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ROLE OF CANCEROUS STEM CELLS IN BREAST CANCER DEVELOPMENT: A BRIEF UPDATE

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Abstract

The cancerous stem cells (CSCs) play important role in tumor progression, metastasis, and relapse of cancer disease. These cells provide resistance to the therapies like chemotherapy, radiation therapy, and hormonal therapy. The breast tissue CSCs (BCSCs) are $CD44^{+}/CD24^{-/low}/ESA^{+}$ cells with high aldehyde dehydrogenase (ALDH) activity and possess the property of mammosphere formation. The ALDH1A1 isoform plays significant role in mammosphere formation and glycolytic restriction is found to enhance the ALDH activity thus supporting the maintenance of BCSCs. Many factors regulate the CSC population and promote their stemness such as protein homeobox-D3 (HOXD3), Sirt-1, B7-H3 etc. Many pathways also regulate the BCSC development like MAPK kinase pathway, STAT3/IL-6 pathway, wnt/β-catenin pathway, sonic hedgehog signaling (shh) pathway etc. This mini-review focuses on the latest developments in the study of CSCs involved in breast cancer (BC) development and the therapies targeting the CSCs.

Key words

Cancerous stem cells, mammary gland, hormone, marker

Introduction

Breast cancer is the second most prevalent cancer in women after skin cancer worldwide with mortality rate of 1 out of 37 women (around 2.7%) [1]. Despite the use of prevailing therapies there is a risk of recurrence involved in the cancer growth [2]. Evidences are there that chemoresistance arises from cancerous stem cells (CSCs) [3-5]. When the small population of CSC survives the chemotherapy they initiate tumor recurrence enhancing the number of CSCs as well as the number of differentiated cancerous cells [6].

The CSCs are the clones derived from normal stem cells with the ability to resist chemotherapy, irradiation, and hormonal therapy. The CSCs were identified for the first time in late 1990s in acute myeloid leukemia and since then it has been the crucial area to focus in the branch of cancer research. The origin of CSCs has been a debate with different hypotheses given by the researchers. One of the hypotheses stress that the accumulation of mutation in the stem cell niche population leads to mutated daughter cell formation which further develop to a cancerous mass in future [7]. Another theory claims the involvement of adult stem cells in

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cancerous growth with accumulation of mutations in those cells in highly dividing tissues which further drive the initiation of cancer [8, 9]. The de-differentiation of mutated cells is also a possibility showing stem cell – like properties and driving the tumor formation in which case any fully differentiated cell undergoing mutation may reprogram to achieve stemness and grow as tumor, as evidenced from prostate cancer model [10]. The CSCs play crucial role in development of different types of cancer, their progression, maintenance, metastasis, and relapse [11]. The CSCs show phenotypic plasticity which is an essential requirement for the tumor mass to be adherent epithelial-like cells and growing fast at a particular location as well as being motile mesenchymal-like cells migrating to other locations in the process of metastasis [12]. There might be existence of strong correlation between the epithelial-mesenchymal transition and attaining the CSC – like properties corresponding to the tumor microenvironment which needs to be established [13].

Difficulties follow the isolation of pure CSCs, impeding the research on these cells. It is found that CSCs could be generated *in vitro* from immortalized human mammary epithelial cells using small molecules like CHIR99021 (glycogen synthase kinase 3 inhibitor), and PD184352 (Mitogen activated protein kinase kinase inhibitor) [14]. These molecules inhibited the glycogen synthase kinase – 3 and mitogen-activated protein kinase kinase synergistically stimulating the CSC development through Wnt and MAPK kinase signaling pathway and these CSCs showed tumorigenicity property with increased mammosphere formation [15]. Study showed that tumorigenic cancer cells could be separated from the nontumorigenic cancer cells through identification of cell-specific surface markers (tumorigenic cells are $CD44^{+}CD24^{-/low}$ lineage). The breast CSCs (BCSCs) were found to be $CD44^{+}/CD24^{-/low}/ESA^{+}$ cells having self-renewal and differentiation ability [15]. They possess high ALDH activity, CD49f and CD133 expression with mammosphere formation property. Novel antibodies were developed to CSC and EMT-associated factors (Goosecoid, Sox9, Slug, Snail, and CD133), further facilitating the *in vitro* studies [12].

This review focuses on the role of CSCs in tumorigenesis, the associated markers, hormonal effects on the CSC population, and possible therapeutic targets which will help the researchers to design further work on CSCs for control and treatment of breast cancer.

CSCs and epithelial mesenchymal transition (EMT)

There is a strong correlation exists between the CSC activity and the state of EMT in cancer progression [16]. The EMT is a physiological process during embryogenesis and also it is required in cancer progression [17]. The epithelial cells attain the mesenchymal property as they loose cell–cell attachment and polarity by cytoskeletal remodeling [18]. This transition is reversible and the mesenchymal to epithelial transition (MET) can occur by expression of E-cadherin and gaining the cell polarity. It is observed that the epithelial BCSCs are similar to the luminal stem cells and the mesenchymal BCSCs are similar to the basal stem cells of normal human mammary gland in their functionality [19]. Many signaling pathways are reported to be involved in regulating the CSC function in the EMT transition process and to mention a few are hedgehog pathway, TGF-β signaling, Wnt pathway, Notch pathway, nuclear factor kappa light chain enhancer of activated B cells (NF-κB) pathway promoting the EMT process [20-22]. Besides, the pathways which induce MET transition through CSC regulation are bone

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morphogenetic protein (BMP) pathway and human epidermal growth factor receptor pathway [23, 24].

Markers of stem cells

The lineage studies of mammary gland (MG) differentiation hierarchy showed the existence of bipotent normal mammary stem cells (MSCs) giving rise to both luminal and myoepithelial cells along with the long-lived unipotent progenitor cells which drive the MG development and homeostasis [25-27]. Reportedly, two populations of BCSCs are present having epithelial like ($ALDH^+$ cells) and mesenchymal like ($CD44^+CD24^-$ cells) phenotypes [28]. These two cell types show high plasticity and are inter-convertible depending on the tumor microenvironment [19]. Another cell population which expresses both $ALDH^+$ and $CD44^+CD24^-$ markers have highest potential of mammosphere formation and express more of genes IDI, SOX2, TWIST1, and ZEB2 associated with stemness and epithelial-mesenchymal transition [29]. The enzyme ALDH has isoforms ALDH1A1, ALDH1A2, and ALDH1A3 out of which the ALDH1A1 seemed to localize in small lobules and ALDH1A3 localize in extra lobular ducts. The ALDH1A1 has significant role in BC development as it promotes the mammosphere formation and its level is found to be higher in HER2 posiive and triple negative breast cancer (TNBC) cases [30]. ALDH normally catalyzes the oxidation of retinal to retinoic acid which has regulatory role in normal development of adult tissues [31]. Glycolytic restriction seemed to enhance the $ALDH^+$ activity and $CD44^+CD24^-$ cell population, thus supporting the stem cell like cell phenotype of BCSCs [28]. On contrary, recent works showed increased glycolysis can be a causative factor of maintaining stem cell phenotype of BCSCs [32]. Evidences show that the undifferentiated proliferative MSCs utilize oxidative phosphorylation as the major pathway for energy production [29]. These above mentioned markers along with $CD49f^{hi}EpCAM^{-/lo}$ are equally useful in isolation and functional characterization of normal human breast stem cells [33-36].

The normal human mammary gland shows four population of cells based on the two markers such as alpha - 6 – integrin (CD49f) and epithelial cell adhesion molecule (EPCAM) [36]. The four populations are $EPCAM^+CD49F^-$ epithelial cells, $EPCAM^+CD49f^+$ luminal progenitor cells, $EPCAM^-CD49f^+$ stem cells, and $EPCAM^-CD49f^-$ stromal cells. The study to correlate BCSC marker with the normal mammary gland cell marker showed that $EPCAM^-CD49f^+$ cells of healthy mammary gland display the BCSC marker $CD24^-CD44^+$ phenotype located in basal layer of mammary duct. Additionally, the EPCAM+CD49f+ cells of healthy mammary gland showed positive for BCSC marker ALDH+ with highly proliferative property and epithelial – like expression profile present at the abluminal location in lobules [19].

Factors regulating CSC population

The pluripotent genes OCT-3/4, SOX-2, NANOG, and Klf-4 are responsible for maintaining the stemness properties of normal stem cells. These key stemness factors are also found to be active in certain growing tumors [37]. The ectopic expression of oct3/4, Sox-2, NANOG, and Klf-4 genes induce pluripotency in differentiated cells which is the basis of iPSC (induced pluripotent

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stem cell technology). Out of these factors Klf-4 is found to be active in growing tumour cells in number of cancer types [38, 39]. Study on mechanism of Klf-4 induced stemness reveals the SIRT-1 – PRRX-1 – Klf-4 – ALDH-1 pathway regulating CSC function [40]. SIRT-1 is a NAD-dependent deacetylase sirtuin-1 protein which deacetylates proteins and contributes in cellular regulation to stress and longevity [41]. The SIRT1 protein belongs to the most conserved longevity genes which connects metabolism to stress, ageing, endocrine regulations, and cancer progression [42]. The PRRX1 gene expresses the protein which is a DNA-associated protein and acts as a transcription coactivator, playing crucial role in mesenchymal stem cell differentiation [43]. Functional analysis of SIRT1 protein by Shi et al. (2018) revealed that this protein acts as a central molecule regulating age – related CSCs in breast cancer [40]. The SIRT1 depletion significantly enhanced the number of $ALDH1^+$ CSCs, showing partial MET, facilitating lung metastasis by up regulating the Klf-4 protein expression in human and mouse breast cancer cells. The underneath molecular mechanism shows that SIRT1 protein deacetylates the PRRX1 protein and stabilizes it which is an EMT inducer. Ideally the destabilization of PRRX1 induces Klf-4 transcription which further enhances the ALDH1 transcription ultimately inducing the CSCs [40].

The protein homeobox D3 (HOXD3, a transcriptional regulator), is observed to be crucial for the stemness of cancer cells through integrin-β3 mediated wnt/β-catenin signaling [1]. The HOXD3 is a member of the homeobox family of genes having role as transcriptional regulator in morphogenesis of multicellular organisms. This protein is found to be overexpressed in breast cancer tissues and showing its function by developing resistance against therapies and also enhancing the stemness of tumor mass as evidenced from expression of stem cell biomarkers. The drug resistance and enhanced stemness seems to be induced through integrin β3 – mediated Wnt/β catenin signaling pathway [1]. Besides, the glycoprotein B7-H3 (immune regulatory and a tumor promoting factor) in higher amount, increases stem cell population by binding to major vault protein (MVP) which ultimately regulates the MAPK Kinase pathway [44]. The Sonic Hedgehog signaling (Shh) pathway also plays an essential role in maintaining CSCs and side populations [45, 46]. The Hedgehog (Hh) signaling pathway is vital during growth and patterning in embryonic development [47]. This pathway involves coordinated network including twelve transmembrane protein patched 1 (Ptch1) inhibition through binding of Hh protein, Smo transmembrane protein activation, release of Gli proteins and their nuclear translocation, and subsequently target gene transcription [47-49]. The Hh pathway is reported to be activated in human breast cancer cells and disruption of this pathway reduced the proliferation of cancer cells [50]. Tanaka et al. (2009) observed that Hh pathway is essential for maintaining the CSC population and this pathway can be a significant therapeutic target for BCSC therapy [45]. Additionally, the compound nicotine (enters body through smoking) is also involved in metastasis and relapse of the disease. It reduces the effectiveness of doxorubicin, promoting $CD44^+CD24^-$ CSC population in the MCF-7 human BC cells [51].

Steroid hormones modulating BCSC population

Estrogen and progesterone are main steroid hormones modulating the CSC activity [11]. However, their effects seem to be indirect mediated through different paracrine and juxtacrine cell-cell signals. Estrogen promotes epithelial cell proliferation through two receptors (ERα and ERβ). The BCSCs respond to estrogen through ERα variant ERα36, and ERβ to maintain the

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$CD44^{+}/CD24^{-/low}$ cell population through activation of mitogenic signaling via AKT/GSK3β pathway [52]. Progesterone signaling mediates proliferation and cell function of stem cells and progenitor cells through CXCR4-CXCL12 signaling axis in normal MG [53]. Progesterone also inhibits the microRNA (miR-29 and miR-141) expression and ultimately de-represses the target genes KLF4 and STAT5A, involved in maintenance and regulation of BCSCs [54-57]. *In vitro* studies showed progesterone stimulating the proliferation of bipotent stem cells, increasing number of progenitor cells and CSC marker expression [58, 59].

Therapies targeting CSCs

The $CD24^{-/low}/CD44^{+}$ CSCs showed reduced activation of death receptor pathways like FasL, TRAIL, and TNF-α as compared to the $CD24+/CD44^{low}$ non-stem cells through radiation therapy [60]. The resistance of BCSCs to ionizing radiation is due to the embryonic stem cell factor Oct-4 [61]. This regulatory molecule suppresses the radiation–induced senescence of cancer cells by STAT3 and NF-κB mediated IL-24 production. So antagonist to IL-24 can be designed for therapy. However, IL-18 can be used as a therapeutic agent as it was observed mesenchymal stem cells overexpressing IL-18 have antitumor effect inhibiting the proliferation and metastasis of breast cancer [62]. Besides, Gli-1 (Glioma–associated oncogene homolog), a downstream molecule of Shh pathway has crucial role in invasion. The Gli inhibitor HPI-1 has profound effect on BCSCs inhibiting their proliferation and invasion [63]. The drugs like SERMs (tamoxifen), SERDs (fulvestrant), and aromatase inhibitors enrich BCSCs through reduction of estrogen synthesis [64]. Ulipristal acetate (selective PR modulator) shows anti-tumor activity by reduction of Ki67 and cyclin-D1 level [65]. The type-I IFNs (IFN-β) play vital role in regulation of CSC activity. IFN-β can be adopted for therapy targeting the BCSCs as the IFN signaling represses the EMT genes and CSCs differentiate into non-CSC epithelial cells [66].

Some plant derived compounds like quercetin-3-methyl ether (inhibits Notch1 and PI3K/Akt signaling pathways), quercetin (reverses multi-drug resistance), esculentoside A and catechol (modulate STAT3/IL6 signaling pathway) are found to suppress/inhibit the BCSCs proliferation [67-70].

Conclusion

The CSCs play vital role in progression, metastasis and relapse of breast cancer. They build resistance to therapies like chemotherapy, radiation therapy and hormonal therapy. Different signaling pathways modulate BCSC population in the tumor mass like Shh pathway, MAPK kinase pathway, STAT3/IL6 pathway, wnt/β-catenin pathway etc. The BCSCs or the signaling molecules involved in BCSC maintenance can be targeted for cancer therapy and relapse can be controlled. Future studies should focus on understanding the markers associated with BCSCs and signaling pathways which will pave the way for targeted drug therapy to curb the breast cancer.

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