Am J Cancer Res 2022;12(7):3208-3222
www.ajcr.us /ISSN:2156-6976/ajcr0142343

Review Article

Research progress in breast cancer stem cells: characterization and future perspectives

Xiao-Dong Mao[1,2*], Xiao Wei[1,2*], Tao Xu[3], Tai-Ping Li[4], Kang-Sheng Liu[5]

[1]Department of Endocrinology, Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210028, Jiangsu, China; [2]Key Laboratory of TCM Syndrome & Treatment of Yingbing of State Administration of Traditional Chinese Medicine, Jiangsu Province Academy of Traditional Chinese Medicine, Nanjing 210028, Jiangsu, China; [3]Xi'an Jiaotong University Global Health Institute, Xi'an 710049, Shanxi, China; [4]Department of Neuro-Psychiatric Institute, The Affiliated Brain Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu, China; [5]Department of Clinical Laboratory, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing 210029, Jiangsu, China. [*]Equal contributors and co-first authors.

Received February 14, 2022; Accepted June 25, 2022; Epub July 15, 2022; Published July 30, 2022

Abstract: More and more studies have proved that there are a small number of cells with self-renewal and differentiation ability in breast tumors, namely breast cancer stem cells. Such cells play a key role in the initiation, development and migration of breast tumors. The properties of breast tumor stem cells are regulated by a range of intracellular and extracellular factors, including important signaling pathways, transcription factors, non-coding RNAs, and cytokines such as Hedgehog, Wnt, Notch, microRNA93, microRNA100, and IL-6. Tumor microenvironment (such as mesenchymal stem cells, macrophages and cytokines) plays an important role in the regulation of breast tumor stem cells. Using the keywords including "breast cancer stem cells", "signal pathway", "chemotherapy tolerance", and "non-coding RNA", "triple negative breast cancer", "inhibitors", this study retrieved the original articles and reviews published before October 3, 2021, from PubMed and WEB OF SCI database and this study performed a comprehensive review of them. After treatment, there is a correlation between the metastasis-prone nature and recurrence with breast cancer stem cells. The signaling pathway of breast cancer stem cells plays a significant role in activating the function of breast cancer cells, regulating the differentiation of breast cancer cells and controlling the division of breast cancer cells. This imbalance leads to the uncontrolled growth and development of breast cancer cells. Targeted therapy that blocks the corresponding pathway may become a new perspective for breast cancer treatment. In addition, corresponding therapeutic strategies can be used according to the expression characteristics of different molecular types of breast cancer stem cells. For ER-positive breast cancer, simultaneous endocrine therapy and targeted therapy of tumor stem cells may improve the efficacy of endocrine therapy. Trastuzumab therapy significantly reduces the risk of recurrence of HER2-positive breast cancer. For drug-resistant patients, combination therapy is required due to the different phenotypes of epithelial-mesenchymal transforming tumor stem cells. This study briefly reviews the research progress of breast cancer stem cell-related signaling pathways and their inhibitors, in order to provide a reference for breast cancer patients to obtain more effective clinical treatment.

Keywords: Breast cancer stem cells, signal pathway, chemotherapy tolerance, regulating cell differentiation, HER2 positive breast cancer, triple negative breast cancer, inhibitors

Introduction

At present, breast cancer is the most common malignant tumor in women. In the past two decades, the incidence of breast cancer has increased year by year, and it has become the number one cancer killer threatening women's health [1]. According to the "Statistics Report of Global Cancer in 2018" released by the World Health Organization's International Agency for Research on Cancer (IARC), there were 2.1 million new cases of breast cancer in women worldwide in 2018, accounting for about a quarter of all cancers in women. The number of deaths accounted for approximately 11.6% of all cancer deaths, ranking among the

top three in both morbidity and mortality [2]. Although great progress has been made in the early diagnosis and comprehensive treatment of breast cancer, some patients still experience local recurrence or distant metastasis [3]. With the further study of tumorigenesis mechanism and stem cell theory, solid tumor stem cell theory has attracted more and more attention. BCSCs are a group of self-renewing, highly tumorigenic pluripotent cells that are closely associated with breast cancer initiation, metastasis, recurrence and emergence of drug resistance [4]. Among the various treatment methods for breast cancer, only targeted elimination of BCSC is the key to curing breast cancer fundamentally. According to the cancer stem cell theory, cancer stem cells play a decisive role in cancer formation and growth, and are the root cause of cancer recurrence, metastasis and chemotherapy resistance. Currently, it is believed that Wnt, Notch, Hedgehog and other signaling pathways are involved in the self-renewal process of breast cancer stem cells, and play an important role in the occurrence, development and drug resistance of breast cancer [5]. Targeted drugs for the above-mentioned pathways will bring new ideas for breast cancer treatment. In view of this, this study reviews the research progress of breast cancer stem cell-related signaling pathways and their inhibitors, and also reports the effect of breast cancer stem cells on the efficacy of breast cancer patients with different molecular types.

Discovery, isolation and purification of breast cancer stem cells

Many cancer tissues, including breast cancer, contain some cells with very high tumorigenic capacity and very low differentiation. These cells are called “cancer stem cells” (CSCs) and they are characterized by self-renewal and multidirectional differentiation as stem cells. The isolation and identification of tumor stem cells are important prerequisites for the development of tumor stem cell research. Since the identification of tumor-initiating $CD34^+/CD38^-$ cells in AML provided conclusive evidence for the existence of tumor stem cells, tumor stem cells have been identified in a variety of other tumors [6]. Al-hajj *et al.* [5] identified solid tumor stem cells in breast tumors for the first time. Self-renewal and differentiation of mammary stem cells were studied by lentiviral vector-mediated RNA interference or gene overexpression and by Sphere Forming assay in vitro suspension culture and NOD/SCID mouse model. At present, it is a method system widely adopted by stem cell researchers [7]. Due to the heterogeneity of tumors, different tumors have different phenotypes and functions. Even within the same tumor, different cells may have different phenotypes and functions, particularly in breast tumors. Breast tumors are composed of many breast tumor cells with different phenotypes. Al-hajj *et al.* [5] isolated multiple cell populations according to different cell surface molecular expressions, and used immunodeficient mice to identify cells with $CD44^+CD24^-$/Low $Lineage^-$ phenotype with ability to initiate tumor growth. Later, Ginestier *et al.* and Liu *et al*. isolated and identified $ALDH^+$ cells from breast tumors with the characteristics of tumor stem cells, indicating that heterogeneity also existed in tumor stem cells [8, 9].

In recent years, breast cancer stem cells (BCSCs) have been closely related to epithelial-mesenchymal transition (EMT). It has been found that breast tumor stem cells have at least two different phenotypic and functional states-epithelial-like states and mesenchymal-like states. Epithelioid BCSCs are dominated by $ALDH^+$ cells, which are distributed in the central region of the tumor and are in a relatively active proliferation state. They can undergo rapid proliferation and self-renewal, thus generating new tumor masses. However, mesenchymal BCSCs are mainly $CD44^+CD24^-$ cells, which are distributed at the edge of the tumor and in a static proliferation state, but have strong invasion ability. They can enter the blood vessels and migrate to the distal organs with the blood. Once they reach the distal organs, these stem cells are transformed into epithelial stem cells. It promotes the rapid proliferation of tumor cells in the distal organs, and the transformation between different states plays a key role in tumor growth and migration [9], and such transformation may be regulated by various signals. These findings provide new ideas for further purification of breast cancer stem cells and more accurate targeting of cancer stem cells.

Tumor microenvironment plays an important role in the regulation of breast tumors stem cells

Tumor microenvironment contains a large number of non-tumor cells such as mesenchymal

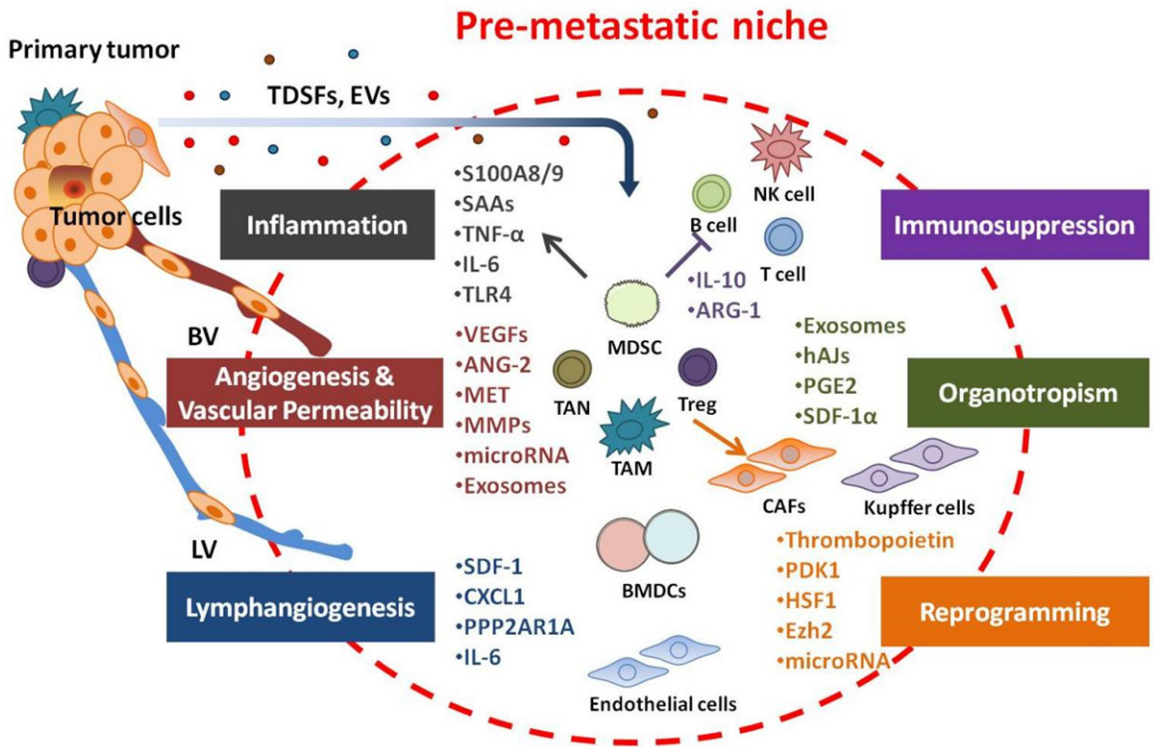

Figure 1. Liu Y and Cao XT. Characteristics and significance of the pre-metastatic niche [10]. The pre-metastatic niche that is created by the TDSFs, BMDCs, regulatory/suppressive immune cells, and stromal components in the distant organ can be endowed with six enabling characteristics which promote tumor cell colonization and metastasis. The characteristics of the premetastatic niche can be summarized as immunosuppression, inflammation, angiogenesis/vascular permeability, lymphangiogenesis, organotropism, and reprogramming.

stem cells (MSCs), immune cells, stromal cells, etc. These cells either secrete specific cytokines or interact with each other directly. It plays a crucial role in the occurrence, development and metastasis of tumors (**Figure 1**) [10]. It has been reported that cytokines IL-8 and IL-6 in the microenvironment have regulatory effects on breast tumor stem cells (**Figure 2**). Among them, the IL-8 pathway plays an important role in the self-renewal of breast tumor stem cells. Since chemotherapeutic drugs can only kill common tumor cells, these apoptotic common tumor cells can release a large amount of IL-8, thereby activating the self-renewal and regeneration of cancer stem cells through the IL-8 CXCR1 pathway. This leads to further development of cancer [11]. In addition, bone marrow mesenchymal stem cells induced by IL-6 can secrete more cytokines such as IL-8 and IL-6 and migrate from the bone marrow to the site of in situ breast cancer, promoting the self-renewal of tumor stem cells and the growth of in situ cancer [12]. These results suggested that the breast tumor microenvironment played a significant role in breast cancer stem cells through some regulatory cytokines related to immune response, providing a new direction for targeted therapy of cancer stem cells. In other words, by interfering with cancer-related tumor microenvironment, such as blocking signal transduction of IL-8 and IL-6 [13], tumor stem cells could be effectively reduced, and tumor growth and metastasis could be inhibited, especially for triple negative breast cancer with high malignancy.

Recently, Liu's team published a research paper on the Cancer Research website, revealing the remodeling and tumour-promoting effect of ALDH1A1 enzyme activity on the immune microenvironment of breast cancer.

It was found that ALDH1A1 reduced the pH value in tumor cells by ALDH1A1 enzyme activity, and then activated the TAK1-NFκB signaling pathway. The secretion of granulocyte-macrophage-stimulating factor (GM-CSF) in tumor cells was increased, which in turn induced an

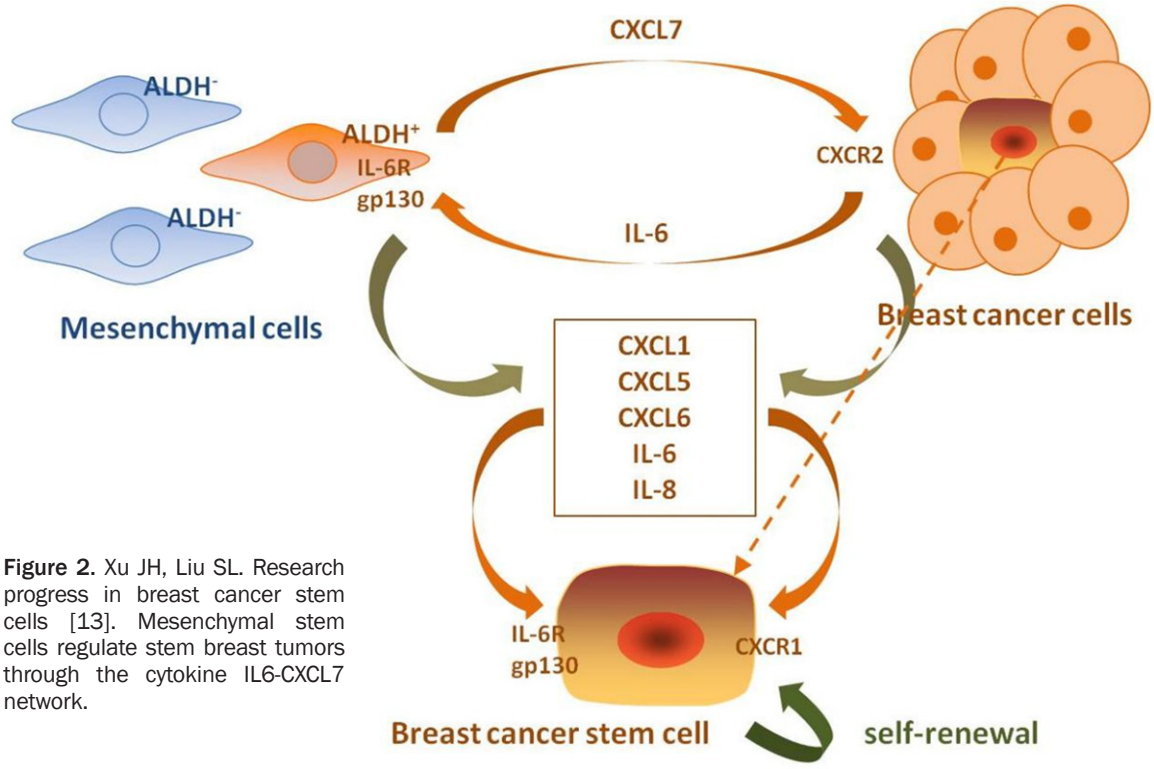

Figure 2. Xu JH, Liu SL. Research progress in breast cancer stem cells [13]. Mesenchymal stem cells regulate stem breast tumors through the cytokine IL6-CXCL7 network.

increase in MDSCs in the tumor microenvironment. The increase of MDSCs ultimately inhibited the anti-tumor immune activity of $CD8^+$ T cells to promote breast tumor growth. The role of ALDH1A1 in breast tumor development was better elucidated, providing a new idea for the clinical treatment of malignant breast cancer [14].

Relationship between breast cancer stem cells and chemotherapy tolerance

After surgical resection and chemotherapy, ordinary breast cancer cells are removed and eliminated, but there are still a small number of malignant cancer cells lurking in the patient's body to resist treatment. Some of these cancer cells are naturally resistant to chemotherapy, and some are induced by chemotherapy. These cells all have characteristics of breast cancer stem cells. These treatment-resistant breast cancer stem cells generate new breast cancer cells through continuous proliferation and differentiation, leading to breast cancer recurrence, or spreading to other tissues and organs through invasion and metastasis, thus resulting in breast cancer metastasis [15]. The causes of drug resistance of tumor stem cells (currently the main mechanisms) include: (1) Tumor stem cells have low differentiation and a high capacity to repair DNA damage. It is able to repair chemotherapy-induced cell damage and survive, and develop tolerance to treatment [16]. (2) Under conventional conditions, tumor stem cells are dormant, that is, trapped in the G0/G1 phase of the cell cycle, thereby evading cell cycle-affecting chemotherapy. (3) The ALDH expression specific to cancer stem cells is not only an important biomarker for cancer stem cells, but also an important factor in inducing chemotherapy tolerance of cancer stem cells [17]. (4) As mentioned above, tumor stem cells, such as SP cells with high expression of ATP-binding transporters, including multidrug-resistant protein 1 and P-glycoprotein, can actively pump hydrophobic chemotherapy drugs out of the cell, reduce intracellular drug accumulation, increase drug efflux, and develop resistance to chemotherapy drugs [18, 19]. (5) Tumor stem cells are usually concentrated in areas where tumor tissue is hypoxic.Oxygen in tumor cell microenvironment is one of the potential sensitization factors of radiation therapy. Hypoxic areas in tumor tissues are enriched with tumor stem cells, which are less sensitive to radiation therapy. (6) The activation of

tumor stem cell signaling pathway promotes the tolerance to chemotherapy and greatly increases the risk of breast cancer recurrence. For example, breast tumor stem cells from mice with activation of the Wnt/β-catenin signaling pathway were more resistant to chemotherapy [20].

Non-coding RNA and breast cancer stem cells

circRNA

At present, many circRNAs have been proved to be related to the proliferation of breast cancer stem cells, indicating their potential role as therapeutic targets for breast cancer [21]. Wang et al. [21] found that circRNA-000911 acted as a miRNAs "sponge" for miRNA-449a, promoting the function of Notch 1 and the nuclear factor kappa-B (NF-κB) signaling pathway. Therefore, circRNA-000911 overexpression would provide new strategies and directions for breast cancer treatment. The ability of circ-Foxo3 to induce apoptosis and inhibit breast cancer progression also makes it a promising therapeutic target for breast cancer [22]. Young et al. [23] preliminarily explored the expression pattern of circRNAs in breast cancer stem cells. Bioinformatics predictions of the circRNAs/miRNA interaction network suggested that circRNAs may regulate the self-renewal of breast cancer stem cells through the spongy action of miRNA. Further studies showed that circRNA vaccinia-related kinase 1 (circVRK1) inhibited the expansion and self-renewal of breast cancer stem cells.

Liang et al. [24] found that circDENND4C was positively correlated with the size of malignant breast tumors, and circDENND4C was closely correlated with Hypoxia inducible fact-1α (HIF1α) in a hypoxia environment. In the hypoxic environment, it can reduce circDENND4C surface level through down-regulated HIF1α, and then inhibit the proliferation of breast tumor cells. Liu et al. [25] believed that hsa_circ_0008039 was an oncogene in breast cancer, which promoted the progression of breast cancer by enhancing the expression of transcription factor cadherin E2F3 through spongy miRNA-432-5p. These results indicated that hsa_circ_0008039 may be a potential target for breast adenocarcinoma treatment. Zhang et al. [26] found that hsa_circ_0052112 was up-regulated in MDA-MB-231 cells, and overexpressed hsa_circ_0052112 acted as a "sponge" for miRNA-125a-5p. Thus contributing to the migration and invasion of breast cancer cells. The research of Yang et al. [27] showed that circRNA ARF-Gap domain and FG repeat-containing protein 1 (Circ-agfg1) were regulated by miRNA-195-5p (G1/S-specific cyclinE1, CCNE1) expression, which led to the occurrence and progression of triple negative breast cancer. CircAGFG1 may be a valuable prognostic marker and is expected to be a marker for the diagnosis and treatment of triple negative breast cancer. Existing studies have demonstrated that many circRNAs are closely related to the proliferation, progression and self-renewal ability of breast cancer stem cells, and as a promising potential therapeutic target, they can provide a fresh therapeutic strategy and direction for breast cancer.

LncRNA

LncRNA acts directly or indirectly on cancer stem cells and regulates the occurrence and development of cancer. Some LncRNAs contain binding sites of stem cell-related miRNAs. The miRNAs can be competitively bound by miRNA sponge engineering to inhibit their regulation of stem cells. LncRNA H19 sequence has multiple potential let-7 family binding sites and is a sponge adsorption molecule of let-7. In muscle cells, deletion of H19 can lead to increased expression of let7 and promote stem cell differentiation [28]. Some lncRNA molecules can interact with dry genes such as OCT4, SOX2, KLF4 and PcG to form a feedback loop and accurately regulate the proliferation and differentiation of stem cells [29]. The regulation of lncRNA on breast cancer stem cells is still in the preliminarystage. $CD24^{-/low}$ $CD44^{+}$ LIN^{-} cell subsets and $ALDH^{+}$ cell subsets in breast cancer cells were confirmed to have stem cell characteristics, and 100 cells with these characteristics were able to regenerate breast tumors when transplanted into mice.

Screening and characterizing the expression of LncRNAs in breast cancer stem cells will certainly reveal the regulatory pathways of breast cancer and provide more potential therapeutic targets to inhibit breast cancer recurrence and metastasis. Hou et al. found that transcription factor LINc-ROR in breast cancer cells induced EMT to promote the invasion and metastasis of

cancer cells [30]. In mammary epithelial cell MCF10A, overexpression of Linc-ROR significantly increases the proportion of $CD44^+CD24^-$ cell subsets with tumor stem cell characteristics and the number of in vitro mammosphere formations. These results indicate that Linc-ROR is involved in self-renewal and regenerative tumor regulation of breast cancer stem cells.

LncRNA and SOX2OT (SOX2 transcript) derived from the intron sequence of dry factor SOX2 gene have strong spatio-temporal specific regulation of embryogenesis. SOX2OT is positively correlated with SOX2 expression in breast cancer tissue samples. Overexpression of SOX2OT can induce the expression of SOX2 [31]. SOX2OT is especially highly expressed in ER-positive breast cancer tissues and is highly sensitive to steroid hormones. Whether SOX2OT regulates the ability of breast cancer stem cells to regenerate tumor by affecting SOX2 expression remains to be further studied.

Micro-RNA

Micro-RNAs (miRNAs) are a group of short RNA sequences that do not encode proteins. Studies have found that miRNA expression at chromosomal vulnerable sites in human breast cancer cells can be changed, indicating that miRNA is closely related to the occurrence of breast cancer [32]. miRNA can regulate the expression of related proteins and signal pathways, thus affecting the invasion and metastasis of breast cancer. Many miRNAs that regulate breast cancer metastasis have been identified in studies in the past few years, such as miR-10b, miR-335, miR-373, miR-520 and miR-31. Recently, miR-33b, miR-34a and miR-let-7 have been studied. It was found that in breast cancer cell lines, the expression level of miR-33b was negatively correlated with the invasion and metastasis ability of breast cancer cells. miR-33b regulates the dryness, invasion and metastasis of breast cancer mainly by directly regulating the 3'UTR of the three downstream IE genes HMGA2, SALL4 and Twist1. The miR-33b can inhibit the breast microglobulin formation and dryness of breast cancer tumor stem cells [33].

Studies have found that overexpression of miR-34a can inhibit the proliferation, invasion and metastasis of tumor stem cells [34]. Inhibition of miR-34a by miR-34a inhibitors can promote the proliferation, invasion and metastasis of tumor stem cells. The miR-34a can inhibit self-renewal of breast cancer stem cells by down-regulating SIRT1, and the mechanism may be related to down-regulating Nanog protein expression. Lin28 is an RNA-binding protein that regulates the function of breast cancer stem cells by regulating let-7 expression [35, 36]. In addition, miR-100 has also been confirmed to be closely related to the metastasis mechanism of breast cancer stem cells, and down-regulation of miR-100 can increase the number of breast cancer stem cells and improve the proliferation ability of breast cancer cells in vitro. Low expression of miRNA-100 can increase acetaldehyde dehydrogenase 1 (ALDH1) expression and reduce the survival rate of breast cancer patients [37]. Therefore, studying the relationship between miRNA and tumor stem cell metastasis and related mechanisms are important for finding new ways of tumor therapy.

Research status of breast cancer stem cell signaling pathway and signaling pathway inhibitors

Breast cancer stem cells grow slowly, and their proliferation and self-renewal require activation of related signaling pathways. The cell signaling pathway plays an important role in regulating the normal growth of stem cells and the stability of their internal environment. Normally, breast cancer stem cells are like normal stem cells and are in a quiescent state, unaffected by the activity of cell division. If mutations or abnormal activation of related pathways occur, the balanced state will be broken, and breast cancer stem cells will directly enter the proliferation cycle and self-renewal, resulting in uncontrolled cell growth and breast cancer. Currently, Hedgehog, Wnt and Notch pathways are the most widely studied signaling pathways of breast cancer stem cells.

Notch signaling pathway

Notch is an indispensable signaling pathway in various animals, regulating multiple life processes of cells. Notch signaling pathway not only controls the tumor cells, including the growth, differentiation, apoptosis, cycle and epithelial cells-mesenchymal cells (EMT), and other series of life processes. Moreover, it also

plays an important role in the growth and maturity of embryos, the formation of blood vessels, the hematopoietic function, and the formation of some tumors, physiological and pathological processes. Notch targets include Hes1, P21CIP1/WAF1, cyclin D and NF-κB [38]. Notch receptors bind to specific downstream genes to transmit the signal, inhibit the directed differentiation of stem cells, and thus promote cell proliferation [39]. Activation of these genes may lead to excessive proliferation of breast cancer stem cells and inhibit apoptosis of breast cancer cells. Notch pathway has been demonstrated in breast cancer stem cells, and Notch antibody can inhibit Notch signaling pathway transduction. Notch receptors have been found to be expressed in breast cancer stem cells [40-42]. Overexpression of Notch1-4 has been shown to inhibit the differentiation of mammary epithelial cells and the development of breast cancer in vitro [43]. In vitro, overexpression of the active form of Notch4 inhibits mammary epithelial differentiation. Insertional mutated Notch4 gene induces malignant transformation of mouse mammary tumor cells and induces mammary epithelial cells to lose their ability to form ducts [42]. The abnormal activation of Notch signaling pathway can lead to excessive proliferation of breast cancer stem cells, thereby promoting the occurrence and metastasis of breast cancer. Several ways to inhibit the Notch pathway have been studied, mainly including two types. One is selective blocking agents, mainly biological agents, including antisense RNA, interfering RNA and monoclonal antibody. Small molecule selective inhibitors acting on the receptor have not been reported. Non-selective inhibitors include ligand blockers, γ-secretory inhibitors and some natural products. Monoclonal antibodies are currently being developed to block Notch1, 2, and 3. Two classes of Notch1 mAb are being developed for the treatment of breast cancer (NRR1, Genentech and Exelixis) and Notch2 mAb (NRR2, Genentech and Exelixis). Studies have shown that pretreatment of breast cancer stem cells with DAPT (novel GSI) or neutralizing antibody Notch4 can reduce the efficiency of microsphere formation [42]. Choy L et al. [43] found that docetaxel combined with MK0752 reduced the number and volume of breast cancer stem cells and inhibited the formation of metastatic breast cancer. Currently, clinical trials of MK0752 in combination with different drugs are also underway, such as MK0752 in combination with endocrine drugs or chemotherapy for breast cancer [44]. PF-03084014 is also currently in phase I clinical trials for advanced breast cancer [45].

The combination of GSI MRK-003 and trastuzumab can inhibit breast cancer growth and avoid the recurrence of metastatic breast cancer [46, 47]. Studies have shown that this drug can significantly reduce Notch activity, inhibit breast cancer cell migration and microsphere formation, and reduce the expression level of Notch target gene. Recent studies have further verified that salamycin can inhibit breast cancer cells and breast cancer stem cells, and the mechanism of action may be through inhibiting Notch1/Hedgehog signal transduction pathway. Through mammary pellet formation test, it has been confirmed that salamycin can inhibit the mammary pellet formation ability of breast cancer stem cells. Flow cytometry showed that halamycin reduced the proportion of $CD44^{+}CD24^{-}$ cells on the surface of breast cancer stem cells, and RT-PCR and Western Blot further showed that both mRNA and protein expression of ALDH1 decreased [48].

Activation of Wnt signaling pathway

Activation of Wnt pathway can interfere with the normal self-renewal of breast stem cells, induce abnormal proliferation and transformation of breast stem cells, and eventually lead to the formation of breast cancer stem cells. The expression of Wnt signaling pathway-related proteins is limited in normal breast stem cells, but increased in breast cancer stem cells. It has been found that inhibition of Wnt/β-catenin signaling pathway can inhibit the proliferation and migration of breast cancer cells [49]. Breast cancer stem cells can also be isolated from breast tumors induced by mouse breast tumor virus carrying Wnt-1 gene [50]. These results suggest that Wnt signaling pathway plays an important role in maintaining self-renewal, undifferentiated state and adult development of breast cancer stem cells. Some studies have revealed that abnormal activation of Wnt signaling pathway can cause abnormal breast cancer stem cells to form breast cancer [51]. The correction of Wnt pathway abnormalities can be accomplished by antagonizing receptors that initiate the Wnt pathway, blocking the

information transfer of key signal transduction proteins in cells or disrupting the interactions of transcription initiation factor complexes, and using adenovirus to express genes encoding cytotoxins in breast cancer stem cells. The secreted FZD-associated protein SFRP and Wnt inhibitor WIF can bind to Wnt-associated receptors on the cell membrane, thereby antagonizing the Wnt signaling pathway. Monoclonal antibody Wnt-1 can inhibit the binding of Wnt ligand to a cell membrane receptor, induce changes in downstream proteins of Wnt pathway, and induce apoptosis of breast cancer stem cells. Using RNA interference technology and antisense RNA can prevent intracellular signal transduction and effectively inhibit the growth of breast cancer. Non-steroidal anti-inflammatory drugs, such as aspirin, indomestacin and sulinic acid, can down-regulate β-catenin in breast cancer stem cells and play an anti-breast cancer effect. Kakarala et al. [52] proposed that polyphenol needles, curcumin and pepper could block the Wnt pathway to reduce the volume of breast cancer stem cell microspheres and the number of breast cancer stem cells. Another substance that inhibits Wnt signaling pathway is pyrvinium, which can significantly reduce the number of breast adenocarcinoma stem cells and inhibit the growth and differentiation of breast cancer in vivo [53]. Xu et al. [54] studied the selective inhibition of breast cancer stem cells by plasma heating mediated by gold nanorods, and found that salamycin applied to gold nanorods for plasma heating could inhibit the formation of microspheres and reduce the number of ALDH1 cells.

Hedgehoge signaling pathway

Hedgehog (Hh) signaling pathway is an indispensable signaling pathway, which plays an important role in the early stage of embryonic development, especially in the process of epithelial mesenchymal transformation [55]. It is a highly conserved signaling pathway. The basic components of Hh signaling pathway are mainly composed of its important ligand SHH, two essential membrane receptors Ptch, SMO and the representative transcription factors Gli family (Glil, Gli2, Gli3) downstream of SMO. The Hh homologous genes include SHH, Ihh, Dhh. Hh signaling begins with the binding of Hh ligand to the transmembrane protein receptor Ptch1 to activate Smo, which then acts on Gli2 and Gli3 to induce transcription of the target gene Gli1. Therefore, Gli1 mRNA expression is a marker of Hh/Gli signal pathway activation. Hh regulates breast cancer-associated fibroblasts through activation of the Hedgehog signaling pathway and inhibits breast cancer stem cell proliferation [56]. Wolf [57] found that Ptch1 protein was highly expressed in normal breast tissue, but decreased in invasive ductal carcinoma. Hui M et al. [58] reported that in invasive ductal carcinoma, the expression levels of Ptch1, Smo, Gli1, Gli2 and Gli3 were positively correlated with the breast cancer proliferation index. It was suggested that Hh pathway was related to the occurrence of breast cancer, and Hh regulation could be used as an early event of breast cancer.

Hh signaling pathway is an important regulatory pathway of breast cancer stem cells, promoting the formation of breast cancer and the occurrence of multidrug resistance of breast cancer. Targeting Hh signaling may be a way to treat breast cancer and reverse drug resistance. Hh pathway inhibitors act directly on Hh ligands, receptors Ptch or Smo, and Gli1 proteins. The main inhibitors of Hh ligand are Shh antibody and PRARβ. Ptch inhibitors include Cur61414 and 5-FU. Smo suppressor genes include cyclopamine, USA-SMO5a, KAAD-cyclopamine, VitD3, SANT1-4, SMO-SiRNA, and Gli suppressor factors including Glii-siRNA, FGF, REN-KCTD11, Numb and PP2A. It is expected to be a new drug for breast cancer treatment. They can inhibit Hh signaling pathway expression in breast cancer at different levels. For example, salamycin can selectively kill breast cancer microspheres by inhibiting the expression of Hh signaling pathway Ptch, Smo, Gli1 and Gli2 [59]. However, cyclopamine (a steroid alkaloid extracted from lilium plants) can bind to the seven-helix cluster region of Smo, thus inhibiting intracellular signal transduction of Smo and further inhibiting transcription and expression of cytokines. While inducing apoptosis of cancer cells, cyclopamine has no effect on the growth of normal cells. It has entered clinical trials as an anti-breast cancer drug [60]. TSPAN8 is involved in tumor cell dry maintenance by activating Sonic Hh signaling. In 2019, Wang's team overexpressed TSPAN8 in a series of tumor cell lines and found enhanced desiccation. It was characterized by increased size and number of microspheres, enhanced colony

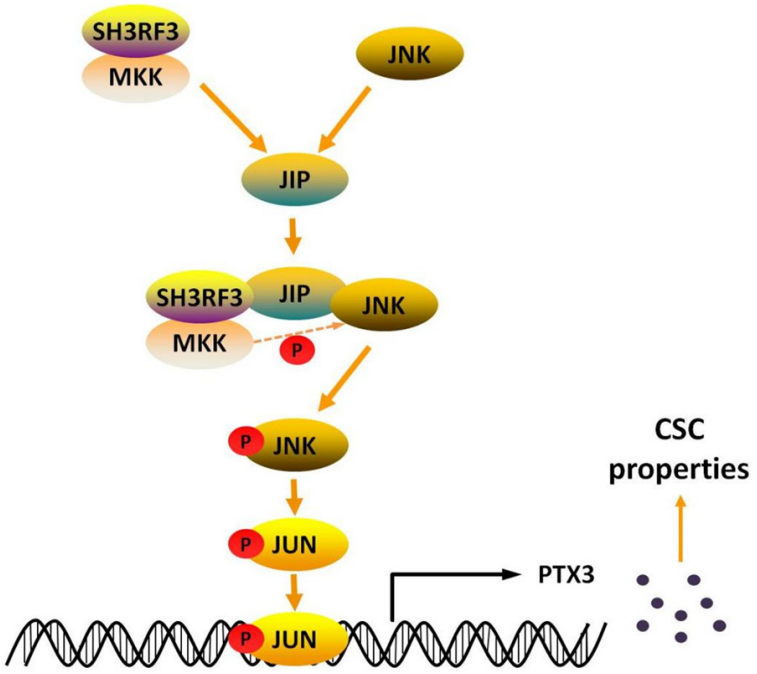

Figure 3. Zhang PY, Liu YJ, Lian C, et al. [68]. SH3RF3 promotes breast cancer stem-like properties via JNK activation and PTX3 upregulation. Nat Commun. Schematic model of the role of SH3RF3 in BCSC regulation.

formation, increased $CD44^{+}/CD24^{-}$ cell ratio, and enhanced cell resistance to the chemotherapeutic drugs doxorubicin (ADR) and paclitaxel (PTX). To evaluate the key signaling pathways through which TSPAN8 exerted this effect, we overexpressed TSPAN8 in tumor cells. It was found that the mRNA and protein expressions of PTCH1, GLI, HHIP and SHH (Hh signaling pathway) were significantly increased, and SMO phosphorylation and GLI1 transcriptional activity were significantly increased. Treatment with Hedgehog pathway inhibitors vismodegib and Ru-ski43 reversed this phenomenon [61].

Other signal pathways

PI3K/Akt/mTOR, Hippo, JAK2/STAT3, and JAK2/STAT3 signaling pathways have also been found to be associated with breast cancer stem cells [62-64]. PI3K is overexpressed in breast cancer [65]. Harvey et al. [66] found that the transcriptional coactivator TAZ of Hippo signaling pathway was closely related to the proliferation, migration and transformation ability of breast cancer stem cells. Also, the increased protein level of Hippo signaling pathway was related to breast cancer. The self-renewal and tumor-initiation abilities of breast cancer stem cells required the activation of TAZ [67].

JNK-JUN signaling pathway

The recent results of Hu's group demonstrated that overexpression of SH3RF3 enhanced the tumorigenicity and metastasis of breast cancer cells in a breast cancer cell model. The role of SH3RF3 in promoting tumor dry features was also demonstrated in organoid bodies of breast cancer patients. Mechanism studies have shown that SH3RF3 can interact with MKK-JNK complex to promote the activation of JNK-JUN signaling pathway in a JIP dependent manner. The expression of PTX3 was thereby promoted and ultimately the stemness of tumor cells was improved and the malignancy of tumor cells was enhanced (**Figure 3**) [68].

PI3k-AKT signaling pathway

Recently, Chen's research team found that a new gene SGCE inhibited the normal degradation of EGFR. In terms of mechanism, SGCE was found to bind to the E3 ubiquitin ligase C-CBL. SGCE deletion promoted the release of C-CBL and the ubiquitination of its substrate protein EGFR. This led to internalization of EGFR to the lattice-mediated and macrocytosis pathways, followed by internalization of EGFR to lysosomal degradation. EGFR degradation leads to the blocking of downstream pathways and ultimately inhibits BCSC self-renewal and extracellular matrix ECM accumulation. When SGCE is highly expressed in BCSC, SGCE interacts with C-CBL, and EGFR can normally activate its downstream signaling pathway PI3K-Akt, promoting dry maintenance of BCSC, tumor cell migration, and resistance to chemotherapy drugs and EGFR-targeted inhibitors [69].

Relationship between tumor stem cells and the efficacy of different molecular types of breast cancer

Advances in breast cancer treatment have significantly improved survival for breast cancer patients. Breast cancer is a heterogeneous disease, regulated by gene expression and epi-

genetics. In ER positive breast cancer, histone methylation is associated with drug resistance to tamoxifen or aromatase inhibitors and is associated with poor prognosis [70]. Studies have shown that the use of histone deacetylase inhibitors can enhance the sensitivity of tamoxifen treatment [71]. Clinical trials have shown that the combination of different histone deacetylase inhibitors can reverse endocrine resistance, whereas the use of one histone deacetylase inhibitor alone cannot reverse endocrine resistance [72]. The tumor stem cell hypothesis has important implications in tumor therapy. Studies have found that tumor stem cells are insensitive to chemotherapy, and in fact, radiotherapy and chemotherapy can stimulate tumor stem cells to enhance their ability to self-renew by secreting cytokines or DNA damage repair mechanisms [73]. Currently, the treatment of tumor is mainly targeted at the primary tumor cells. Gene mutations or epigenetic changes may cause the occurrence of distant metastases and treatment failure. Although tumor stem cells and their differentiated tumor cells share some of the same genetic mutation sites, tumor stem cells have some independent signaling pathways and are independent of the molecular typing of breast cancer. Therefore, combined with individualized treatment for molecular typing, targeted therapy for tumor stem cells may significantly improve the efficacy of breast cancer treatment [74].

ER positive breast cancer

ER positive breast cancer can be divided into Luminal A and Luminal B based on ki-67 index. Although all patients can be treated with endocrine therapy, the prognosis of Luminal A patients is generally better than that of Luminal B patients. This may be related to the low proportion of Luminal A tumor stem cells, low heterogeneity of differentiated tumor cells and sensitivity to endocrine therapy.

However, Luminal B breast cancer may express a certain proportion of ER/PR negative tumor stem cells, and therefore endocrine therapy is less effective [75]. Studies have found that ALDH positive tumor stem cells do not express normal ERα, but express another subtype of ERα, thus affecting the effect of endocrine therapy. Vitro experiments have confirmed that endocrine therapy resistance is associated with an increased proportion of accompanying tumor stem cells [76]. Clinical treatment also found that neoadjuvant endocrine therapy caused an increased proportion of $CD44^+/CD24^-$ tumor stem cells.

These results suggest that the combination of endocrine therapy and targeted therapy of tumor stem cells may improve the efficacy of endocrine therapy. In addition to estrogen regulation, hormone receptor-positive tumor stem cells are also regulated by a number of growth factor-related signaling pathways, such as phosphatidylinositol 3-kinase, mammalian target of rapamycin (mTOR) and HER2. Previous studies have demonstrated that endocrine therapy combined with mTOR inhibitors or CDK4/6 inhibitors can prolong disease-free survival in patients with ER-positive breast cancer [77]. These results suggest that mTOR or CDK4/6 inhibitors may enhance the efficacy of endocrine therapy in patients with ER-positive breast cancer.

HER2 positive breast cancer

Trastuzumab, a monoclonal antibody against human epidermal growth factor receptor 2 (HER2), is widely used to treat HER2-positive breast cancer. Patients with HER2-positive breast cancer should be treated with trastuzumab, which can significantly reduce the risk of recurrence. In addition to trastuzumab, there are other targeted drugs available, such as pertuzumab and TDM-1.

HER2 signaling pathway plays an important role in the pathogenesis and prognosis of breast cancer patients, and it is related to self-renewal of breast cancer tumor stem cells [78]. Amplification of HER2 gene can increase the proportion of tumor stem cells, whereas trastuzumab can decrease the ability of cells to proliferate. Although trastuzumab is currently approved for use only in patients with HER2-positive breast cancer, retrospective clinical studies have shown that trastuzumab also improves prognosis in patients with HER2-negative breast cancer [79]. Although HER2-targeted therapy can improve the prognosis of patients with HER2-positive breast cancer, the drug resistance of breast cancer cells to trastuzumab remains an important factor affecting the therapeutic effect.

At the same time, deletion of the PTEN gene usually stimulates the production of epithelial-mesenchymal transform-like tumor stem cells in HER2-positive breast cancers. Due to the different phenotypes of epithelial-mesenchymal transformed tumor stem cells, a combination therapy strategy is required. To some extent, targeting Notch1 can reduce the dryness of breast cancer cells and tumorigenesis ability of animals, suggesting that inhibition of the Notch1 signaling pathway may have a promising applications in tumor therapy.

Triple negative breast cancer

Triple negative breast cancer (TNBC) lacks the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), and is a type of breast cancer with a high malignant degree, easy metastasis, high recurrence and poor prognosis. Due to the lack of effective therapeutic targets, chemotherapy is one of the main treatment methods for TNBC. However, chemotherapy drugs can kill the dividing tumor cells, but cannot effectively act on the tumor stem cells, resulting in the enrichment of tumor stem cells, and then the drug resistance of TNBC. As a result, drug resistance mediated by tumor stem cells is one of the difficulties in the treatment of TNBC. Studies have reported that differentiation inhibitor-4 is a significant factor regulating breast stem cell self-renewal, and is one of the indicators of poor prognosis in triple-negative breast cancer patients [80]. There are many other signaling pathways that regulate triple negative breast cancer stem cells, such as Notch, Hedgehog, and IL-8-CXCR1. A number of clinical trials are currently examining whether inhibitors of these pathways can improve outcomes in patients with triple-negative breast cancer. Currently, cellular immunotherapy is also a hot direction in the treatment of triple negative breast cancer. Although recent studies suggest that immunotherapy such as programmed cell death Egg-1 inhibitors can improve the prognosis of some triple negative breast cancer patients, tumor stem cells can escape immune surveillance through various mechanisms. Therefore, immunotherapy of tumor stem cells remains a major challenge.

Recently, Chen's team found that EGFR was highly expressed in more than 50% of TNBC patients, which was closely related to breast cancer cell proliferation, metastasis and dry maintenance of BCSC. However, EGFR inhibitors (such as gefitinib and lapatinib) were not effective in the clinical treatment of breast cancer. SGCE molecules contribute to maintaining high EGFR expression in BCSC, and the removal of SGCE expression can promote the effect of EGFR-targeted therapy in TNBC, thus providing a new strategy for the combination therapy of EGFR with other targets [69].

Conclusion and prospects

Since the 21st century, with the development of molecular biology and genomics, global precision medicine has opened a new era of precision cancer treatment. Molecular targeted therapy of breast cancer stem cells is the most active field in breast cancer treatment research. The study of breast cancer stem cell signaling pathway and the inhibitors provides a theoretical basis for the development of anti-breast cancer drugs targeting breast cancer stem cell-related signaling pathways. In the future, more prospective studies are needed to kill breast cancer stem cells, protect normal stem cells, and provide safer and more effective individualized treatment for breast cancer patients. Identifying predictors of effectiveness, optimizing the beneficiary population, and further investigating the efficacy and safety of targeted drugs in combination with chemotherapeutic agents will require sustained efforts. In order to maximize the efficacy of targeted breast cancer therapy, targeting breast cancer stem cells to benefit more breast cancer patients is the future direction of treatment.

Acknowledgements

We thank Dr. Kang-Sheng Liu, Department of Clinical Laboratory, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing 210029, P. R., for his great efforts in revising the article in English.

All authors have given their consent for publication.

Disclosure of conflict of interest

None.

Abbreviations

IARC, International Agency for Research on Cancer; CSCs, cancer stem cells; BCSCs, breast cancer stem cells; EMT, epithelial-mesenchymal transition; MSCs, mesenchymal stem cells; GM-CSF, granulocyte macrophage stimulating factor; Hh, Hedgehog; mTOR, mammalian target of rapamycin; TNBC, Triple negative breast cancer; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; ADR, Adriamycin; PTX, paclitaxel; NF-κB, nuclear factor kappa-B; CircVRK1, circRNA vaccinia-related kinase 1; HIF-1α, Hypoxia inducible fact-1α; Circ-agfg1, circRNA ARF-Gap domain and FG repeat-containing protein 1; ALDH1, acetaldehyde dehydrogenase 1; miRNA, Micro-RNA.

Address correspondence to: Kang-Sheng Liu, Department of Clinical Laboratory, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing 210029, Jiangsu, China. E-mail: Kanshengliu@163.com

References

[1] Harbeck N and Gnant M. Breast cancer. Lancet 2017; 389: 1134-1150.

[2] Bray F, Ferlay J, Soerjomataram, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

[3] Calcagno AM, Salcido CD, Gillet JP, Wu CP, Fostel JM, Mumau MD, Gottesman MM, Varticovski L and Ambudkar SV. Prolonged drug selection of breast cancer cells and enrichment of cancer stem cell characteristics. J Natl Cancer Inst 2010; 102: 1637-1652.

[4] Du B, Qian M, Zhou Z, Wang P, Wang L, Zhang X, Wu M, Zhang P and Mei B. In vitropanning of a targeting peptide to hepatocarcinoma from a phage display peptide library. Biochem Biophys Res Commun 2006; 342: 956-962.

[5] Al-hajj M, Wicha MS, Benito-hernandez A, Morrison SJ and Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100: 3983-3988.

[6] Bonnet D and Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3: 730-737.

[7] Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, Suri P and Wicha MS. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res 2006; 66: 6063-6071.

[8] Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu SL, Schott A, Hayes D, Birnbaum D, Wicha MS and Dontu G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1: 555-567.

[9] Liu S, Cong Y, Wang D, Sun Y, Deng L, Liu Y, Martin-Trevino R, Shang L, McDermott SP, Landis MD, Hong S, Adams A, D'Angelo R, Ginestier C, Charafe-Jauffret E, Clouthier SG, Birnbaum D, Wong ST, Zhan M, Chang JC and Wicha MS. Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. Stem Cell Rep 2013; 2: 78-91.

[10] Liu Y and Cao X. Characteristics and significance of the pre-metastatic niche. Cancer Cell 2016; 30: 668-681.

[11] Ginestier C, Liu S, Diebel ME, Korkaya H, Luo M, Brown M, Wicinski J, Cabaud O, Jauffret EC, Birnbaum D, Guan JL, Dontu G and Wicha MS. CXCR1 blockade selectively tar gets human breast cancer stem cells in vitro and in xenografts. J Clin Invest 2010; 120: 485-497.

[12] Liu S, Ginestier C, Ou SJ, Clouthier SG, Patel SH, Monville F, Korkaya H, Heath A, Dutcher J, Kleer CG, Jung YH, Dontu G, Taichman R and Wicha MS. Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. Cancer Res 2011; 71: 614-624.

[13] Xu JH and Liu SL. Research progress in breast cancer stem cells. Sci Sin Vitae 2017; 47: 140-144.

[14] Liu C, Qiang J, Deng Q, Xia J, Deng L, Zhou L, Wang D, He X, Liu Y, Zhao B, Lv J, Yu Z, Lei QY, Shao ZM, Zhang XY, Zhang L and Liu S. ALDH1A1 activity in tumor-initiating cells remodels myeloid-derived suppressor cells to promote breast cancer progression. Cancer Res 2021; 81: 5919-5934.

[15] Tuasha N and Petros B. Heterogeneity of tumors in breast cancer: implications and prospects for prognosis and therapeutics. Scientifica (Cairo) 2020; 2020: 4736091.

[16] Olivares-Urbano MA, Griñán-Lisón C, Marchal JA and Núñez MI. CSC radioresistance: a therapeutic challenge to improve radiotherapy effectiveness in cancer. Cells 2020; 9: 1651.

[17] Chen J, Liu S, Su Y and Zhang X. ALDH1+ stem cells demonstrate more stem cell-like characteristics than CD44+/CD24-/low stem cells in different molecular subtypes of breast cancer. Transl Cancer Res 2020; 9: 1652-1659.

[18] Wang JQ, Wu ZX, Yang YQ, Teng QX, Li YD, Lei ZN, Jani KA, Kaushal N and Chen ZS. ATP-binding cassette (ABC) transporters in cancer: a

review of recent updates. J Evid Based Med 2021; 14: 232-256.
[19] Kathawala RJ, Gupta P, Ashby CR and Chen ZS. The modulation of ABC transporter-mediated multidrug resistance in cancer: a review of the past decade. Drug Resist Updat 2015; 18: 1-17.
[20] Singh S, Brocker C, Koppaka V, Chen Y, Jackson BC, Matsumoto A, Thompson DC and Vasiliou V. Aldehyde dehydrogenases in cellular responses to oxidative/electrophilic stress. Free Radic Biol Med 2013; 56: 89-101.
[21] Wang H, Xiao Y, Wu L and Ma DC. Comprehensive circular RNA profiling reveals the regulatory role of the circRNA-000911/miR-449a pathway in breast carcinogenesis. Int J Oncol 2018; 52: 743-754.
[22] Ma I and Allan AL. The role of human aldehyde dehydrogenase in normal and cancer stem cells. Stem Cell Rev 2011; 7: 292-306.
[23] Young SZ and Bordey A. GABA's control of stem and cancer cell proliferation in adult neural and peripheral niches. Physiology (Bethesda) 2009; 24: 171-185.
[24] Liang G, Liu Z, Tan L, Su AN, Jiang WG and Gong C. HIF1α-associated circDENND4C promotes proliferation of breast cancer cells in hypoxic environment. Anticancer Res 2017; 37: 4337-4343.
[25] Liu Y, Lu C, Zhou Y, Zhang ZH and Sun L. Circular RNA hsa_circ_0008039 promotes breast cancer cell proliferation and migration by regulating miR-432-5p/E2F3 axis. Biochem Biophys Res Commun 2018; 502: 358-363.
[26] Zhang HD, Jiang LH, Hou JC, Zhong SL, Zhou SY, Zhu LP, Li J, Wang DD, Sun DW, Ji ZL and Tang JH. Circular RNA hsa_circ_0052112 promotes cell migration and invasion by acting as sponge for miR-125a-5p in breast cancer. Biomed Pharmacother 2018; 107: 1342-1353.
[27] Yang R, Xing L, Zheng X, Sun Y, Wang XS and Chen JX. The circRNA circAGFG1 acts as a sponge of miR-195-5p to promote triple-negative breast cancer progression through regulating CCNE1 expression. Mol Cancer 2019; 18: 4.
[28] Kallen AN, Zhou XB, Xu J, Qiao C, Ma J, Yan L, Lu L, Liu C, Yi JS, Zhang H, Min W, Bennett AM, Gregory RI, Ding Y and Huang Y. The imprinted H19 lncRNA antagonizes let-7 microRNAs. Mol Cell 2013; 52: 101-112.
[29] Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, Wu M, Xiong J, Guo X and Liu H. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. Dev Cell 2013; 25: 69-80.
[30] Hou P, Zhao Y, Li Z, Yao R, Ma M, Gao Y, Zhao L, Zhang Y, Huang B and Lu J. LincRNA-ROR induces epithelial-to-mesenchymal transition and contributes to breast cancer tumorigenesis and metastasis. Cell Death Dis 2014; 5: e1287.
[31] Amaral PP, Neyt C, Wilkins SJ, Askarian-Amiri ME, Sunkin SM, Perkins AC and Mattick JS. Complex architecture and regulated expression of the Sox2ot locus during vertebrate development. RNA 2009; 15: 2013-2027.
[32] Petri BJ and Klinge CM. Regulation of breast cancer metastasis signaling by miRNAs. Cancer Metastasis Rev 2020; 39: 837-886.
[33] Sun M, Gomes S, Chen P, Frankenberger CA, Sankarasharma D, Chung CH, Chada KK and Rosner MR. RKIP and HMGA2 regulate breast tumor survival and metastasis through lysyl oxidase and syndecan-2. Oncogene 2014; 33: 3528-3537.
[34] Malla RR and Kiran P. Tumor microenvironment pathways: cross regulation in breast cancer metastasis. Genes Dis 2020; 9: 310-324.
[35] Sun X, Qin S, Fan C, Xu CW, Du N and Ren H. Let-7: a regulator of the ERα signaling pathway in human breast tumors and breast cancer stem cells. Oncol Rep 2013; 29: 2079-2087.
[36] Yang X, Lin X, Zhong X, Kaur S, Li N, Liang S, Lassus H, Wang LP, Katsaros D, Montone K, Zhao X, Zhang YC, Bützow R, Coukos G and Zhang L. Double-negative feedback loop between reprogramming factor LIN28 and microRNA let-7 regulates aldehyde dehydrogenase 1-positive cancer stem cells. Cancer Res 2010; 70: 9463-9472.
[37] Deng L, Shang L, Bai S, Chen J, He X, Martin-Trevino R, Chen S, Li XY, Meng X, Yu B, Wang X, Liu Y, McDermott SP, Ariazi AE, Ginestier C, Ibarra I, Ke J, Luther T, Clouthier SG, Xu L, Shan G, Song E, Yao H, Hannon GJ, Weiss SJ, Wicha MS and Liu S. MicroRNA100 inhibits self-renewal of breast cancer stem-like cells and breast tumor development. Cancer Res 2014; 74: 6648-6660.
[38] Chai JY, Sugumar V, Alshawsh MA, Wong WF, Arya A, Chong PP and Looi CY. The role of smoothened-dependent and -independent hedgehog signaling pathway in tumorigenesis. Biomedicines 2021; 9: 1188.
[39] Suman S, Das TP and Damodaran C. Silencing NOTCH signaling causes growth arrest in both breast cancer stem cells and breast cancer cells. Br J Cancer 2013; 109: 2587-2596.
[40] Bolós V, Mira E, Martínez-Poveda B, Luxán G, Cañamero M, Carlos Martínez-A, Mañes S and de la Pompa JL. Notch activation stimulates migration of breast cancer cells and promotes tumor growth. Breast Cancer Res 2013; 15: R54.
[41] Pal D, Kolluru V, Chandrasekaran B, Baby BV, Aman M, Suman S, Sirimulla S, Sanders MA,

Alatassi H, Ankem MK and Damodaran C. Targeting aberrant expression of Notch-1 in ALDH+ cancer stem cells in breast cancer. Mol Carcinog 2017; 56: 1127-1136.
[42] D'Angelo RC, Ouzounova M, Davis A, Choi D, Tchuenkam SM, Kim G, Luther T, Quraishi AA, Senbabaoglu Y, Conley SJ, Clouthier SG, Hassan KA, Wicha MS and Korkaya H. Notch reporter activity in breast cancer cell lines identifies a subset of cells with stem cell activity. Mol Cancer Ther 2015; 14: 779-787.
[43] Choy L, Hagenbeek TJ, Solon M, French D, Finkle D, Shelton A, Venook R, Brauer MJ and Siebel CW. Constitutive NOTCH3 signaling promotes the growth of basal breast cancers. Cancer Res 2017; 77: 1439-1452.
[44] Sim S, Bergh J, Hellström M, Hatschek T and Xie H. Pharmacogenetic impact of docetaxel on neoadjuvant treatment of breast cancer patients. Pharmacogenomics 2018; 19: 1259-1268.
[45] Olsauskas-Kuprys R, Zlobin A and Osipo C. Gamma secretase inhibitors of Notch signaling. Onco Targets Ther 2016; 6: 943-955.
[46] Zhang CC, Pavlicek A, Zhang Q, Lira ME, Painter CL, Yan Z, Zheng X, Lee NV, Ozeck M, Qiu M, Zong Q, Lappin PB, Wong A, Rejto PA, Smeal T and Christensen JG. Biomarker and pharmacologic evaluation of the gamma secretase inhibitor PF 03084014 in breast cancer models. Clin Cancer Res 2012; 18: 5008-5019.
[47] Edwards A and Brennan K. Notch signalling in breast development and cancer. Front Cell Dev Biol 2021; 9: 692173.
[48] Qiu M, Peng Q, Jiang I, Carroll C, Han G, Rymer I, Lippincott J, Zachwieja J, Gajiwala K, Kraynov E, Thibault S, Stone D, Gao Y, Sofia S, Gallo J, Li G, Yang J, Li K and Weil P. Specific inhibition of Notch1 signaling enhances the antitumor efficacy of chemotherapy in triple negative breast cancer through reduction of cancer stem cells. Cancer Lett 2013; 328: 261-270.
[49] Klaus A and Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer 2008; 8: 387-398.
[50] Yousefnia S, Seyed Forootan F, Seyed Forootan S, Nasr Esfahani MH, Gure AO and Ghaedi K. Mechanistic pathways of malignancy in breast cancer stem cells. Front Oncol 2020; 10: 452.
[51] Morrow KA, Das S, Meng E, Menezes ME, Bailey SK, Metge BJ, Buchsbaum DJ, Samant RS and Shevde LA. Loss of tumor suppressor Merlin results in aberrant activation of Wnt/β-catenin signaling in cancer. Oncotarget 2016; 7: 17991-18005.
[52] Kakarala M, Brenner DE, Korkaya H, Cheng C, Tazi K, Ginestier C, Liu S, Dontu G and Wicha MS. Targeting breast stem cells with the cancer preventive compounds curcumin and piperine. Breast Cancer Res Treat 2010; 122: 777-785.
[53] Xu L, Zhang L, Hu C, Liang S, Fei X, Yan N, Zhang Y and Zhang F. WNT pathway inhibitor pyrvinium pamoate inhibits the self-renewal and metastasis of breast cancer stem cells. Int J Oncol 2016; 48: 1175-1186.
[54] Xu Y, Wang J, Li X, Liu Y, Dai L, Wu X and Chen C. Selective inhibition of breast cancer stem cells by gold nanorods mediated plasmonic hyperthermia. Biomaterials 2014; 35: 4667-4677.
[55] Rokkam P, Gugalavath S, Gift Kumar DK, Vempati RK and Malla RR. Prognostic role of Hedgehog-GLI1 signaling pathway in aggressive and metastatic breast cancers. Curr Drug Metab 2020; 21: 33-43.
[56] Valenti G, Quinn HM, Heynen GJJE, Lan L, Holland JD, Vogel R, Wulf-Goldenberg A and Birchmeier W. Cancer stem cells regulate cancer-associated fibroblasts via activation of hedgehog signaling in mammary gland tumors. Cancer Res 2017; 778: 2134-2147.
[57] Wolf I, Bose S, Desmond JC, Lin BT, Williamson EA, Karlan BY and Koeffler HP. Unmasking of epigenetically silenced genes reveals DNA promoter methylation and reduced expression of PTCH in breast cancer. Breast Cancer Res Treat 2007; 105: 139-155.
[58] Hui M, Cazet A, Nair R, Nair R, Watkins DN, O'Toole SA and Swarbrick A. The Hedgehog signaling pathway in breast development carcinogenesis and cancer therapy. Breast Cancer Res 2013; 15: 203.
[59] Fu YZ, Yan YY, He M, Xiao QH, Yao WF, Zhao L, Wu HZ, Yu ZJ, Zhou MY, Lv MT, Zhang SS, Chen JJ and Wei MJ. Salinomycin induces selective cytotoxicity to MCF-7 mammosphere cells through targeting the Hedgehog signaling pathway. Oncol Rep 2016; 35: 912-922.
[60] He M, Fu Y, Yan Y, Xiao Q, Wu H, Yao W, Zhao H, Zhao L, Jiang Q, Yu Z, Jin F, Mi X, Wang E, Cui Z, Fu L, Chen J and Wei M. The Hedgehog signalling pathway mediates drug response of MCF-7 mammosphere cells in breast cancer patients. Clin Sci (Lond) 2015; 129: 809-822.
[61] Zhu R, Gires O, Zhu L, Liu J, Li J, Yang H, Ju G, Huang J, Ge W, Chen Y, Lu Z and Wang H. TSPAN8 promotes cancer cell stemness via activation of sonic Hedgehog signaling. Nat Commun 2019; 10: 2863.
[62] Marotta LL, Almendro V, Marusyk A, Shipitsin M, Schemme J, Walker SR, Qimron NB, Kim JJ, Choudhury SA, Maruyama R, Wu ZH, Gönen M, Mulvey LA, Bessarabova MO, Huh SJ, Silver SJ, Kim SY, Park SY, Lee HE, Anderson KS, Richardson AL, Nikolskaya T, Nikolsky Y, Liu XS, Root DE, Hahn WC, Frank DA and Polyak K. The JAK2/STAT3 signaling pathway is required for

growth of CD44+CD24- stem cell-like breast cancer cells in human tumors. J Clin Invest 2011; 1217: 2723-2735.
[63] Wei W, Tweardy DJ, Zhang M, Zhang XM, Landua J, Petrovic I, Bu W, Roarty K, Hilsenbeck SG, Rosen JM and Lewis MT. STAT3 signaling is activated preferentially in tumor initiating cells in claudin low models of human breast cancer. Stem Cells 2014; 32: 2571-2582.
[64] Hardt O, Wild S, Oerlecke I, Hofmann K, Luo S, Wiencek Y, Kantelhardt E, Vess C, Smith G, Schroth G, Bosio A and Dittmer J. Highly sensitive profiling of CD44+/CD24- breast cancer stem cells by combining global mRNA amplification and next generation sequencing evidence for a hyperactive PI3K pathway. Cancer Lett 2012; 325: 165-174.
[65] Park JH, Shin JE and Park HW. The role of Hippo pathway in cancer stem cell biology. Mol Cells 2018; 41: 83-92.
[66] Harvey KF, Zhang X and Thomas DM. The Hippo pathway and human cancer. Nat Rev Cancer 2013; 13: 246-257.
[67] Bartucci M, Dattilo R, Moriconi C, Pagliuca A, Mottolese M, Federici G, Di Benedetto A, Todaro M, Stassi G, Sperati F, I Amabile M, Pilozzi E, Patrizii M, Biffoni M, Maugeri-Saccà M, Piccolo S and De Maria R. TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells. Oncogene 2015; 34: 681-690.
[68] Zhang P, Liu Y, Lian C, Cao X, Wang Y, Li X, Cong M, Tian P, Zhang X, Wei G, Liu T and Hu G. SH3RF3 promotes breast cancer stem-like properties via JNK activation and PTX3 upregulation. Nat Commun 2020; 11: 2487.
[69] Zhao L, Qiu T, Jiang D, Xu H, Zou L, Yang Q, Chen C and Jiao B. SGCE promotes breast cancer stem cells by stabilizing EGFR. Adv Sci (Weinh) 2020; 7: 1903700.
[70] Holm K, Grabau D, Lövgren K, Aradottir S, Gruvberger-Saal S, Howlin J, Saal LH, Ethier SP, Bendahl PO, Stål O, Malmström P, Fernö M, Rydén L, Hegardt C, Borg A and Ringnér M. Global H3K27 trimethylation and EZH2 abundance in breast tumor subtypes. Mol Oncol 2012; 6: 494-506.
[71] Thomas S, Thurn KT, Biçaku E, Marchion DC and Münster PN. Addition of a histone deacetylase inhibitor redirects tamoxifen-treated breast cancer cells into apoptosis, which is opposed by the induction of autophagy. Breast Cancer Res Treat 2011; 130: 437-447.
[72] Munster PN, Thurn KT, Thomas S, Raha P, Lacevic M, Miller A, Melisko M, Ismail-Khan R, Rugo H, Moasser M and Minton SE. A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. Br J Cancer 2011; 104: 1828-1835.
[73] Maugeri-Saccà M, Bartucci M and De Maria R. DNA damage repair pathways in cancer stem cells. Mol Cancer Ther 2012; 11: 1627-1636.
[74] Marusyk A, Tabassum DP, Altrock PM, Almendro V, Michor F and Polyak K. Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity. Nature 2014; 514: 54-58.
[75] Harrison H, Simões BM, Rogerson L, Howell SJ, Landberg G and Clarke RB. Oestrogen increases the activity of oestrogen receptor negative breast cancer stem cells through paracrine EGFR and Notch signalling. Breast Cancer Res 2013; 15: R21.
[76] Piva M, Domenici G, Iriondo O, Rábano M, Simões BM, Comaills V, Barredo I, López-Ruiz JA, Zabalza L, Kypta R and Vivanco Md. Sox2 promotes tamoxifen resistance in breast cancer cells. Embo Mol Med 2014; 6: 66-79.
[77] Finn RS, Crown JP, Lang I, Boer K, Bondarenko LM, Kulyk SO, Ettl J, Patel R, Pinter T, Schmidt M, Shparyk Y, Thummala AR, Voytko NL, Fowst C, Huang X, Kim ST, Randolph S and Slamon DJ. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015; 16: 25-35.
[78] Giordano A, Gao H, Anfossi S, Cohen E, Mego M, Lee BN, Tin S, Laurentiis MD, Parker CA, Alvarez RH, Valero V, Ueno NT, Placido SD, Mani SA, Esteva FJ, Cristofanilli M and Reuben JM. Epithelial-mesenchymal transition and stem cell markers in patients with HER2-positive metastatic breast cancer. Mol Cancer Ther 2012; 11: 2526-2534.
[79] Early Breast Cancer Trialists' Collaborative group (EBCTCG). Trastuzumab for early-stage, HER2-positive breast cancer: a meta-analysis of 13864 women in seven randomised trials. Lancet Oncol 2021; 22: 1139-1150.
[80] Junankar S, Baker LA, Roden DL, Nair R, Elsworth B, Gallego-Ortega D, Lacaze P, Cazet A, Nikolic I, Teo WS, Yang J, McFarland A, Harvey K, Naylor MJ, Lakhani SR, Simpson PT, Raghavendra A, Saunus J, Madore J, Kaplan W, Ormandy C, Millar EK, O'Toole S, Yun K and Swarbrick A. ID4 controls mammary stem cells and marks breast cancers with a stem cell-like phenotype. Nat Commun 2015; 6: 6548.