can even induce DNA methylation. Importantly, every mechanism mentioned above is reversible by an opposing action and is catalyzed by the enzyme.[8]

Another notable feature of epigenetics is its transgenerational inheritance. Several mechanisms are observed to carry out

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epigenetic inheritance in multiple species. Indeed, epigenetic inheritance has been recorded in corn, roundworms (*Caenorhabditis elegans*), fruit flies (*Drosophila*), and the plant Arabidopsis.[12] Epigenetics maintains the stability and state of the gene transcription during the cell cycle.[13,14] Adverse effects arise when those regulations are disrupted.[15] Cancer is the wrongful turning on of some genes (oncogenes) to induce detrimental activities like angiogenesis and tumor formation and turning off of the protective genes (tumor suppressor gene) that perform apoptosis and tumor suppression.

A paper published in 1983 by Andrew P. Feinberg and Bert Vogelstein reported the discovery of hypomethylation as a distinctive marker in cancer genes, making cancer the first disease connected to epigenetics.[16] Since then, epigenetic mechanisms have become successful factors in diagnosing and distinguishing between breast cancer. For example, high levels of methylation distinguish ER+ breast cancer from ER- breast cancer, and the methylation level of different genes fluctuates in different subtypes. Overexpression of one histone acetylation enzyme can indicate lower survival rates.[17] With all being said, the significance of genetic factors, besides epigenetic factors, remains unignorable. Mutations in BRCA genes and other genes largely elevate the likelihood of getting breast cancer, especially in the incidence of familial breast cancer.[18] In light of the transgenerational aspect, diet, environment, and habitual behaviors of the paternal germline can contribute to the cancer risk of future generations through epigenetic inheritance.[19] Therefore, this paper will investigate the epigenetic mechanisms behind the diagnosis, development, and treatment of breast cancer combined with the transgenerational perspective.

Results and Discussion

Development of Breast Cancer and Transgenerational Epigenetics :

Overall, the development of breast cancer can be simplified as the epigenetic dysregulation of cancer genes, leading to cancerous hallmarks like metastasis, tumorigenesis, and apoptosis resistance.[20] Metastasis occurs when the original tumor breaks apart and spreads to secondary locations to develop new tumors. E-cadherin is a transmembrane protein that allows cells to stick together.[21] The hypermethylation (more silencing) and hypoacetylation (less transcribing) of the E-cadherin gene's promoter ultimately reduce its production of such protein and elevate transcription factors (TFs). TFs accelerate EMT (transformation from epithelial cell to a less adhesive cell type), a fundamental process of metastasis.[22] NcRNA epigenetic dysregulation is also involved in metastasis. There are mainly two types of ncRNA strongly correlated with cancer: microRNA (miRNA, >200 nucleotides) and long-noncoding RNA (lncRNA, <200 nucleotides). They work with RNA-binding proteins (RBPs) to modulate cancer genes during and after the transcription of the genes by epigenetic mechanisms.[23] Particularly, HOTAIR (lncRNA) is responsible for chromatin state reprogramming, usually as a silencer that suppresses gene transcription.[24] In a cancerous context, HOTAIR utilizes PRC2, the catalyzer for the methylation of a specific histone, to silence metastasis-suppressor genes, promoting cell motility.[25,26]

Methylation of another type of RNA transfer RNA (tRNA) is associated with tumorigenesis. tRNA methylation is directed by the enzyme tRNA methyltransferase and plays a critical role in cell proliferation and differentiation.[27] In breast cancer, the loss of gene (hTRM9L) that codes for a tRNA methyltransferase leads to decreased tRNA methylation and disrupts the cell cycle, promoting tumor cell formation.

Apoptosis is the programmed cell death that clears unwanted and abnormal cells.[28] A report written in 2003 by David Mu *et al*. established the direct relationship between overexpression of *KCNK9*–an oncogene that can activate ion channels–and the apoptosis resistance hallmark in cancer.[29] In a newer report written in 2021 by David. A. Skarr *et al*., *KCNK9* is observed with hypomethylation in TNBC and its high-risk patients.[30] Hypomethylation of *KCNK9* increases the expression of the protein it encodes–*TASK3*, which regulates mitochondria membrane potential, a factor initiating apoptosis. Overexpression of *TASK3* decreases apoptosis, hence bolstering cancer cell survival chance.[30,31]

More importantly, *KCNK9* methylation is a form of genomic imprinting in breast tissue.[30] Genomic imprinting occurs in the germline cells. It passes the epigenetic dysregulations that the environment stimulates in the parents to the offspring, making them susceptible to the parental environment without direct interaction. The dysregulation of those imprints can induce cancer risk and other disease risks, so such imprints reflect environmental risk on cancer susceptibility. For example, exposure to the environmental toxicant endocrine disruptor vinclozolin (commonly used as a fungicide) induced transgenerational breast cancer in rats.[32,33] Additionally, breastfeeding in mice was discovered to transmit miRNA with epigenetic alteration roles to the offspring.[34] Exposure to certain toxins has known effects on the maternal germline but not on the paternal germline.[33] That said, which human genes promote disease susceptibility with genome imprints is unknown.[35] Transgenerational epigenetic inheritance remains a big area of interest for future research as its unanswered questions abound: what is the purpose of transgenerational inheritance in evolution since it provides disadvantages like promoting breast cancer and other cancers, and what can we utilize from it for the treatment and prediction of breast cancer. More research on the molecular basis of such mechanisms instead of the pathological behaviors of the subjects is needed to support the occurrence of epigenetics in mammals, especially humans.[36]

The effect of mutation and epigenetic regulation of genes on breast cancer will be incomplete without that of *BRCA1/BRCA2*. *BRCA1/2* (BReast-CAncer susceptibility gene 1/2) are tumor-suppressor and DNA repair genes.[37] They maintain the progression of the cell cycle and protect the genome's stability and integrity. Hyperphosphorylation of *BRCA1* is often observed in DNA damage, though *BRCA2* is more directly involved in DNA repair. *BRCA1* is hyperphosphorylated by the bonding of ataxia-telangiectasia mutated (ATM) to detect the Double-Strand Break, DBS, a threatening type of DNA damage. *BRCA2* utilizes DNA repair protein RAD51 to amend

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the DBS by homologous recombinant (HR), a preferable and error-free process that employs the intact sister chromatid as a template strand for reparation.[38] The hyperphosphorylation of *BRCA1* also activates cell cycle checkpoints G1/S checkpoint (check before and during replication) and G2/M checkpoint (check before cell division). Since *BRCA1* phosphorylation participates in cell cycle checkpoints and DNA repairs, mutation in the sites where phosphorylation occurs initiates tumorigenesis, uncontrolled cell division, and increased proliferation. 15-20% of hereditary breast cancer showcased mutation in the *BRCA* gene.[38,39]

Cancer can also occur with normal *BRCA* due to epigenetic silencing and suppression. Hypermethylation of *BRCA1* silences the gene expression and stunts the DNA repair and checkpoints.[39] In 2019, research investigating the abnormal epigenetic regulation on histone markers and tumor suppressor genes in breast cancer revealed that promoter methylation (prohibiting the gene from being transcribed) of *BRCA1* occurs in 44% of 58 patients.[40] In research conducted three years later focusing on the different methylation of *BRCA1* and *BRCA2* genes, the *BRCA2* gene (50%) is more frequently methylated than the *BRCA1* gene (34%) in the 50-patient sample.[41] In an aspect of post-transcriptional regulation by ncRNA, miRNA-146a and miRNA-146b-5p downregulates *BRCA1* while miRNA-19a and miRNA-19b downregulates *BRCA2*, increasing cell proliferation and decreasing homologous recombinant, the process that restores the damaged gene.[39]

There is a lot of research supporting the link between hereditary breast cancer and *BRCA* mutation.[42] However, there is a lack of solid link between the epigenetic dysregulation of *BRCA* and hereditary breast cancer in mammals and especially humans, consequently suggesting potential future direction.

Diagnosis of Breast Cancer Epigenetic Factors:

The use of epigenetic factors to diagnose disease is a relatively new idea.[43] As mentioned, it was in 1948 that the first "epigenetic mark" (the methyl group added to a cytosine) was detected by Rollin Hotchkiss.[43] Later, in 1983, DNA methylation patterns were demonstrated to distinctly differentiate cancer cells from normal cells in human tissue samples in a study of A. P. Feinberg and B. Vogelstein.[16] This was a landmark study because it made cancer one of the first diseases that epigenetic factors could diagnose. Besides DNA methylation, other epigenetic factors like histone modification and miRNA can be used as biomarkers for cancer diagnosis.[44] Those epigenetic alterations often occur earlier than observable symptoms and before cancer is diagnosed. This means detection of epigenetic factors can be a preliminary diagnosable approach for high-risk individuals who are at an older age, with a family history of breast cancer, or those who have close relatives with BRCA mutation.[23] Different panels can be used to detect epigenetic factors like miRNA and methylation in blood, serum, urine, and other body liquids collected by liquid biopsy. Detection of breast cancer with epigenetic factors is arguably less costly and more on time compared with the traditional approaches, though it showcased inconsistency in the lab.[23,45] Overall, epigenetics sheds light on diagnosing breast cancer since the discovery of its exclusive appearance in cancer cells.

Methylation-specific PCR (MSP) is used to analyze DNA methylation sites on strands of genes.[46] The MSP can be divided into two steps. The first is detecting methylation by bisulfite conversion, where methylation adds a methyl group to the cytosine amino group of DNA. When the same sequence of single-stranded DNA with different methylation states reacts with sodium bisulfite, two different sequences of DNA will be produced because the unmethylated cytosine will be converted to a uracil. Uracil and cytosine are all nitrogen bases that determine the sequence of nucleic acids. A conversion to uracil creates a discrepancy in the differentially methylated DNAs, which is detectable in later steps. After the different methylation state is manifested, PCR is used to amplify the result by two "primers" --primer U to amplify unmethylated cytosines and primer M to amplify unmethylated cytosines. Under visualization approaches like fluorescence or gel electrophoresis, primer U will showcase marks when both alleles are methylated. On the other hand, primer M showcases marks when none are methylated, and both primers will amplify when both conditions occur.[46] Overall, PCR is a quantitative and rapid approach with high accuracy and sensitivity in various genes from sources like blood and serum.[47] PCR panels are done to look for hypermethylation of specific genes designated as "biomarkers" because they are usually methylated in promoter regions in breast cancer.[46] In an experiment comparing biomarkers in the genes *APC*, *BRCA1*, *CCND2*, *FOXA1*, *PSAT1*, *RASSF1A*, and *SCGB3A*, the most accurate genes containing biomarkers for the detection of breast cancer were APC, FOXA1, and *RASSF1A* with an accuracy, sensitivity, and specificity over 70%.[48] The high percentage in those parameters indicates accuracy in differentiating the cancer cells from healthy cells. MSP's success in finding methylation marks in cancer cells renders DNA methylation competent as a diagnosing mark.

In addition to DNA biomarkers, miRNAs are qualitative biomarkers for breast cancer.[39] MiRNA is one kind of non-coding RNA that regulates the expression of genes during or after its transcription. Aberrant levels of miRNAs can be observed in reduced tumor suppressor genes. Indeed, significant overexpression of miRNA appears exclusively in breast cancer; meanwhile, some miRNAs are downregulated as oncogene suppressors that subdue genes that lead to cancer activities like metastasis and cell proliferation.[49] Approaches to detect abnormal miRNA levels are qPCR, microarrays, and miRNA Next Generation sequencing (NGS). In a microarray, samples of miRNA are first dyed by fluorescence to a specific color. The colored miRNA is then put into thousands of lined probes and binds to the probe with its complementary strand. This prompts the sample miRNA to be stationed to the solid probes that indicate its type. The array of probes with different spectra of coloring (due to the coloring of miRNA) is then analyzed. The high intensity of color in one miRNA implies the overexpression of the miRNA in this sample.[49]

Another tool for finding miRNA biomarkers for breast cancer is PCR. First, the RNA samples (e.g., miRNA) are reve-

rsely transcribed into their complementary DNA (cDNA) strands, which will then gather their matching complementary strand to form the double helix structure of normal DNA. This step alone is called reverse transcription PCR (RT-PCR), but more steps are needed for sequencing miRNA. The next step is called quantitative real-time PCR. cDNA is put into probes containing fluorescence dye that can bind to them. Because the reaction happens in "real-time," the reaction time is measured and plotted on the graph, which amplifies the sensitivity and quantity of miRNA samples for analysis.

NGS is one application of PCR, which uses the expression rate of a specific sequence of genes in one miRNA to predict its abundance in the entire sample with many more times of miRNA. This is a very simplified method that can quantify miRNA expression like RT-qPCR, though more inputs of RNA are needed for accuracy.[50] For example, upregulation of the miRNAs miR-185-5p and miR-362-5p are sensitive indicators (sensitivity 92.65%) that specifically (specificity of 92.31%) occur in breast cancer, being a distinctive marker for breast cancer cells.[51] In addition, panels on miR-21 and miR-27 in blood plasma achieved a sensitivity of 90.62%, agreeing with the sample donor's breast cancer diagnosis.[51] Particularly, a higher level of miR-21 is a sign of poor prognostic results, with a higher tendency to develop metastasis and aggressive cancer activities because it directly downregulates several tumor-suppressor genes.[52] More importantly, the previously mentioned miRNAs are demonstrated to regulate the epithelial-mesenchymal transition (EMT), a metastasis pathway.[51] This means levels of different miRNAs might predict the potential secondary location of metastasis (e.g., miR-21 indicates metastasis to bone). Specific miRNAs are even associated with the development of TNBC.[53] To conclude, the sequencing of miRNA creates another option for diagnosing breast cancer by epigenetic factor and potentially predicting the survival rate of patients.

Liquid biopsy is not a technology used to sequence the epigenetic factor at the molecular level but rather a method to collect the samples for subsequent molecular sequencing.[54] Compared to mammography and tissue biopsy, liquid biopsy is less invasive and more comprehensive in examining the genomic landscape. Indeed, liquid biopsy is the sampling of minuscule materials that broke off from tumors and are circulating in body fluid like urine and blood; examples are tumor DNA, circulating tumor DNA (ctDNA), cell-free DNA (cfDNA or ccfDNA), and miRNA.[55] CfDNA is DNA released into the bloodstream when cells die and can appear in healthy patients. CtDNA is the cfDNA released by dead tumor cells, betokening the presence of tumors, and can be detected by liquid biopsy. In a study detecting TNBC with cfDNA derived by liquid biopsy, protein kinase *CDKL2* hypermethylation is suggested to correlate with *HER*+ breast cancer.[56] Additionally, epigenetic factors in serum can help determine breast cancer patients' grade, type, and survival rate. Serum of patients with invasive breast cancer might have *SPAG16* gene promoter methylation; elevated methylation of *SFRP5* often appears in the serum of patients with TNBC, a cancer type with poor prognosis;[10] Differentially Methylated Regions (DMR, where the region of gene shows discrepancy in methylation between BC cells and healthy cells) in breast cancer are furtherly diversified between low, intermediate, and high level of tumor grade. Overall, the methylation score of cfDNA is higher in breast cancer patients compared to normal people and increases as the stage of cancer progresses. Those discoveries reveal that liquid biopsy is a competent sampling approach for its less invasive nature, and the sample collected (like cfDNA) can be utilized for not only diagnosis but also prognosis of breast cancer. The utilization of epigenetic markers as diagnostic factors presents an advanced, accurate, and noninvasive detection of breast cancer.

Therapeutic Approaches for Treating Epigenetic Markers of Breast Cancer:

Epigenetic marks in cancer lead to erroneous expression levels of specific genes, differentiating healthy cells from cancerous ones. Using epidrugs to fight cancer is a contemporary idea, and started roughly around 2006 (when the first epidrug Vorinostat was FDA approved).[57] Epigenetic mechanisms are catalyzed by enzymes and reversible, which is the underlying rationale of how the drug works: disrupting the enzyme to stop/boost the reactions. DNA methylation is catalyzed by the enzyme DNA methyltransferase (DNMT), and histone acetyltransferases HATs and deacetylases HDACs catalyze histone acetylation. As a result, the epidrug can be divided into two groups: DNMT inhibitor (DNMTi) and HDAC inhibitor (HDACi), plus some newer drugs that regulate ncRNAs.[57] The use of chemicals as an inhibitor to the enzyme can successfully disrupt the epigenetic mechanism it regulates, therefore reactivating or suppressing genes. To exemplify, if a tumor-suppressor gene is silenced by methylation on its promoter region, epidrug blocking the methylation can hopefully reactivate the transcription of this gene and alleviate tumor progression.[58]

One big group of epidrug is DNA methylation inhibitors (DNMTi) and histone acetylation (HDACi). Generally, the DNMTi works by targeting the two integral parts of DNA methylation: the DNMT that binds to the CpG island to catalyze the process and the S-adenosyl-L-methio-nine (SAM) that donates the methyl group, which will be added to the DNA to complete the methylation. Both azacitidine and decitabine are drugs that interrupt the binding of DNA and the DNMTs.[59] The decitabine works by replacing the energy-providing molecule and irreversibly binds to the catalytic site of the DNMT, achieving demethylation. Low doses of decitabine induce promoter demethylation of tumor-suppressor genes like *TIMP3*, *p15*, *p16*, e-cadherin, and *RASSF*. Interestingly, higher doses of decitabine can kill cancer cells by its cytotoxic effects. The other drug, Azacitidine, is less effective in disturbing DNA methylation than decitabine because it is mainly incorporated into the RNA and dysregulates its metabolism, protein synthesis, and methylation, killing the cancer cell. Only 10-20% of azacitidine will be bound to DNA for demethylation by converting to decitabine.[60] Another limitation of azacitidine is that it binds to the whole DNA instead of binding to a specific region of DNA, thus lacking specificity in targeting genome region or CpG islands, and may raise un

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wanted side effects. The HDACi works by depriving the zinc of the HDAC's active site to disable the binding.[61] Vorinostat (SAHA) and panobinostat are all hydroxamic acid-based and FDA-approved inhibitors. They are all broad spectrum, meaning they target multiple HDACs. Hydroxamic acid is their primary functional group and interacts with zinc ions.[62] While hydroxamic acid-based inhibitors have tumor-suppressing effects and can enhance chemotherapy efficacy, their limitation, on the other hand, is their disappointing effectiveness on solid tumors.[60]

Bromodomain (BRD) is a protein that recognizes the acetylated markers on the lysine to employ the needed proteins at the acetylation site.[63] They are not enzymes that catalyze this process (like HDAC, the "writer"), but they still play a significant role as a "reader." Several proteins related to chromatin modification contain BRD, including the BET proteins, which are considered transcription regulators. Above all, Brd 2, 3, T, and 4 are correlated with breast cancer. In particular, Brd 4 enrolls a protein complex that regulates transcription to the transcription site of MYC, a growth-promoting gene that is oncogenetic and breast cancer-provoking. Brd 4 binds with acetylated histones and other transcriptional activators to form a super-enhancer complex that can activate the transcription of super-enhancer-driven oncogenes like MYC. This relationship is the target of BET inhibitors, which repress the activation of such oncogenes by disrupting the binding of Brd 4 and the other components of the super-enhancer. In general, BET inhibitors' adverse effects are unignorable, especially when combined with radiation therapy.

Conversely, when combined with chemotherapy, BET inhibitors demonstrated a satisfying anticancer effect in TNBC. More research should focus on the rising potential of its anticancer effect with other epidrugs like HDACi. Moreover, currently, no drugs targeting MYC are developed, which remains a blank yet to be filled.

Histone methylation is the addition of methyl groups (varies in number) on the lysine protein or arginine protein. Specifically, histone methylation of lysine is catalyzed by lysine methyltransferases (KMTs) and reversed by lysine demethylase (KDMs), keeping the transcription rate of genes at balance.[64] Different lysine methylation is done through different KMTs, and different inhibitors are developed. Some KMTs targeted for inhibition in anticancer therapy are EHMT1/2, EZH, and SMYD2. EHMT1/2 methylates H3K9 (Histone h3 Lysine 9) and lysine 373 of p53, a tumor suppressor gene.[65] G9a is one kind of EHMT that methylates H3K9. Its inhibition stunts cancer cell growth and migration in breast cancer.[66] BIX01294 inhibits G9a by binding to the substrate side of the enzyme that H3K9 would otherwise bind. It showcases acceptable toxicity as drugs treating breast cancer. EZH plays an enzymatic role in the PRC2 that methylates H3K27 to silence tumor-suppressor genes.[65] A variety of its inhibitors had been developed, but none was directed to the treatment of breast cancer. A study in 2019 by Ma *et al.* first discovered an EZH inhibitor, MS1943, effective in treating TNBC. It disrupts the 3D folding of the EZH polypeptide chains, so the protein loses its functional form. MS1943 was effective in several cell types and showcased acceptable toxicity in mice.[67] The last KMT linked to breast cancer is SMYD2. In the study by Li *et al.*, 2018, "SMYD2 methylates [silence not only] histone H3K4 and H3K36, [but also tumor suppressor gene] p53, [breast-cancer related proteins] *Rb, HSP90* and estrogen receptor α (ERα)". AZ505 can inhibit SMYD2 to decrease tumor size, suppress cancer cell growth, and induce apoptosis by demethylating the *p53* gene.[68] AZ505 is an inhibitor highly selective to SMYD2 and works by binding to the substrate site of the enzyme to prohibit future catalytic reactions.[69] Besides enzymes that help methylation, enzymes that promote demethylation can also be the targets of drugs. LSD1 is a histone demethylase reversing the methylation on histone H3K4 and is overexpressed in ER- breast cancer. Despite the conventional knowledge that demethylation is often linked to the expression of genes, demethylation of H3K4 is an indispensable process for subsequent methylation, which ultimately silences the gene. Demethylation of genes like p21 and *p53* disrupt their proper functioning.[70] Phenelzine is an inhibitor of LSD1 that alleviates metastasis in breast cancer. It achieves inhibition by binding to the active site of the LSD1.[71]

Grimaldi *et al.* did an impressive job in organizing the recent studies that discovered possible miRNAs as therapeutic targets for breast cancer. MiRNA treatment is usually done by restoring or inhibiting the trouble-causing RNA. For example, deficiency in miRNA-26a is found to have a low survival rate, so delivery of this miRNA to TNBC cells can suppress cancer cell growth and reinforce chemotherapy efficacy. Injection of miR-34a increased survival rate in mice. Injection of liposomes containing miRNA-203 and liposomes with chemotherapy drugs can nearly entirely inhibit cancer cell growth. As mentioned, miRNA-21 is a major biomarker for breast cancer. Inhibition treatment on miRNA-21 induces apoptosis and restrains tumor formation. The two limitations of this method are the specificity of the miRNA targets and the stability of the delivery method. miRNA tends to target numerous genes simultaneously, so unwanted or enlarged reactions might arise as adverse effects.[72] Some examples of transportation nanoparticles are liposomes, exosomes, quantum dots, dendrimers, and polymeric nanoparticles.[73] Granted, there are limitations and adverse effects in the newly developed drugs, but still, they should be considered as prospective breast cancer therapeutic. They are shown to have impressive efficacy in hampering cancer development by interfering with the critical enzymatic process or killing cancer cells by the drug's cytotoxicity. The future direction of epigenetic therapeutics should experiment with more combinations of traditional therapy and drugs (or a combination of drug and drug) to decrease cancer cells' drug resistance and chemotherapy resistance.

Conclusion

Alongside the rising research on epigenetics, its medical implementation is starting to surface. Epigenetics plays a significant role in the development, diagnosis, and treatment of breast cancer, a fatal and incurable disease common in women. It determines the expression state of genes by turning it on or off, resulting in phenotypic changes without altering the genetic sequence. The improper epigenetic regulations

of oncogenes and tumor suppressor genes cause cancerous activities such as metastasis, inhibited apoptosis, cell proliferation, and tumorigenesis, depending on the activity the gene directs. *BRCA1* and *BRCA2* are two genes whose mutations are most broadly discussed in the causation and incidence of the most precarious breast cancer subtype. Studies are showing that unmutated *BRCAs* can also cause breast cancer due to aberrant methylation and miRNA regulation. Importantly, epigenetic alterations appear before cancer diagnosis in high-risk individuals, enabling more on-time intervention. MiRNA and methylation levels of cells collected by liquid biopsy, a less invasive technology, from breast cancer patients can be discriminated from those of healthy people and patients with different severity by molecular sequencing technologies. Diagnosing by epigenetic factors allows accurate detection of breast cancer in advance of severe deterioration without intrusive damage to the patients, making this method an advisable option for doctors.

The therapeutic value of epigenetic factors is even more compelling. Since epigenetic mechanisms can be undone, their cancerous impact can theoretically be reversed. The epigenetic pathway can be reversed by its antagonistic mechanism or inhibition of its specific enzyme by applying "epidrugs." Some drugs target DNA methylation and histone acetylation, which are FDA-approved, while others targeting DNA demethylation are not yet in application. The newest ones are targeting miRNA. For future directions, research should investigate a more stable approach for the precise transportation of drugs to cancer cells, aiming to reduce drug resistance and adverse effects on humans. More experiments should combine epidrugs with other epidrugs or traditional approaches. Bigger ambitions could focus on the transgenerational effects of epigenetics. Epigenetic alterations in some genes can be sustained in the next generation, though these inheritances are not observed in genes like *BRCA*. Identifying which genes' epigenetic modifications are likely to be inherited and how is crucial. Inherited epigenetic alterations could potentially be a considerable precursor of breast cancer that is neglected when profiling high-risk individuals. Nonetheless, before such research is initiated, more evidence corroborating the transgenerational effect of epigenetics on humans should be established, and the exact molecular mechanism behind it should be scrutinized. This will provide a refreshing perspective in the fight against breast cancer.

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