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REVIEW

Research Progresses in Cancer Stem Cells of Three Common Fertility-Related Female Malignancies

Xi-ping Zhang[1] · Qi-hui Cheng[2] · Hong-jian Yang[1] · En-qi Qiao[1]

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Abstract

With abilities to renew themselves and lead to heterogeneity of tumors, cancer stem cells (CSCs) are similar to stem cells. As three leading causes of death that endanger women's health, breast cancer, ovarian cancer and cervical cancer are characterized by high degree of malignancy, metastasis and recurrence. Associated with women's fertility, these three malignancies are common and representative among females. These years, research findings have suggested that CSCs are closely connected with many cancers (including aforementioned three malignancies) and several processes of tumors such as their genesis and development. CSCs have become great concerns for current cancer treatment and interventions. This paper does not only summarize roles of CSCs in genesis, development, drug resistance, metastasis and recurrence of breast cancer, ovarian cancer and cervical cancer, but also proposes potential methods of treatment and intervention, in hope of inspiring readers and researchers.

Keywords Cancer stem cells · Female · Malignant tumors · Breast Cancer · Ovarian Cancer · Cervical Cancer

Introduction

Some results have been achieved in treating malignant tumors by traditional methods, including radiotherapies, chemotherapies and surgeries, whereas these methods can hardly effectively avoid recurrence. According to the latest research findings, the genesis and recurrence of cancer are related to a subpopulation of "cancer stem cells (CSCs) [1]. The American Association for Cancer Research (AACR) has reached a consensus in 2006 that CSCs are a group of cells that can renew themselves and lead to heterogeneity of cancer cells in tumors [2]. Although CSCs have something in common with the stem cells in the traditional sense, they also have their own unique characteristics. CSCs play important roles in genesis, drug resistance and metastasis of tumors. Tumors are considered to originate from a minority of CSCs with self-renewal abilities and strong tumorigenicity [3].

In the past, it was generally acknowledged that all cells of tumor tissues were able to renew themselves. However, only CSCs have been detected to be capable of self-renewal, highly proliferative and resistant to lots of anti-cancer drugs, so they play important roles in drug resistance of tumors. Now, the metastasis of tumors is thought to result from migration of highly carcinogenic CSCs, which exhibit strong migration ability [4]. It is of great significance for treating tumors more effectively by developing theories about CSCs. With these theories, the purposes and focuses will be more evident in human research and development of anti-cancer drugs. It will be more effective if new anti-cancer drugs and new therapies can be developed according to characteristics of CSCs.

Common fertility-related female malignancies include breast cancer, ovarian cancer, cervical cancer and endometrial cancer and so on. These years, related research has focused on breast cancer, ovarian cancer and cervical cancer. Priority has been given to breast cancer, followed by ovarian cancer. To be specific, the research about CSCs of aforementioned malignancies, including breast cancer, ovarian cancer and cervical cancer, has particularly discussed markers of CSCs, correlations between CSCs and drug resistance of tumors, how CSCs are related to recurrence and metastasis of tumors, CSCs and microenvironment of tumors as well as human intervention with CSCs for treating tumors. The following sections will place an emphasis on introducing the research progress in breast cancer, ovarian cancer and cervical cancer stem cells.

✉ Xi-ping Zhang
zxp99688@sina.com

[1] Department of Breast Surgery, Zhejiang Cancer Hospital, Hangzhou 310022 ZhejiangProvince, People's Republic of China

[2] Department of Obstetrics and Gynecology, Hangzhou First People's Hospital, Province, Zhejiang 310006, Hangzhou, China

The epithelial-mesenchymal transition (EMT) is often activated during cancer invasion and metastasis. CSCs constitute a small minority of neoplastic cells within a tumor and are defined operationally by their ability to seed new tumors. EMTs generate stem cell-like cells, which express markers associated with EMT. Normal and neoplastic human breast stem-like cells express markers associated with an EMT,which promotes the generation of cancer stem cells [5].

Breast Cancer

As one of the most common female malignancies, breast cancer has been studied in depth. The first reported CSCs were obtained from breast cancer, and many research progresses have been made in examining breast cancer stem cells. Yang et al. have reviewed the research progresses in CSCs of breast cancer that had been made by the first half of 2016, particularly different biomarkers of CSCs in breast cancer, roles of these CSCs in development of breast cancer and regulatory mechanism of such CSCs [6] To sum up, (1) markers of breast cancer mainly include CD44 (cluster of differention44), ALDH1 (aldehyde dehydrogenase 1), CD133 (prominin-1), CD24 (cluster of differention24) and CK18 (cytokeratin18). (2) Breast cancer stem cells play important roles in tumorigenesis, EMT, mesenchymal-epithelial transition (MET), drug resistance and metastasis. (3) Notch (*Drosophila melanogaster* (fruit fly)), Wnt and Hedgehog signaling pathways are major pathways for regulating characteristics of CSCs in breast cancer. (4) Tumor micro-environment and ncRNAs get involved in regulating Breast cancer stem cells. Besides, further progresses have been made in breast cancer stem cells these years and will be particularly introduced hereunder.

Breast Cancer Stem Cells and Tumorigenesis

Kwat et al. have studied the impacts of HIF2α/EFEMP1 (hypoxia inducible factor 2α/epidermal growth factor-containing fibulin-like extracellular matrix protein 1) cascade upon grade of CSCs in breast cancer [7]. They have discovered that HIF2α, as a hypoxia-inducible factor, specifically strengthens abilities of these CSCs to form spheres, upregulate subcategories of CD44+/CD24−/low CSCs and remove HIF2α to suppress CSC-like phenotypes and CSCs-mediated drug resistance. It has been found in further research that the stimulus of hypoxia-induced HIF2α's stimulus of the abilities of breast CSCs to form spheres may be weakened by knocking out EFEMP1. Hence, the author considers that HIF2α mediates hypoxia-induced growth and metastasis of breast cancer. Besides, EFEMP1 is the downstream executor of hypoxia-induced HIF2α during the carcinogenesis of mammary glands. This report focuses on elaborating how microenvironment changes to breast cancer stem cells promote carcinogenesis of mammary glands and further enhances people's understanding about the genesis of breast cancer.

Li et al. have investigated the impacts of Tafazzin (TAZ) of Hippo signaling pathway upon characteristics and tumorigenicity [8]. According to their research, Hippo signaling pathway, impacting development and regeneration of organisms, is dysregulated in multiple types of tumors. As a category of transcriptional coactivator and downstream executor of the Hippo signaling pathway, TAZ is also an important stimulus of triple negative breast cancer (i.e. estrogen negative, progestin negative and HER-2 negative). The author has found that TAZ with constitutive expression may endow untransformed human mammary basal epithelial cells with characteristics of CSCs, which depends upon the structural domain where TAZ and TEAD (transcriptional enhanced associate domain) interact with each other. WW domain and structural activation domain of TAZ are critical for characteristics and oncogenicity of CSCs. At last, the author considers that TAZ inside tumors has made a new targeted therapy available for removing breast cancer stem cells and treating breast cancer.

Markers of Breast Cancer Stem Cells

At present, research has been conducted to explore the roles of markers of breast cancer stem cells in predicting carcinoma treatment prognosis and chemotherapy resistance. Miyoshi and others have reported ALDH1 (a marker of these CSCs) in above aspects [9]. By detecting related cases, it has been discovered that the expression of ALDH1 is positively correlated to early recurrence and chemotherapy resistance of ER-positive and HER-2 negative breast cancer.

Previous studies have suggested that breast cancer stem cells (BCSCs) mediate metastasis, are resistant to radiation and chemotherapy, and contribute to relapse. Liu S et al. showed that breast cancer stem cells (BCSCs) exist in distinct mesenchymal-like (EMT) and epithelial-like (MET) states. Mesenchymal-like BCSCs characterized as CD24(−)CD44(+) are primarily quiescent and localized at the tumor invasive front, whereas epithelial-like BCSCs express aldehyde dehydrogenase (ALDH), are proliferative, and are located more centrally. The plasticity of BCSCs that allows them to undergo reversible EMT/MET transitions regulated by the tumor microenvironment may be necessary for successful metastatic colonization [10].

Breast Cancer Stem Cells and Drug Resistance of Tumors

Some new progresses have been achieved in research about drug resistance of breast cancer. Barnawi has reported a mechanism for bodies to maintain the pool of CSCs in breast cancer [11], and fascin has been detected to be critical for maintaining the stem cell pool of breast cancer. As a type of actin-binding protein, fascin is the major regulator of chemotherapy

resistance and metastasis of breast cancer. It has been discovered that after the knockout of fascin expression, the properties of the stem cell-like breast cancer cell lines ($CD44^{hi}/CD24^{lo}/ALDH^{+}$) are weakened significantly, and EMT is reversed. Meanwhile, the expressions of transcription factors such as Oct4 (octamer-binding transcription factor4), Nanog, transcription factor Sox2 (SRY-box2) and Klf4 (Krüppel-like factor 4) are downregulated in embryonic stem cells. In addition, the colony-forming abilities of breast cancer cells and sphere-forming abilities of tumor cells are impaired after the knockout of fascin. Further research has suggested that the impacts of fascin upon chemotherapy resistance and self-renewal of CSCs are related to the Notch signaling pathway. It has revealed that fascin would become a new target for treating breast cancer.

Breast Cancer Stem Cells and Metastasis of Tumors

At present, some signaling pathways have been detected to be related to cell expansion and metastasis of breast cancer stem cells. Wang and others have found in their research that butyl benzyl phthalate (BBP) stimulates SPHK1/S1P/S1PR3 signaling pathways and enhances the formation of metastasis-initiating breast cancer stem cells [12]. BBP induces histone modifications of SIPR3 genes in breast cancer stem cells, but doesn't play such roles in non-breast cancer stem cells. This study indicates that SIPR3, as a signal molecule, is not only a key molecule for PAEs-driven metastasis of breast cancer, but also a potential therapeutic target for regulating the population of breast cancer stem cells. Transplantation of stem-like metastatic cells from low-burden tissues showed that they have considerable tumour-initiating capacity, and can differentiate to produce luminal-like cancer cells. Progression to high metastatic burden was associated with increased proliferation and MYC expression, which could be attenuated by treatment with cyclin-dependent kinase (CDK) inhibitors [13].

Markers that Intervene with Breast Cancer Stem Cells

Witt and some others have discussed new markers of breast cancer and possibilities for treating such cancer by intervening with these markers [14]. It has been discovered in research that the expressions of HDAC1 (histone deacetylase 1) and HDAC7 (histone deacetylase 7) are higher in breast cancer stem cells than those in non-breast cancer stem cells. If the expression of HDAC7 is upregulated significantly, the breast cancer stem cells will be more carcinogenic. Hence, HDAC inhibitors clinically used for intervening with HDAC1 and HDAC7 are deemed to be preferred choices for breast cancer stem cells. This study has suggested that HDAC1 and HDAC7 are possibly the targets for treating breast cancer as markers that intervene with breast cancer.

Micro-Environment that Intervenes with Breast Cancer Stem Cells

Meng et al. have explored how to avoid the enrichment of CSCs during chemotherapies [15]. Using CSOSA (Chitosan oligosaccharide-g-stearic acid) as raw material, the author prepared CSOSA/DOX as a new Doxorubicin translocation system. First of all, it was discovered in the in vitro experiment that CSOSA/DOX inhibited growth of breast cancer stem cells. Next, it was detected in the in vivo experiment that CSOSA/DOX hindered tumors from development resulted in a significant decline in the proportion of CSCs and weakened their drug resistance of CSCs. Furthermore, it was helpful for dissociating the micro-environment of breast cancer stem cells and reducing the systemic side effect. This study has indicated that the breast cancer stem cells may be inhibited from aggravation into malignancies by transporting energy via special drugs, and the micro-environment that impacts breast cancer stem cells is also one of the important mechanisms.

Other Research Progresses in Intervention with Breast Cancer Stem Cells

At present, metabolic products of some plants are found to play important roles in treating breast cancer as well. Kim and some other researchers have reported that breast cancer stem cells may be inhibited by the metabolic product of garlic known as diallyl trisulfide (DATs) [16], which has been discovered to downregulate FoxQ1 (forkhead box Q1) of breast cancer stem cells to intervene with the cancer. FoxQ1 is a member of the FOX gene family, where FOX genes are found to play certain roles in embryo development, cell cycle regulation, tissue-specific gene expression, signal transduction and tumorigenesis. According to this paper, DATs are potent for treating breast cancer by inhibiting FoxQ1 of breast cancer stem cells.

In addition, some drugs previously used for treating other diseases are discovered to be effective for intervening with breast cancer. Lee and some others have reported the therapeutic effects of metformin (which was utilized for treating diabetes mellitus type 2 (T2DM)) for treating breast cancer by intervening with the stem cells [17]. As a derivative of metformin, metformin-butyrate (MFB) is found to be more effective for activating AMPK (AMP-activated protein kinase) to inhibit mTOR (mechanistic target of rapamycin kinase) and interfere with cell cycle progression in S and G2/M phases. It also imposes toxic effects on cells in concert with docetaxel and cisplatin. Besides, MFB is more effective for impacting G2/M phase and targeting breast cancer stem cells than metformin-HCl. This means that it would be more effective for inhibiting invasion and resistance of breast cancer. The research suggests that perhaps MFB is a promising drug for treating breast cancer.

Above all, more and more new markers, signaling pathways, signaling molecules and micro-environmental factors of breast cancer stem cells have been detected in research. Above new findings are favorable for human beings to acquire a deeper understanding about genesis, development, drug resistance and micro-environment of breast cancer. Furthermore, they are helpful for human beings to explore different ways for intervening with breast cancer and ultimately facilitate the treatment of breast cancer.

Autophagy plays a significant role in cancer stem-like cells, and distinct cancer stem-like cells within a tumor may require different treatment modalities. The role of autophagy in breast cancers appears to be dependent on the context of the tumor subtype and this is conceivable, considering different mammary epithelial cells (MECs) utilize autophagy dissimilarly. Autophagy appears to impinge on the traits of these distinct CSC populations through the regulation of TGFB-SMAD and EGFR-STAT3 pathways [18]. Tianyi Wang et al. demonstrate that JAK/STAT3 regulates lipid metabolism, which promotes BCSCs and cancer chemoresistance. Inhibiting JAK/STAT3 blocks BCSC self-renewal and expression of diverse lipid metabolic genes, including carnitine palmitoyltransferase 1B (CPT1B), which encodes the critical enzyme for fatty acid β-oxidation (FAO) [19].

Nuclear factor-kappa B (NF-κB) signalling has been shown to regulate properties of breast cancer stem cells. Through shRNA silencing of the NF-κB-2 gene, the role of p100/p52 in 4 T1 and N202.1A cell lines were assessed by NF-κB reporter, invasion, tumoursphere and orthotopic transplantation assays. Yeo SKet al's findings indicate that inhibiting the processing of p100 may be a potential therapeutic strategy to suppress CSC activity in a subset of breast tumours [20].

Ovarian Cancer Stem Cells

Markers of Ovarian Cancer Stem Cells

CSCs of ovarian cancer have some common markers, including CD44, CD24 and ALDH1, which also exist in other tumors. These markers have symbolic meanings for genesis, metastasis, recurrence and therapeutic resistance of ovarian cancer. Apart from common tumor markers, new ones have been constantly discovered. For instance, Yu and some others have explored the expression of a new potential marker of CSCs in ovarian cancer [21]. They have discovered that the expression of Cacna2d1 is not only positively correlated to clinical manifestations of epithelial ovarian cancer (EOC), but also has negative correlations with prognosis of EOC and survival of patients. At last, that paper has suggested that Cacna2d1 (calcium voltage-gated channel auxiliary subunit alpha2delta 1) would be useful for predicting prognosis of tumors and become a potential target for intervention. Yin et al. have reported the associations of VEGF-dependent gene signature (VDGs) with subtypes of ovarian cancer and patients' prognosis [22]. The results have suggested that the expression of VDGs is too high in patients with ovarian cancer and mesenchymal subtypes, negatively correlated to therapeutic effects. This study reports that VEGF-regulated angiogenesis is effective for prognosis of ovarian cancer. It is proposed in this paper that VDGs could be molecular basis for developing novel diagnostic strategies to aid patients' selection of VEGF-targeted agents.

It is generally acknowledged that the genesis of ovarian cancer will be promoted once the expressions of markers of CSCs are upregulated. Nevertheless, there are also some counterexamples. For instance, Chen et al. have discovered in studying CD90 (Thy-1 cell surface antigen) (i.e. a marker) of CSCs [23]. that the mRNA expressions of CD90 are positively correlated to its DNA copy number, and the prognosis of patients with ovarian cancer whose CD90 expressions are high is better than others with low expressions of CD90. CD90 inhibits anchorage-dependent growth of SKOV3 in human cells of ovarian cancer in vitro, tumorigenesis arising from SKOV3 in vivo, sphere forming ability of SKOV3 and activity of ALDH, but promotes apoptosis of SKOV3. The research has suggested that CD90 affects the characteristics of CSCs and thereby inhibits the growth of cells with ovarian cancer. Furthermore, it down regulates the expressions of other CSC markers such as CD133 and CD24. CD90 will resist tumorigenesis once it acts in combination with β3 integrin. This study provides a new insight that CD90 has great potential for its application in development of therapeutic strategies for ovarian cancer.

Ovarian Cancer CSCs and Drug Resistance of Tumors

Over the past years, more and more genes have been found to be related to ovarian cancer stem cells and drug resistance of tumors have been constantly discovered. They have become new potential targets for intervening with ovarian cancer. For example, Kim and some others have investigated the impacts of HMGA1 (i.e. a factor for chromosome remodeling) upon ovarian cancer stem cells [24]. They have discovered that when HMGA1 expressions are upregulated, genes related to stemness of cancer stem cells, including SOX, KLF4, ALDH, ABCB1 (ATP binding cassette subfamily B member 1) and ABCG2 (ATP binding cassette subfamily B member 2), will also show higher expressions while getting more drug-resistant. It has been also found that the knockout of HMGA1 (high mobility group AT-hook 1) results in weaker proliferation ability, poorer sphere forming ability and lower expressions of stemness-related genes. According to further research, after it is highly expressed in adherent ovarian cancer, HMGA1 is detected to be a potential and

important target for intervening with ovarian cancer as indicated from the characteristics of ovarian cancer stem cells (including stronger drug resistance).

In addition, the drug resistance of ovarian cancer is considered to be promoted by high expressions of certain genes in ovarian cancer. Although these genes are not targets for intervening with ovarian cancer, they are significant for clarifying mechanisms of drug resistance to ovarian cancer. For instance, Januchowski and some others have investigated the expressions of collagen genes in drug-resistant ovarian cancer cells [25], which are deemed to represent ovarian cancer stem cells. Their research findings have suggested that several types of collagen genes are highly expressed in these drug-resistant ovarian cancer cells, and carcinoma tissues may become more drug resistant with the increase of collagen expressions, because drugs may be hindered from entering carcinoma tissues with the increase of collagens, and ovarian cancer cells become more resistant to apoptosis.

Markers that Intervene with Ovarian Cancer Stem Cells

Markers of CSCs are often used for intervening with tumors, including ovarian cancer. Witt and some others have studied markers of ovarian cancer stem cells and developed targets for treating ovarian cancer. They have discovered in their research that HDAC1 (histone deacetylase 1) and HDAC7 (histone deacetylase 7), as two types of histone deacetylases, exhibit higher expressions in ovarian cancer stem cells than non-cancer stem cells, so these two kinds of enzymes are inferred to be markers of these stem cells. After further research, it has been found in this paper that overexpressed HDAC7 increases the degree of malignancy of ovarian cancer stem cells and HDAC inhibitors used for clinical purposes effectively act upon ovarian cancer stem cells, which indicates the development prospects of HDAC as a target for treating ovarian cancer. Gu et al. have examined the expressions of Galectin-3 and molecular mechanism of its functions in ovarian cancer stem cells [26]. According to their research, overexpressed Galectin-3 makes ovarian cancer stem cells more drug-resistant to cisplatin and paclitaxel. Meanwhile, Galectin-3 has been found to activate the Notch signaling pathway to play its roles, which reveals that Galectin-3 may be employed as a marker of ovarian cancer stem cells and possibly become a target for treating ovarian cancer.

Signaling Pathways and Molecules that Intervene with Ovarian Cancer Stem Cells

Signaling pathways concerning ovarian cancer stem cells are one of therapeutic targets. For instance, current research has discovered that [27, 28] RhoC, as a type of G-protein (GTPase) with small molecular weight (21KDa), is an important factor that impacts malignancy of ovarian cancer cells. This paper reports that in more malignant ovarian cancer cell lines, CD117 and CD133exhibit higher expressions as markers of CSCs. After RhoC is interrupted by si-RhoC through gene silencing, the RNA expressions will be significantly inhibited in RhoC, CD117 (proto-oncogene receptor tyrosine kinase), CD133, MDR1 (p-glycoprotein) and MMP9 (matrix metallopeptidase 9). They are positively correlated to stemness of ovarian cancer. Besides, it has been also discovered that the silencing of above genes also inhibits proliferation, invasion, and metastasis and drug resistance of more malignant ovarian cancer cells. This report has not only suggested that RhoC may be used as a target for intervening with genesis, metastasis and drug resistance of ovarian cancer, but also indicated that important signaling pathways and molecules intervening with ovarian cancer stem cells would be new means for treating ovarian cancer. Hence, more signaling pathways and molecules have been found to be related to ovarian cancer stem cells. It will be a new strategy for treating ovarian cancer by developing the pathways and molecules into objects of intervention.

Micro-Environment that Intervenes with Ovarian Cancer Stem Cells

Although ovarian cancer cells, including the stem cells, are research objects of most present reports concerning intervention with ovarian cancer, plenty of researchers attempt to treat ovarian cancer by intervening with micro-environment of the ovarian cancer cells. Yeung and some others have reviewed studies about how to intervene with ovarian cancer by interfering with interactions between interstitial and cancer cells [29]. They have particularly discussed the intervention with cancer associated fibroblasts (CAFs) and finally pointed out that ovarian cancer can be also treated by interfering with its micro-environment.

Above all, a growing amount of new markers, signaling pathways, signaling molecules and micro-environmental factors of ovarian cancer stem cells have been discovered. These new findings are closely associated with the genesis, development and drug resistance of ovarian cancer. Based on their understanding of ovarian cancer stem cells, people take initiatives to explore different intervention methods, including important markers for intervening with CSCs, significant signaling pathways/molecules and critical micro-environmental factors. These research progresses will be helpful for treating ovarian cancer.

Cervical Cancer Stem Cells

Cervical cancer is a kind of gynecologic tumors with relatively high incidence and its stem cells have become hot research topics. Yao and some other researcher have summarized the

major research progresses that had been made in cervical cancer stem cells by 2015 [30] as follows: (1) The markers of cervical cancer stem cells mainly include transcription factors of embryonic stem cells like Nanog, cytokeratins (including CK8 and CK17), cell adhesion molecules (e.g. CD13, CD29, CD44, CD105 and HLA-I), Oct4, ALDH1, Nucleostemin (NS), Musashi1, p16 (hypothetical protein) and p63 (hypothetical protein). (2) SHL, Notch and Wnt signaling pathways are major pathways related to cervical cancer stem cells. (3) There are generally four strategies for treating cervical cancer stem cells, including directly attacking CSCs, killing CSCs or promoting differentiation of CSCs, stimulating CSCs to enter the cell cycle to be not inactive, attacking the microenvironment of CSCs and inhibiting transition of CSCs into non-CSCs. Similar research findings have been also gained in other gynecologic cancers. Like CSCs of other sources, some research progresses are being made in cervical cancer stem cells and briefly summarized as follows.

Markers of Cervical Cancer Stem Cells

Concerning markers of cervical cancer stem cells, Sato and some other researchers have explored relationships between Gremlin 1 and cervical cancer stem cells [31]. As a type of antagonists against bone morphogenetic protein (BMP), Gremlin 1 is related to cellular differentiation like BMPs and found to be connected with several diseases. In this paper, the expression of Gremlin 1 is found to be higher in cervical cancer tissues and negatively correlated to the "progression-free survival" (PFS, a prognostic indicator). Once CaSki (i.e. a cervical cancer cell) is treated with Gremlin 1, the expression of Nanog will significantly increase as a stemness factor, so will the percentage of ALDH positive cells. Like CSCs, Nanog is found to show higher expressions and be ALDH-positive, so it is deduced in this paper that Gremlin 1 may be used as a marker of cervical cancer. Liu and some others have studied the characteristics of Hiwi as a marker of cervical cancer stem cells [32]. HiWi, also known as PiwiL 1, is an anthropogenic homologue of the PiWi family of proteins. It has been detected to be related to stem cells and overexpressed in multiple types of cancer. In this paper, the dye strike rate of HiWi is detected to be higher in cervical cancer tissues than that in normal cervixes. In particular, it is limited in basal cells of normal cervixes, related to development, progression and chemotherapy resistance of cervical cancer. On the contrary, silencing HiWi may decrease the survival rate of cervical cancer cells. Discovered to promote tumor sphere-forming abilities in vitro, HiWi increases tumorigenicity and upregulates the expressions of transcription factors related to self-renewal in vivo. Hence, it may be used as a marker of cervical cancer stem cells and a potential target for treating cervical cancer.

ALDH1 in Cervical Cancer Stem Cells and Drug Resistance of Tumors

Like treating other tumors, the resistance to chemotherapy drugs is a critical for treating cervical cancer. This paper predicts if cervical cancer is resistant to certain chemotherapies by measuring related specific indicators, which is also one of current research interests. Xie and some others have reported how ALDH1 is associated with drug resistance to neoadjuvant chemotherapies (NAC) and prognosis in treating cervical cancer [33]. ALDH1 is one of important markers of cervical cancer stem cells. Once ALDH1 is positive, the "disease-free survival" (DFS) and the overall survival (OS) would be poor and low. Hence, the expression level of ALDH1 is somewhat effective for forecasting the responses to NAC before such treatment. After NAC, ALDH1 may be used as another potential prognostic marker of cervical cancer.

Cervical Cancer Stem Cells and Resistance to Radiation Therapies of Tumors

Resistance to radiation therapies is another key factor for treating cervical cancer. For example, Pranatharthi and some other researchers have reported the roles of Rho/ROCK signaling pathways in keeping cervical cancer stem cells resistant to radiation therapies [34]. They consider that these signaling pathways are associated with the resistance of tumors to radiation therapies and play important roles. It will be more helpful for exploring resistance to radiation therapies and developing better therapeutic regimes by deeply investigating these signaling pathways.

Intervention with Cervical Cancer Stem Cells

Based on their understanding of CSCs and cervical cancer stem cells, researchers are developing regimes for treating cervical cancer from multiple perspectives. Kwon and some others have proposed intervening with HPV16 (Human papillomavirus type 16)-positive cervical cancer cells by A1E, which is a compound prescription of traditional Chinese medicine [35]. HPV infection is a major factor that induces cervical cancer. It is discovered in this paper that A1E inhibits growth of HPV16-positive cervical cancer cells instead of HPV16-negative cervical cancer cells. OCT-3/4 and Sox2 will show much lower expressions in HPV16-positive cervical cancer cells after they are treated by A1E. Meanwhile, the sphere-forming abilities of these cells are dramatically weakened. After the intervention of A1E, both ALDH and CD133 positive cells decrease drastically. These indicators are important symbols of cervical cancer stem cells and account for a relatively high proportion in these cells. The research has shown that A1E, as a compound prescription of traditional Chinese medicine, is somewhat effective for treating cervical

cancer stem cells. It is undoubtedly significant for developing drugs against cervical cancer stem cells in clinical practices.

In a word, human beings have constantly enriched their knowledge about markers of cervical cancer stem cells, drug resistance and resistance to chemotherapies. Based on their understanding, they are exploring how to intervene with cervical cancer from different perspectives, achieved certain encouraging research progresses, which will be helpful for treating cervical cancer.

Relationships between CSCs and Recurrence of Three Fertility-Related Malignancies

Above three fertility-related malignancies are highly recurrent, and CSCs are closely connected with the recurrence of these malignant tumors [36]. It has been a hot research topic for discovering new factors that promote recurrence and inhibit the recurrence of such malignant tumors by intervening with CSCs. Chefetz I et al. [37] have discovered that TLR2-MyD88-NFκB signaling pathway (i.e. a type of proinflammatory cytokine) facilitates CSCs-driven repair of tumor tissues and self-renewal of tumor cells for epithelial ovarian cancer, which is a subtype of oval cancer. However, the repair and self-renewal promotes the recurrence of epithelial ovarian cancer. The implications of this study consist in its discovery of new factors that stimulate the recurrence of ovarian cancer. At last, similar studies are helpful for developing new therapeutic strategies for preventing the recurrence of ovarian cancer. Alvero AB et al. [38] have reported that TRX-E-002-1, as a type of super benzopyran derivative, induces apoptosis and formation efficiency of ovarian cancer stem cells resistant to chemotherapies. In addition, animal experiments suggest that it is favorable for significantly postponing recurrence of the disease by consecutively administering TRX-E-002-1 after the treatment of paclitaxel. This research finding makes a new option available to prevent the recurrence of ovarian cancer, and new drugs are suggested to be explored with CSCs. In other words, CSCs are interposed to achieve preliminary therapeutic effects before further exploration in animal experiments. Miyoshi Y et al. [9] have studied ALDH1 (alcohol dehydrogenase 1, a type of CSC markers) for predicting the recurrence of ER-positive/HER-2 negative breast cancer. They have found that compared with non-recurrent patients, ALDH1 is more common in patients with breast cancer whose disease recurs in the early stage, and connected with more malignant breast cancer. This indicates that ALDH1 would be used for predicting the recurrence of ER-positive/HER-2 negative breast cancer as a marker and further become a target for intervening with treatment. Martinez-Outschoorn UE et al. [39] have discovered in their research that high-energy metabolites, including lactic acid and ketone body, stimulate expressions of breast cancer cells and "stemness" related genes, which are generally expressed by CSCs and finally cause poor prognosis (recurrence and metastasis included). This study has suggested that high-energy metabolites promote the development of breast cancer by increasing "stemness" of breast cancer cells. In this paper, tumor development is reckoned to be associated with patients' metabolic status, which is regarded as a new insightful vision about the development of breast cancer.

Thus, it is clear that more and more breakthroughs may be made in exploring how CSCs are related to the recurrence of above three fertility-related malignancies and possible interventions from multiple perspectives.

Relationships between CSCs and Metastasis of above Three Fertility-Related Malignancies

CSCs have been considered as important originators of three fertility-related malignancies. At present, it is a hot research topic to explore how to treat these tumors by intervening with corresponding CSCs. Wang J et al. [40] have discovered that tumor growth and pulmonary metastasis of xenogeneically transplanted mice may be significantly inhibited by downregulating HOTAIR (a type of long non-coding RNA, lncRNA) of CD117-positive and CD44+ CSCs for ovarian cancer with RNA interference technologies. According to this study, it would be a new way for intervening with ovarian cancer by interfering with RNA of HOTAIR in CD117-positive and CD44+ CSCs. Kakar SS [41] has reported that WFA, extracted from biologically active plants, will significantly inhibit tumor growth and metastasis when it is separately used in combination with cisplatin which is a kind of chemical anticancer drugs. After further research of the mechanisms, it is found that the cells expressing several types of CSC markers (including CD44, CD24, CD34, CD117 and Oct4) are extensively removed, while the expressions of Notch1 and Hes1 genes are downregulated. This suggests that WFA acts against ovarian cancer stem cells, which indirectly demonstrates that metastasis of ovarian cancer is related to CSCs. Samanta D et al. [42] have discovered that the expression of PHGDH (phosphoglycerate dehydrogenase) is necessary for dynamic redox balance of mitochondria, maintenance of breast cancer stem cells and pulmonary metastasis of breast cancer. For example, the silenced expression of PHGDH (phosphoglycerate dehydrogenase) in ER positive and negative breast cancer cells with RNAi techniques downregulates the level of NAPDH (coenzyme II), interrupts the dynamic redox balance of mitochondria, increases apoptosis and further removes the enrichment of breast cancer stem cells under hypoxic conditions. Furthermore, the PHGDH-deficient breast cancer cells are not quite neoplastic and the amount of breast cancer stem cells decline in tumors. As a consequence, the metastasis is eliminated. This study shows that PHGDH plays some roles in the formation of Grade-II tumors (recurrence or metastasis) and possibly becomes a target for intervening with tumor development. Furthermore, the research

suggests that it is generally correct to treat breast cancer by intervening with its cancer stem cells.

Thus, it is clear that perhaps more and more new breakthroughs could be made by exploring relationships between CSCs and metastasis of three major fertility-related malignancies and possible interventions from multiple perspectives.

Immunological Approaches to Targeting CSCs

In recent years, multiple studies have demonstrated that aldehyde dehydrogenase (ALDH) can serve as a specific marker for cancer stem cells in a variety of cancers. Lin M et al. characterized the tumourigenicity and stemness of ALDH (high) enriched cancer cells in immunocompetent animal models, and developed a dendritic-cell based cancer stem cell vaccine (CSC-DC) in murine melanoma (D5) and murine squamous cell carcinoma (SCC7) tumour models. Their experiments have offered direct evidence that CSC-DC vaccine could induce significant antitumour effect by immunologically targeting cancer stem cells [43]. Tumor therapy resistance has been attributed to CSC. Immunotherapy targeting of ALDH(+) CSC may therefore be a promising approach. The results of Liao T et al.'s experment may be helpful for the development and optimization of adjuvants, as here exemplified for INF-γ, for CSC-targeted vaccines, independent of the availability of CSC-specific antigens [44].

Conclusion

Above three fertility-related female malignancies, namely breast cancer, ovarian cancer and cervical cancer, have much in common in terms of their markers and signaling pathways. For instance, markers such as CD44, CD133 and ALDH1 are common in CSCs of several malignancies. Furthermore, all these types of malignancies have Notch and wnt signaling pathways, so there would be some similarities among final strategies for treating these tumors.

Concerning above three important female malignanies, more and more new markers, signaling pathways, signaling molecules and micro-environmental factors have been discovered. These new research findings are closely associated with genesis, development, drug resistance, recurrence and metastasis of these tumors. According to aforementioned progresses and theories on CSCs, people mainly think of treating these tumors by following therapeutic strategies: (1) intervene with markers of CSCs and directly attack these stem cells; (2) interfere with molecules of important signaling pathways in CSCs; (3) intervene with micro-environment of CSCs.

So far, lots of research progresses have been made based on aforementioned ideas and some of them are extremely encouraging. Nevertheless, further research and development are necessary for exploring stable and effective regimes of treatment.

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Compliance with Ethical Standards

Claim We claimed that this paper was original and would not have any financial interest in a company or its competitor, and that all authors meet criteria for authorship.

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