Breast Cancer Stem Cells

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Breast cancer is a highly heterogeneous and phenotypically diverse group of diseases, which require different selection of treatments. Accurately being able to distinguish between the various subtypes of breast cancer is vital as they have different prognoses and responses to therapy. Gene expression studies have identified six distinct molecular subtypes for breast cancer, which characterize distinct physiological presentation, gene expression profile, prognosis and clinical outcomes. These subtypes are classified according to the presence or absence of hormone (estrogen (ER) or progesterone (PR)) receptors (HR+/HR-) and human epidermal growth factor receptor 2 (HER2+/HER2-). Luminal A (HR+/HER2-) represents a slow-growing and less aggressive subtype, while luminal B (HR+/HER2+) seems to be more aggressive than luminal A. HER2-positive (HR-/HER2+) breast cancers, which express excess HER2 and do not express hormone receptors, grow and spread more aggressively than other breast cancers and are correlated with poorer prognosis than ER+ breast cancers.

Keywords: breast cancer ; breast cancer stem cells ; epigenetic regulation ; therapies

1. Overview

Globally, breast cancer has remained the most commonly diagnosed cancer and the leading cause of cancer death among women. Breast cancer is a highly heterogeneous and phenotypically diverse group of diseases, which require different selection of treatments. Breast cancer stem cells (BCSCs), a small subset of cancer cells with stem cell-like properties, play essential roles in breast cancer progression, recurrence, metastasis, chemoresistance and treatments. Epigenetics is defined as inheritable changes in gene expression without alteration in DNA sequence. Epigenetic regulation includes DNA methylation and demethylation, as well as histone modifications. Aberrant epigenetic regulation results in carcinogenesis. In this review, the mechanism of epigenetic regulation involved in carcinogenesis, therapeutic resistance and metastasis of BCSCs will be discussed, and finally, the therapies targeting these biomarkers will be presented.

2. Breast Cancer

Globally, breast cancer has remained the most commonly diagnosed cancer and the leading cause of cancer death among women [1]. In 2021, 281,550 new cases of breast cancer were estimated to be diagnosed in women, and 43,600 deaths were predicted from breast cancer in the USA. Therefore, breast cancer has the second highest cancer-related death rate, and is among the most commonly diagnosed cancers in US women [2].

Breast cancer is a highly heterogeneous and phenotypically diverse group of diseases, which require different selection of treatments [3][4][5]. Accurately being able to distinguish between the various subtypes of breast cancer is vital as they have different prognoses and responses to therapy [6]. Gene expression studies have identified six distinct molecular subtypes for breast cancer, which characterize distinct physiological presentation, gene expression profile, prognosis and clinical outcomes [7][8][9]. These subtypes are classified according to the presence or absence of hormone (estrogen (ER) or progesterone (PR)) receptors (HR+/HR-) and human epidermal growth factor receptor 2 (HER2+/HER2-). Luminal A (HR+/HER2-) represents a slow-growing and less aggressive subtype, while luminal B (HR+/HER2+) seems to be more aggressive than luminal A. HER2-positive (HR-/HER2+) breast cancers, which express excess HER2 and do not express hormone receptors, grow and spread more aggressively than other breast cancers and are correlated with poorer prognosis than ER+ breast cancers. Triple-negative or basal-like (HR-/HER2-) breast cancer, with no expression of ER and PR (ER-, PR-) or HER2 (HER2-), represents the worst prognosis subtype. Normal-like breast cancer (HR+/HER2-) is similar to luminal A disease. Although normal-like breast cancer has a good prognosis, its prognosis is still slightly worse than that of luminal A. Lastly, claudin-low tumors are characterized by low genomic instability, mutational burden and proliferation levels [3][4][10][11].

2.1. Breast Cancer Stem Cells (BCSCs)

Cancer stem cells (CSCs) are a subpopulation of tumor cells that are endowed with self-renewal and multi-lineage differentiation capacities and play a crucial role in initiation, tumorigenesis, metastasis, chemoresistance and relapse of tumors [12][13][14]. BCSCs are characterized by the expression of cell surface markers, such as CD24−/low, CD44+ and epithelial cell adhesion molecule (EpCAM+) [15][16]. Other surface markers, such as CD133, CD49f, CD90, nestin, ganglioside GD2, C-X-C chemokine receptor type 4 (CXCR4), C-X-C motif chemokine ligand 1 (CXCL1), hydroxymethylglutaryl-CoA synthase (HMGCS), CD166, CD47, aldehyde dehydrogenase 1 (ALDH1) and ATP-binding cassette super-family G member 2 (ABCG2), have also been identified to be associated with BCSCs [17][18][19]. It is now becoming evident that BCSCs can generate different breast cancer subtypes, which express different surface markers due to limited or aberrant differentiation [20][21][22].

Compared to normal cells, BCSCs initiate the multiple changes in gene expression involved in the invasion–metastasis cascade as a result of several mechanisms, including EMT induction and abnormal miRNA biogenesis [23][24][25]. EMT is a complex process that involves many transcription factors, including but not limited to, TWIST, ZEB1, SNAIL, SLUG, Smad interacting protein 1 (SIP1) and E47, and many signaling pathways, such as Wnt/β-catenin, Notch, Hedgehog (HH), nuclear factor-κB (NF-κB)/Akt and transforming growth factor-β (TGF-β)/Smad pathways [26]. Cells undergoing EMT can acquire stem cell-like properties to become CSCs [27]. Intriguingly, BCSCs with a CD44+/CD24−/low phenotype also possess EMT characteristics, such as low expression of E-cadherin (*CDH1*) and high expression of vimentin, N-cadherin (*CDH2*), fibronectin and EMT inducers (Twist, Snail and Slug) [28][29][30]. Since BCSCs play a critical role in carcinogenesis, proliferation and metastasis of breast cancer, targeting BCSCs represents an attractive therapeutic strategy for breast cancer.

2.2. Epigenetic Regulation in Normal Function

It has been proven that epigenetic regulation and non-coding RNAs (ncRNAs) are master gene regulators of EMT and CSCs for invasiveness and metastasis of cancer cells [31][32]. Therefore, deciphering the molecular mechanisms that regulate the CSCs' self-renewal/differentiation balance is urgently required for developing new treatments [33]. In contrast to genetics, epigenetics is defined as inheritable changes in gene expression without alteration in DNA sequence [34]. DNA winds around histone protein to form larger order structural units, nucleosomes, the basic structural units of chromatin. There are two levels of chromatin organization, "open, euchromatin", which permits active transcription, or "closed, heterochromatin", which represses transcription. The homeostasis between euchromatin and heterochromatin is determined by epigenetic regulations, including DNA methylation, post-translational histone modifications and alteration of ncRNA expression [35][36].

2.2.1. DNA Methylation and Demethylation

DNA methylation is the most important epigenetic regulation for mRNA and microRNA (miRNA) expression in mammalian cells to ensure normal development and growth [37]; conversely, it is dysregulated in cancer cells [38][39]. In the process of DNA methylation, it creates a 'fifth base' from cytosine, 5-methylcytosine (5mC), mostly occurring in CpG islands (CGIs), which act as regulatory hotspots found upstream of the promoter region [40]. There are three types of proteins for DNA methylation and demethylation, including DNA methyltransferases (DNMTs), ten-eleven translocation (TET) enzymes and methyl-binding domain (MBD) proteins [41][42]. Three DNMTs controlling methyl group transfer or CGI methylation consist of DNMT1, responsible for methylation maintenance, and DNMT3A and DNMT3B, capable of de novo methylation, which play critical physiological roles in mammalian genome stability, cellular proliferation and development and cell fate determination [43][44]. Recently, DNMT2 has been identified as a methyltransferase, but for methylation of tRNA instead [45]. The methylated DNA can be recognized by binding MBD proteins to recruit histone-modifying complexes, such as histone methyltransferases (HMTs), for regulating gene transcription and chromatin remodeling [46][47]. It is estimated that 70% of all CGIs in humans are hypermethylated and are found in heterochromatin, which represses transcription. In contrast, hypomethylated CGIs are located in euchromatin, which activates gene expression [48]. Conversely, demethylation is catalyzed by TET family enzymes, TET1, TET2 and TET3, oxygenase enzymes that convert 5mC to 5-hydroxymethylcytosine (5hmC), 5-formyl cytosine (5fmC) and 5-carboxyl methyl cytosine (5CamC) [49][50][51][52].

2.2.2. Histone Modifications

Covalent post-translational modifications (PTMs) of histone tails, including methylation, acetylation, phosphorylation, ubiquitination and SUMOylation, play a pivotal role in modifying gene expression [53]. In contrast to DNA methylation, associating with gene-silencing, histone methylation, acetylation and phosphorylation can change the secondary structure

of DNA and result in either induction or prevention of access by transcription factors to gene promoter regions in order to inhibit or activate transcription [53][54].

Histone methylation plays important roles in gene transcription, DNA replication and repair, chromatin organization and developmental and differentiation processes [55][56][57]. Histone methylation, defined as the transfer of one, two or three methyl groups to lysine or arginine residues of histone proteins, is regulated by HMTs and histone demethylases (HDMs) [58]. Transcription silencing is associated with methylation of histone 3 lysine 9, 20 or 27 (H3K9, H3K20 or H3K27), while methylation of histone 3 lysine 4, 36 or 79 (H3K4, H3K36 or H3K79) is involved in transcription activation [59]. Three families of HMTs have been discovered that are specific for the lysine or arginine residue which they modify: the set domain-containing protein family, the non-set domain protein family and the protein arginine methyltransferases (PRMT1) family [57]. A polycomb repressive complex 2 (PRC2) group protein, Enhancer of zeste homolog 2 (EZH2), methylates H3K27 and is a transcriptional repressor [60]. H3K9 methylation occurring in euchromatin causes mono- and di-methylation (H3K9me1 and H3K9me2) catalyzed mainly by G9a, and in heterochromatin, which requires trimethylation (H3K9me3) mostly catalyzed by Suv39H1 and Suv39H2 and results in transcriptional silencing [55][56]. Furthermore, a novel histone lysine methyltransferase, the set and MYND domain-containing protein 3 (SMYD3), methylates H3K4 [61]. On the other hand, two major families of demethylases have been identified, lysine-specific demethylase 1 (LSD1) and Jumonji domain-containing HDMs (JMJD2, JMJD3/UTX and JARIDs). LSD1 specifically demethylates mono- or di-methylated H3K4 or H3K9 and non-histone proteins, such as p53 and DNMT1, indicating that it plays a vital role in a number of normal biological functions and in carcinogenesis, as described in the following section [62]. Similarly, H3K9me3/me2 demethylation is catalyzed by JMJD2C, also known as histone lysine demethylases 4C (KDM4C) [63]. Additionally, JMJD2C demethylates the second methylated histone substrate, H3K36me3 [64].

Histone acetylation occurs via the modifying enzymes, histone acetyltransferases (HATs) or histone deacetylases (HDACs). An acetyl group is added by HATs to ε-amino groups of lysine residues in the histone N-terminal tails, making euchromatin, which allows transcription factor binding and results in gene activation. Conversely, HDACs catalyze the hydrolytic removal of acetyl groups from histone lysine residues, which compact chromatin into heterochromatin, preventing transcription factor binding to DNA and subsequent gene expression [65].

3. Conclusions

There is a growing list of proteins and ncRNAs identified in epigenetic regulation that may represent useful biomarkers for diagnosis and/or prognosis for breast cancer. The major challenges in cancer therapy are tumor recurrence and resistance to conventional therapies, such as chemotherapy and radiotherapy, and CSCs are the major players in these events. Therefore, comprehensive elucidation of regulatory mechanisms in BCSCs will definitely help to develop more effective precision medicine. There is emerging data on dysregulation of ncRNAs, and ncRNA hyper/hypomethylation contributes to cancer stemness. There are currently not many miRNA-based therapies for breast cancer; therefore, these represent a great opportunity in developing novel therapeutic strategies for breast cancer. Additionally, ncRNAs have the advantage of multi-target characteristics, which should minimize the possibility of drug resistance. However, the major hurdle for miRNA-based therapies lies in the lack of a specific delivery system, a problem shared with all forms of gene therapy in cancer. In spite of the enormous amounts of biomarkers identified in epigenetic regulation of breast cancer and BCSCs, currently, there are only a few drugs available, and even less entering clinical trials. Therefore, in the future, the development of novel drugs or combination regimens are urgently required.

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