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Review Article

Research progress on miRNA, miRNA-regulated signaling pathways and breast cancer

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Abstract: As one of the most common malignant tumors of women, breast cancer is a leading cause of death in females. At present, research findings have suggested that several signaling pathways do not only act together to keep the stem cell population unchanged during progression of breast cancer, but also regulate proliferation, differentiation and multiple other oncologic characteristics of these cells. Therefore, it is of great significance for monitoring diagnosis, treatment and prognosis of breast cancer by exploring how related signaling pathways regulate breast cancer cells. As a small non-coding and single-chain RNA, miRNA has been extensively reported to be involved in regulating multiple signaling pathways in tumors, including Hedgehog, Wnt and Notch signaling pathways. This paper will elaborate how miRNA regulates functions of breast cancer stem cells by regulating some of their signaling pathways and target genes.

Keywords: Breast cancer, stem cells, miRNA, signaling pathway

Introduction

Breast cancer is one of the most common malignant tumors in women and its incidence is the highest for women among all malignant tumors. Thus, it greatly endangers health and life of women. A statistic of the United States suggests that breast cancer is still the leading cause of death for women whose age ranges from 20 to 59 although mortality of cancer has constantly declined among women and mortality of breast cancer has even decreased by 36% over the past few years with the improvement of early diagnosis and treatment [1]. According to this statistic, cases with breast cancer and deaths of breast cancer increased by about 250,000 and over 40,000 respectively all over the United States in 2016 [1]. Hence, it is important to intensively study pathogenesis and progression of breast cancer from a molecular perspective for diagnosing/treating breast cancer and preventing its metastasis and recurrence.

Cancer stem cells (CSC), accounting for a small proportion of cells in tumors and having characteristics of stem cells, can renew themselves and lead to heterogeneity of tumor cells. Usually seen inside tumors, they are not easily affected by drugs under such special microenvironment. In essence, cancer stem cells are quite tolerant to chemotherapy and radiotherapy. A research finding has suggested that the breast cancer stem cells which are separated from breast cancer tissues belong to the $CD44^{+}/CD24^{-/low}$ subpopulation, and tumors may grow in immunodeficient mice when the number of the stem cells is up to 1×10^{3} [2]. In spite of their scarcity, breast cancer stem cells are important for drug resistance, recurrence and metastasis of breast cancer [3, 4]. Hence, how to eradicate breast cancer stem cells is essential for treating breast cancer. After a study of clinical samples, it is discovered that the activity of multiple signaling pathways, including Hedgehog, Wnt, Notch, EMT and AMPK, is abnormal in breast cancer. Further research has indicated that these signaling pathways act together to keep the size of stem cell populations unchanged. Besides, they regulate proliferation, differentiation and some other oncologic characteristics

of these stem cells. The abnormal protein expression in these signaling pathways is directly connected with pathogenesis and development of breast cancer. Therefore, it is important to explore how above signaling pathways regulate breast cancer cells, in order to deeply understand cytological characteristics of breast cancer, scientifically evaluate clinical progresses in breast cancer, assess prognosis of the cancer and develop more scientific drugs and reasonable methods for treating breast cancer. Small or micro RNA (miRNA), which is about 20nt long, small and non-coding with a single chain, It does not only suppress expression of target genes by complementing and pairing with some segments of their mRNA inside animal cells but also gets involved in epigenetic regulation [5]. Widely regulating activity of multiple signaling pathways, miRNA is closely associated with formation and development of tumors and cancer stem cells. For instance, the expression of miR-200c is lower in breast cancer stem cells than that in breast cancer cells, so it promotes proliferation, invasion, metastasis and drug resistance of breast cancer cells [6, 7]. The overexpression of miR-373 and miR-520c promotes metastasis of breast cancer as well [8]. In addition, miR-96/miR-182 may control metastasis of breast cancer when they are overexpressed; they are particularly effective for treating the cancer together with chemotherapy drugs [9]. In this paper, the importance of miRNA for regulating functions of breast cancer stem cells is illustrated based on its regulation of signaling pathways.

Hedgehog signaling pathways

Hedgehog signaling pathways are necessary for regulating correct embryonic development. Each part of embryos is differentiated into different tissues and organs because the concentrations of Hedgehog signaling proteins differ [10]. Hedgehog signaling pathways are also important for multiple types of tumors. In breast cancer cells, Hedgehog signal transduction pathways are extremely active and the expression of their members (including Shh, Ihh, Dhh, PTCH1/2, SMO, GLI1/2 and Bmi1) is significantly higher than that in highly differentiated breast cancer cells. The overexpressed members of Hedgehog signaling pathways can significantly promote tumorsphere formation in breast cancer cells [11], breast cancer differentiation, self-renewal [12] and metastasis of tumor cells [13]. Hence, drug-targeted Hedgehog signaling pathways are promising strategies for treating breast cancer. As an important stem cell factor regulated by Hedgehog signaling pathways, Bmil may promote proliferation of breast cancer [14], invasion [15], metastasis [16] and formation of stem cell spheres [11] if it is overexpressed.

At present, miRNA is discovered to mainly regulate Hedgehog signaling pathways by regulating Bmil. For instance, miR-200b/c is significantly downregulated in breast cancer stem cells, and the expression of Bmil may be suppressed by overexpressed miR-200b or miR-200c [6, 17]. Likewise, the expression of miR-203 is downregulated in breast cancer stem cells and sphere formation in breast cancer cells is promoted when Bmil is not suppressed [18]. Some other research suggests that miR-203 may inhibit stemness of stem cells by suppressing ΔNp63 [19]. In consideration that the expression of miR-203 is downregulated in breast cancer stem cells, its abnormal regulation of ΔNp63 is possibly another important pathway for regulating stemness of breast cancer stem cells, incidence and development of breast cancer.

Wnt signaling pathways

As canonical pathways for tumor genesis, Wnt signaling pathways are essential for regulating proliferation, adhesion, metastasis and stemness maintenance of tumor cells [20]. A Wnt signaling pathway is made up of Wnt ligand, Frizzled (Fz) receptor, β-catenin, GSK-3β, APC and Axin. When Wnt protein is bound to Fz, the whole Wnt pathway may be activated. As a key node of the pathway, β-catenin regulates different downstream molecules. Several members of a Wnt signaling pathway are involved in regulating incidence and progression of breast cancer; the number of stem cells will increase significantly in breast cancer cells when members of the Wnt signaling pathway are overexpressed [21, 22].

As a member of the Wnt signaling pathway in breast cancer, APC may cause ectopic accumulation of β-catenin when its expression is downregulated; as a result, it may not only thereby induce overexpression of cyclinD1 and c-myc, but also promote abnormal cell proliferation [23]. In breast cancer stem cells, miRNA regulates activity of Wnt signaling pathways. For

example, the expression of miR-142 is relatively high in human breast cancer cells [6, 24], so, this gene may suppress the expression of APC as a targeted inhibitor and activate Wnt signaling pathways. Some researchers have also reported interactions between miR-142 and APC and miR-142 binding sites on APC [25]. Once miR-142 is knocked out of human breast cancer stem cells, the APC expression will be upregulated significantly, whereas cell cloning and proliferation are greatly weakened [24]. In addition, the expression of miR-141 is found to be much lower in human breast cancer stem cells [26]. Some research suggests that miR-141 may act upon targeted β-catenin to inhibit Wnt signaling pathways [26, 27]. The expression of let-7 may be further suppressed by activating Wnt signaling pathways to increase the expression of RNA-binding Lin28 [28]. As an important tumor suppressor miRNA [29], Let-7 may affect malignancy of multiple types of tumors including breast cancer through its expression level, so the proliferation of breast cancer may be further regulated by the ways mentioned above.

EMT

EMT refers to the transition of epithelial cells into mesenchymal stem cells. After EMT, both cell motility and invasion are enhanced. Once their EMT is induced, the mammary epithelial cells may not only express stem cell markers, but also generate more mammospheres and become more tumorigenic [30]. Being related to tumor progression, EMT may enhance characteristics of breast cancer stem cells. During EMT, cells are mainly regulated by TGF-β signaling pathways, which are Smad-dependent or independent [31]. Being abnormal in breast cancer, TGF-β signaling pathways are particularly active in many cases with advanced metastatic breast cancer, and suppress Bim by upregulating miR-181a to promote the metastasis of breast cancer [32]. For example, miR-106b/93/25 cluster may be Smad7-targeted to promote EMT of breast cancer cells and increase stemness of breast cancer stem cells [33]. In addition, low expression of let-7, as a sign of poor differentiation for multiple types of tumors like breast cancer, signifies tumor interstitialization and poor prognosis [29].

Research also suggests that E12/E47 may upregulate miR-495 in breast cancer stem cells and suppress E-cadherin (i.e. an epithelial cell marker) after transcription to promote invasion of breast cancer cells. Meanwhile, miR-495 may suppress expression of REDD1 and promote proliferation of breast cancer stem cells [34]. Furthermore, MCF-7 with overexpressed miR-21 may contribute to generation of more $ALDH1^{+}/CD44^{+}/CD24^{-/low}$ cells (i.e. breast cancer stem cells) by regulating EMT, and promote the formation of spheres in breast cancer cells [35]. Above all, miRNA may make the breast cancer stem cells more invasive and metastatic than other breast cancer cells by regulating signaling pathways of EMT.

Notch signaling pathways

Being closely associated with mammary gland development, genesis and progression of breast cancer, Notch signaling pathways are activated in several types of tumors [36, 37]. For mammals, apart from Notch and its ligands like Delta/Jagged, Su (H)/CBF and LAG-I1also are also involved in Notch signal transduction. Once they are bound to ligands, Notch signaling pathways will be activated and notch intracellular domain will be translocated to nucleus to further get involved in multiple intracellular processes [38]. Notch4 is 8 times more active in human breast cancer stem cells compared with differentiated tumor cells, which indicates that Notch signaling pathway are critical for regulating incidence and development of breast cancer by regulating breast cancer stem cell populations [39]. Nevertheless, proteins involved in Notch signaling pathways seldom mutate in solid tumors, which reveal that epigenetic modification is possibly important for regulating the Notch signaling pathways in tumors [40]. As a stem cell factor, ZEB1 does not only activate Notch signaling pathways, but is also important for tumor metastasis and tolerance to chemotherapy. In breast cancer stem cells, ZEB1 may suppress the expression of miR-200 family, and members of this family can target at multiple members of the Notch signaling pathway, including Jagged1 (Jag1), Maml2 and Maml3 [40]. Therefore, disorderly expression of miRNA may fully explain why members of the Notch signaling pathway are extremely active in breast cancer stem cells in spite of no gene mutation. On the other hand, the Notch signaling pathway may also target at miRNA (like suppressing miR-205) [41], to better maintain stemness of breast cancer stem cells and promote genesis of breast cancer. Above all, it is a new and potential way for treating breast cancer by

intervening with miRNA related to Notch signaling pathways.

AMPK/Sirt1 signaling pathways

As receptors of energy metabolism in cells, AMPK (AMP-activated protein kinase) and Sirt1 (silencing information retulator1) can regulate energy metabolism of bodies [42]. Sirt1 is a NAD+-dependent histone deacetylase that regulates transcriptional activity by regulating levels of histone deacetylation, and AMPK may activate the expression of Sirt1, so they are collectively referred to as AMPK/Sirt1 signaling pathway. This pathway regulates autophagy, apoptosis, proliferation, differentiation, protein synthesis, protein degradation and inflammatory responses [43-45]. Certain research findings suggest that AMPK suppresses the expression of MTDH by activating Sirt1 to promote the proliferation of triple-negative breast cancer cells, and Sirt1 is necessary for maintaining stemness of stem cells [46, 47]. The prognosis is relatively poor in breast cancer patients with high expression of Sirt1; distant metastasis and recurrence are more common for these patients, whose overall survival is much lower [48]. This means that it is critical to regulate the expression of Sirt1 in breast cancer stem cells during the progression of breast cancer. According to some studies, miR-34a can suppress breast cancer stem cells by downregulating the expression of Sirt1 to control proliferation and metastasis of tumor cells [49, 50]. Hence, it is important to further explore miR-34a and miRNA related to other AMPK/Sirt1 pathways, in order to find out new ways for monitoring diagnosis, treatment and prognosis of breast cancer.

Summary and prospect

To sum up, miRNA is important for regulating multiple signaling pathways and affects processes of breast cancer and corresponding stem cells. Hence, it is of great significance for correctly understanding, diagnosing, preventing and treating breast cancer by systematically investigating miRNA-regulated target genes and their signaling pathways and clarifying how to regulate disorderly expression of miRNA in breast cancer stem cells.

Breast cancer is refractory, recurrent and metastatic, so more importance shall be attached to treat breast cancer by targeting at stem cells, because breast cancer is refractory with high risks of recurrence and metastasis. Therefore, it is advisable to treat breast cancer by intervening with miRNA and further regulating the signaling pathways in miRNA-mediated tumors. Unlike traditional drugs which can only have a single target, miRNA generally intervenes with genesis and development of tumors systemically by targeting at one or more functionally related signaling pathways. For example, miR-200 family is important for regulating physiological functions of breast cancer stem cells, as its members can target at Bmil of the Hedgehog signaling pathway, β-catenin of the Wnt signaling pathway and ZEB1 of the Notch signaling pathway. Therefore, miRNA-targeted drugs are likely to regulate multiple signaling pathways at the same time to jointly suppress breast cancer cells. However, there are still no appropriate oligonucleotide drugs for treating breast cancer in clinical practices owing to limited drug delivery efficiency and side effects. By deeply investigating how miRNA regulates breast cancer cells, it is believed that more effective miRNA targets with fewer toxic side effects will be made available and corresponding drugs will be developed for effective clinical treatment of tumors like breast cancer with miRNA.

Although there have been numerous reports about regulating breast cancer with miRNA, the regulation of breast cancer stem cells with miRNA has been rarely studied, mainly because it is apparently much more inefficient to separate breast cancer stem cells from tumor tissues for carrying out pertinent research than to directly investigate tumor cells. Nonetheless, cancer stem cells can better reflect some characteristics of tumors than the whole tumor population, particularly during radiotherapy, chemotherapy, escape and metastasis. Hence, it is more realistic to reckon miRNA inside cancer stem cells as a prognostic indicator and therapeutic target.

Although current research suggests that miRNA can impact different physiological functions of breast cancer by regulating multiple signaling pathways, its regulation of a signaling pathway often affects another pathway due to “crosstalk” among several signaling pathways. Thus, related research has always discovered the joint effects of multiple signaling pathways. However, what are roles of specific signaling pathway in breast cancer stem cells? How does

miRNA divide labor to signaling pathways or make them cooperative? Do signaling pathways particularly regulate certain stage or certain type of breast cancer? Since gene mutation mechanism and pathogenic mechanism differ among luminal, HER-2 (+) and Basal-like breast cancer, do their cancer stem cells have different characteristics? Do priorities differ between regulation of miRNA and signaling pathways? Up till now, all these questions still have no definite answers and remain to be further demonstrated.

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Disclosure of conflict of interest

None.

Authors' contribution

Zhang Xiping and Tang Binbin wrote this paper; other authors participated in the translation and revision of this paper. All authors contributed to the intellectual context and approved the final version.

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