

miRNA: A promising tool to target breast cancer stem cells

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Abstract. Breast cancer is one of the most common cancers among women worldwide, and its treatment has made some progress but still faces challenges such as recurrence, metastasis and drug resistance. Breast cancer stem cells (BCSCs) play a key role in breast cancer recurrence and metastasis due to their properties such as self-renewal, differentiation potential and drug resistance. They can evade the attack of the immune system. They are resistant to various chemotherapeutic drugs, which makes it challenging to eliminate them by conventional treatment, resulting in poor therapeutic effects. miRNAs, as a class of non-coding RNA molecules, can influence the biological characteristics of BCSCs through fine-regulating gene expression, which can provide a new idea and method for treating breast cancer. In this paper, we investigated the effects of miRNAs on BCSCs through various mechanisms, such as regulating key signalling pathways, influencing the expression of cell surface markers, and inducing apoptosis. We demonstrated that miRNAs can reduce the stemness of BCSCs and increase the therapeutic sensitivity. Meanwhile, this paper also discusses the current challenges of miRNA therapy, such as delivery efficiency, targeting and in vivo stability. By optimising the miRNA delivery system, improving targeting accuracy and exploring combined treatment options, it is expected to overcome the existing limitations, promote the translation of miRNA therapy to clinical applications and provide more effective and personalised therapeutic options for breast cancer patients.

Keywords: Breast cancer, BCSCs, miRNA.

1. Introduction

According to the World Health Organization (2024), breast cancer is a disease where malignant breast cells grow uncontrollably, forming tumours. It is commonly derived when the BRCA1 and BRCA2 oncogenes in the breast tissue have undergone mutation [1]. It was also implied that breast cancer may have been due to the promotion of growth from hormones or human growth factors, with ligands for these receptors frequently found in breast cancer cells [2].

From the studies of Crabtree and Miele (2018), a small but significant population of cancer cells in breast cancer were found to be breast cancer stem cells (BCSCs) expressive of stem cell-associated markers [3]. Accumulating evidence shows that BCSCs are cells with self-renewal and tumour initiation abilities, allowing them to carry out metastases and tumour growth [4]. In breast cancer models of

patients, animals and cell lines were identified to be different biomarkers for BCSCs, which suggests a diversity of subgroups in association with varying outcomes of therapy [5].

These tumour cells can escape the immune system in multiple ways, for example, the interaction of PD-1 and PD-L1, which hinders the anti-tumour abilities of cytotoxic T cells. BCSCs play a vital role in immune evasion; they can reduce the expression of MHC 1, which stops their interaction with immune cells in advance and also changes their behaviour, weakening the immune response. BSCSs are involved in drug resistance, such as the drug trastuzumab, by unique mechanisms that decrease their efficiency.

However, small molecules of RNA that do not code for proteins, such as microRNAs (miRNA), were shown to strongly associate these properties with BSCSs. They can be actively involved in down regulations of certain gene expressions, associating with phenotypic markers and signalling pathways to induce apoptosis, acting like a tumour suppressor. Hence, their deregulation results in increased numbers of cancer cells. Therefore, miRNA holds promise to become a targeted tool in the combat of breast cancer, potentially overcoming drug resistance and immune escape and thereby improving treatment outcomes.

2. An overview of breast cancer

2.1. Gene mutations

Breast cancer can result from a mix of genetic, environmental and lifestyle factors. Mutations of specific tumour-suppressive genes increase the risk of breast cancer. Two central genes involved in breast cancer, breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2), are tumour suppressor genes which participate in the production of proteins that repair damaged DNA [6]. They are located on chromosome 17q21 and chromosome 13q13. Tumor protein 53 (TP53) is another tumor-suppressive gene on chromosome 17p13. Its function includes the activation of a protein which inhibits the cell cycle and induces apoptosis and DNA repair [7]. Mutations of these genes can cause the disruption of DNA repairing, which increases the incidence of genetic errors in cell replication and the loss of regulation of the cell cycle, which, in consequence, causes uncontrolled cell division.

2.2. Subtypes

Breast cancer can be classified into subtypes depending on the type of receptor proteins on the cell surface, each requiring unique treatment approaches, as shown in Figure 1.

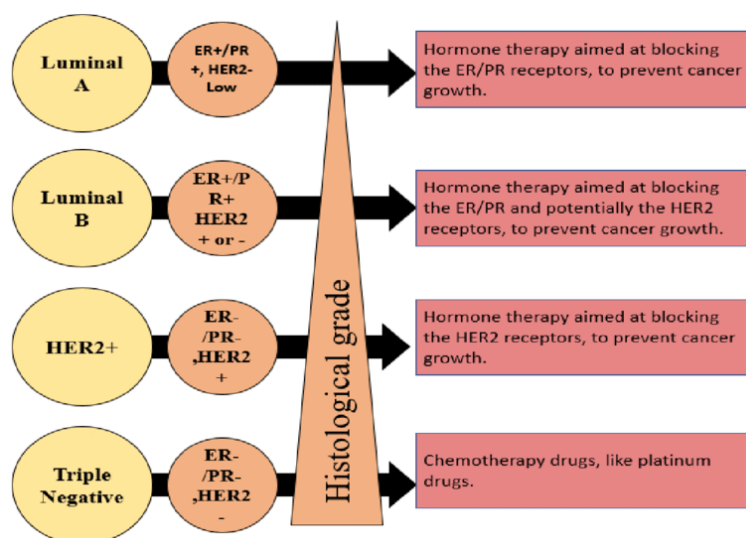


Figure 1. Treatment of different subtypes of breast cancer [5].

Luminal A breast cancer, termed by the luminal group as high expressions of luminal associated markers expressed by luminal cells of the breast, is defined by progesterone and oestrogen receptor-positive lacking HER-2, as one of the four main entities of breast cancer [8]. Luminal A is regarded as the most common, accounting for 50-60% of total cases diagnosed, with patients well responsive to treatment and has a generally favourable prognosis, according to Eroles et al. [9], having a relapse rate of 27.8%. This is associated with luminal A's low proliferation properties measured by a low level of the protein Ki-67 that contributes to the proper segregation of chromosomes during cell division [10]. In luminal A, oestrogen and progesterone bind to receptors which function as transcription factors that alter gene expression, promoting the proliferation of cancer cells. Some common methods of therapy include surgery, radiation therapy, chemotherapy and hormonal therapy, which is best responded to due to the presence of both hormonal receptors.

Luminal B breast cancer, one of the other luminal group cancers, is characterized as oestrogen-positive and/or progesterone-positive and can either be HER-2 positive or negative. Luminal B accounts for 10 to 20% of breast cancers; in comparison with luminal A, it is faster growing, more aggressive, larger, has a higher grade, poorer prognosis, a more likely recurrence and is considered more malignant than luminal A. Behind such differences, luminal B measures the higher expression of proliferation genes such as the Marker of Proliferation of Ki-67 (MKI-67) and cyclin B1, which plays a crucial role in the cell cycle and exhibits higher levels of genomic instability which implies more mutations and chromosomal aberration, suggesting faster tumour growth and progression with more aggressive behaviour and treatment resistance [9,11]. Luminal B's lower level of hormone receptor expression implies it is less responsive to hormonal therapies compared to luminal A breast cancer. Common treatments of luminal B are mostly the same as luminal A except that due to possible HER2 positivity, luminal B often requires additional treatments such as chemotherapy or HER2-targeted therapies (e.g., trastuzumab) [11].

HER 2 enriched breast cancer, also known as HER 2 positive breast cancer, is identified by the overexpression of human epidermal growth factor receptor 2 and can be hormone receptors positive or negative. HER 2 comprises 10-15% of breast cancer diagnoses, with generally worse prognosis than luminal cancers as it grows and divides at a more rapid pace, has a high tendency to spread and may show resistance to hormonal treatment. However, outside of traditional cancer treatment approaches, HER 2 enriched breast cancer can be targeted explicitly by certain medicines that block pathways. One of them is Pembrolizumab, a monoclonal antibody that targets the binding of programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PDL-1) and Trastuzumab, another monoclonal antibody that competitively inhibits the activity of HER2 protein [12,13].

Triple-negative breast cancer (TNBC), otherwise called basal-like breast cancer, is a unique subtype with hormone and HER 2-receptors negative. TNBC has the worst prognosis compared to other subtypes; it tends to grow and spread more quickly than other types of breast cancer and presents as higher-grade tumours because of high levels of Ki-67 [14,15]. TNBC also has a higher risk of recurrence, attributed to its aggressive nature and the limited effectiveness of treatment options as a lack of expression of receptors. Common treatments include surgeries, chemotherapy, radiation therapy, and immunotherapy, often before and after surgeries [16].

3. Role of BCSCs in immune escape

3.1. Breast cancer stem cells (BCSCs)

Breast cancer stem cells (BCSCs) are a minor population of breast cancer cells which carry a major role in the initiation, progression, metastasis, and recurrence of breast cancer. It is often identified by specific surface markers such as high expressions of cluster of differentiation 44 (CD44+), low expressions of cluster of differentiation 24 (CD24-) and aldehyde dehydrogenase 1 (ALDH1), as shown in Figure 2 [5,17]. Cells with these characteristics display the greatest tumorigenic and invasive capacity [18]. ALDH1 is a common marker, an enzyme that plays a critical role in cellular detoxification. ALDH1-positive cells can differentiate into various cell types found within the tumour mass, hence why BCSCs

show the ability of tumour initiation and heterogeneity [18,19]. CD44, a cell surface glycoprotein, is involved in cell interactions, adhesion, and migration. It also maintains stem cell characteristics by interacting with various signaling pathways, such as activating the Wnt/ β -catenin pathway, increasing the expression of stemness-related genes that push tumor progression [20]. CD24 is another glycosylated cell surface protein, and research has shown it to enhance the metastatic potential of tumor cells [21]. Interestingly, CD24 can regulate the motility of BCSCs by interacting with remodelling pathways of the cytoskeleton, helping the cell to migrate. With the combination of high CD44 and low CD24, BCSCs show plasticity; the going through of epithelial-mesenchymal transition (EMT) enables it to acquire mesenchymal traits, allowing the transition between stem-like and differentiated states in relevance to their metastasis [22].

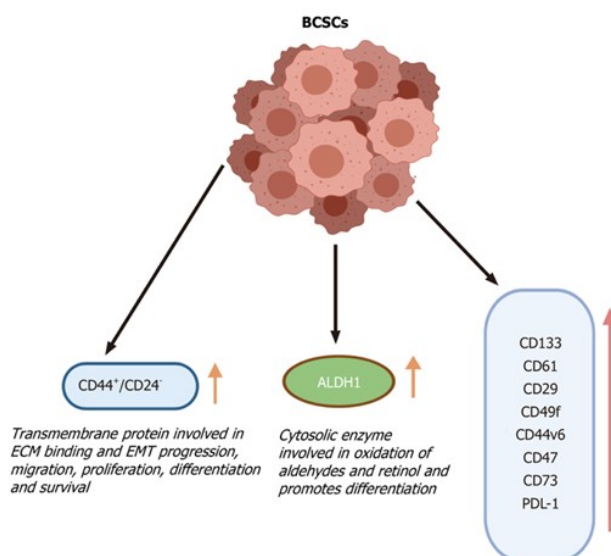


Figure 2. High expressions of cluster in BCSCs [5].

3.2. Approaches of BCSCs in immune escape

There are three main ways BCSC escapes the immune system and thus prevents T-cell attack, as shown in Figure 3 [23].

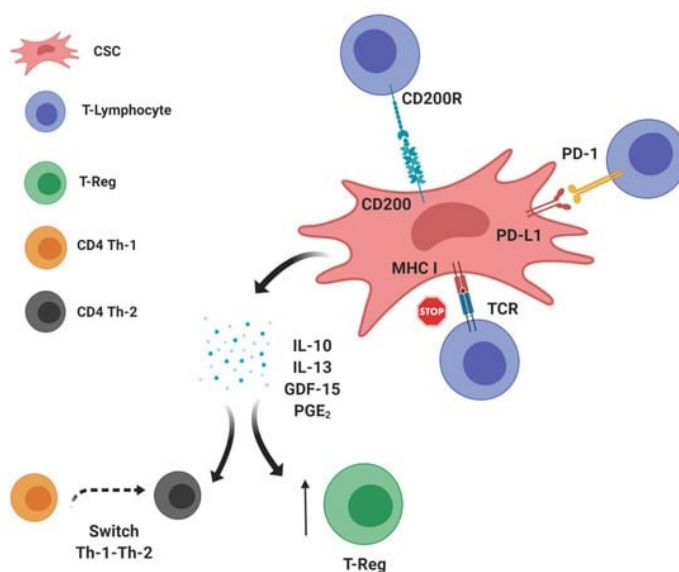


Figure 3. Expression of molecules by cancer stem cells [23].

3.2.1. Expression of Immune Checkpoints. The PD-1/PD-L1 receptor pairing is an essential immune escape mechanism utilized by BCSCs. They can upregulate PD-L1 on their surface and PD-L1 interacts with programmed cell death protein 1, PD-1, receptors on cytotoxic T cells, effectively turning off their anti-tumor ability and allowing the cancer cells to evade immune detection and destruction [24]. Besides, BCSCs may also exploit the CTLA-4 pathway by expressing cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which competes with the co-stimulatory molecule CD28 for binding to B7 ligands on antigen-presenting cells. This competition reduces T cells' activation and effector function, further aiding BCSCs in evading the immune response and contributing to tumor progression and resistance to immunotherapy.

3.2.2. Attraction of Immune Cells. BCSCs can also use two ways to avoid the immune system: attracting certain immune cells and changing the behavior of macrophages, as shown in Figure 4 [25]. Firstly, BCSCs will release chemicals to regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs). Tregs thus suppress the activity of effector T cells and natural killer cells, reducing the immune system's ability to attack BCSCs. MDSCs inhibit T cell activation and promote T cell apoptosis, weakening the immune response. TAMs, especially those polarized towards the M2 phenotype, release anti-inflammatory cytokines and growth factors and create an immunosuppressive microenvironment which supports tumour growth and immune escape. BCSCs can also release IL-10, TGF- β , CSF, and other molecules that promote the polarization of macrophages to the M2-like phenotype. M2 macrophages strongly interact with tumor growth and metastasis as they can repair tissues independently, stimulate vessel formation, and suppress adaptive immunity. This kind of polarization creates an environment that allows tumor cell proliferation while avoiding immune response.

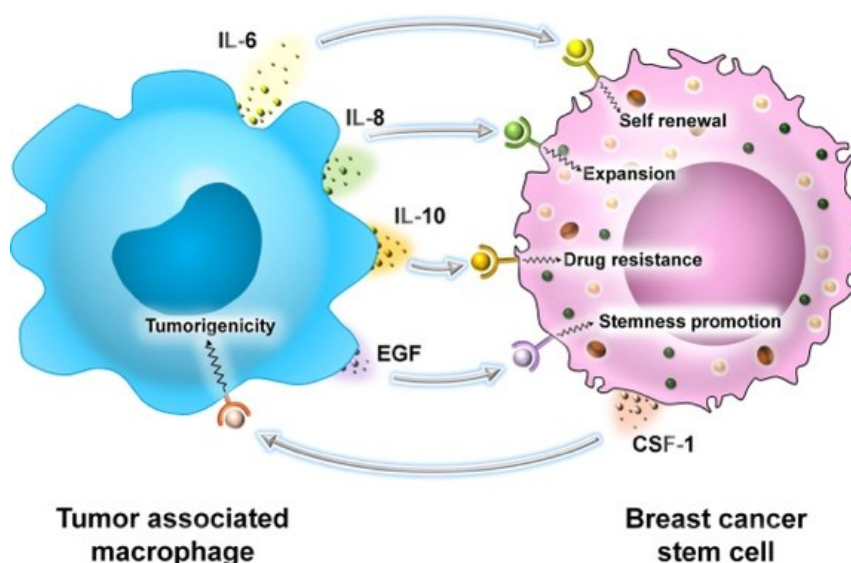


Figure 4. The interaction between TAMs and BCSCs [25].

3.2.3. Impact on Antigen Presentation and Recognition. BCSCs have a crucial strategy: to down-regulate the expression of MHC I on its surface. MHC I molecule is key for APCs to present antigens to cytotoxic T cells, which they can then use to recognize and kill pathogens precisely. By expressing less MHC-I, BCSCs will cause cytotoxic T cells to make it more difficult to recognize some potential threats that can escape from immune surveillance [26]. BCSCs can also manipulate the pathways involved in antigen processing. Normally, tumor antigens are processed within the cell and presented on the cell surface by MHC molecules. This allows immune cells to detect and respond to them. However, BCSCs can disrupt this process, impairing tumour-associated antigens' presentation. This manipulation hinders the ability of effector immune cells, such as cytotoxic T cells and natural killer cells, to recognize and attack the

cancer cells effectively. Thus, using these methods, BCSCs create a protective environment that helps them survive and grow while avoiding the immune system's attacks.

4. BCSCs and drug resistance

As mentioned before, Trastuzumab targeted HER2 receptors. But BCSCs also evolve in different ways to resist the drugs. For instance, it can inhibit the expression of HER2 in most tumour cells, making targeting trastuzumab to the HER2 receptor decrease effectiveness. BCSCs can activate alternative signalling pathways, such as the PI3K/AKT or MAPK pathways, which promote survival and proliferation independently of HER2 signalling. This makes them less reliant on HER2 for growth and survival, as shown in Figure 5 [27].

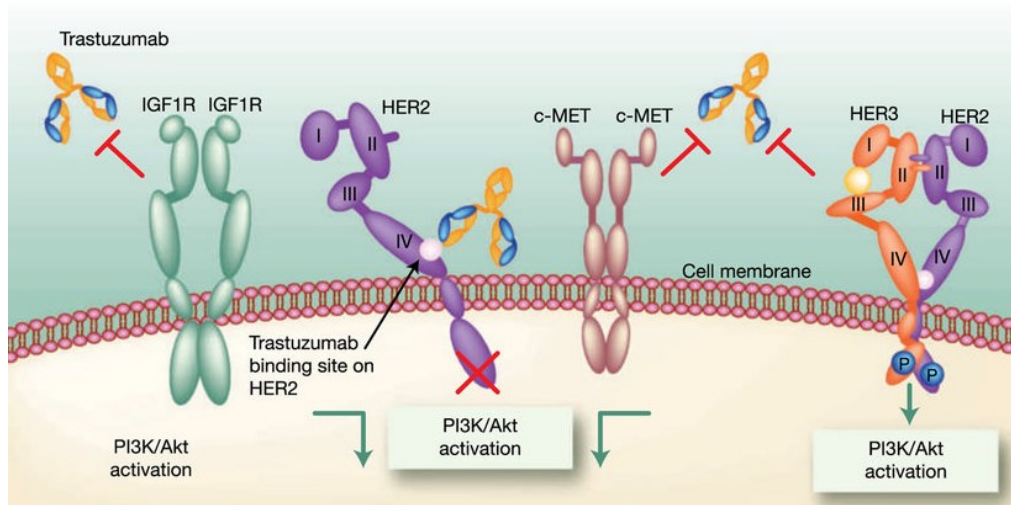


Figure 5. Mechanism of BCSCs resistant to Trastuzumab [27].

In addition, BCSCs can resist trastuzumab through two key mechanisms. First, they often have higher activity of drug efflux pumps, such as ABCG2, which pump out trastuzumab, lowering its effectiveness, as shown in Figure 6 [28]. BCSCs can change their cell surface markers, reducing trastuzumab's ability to bind to them. BCSCs also produce detoxifying enzymes like aldehyde dehydrogenase 1 (ALDH1) to minimize oxidative stress, and they have a highly efficient DNA repair system that quickly fixes DNA damage caused by therapies. These attributes make BCSCs largely insensitive to both chemotherapy and radiotherapy. Studies have shown that the number of BCSCs often increases rather than decreases following conventional treatments, potentially leading to breast cancer recurrence [29].

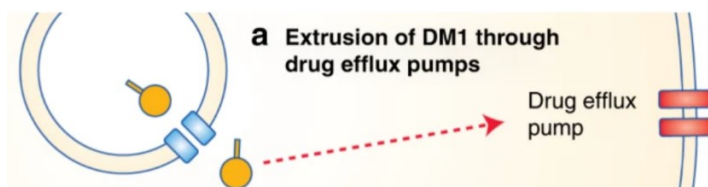


Figure 6. Efflux pump to Trastuzumab [28].

5. MicroRNA (miRNA) as a Promising Tool

MicroRNAs are small, non-coding RNA molecules crucial in regulating gene expression. Typically, about 22 nucleotides long, miRNAs function by binding to complementary sequences on messenger RNAs (mRNAs), leading to mRNA degradation or inhibition of translation.

Breast cancer stem cells can perform immune escape and drug immunity in various ways, which has become a huge problem in the clinical elimination of cancer. While there is tremendous progress today

in targeting breast cancer stem cells through miRNAs. MiRNAs are small, non-coding RNAs, and they can influence gene expression. They have emerged as potential tools for targeting BCSCs through the following approaches, as shown in Figure 7.

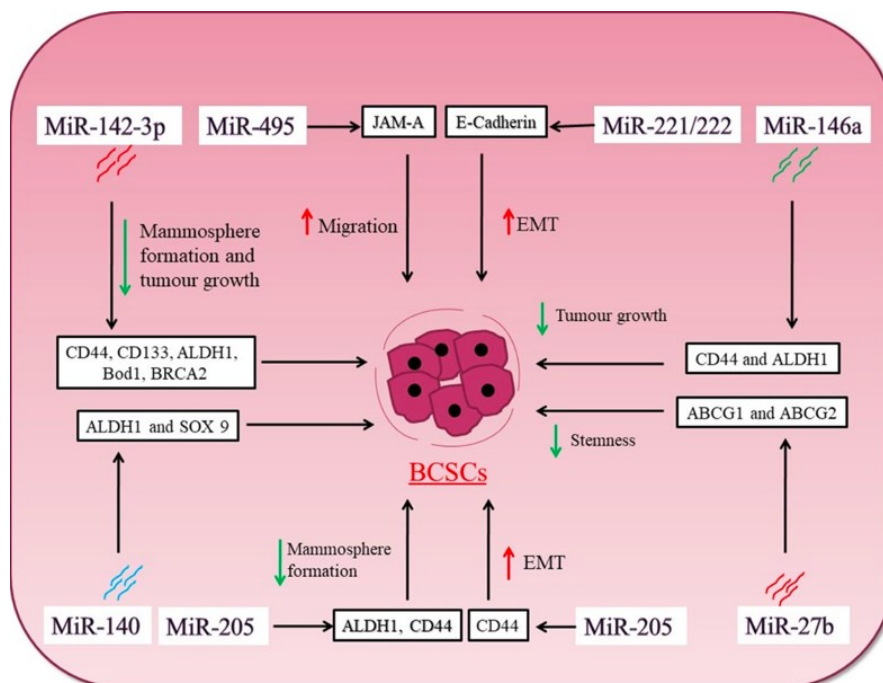


Figure 7. How miRNAs regulate BCSCs [4].

One way is that miRNAs can regulate the phenotypic markers on BCSCs. For miR-142-3p and miR-140, they target and downregulate the expression of surface markers like CD44 and CD133, which are crucial for maintaining the stemness and self-renewal abilities of BCSCs. By reducing the levels of these receptors, miR-142-3p and miR-140 can decrease the stem cell-like characteristics of BCSCs, making them less tumorigenic and more susceptible to therapies. For miR-200c, it targets and inhibits the expression of genes associated with epithelial-to-mesenchymal transition (EMT), such as ZEB1 and ZEB2. EMT is a process that enhances the invasive and metastatic potential of cancer cells. By inhibiting EMT, miR-200c can thus reduce the aggressiveness of BCSCs [4]. For miR-34a, it has the same ability as miR-142-3p, and it promotes the differentiation of BCSCs into more mature, less tumorigenic cell types.

Another mechanism by which miRNAs can regulate the signaling pathways in BCSCs. There are five pathways: 1. miRNAs like miR-600 and miR-29b target components of the Wnt/ β -catenin signaling pathway, crucial for BCSC self-renewal and proliferation. By inhibiting this pathway, these miRNAs reduce the stemness and tumorigenic potential of BCSCs. For instance, miR-600 targets and downregulates the expression of β -catenin, a key component of the Wnt pathway. 2. miRNAs such as miR-34a and miR-200b target the Notch signalling pathway, maintaining BCSC populations. 3. miR-326 targets components of the Hedgehog signalling pathway, such as Smoothened (SMO), thereby reducing BCSC proliferation and promoting apoptosis. 4. miR-124 and miR-146b target the STAT3 signalling pathway. This is involved in promoting BCSC survival and resistance to apoptosis. 5. miRNAs, such as miR-29b, target components of the AKT/ERK1/2 signalling pathways. This pathway works similarly to the Hedgehog signalling pathway.

A group of miRNAs are also capable of regulating the apoptosis of BCSCs. 1. miR-34a targets anti-apoptotic genes such as BCL-2 and survivin, thus promoting cell death in BCSCs. 2. miR-15a and miR-16-1 target and inhibit the expression of anti-apoptotic proteins like BCL-2 and MCL-1. 3. Although

miR-21 is typically associated with promoting survival and proliferation in cancer cells, its inhibition can lead to increased apoptosis in BCSCs.

The final way is to use miRNAs as therapeutic tools: miRNAs can be delivered directly to BCSCs using nanoparticles or viral vectors that specifically target these cells. This targeted delivery ensures that miRNAs reach the intended cells and exert their therapeutic effects. They can be used in combination with conventional therapies to enhance their efficacy. For example, miR-34a can be combined with chemotherapy to induce apoptosis in BCSCs and reduce tumor recurrence. Some miRNAs also act as oncogenes and promote BCSC survival and proliferation. Inhibitors of these oncogenic miRNAs, known as antagomiRs, can block their function and reduce the tumorigenic potential of BCSCs. Besides, miRNAs that act as tumor suppressors can be stabilized and delivered to BCSCs to inhibit their growth and survival. miR-34a mimics can restore tumor-suppressive function in BCSCs [4].

6. Conclusion

This paper reviews the application of miRNAs in targeting breast cancer stem cells (BCSCs) therapeutically. It provides insights into the role of BCSCs in immune escape and drug resistance, as well as the potential of miRNAs as an emerging tool in breast cancer therapy. BCSCs, as a key driver of breast cancer recurrence and metastasis, are characterised by their self-renewal, differentiation potential, and drug resistance properties. These characteristics make it difficult to eliminate BCSCs by conventional therapies completely, thus limiting the long-term therapeutic effects of breast cancer. As non-coding RNAs, miRNAs show great potential in treating breast cancer stem cells by regulating gene expression. miRNAs can target BCSCs through various mechanisms, including down-regulation of surface markers, modulation of signalling pathways, promotion of apoptosis, etc. These mechanisms of action not only reduce the number of BCSCs but also reduce their risk of recurrence and metastasis, which is a key driver of breast cancer recurrence and metastasis. These mechanisms of action reduce the stem cell properties of BCSCs and enhance their sensitivity to conventional treatments, which is expected to improve the cure rate and survival rate of breast cancer.

Despite the remarkable progress in the study of miRNA in targeting BCSCs therapy, some limitations still exist. Firstly, the delivery system of miRNA is not yet perfect, and the delivery efficiency and safety of the currently commonly used methods, such as nanoparticles, liposomes and viral vectors, still need to be improved. Secondly, targeting miRNAs needs to be further optimised to ensure that they can accurately act on BCSCs without causing damage to normal cells. miRNA stability in vivo is also one of the important factors affecting its efficacy, and how to maintain the activity of miRNAs in complex biological environments still needs to be investigated in depth. In addition, most of the current studies on miRNAs in breast cancer treatment remain in the cell and animal experimental stage, lacking the support of large-scale clinical trial data. Therefore, the effectiveness and safety of miRNAs in clinical applications still need to be further verified.

Despite many challenges and limitations, the potential of miRNAs in targeting BCSCs therapy is still enormous. Future research should focus on the following aspects: first, developing more efficient miRNA delivery systems to improve their concentration and stability at the tumour site; second, optimizing the targeting of miRNAs to achieve more precise gene regulation through gene editing and other technologies; and third, strengthening the research on the combined application of miRNAs and traditional therapeutic approaches to explore more effective treatment options. In addition, with the development of precision medicine and personalised treatment, the future application of miRNA in breast cancer treatment will be more precise and personalised. Through the comprehensive analysis of patient genotype, tumour type and staging, the optimal miRNA treatment plan can be tailored for the patient, thus improving the therapeutic efficacy and patient quality of life.

In conclusion, miRNA, as an emerging therapeutic tool, has a broad application prospect in targeted breast cancer stem cell therapy. Although there are still many challenges and limitations, with the deepening of research and the continuous progress of technology, it is believed that miRNA will bring new hope and gospel to breast cancer patients.

Authors Contribution

All the authors contributed equally, and their names were listed alphabetically.

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