Cancer Letters 349 (2014) 1–7

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Breast cancer stem cells: Multiple capacities in tumor metastasis

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ARTICLE INFO

Article history:
Received 27 December 2013
Received in revised form 27 March 2014
Accepted 28 March 2014

Keywords:
Breast cancer
Cancer stem cells
HER2
Therapeutic resistance
EMT
Metastasis

ABSTRACT

Breast cancer is the leading cause of cancer death among women worldwide. Accumulating evidence indicates that the local recurrent and/or distant metastatic tumors, the major causes of lethality in the clinic, are related to the aggressive phenotype of a small fraction of cancer cells loosely termed as cancer stem cells (CSCs), tumor initiating cells (TICs), or cancer metastasis-initiating cells (CMICs). Breast cancer stem cells (BCSCs) are shown to exhibit unique growth abilities including self-renewal, differentiation potential, and resistance to most anti-cancer agents including chemo- and/or radiotherapy, all of which are believed to contribute to the development and overall aggressiveness of the recurrent or metastatic lesions. It is in the urgent need not only to further define the nature of heterogeneity in each tumor but also to characterize the precise mechanisms governing tumor–host cross-talk which is assumed to be initiated by BCSCs. In this review, we will focus on recently identified key factors, including the BCSCs among circulating tumor cells, interaction of BCSCs with the host, epithelial mesenchymal transition (EMT), tumor microenvironment, the intrinsic resistance due to HER2 expression, potential biomarkers of BCSCs and cancer cell immune signaling. We believe that new evidence coming from both bench and clinical research will help to develop more effective approaches to control or significantly reduce the aggressiveness of metastatic tumors.

Introduction

Breast cancer is genetically and clinically a heterogeneous disease [1–3], and metastatic lesions are the leading cause of death in patients [4–7]. Accumulating evidence suggests that the tumor bulk of breast cancer contains a heterogeneous tumor cell population that is derived from a subset of cells that show the characteristics of stem cells, termed as tumor-initiating cells or cancer stem cells (CSCs) [8,9]. The whole course of tumor metastasis is a complex procedure requiring the most aggressive cancer cells rather than all tumor cells to be able to survive the long time circulation and to form new local lesions by extravasation and migration. Accumulating evidence indicates that CSCs play a key role in not only the original tumorigenicity but also in their ability for local invasion and migration [10–12]. Overlapping with some features of normal stem cells, CSCs are shown to be resistant to proapoptotic factors, rendering them a formidable adversary to current anticancer modalities [13–15]. Extensive studies have demonstrated that breast cancer stem cells (BCSCs) exhibit the ability to metastasize to specific parts of the body and are believed to be a cause for metastatic lesions. Although it is expected that the tumor heterogeneity and BCSCs may be the last obstacles for effective breast cancer treatment, the molecular insights and potential specific biomarkers for the therapy-resistant BCSCs need to be further elucidated before potential clinical benefits could be achievable.

The concept of CSCs and self-renewal

The concept of the CSC was first hypothesized in the 20th century by Bonnet and Dick in their studies of human acute myeloid leukemia (AML) [16]. Their study indicated the presence of a unique cellular hierarchy in AML, reflecting the similar order identified in normal hematopoiesis. Leukemic stem cells identified in this hierarchy, originally termed as CSCs, were categorized as $CD34^+/CD38^-$. Recent studies demonstrate that most $CD34^+$ AMLs are derived from progenitor cells but not hematopoietic stem cells [17]. Subsequently, the CSC concept has been described and extended to many solid tumors, including breast cancer, prostate cancer, colorectal cancer, lung and brain cancers [16]. In particular, breast cancer is shown to be heterogeneous, and the tumor bulk is derived from BCSCs [18–20].

The CSC theory challenges the traditional concept of tumorigenesis. Studies show that some CSCs may be derived from normal

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http://dx.doi.org/10.1016/j.canlet.2014.03.036
0304-3835/

stem cell transformation, leading to tumor growth [21–27]. Other studies indicate that mesenchymal stem cells can accelerate cancer cell metastasis [28–32]. It is even proposed that normal stem cells promote the process of tumorigenesis, tumor metastasis, as well as CSCs dynamic change [28]. In 2003, Al-Hajj and colleagues reported for the first time that breast cancer can originate from BCSCs [33]. They identified and isolated a small subset of cells within primary breast cancer cells of which a few cancer cells were able to form palpable tumors in the mammary fat pad of non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. Such cells express CD44 or CD44 with epithelial specific antigen (ESA), but not CD24, consistent with the phenotypic characteristics of mammary stem cells with multipotent differentiation ability [33,34]. Nevertheless, although these results demonstrate the possibility that BCSCs originate from the normal mammary stem cells, it is generally accepted that further genomic profiling and comparison of the normal stem cells versus BCSCs should provide insightful information on the aggressive phenotype of BCSCs.

CSCs are featured by their potential to self-renew and initiate tumors in immunocompromised mice upon serial passage. Several studies reveal that CSCs are responsible for tumor metastasis and the recurrent lesions following chemotherapy and radiotherapy [35–37]. The tumor microenvironment plays a significant role in regulating chemoresistance and radiation resistance via inflammatory cytokines to stimulate CSC self-renewal, which may promote tumor proliferation and metastasis [36,38]. Different cells in the tumor microenvironment produce several factors, including PDGF, IGF, Notch, Wnt, MMPs, and Hedgehog (Hh) to regulate tumor growth, invasion, and metastasis [39–43] (Wnt, Notch and Hh are shown in Fig. 1). Among them, Wnt is known to cause self-renewal of CSCs at early phases and plays a significant role in the initiation and maintenance of CSCs [44–46]. Hh signaling of CSCs was shown to be active not only in a pancreatic xenograft mouse model [47] but also in regulating the maintenance of human leukemic stem cells [48,49]. Ablation of the Hh pathway effector smoothened (Smo) caused elimination of chronic myeloid leukemia (CML) stem cells [48]. In addition to the Hh pathway, activated Notch signaling was shown in colon CSCs [50]. Antibodies targeting Delta-like 4 ligand (DLL4), another downstream effector of Notch signaling, inhibited growth of human colon cancer in xenograft mouse model [50]. Therefore, similar to Notch inhibition, therapeutic agents identified to inhibit these self-renewal signaling pathways may have potential effectiveness to decrease the number of CSCs and suppress tumorigenicity and metastasis.

BCSCs and circulating tumor cells (CTCs)

A subpopulation of circulating tumor cells (CTCs) identified in patient blood has been hypothesized to initiate metastatic carcinoma [51–54]. However, although the presence of increased CTCs is an indicator of poor prognosis and cancer progression, the phenotype of CSCs (or metastasis-initiating cells; MICs) among CTCs has not been elucidated. Since CTCs are able to initiate metastatic growth in distant organs, resembling the same behavior as MICs [55], it is reasonable to believe that stem cell-like CTCs are a subpopulation of cells with metastatic ability to migrate to distant sites from the primary tumor [56]. A recent study showed that MIC-containing CTC populations originating from primary human luminal breast cancer expressing EPAM, CD44, CD47, and MET caused lung, liver, and bone metastasis in mice [51]. In a small patient cohort exhibiting tumor metastasis, the population of $EPAM^{+}CD44^{+}CD47^{+}MET^{+}$ correlated with increased metastasis and low overall survival [51]. An additional study identified BCSCs in a CTC population among patient peripheral blood samples [55]. This study shows that among a total of 1439 CTCs, 35% of the CTCs in 20 out of 30 patients exhibited the BCSC $CD44^{+}/CD24^{-/low}$ phenotype, whereas 17.7% of the CTCs identified in seven patients were $ADLH1^{high}/CD24^{-/low}$ [55]. Such data support the conclusion that functional BCSCs and MICs within CTCs may lead to early diagnosis and treatment of metastatic breast cancer. However, more detailed studies on the characteristics of CTCs are needed to elucidate molecular mechanisms of tumor metastasis initiated by dissemination, seeding, and engraftment of CTCs.

BCSCs and EMT

The epithelial–mesenchymal transition (EMT) is a phenotypic process converting polarized and adjacent epithelial cells to mesenchymal cells conferring motile and migratory properties [57]. It has been proposed that CSCs in primary tumors can metastasize to distant tissues or organs to disseminate and form metastatic colonies via EMT [58]. A recent study has revealed a dynamic in vivo pattern of epithelial-to-mesenchymal transitions in circulating tumor cells and metastases of breast cancer [59]. The $CD44^{+}/CD24^{-}$ subpopulation in RAS or HER2 overexpressing tumor cells is considered to be the phenotype with increased EMT potential [60,61]. The gene expression signature, $CD44^{+}/CD24^{-/low}$, is also found to occur mainly in human breast tumors of the recently identified "claudin-low" molecular subtype [62]. This subtype is characterized by expression of many EMT-associated genes, such as FoxC2, Zeb, and N-cadherin [62,63].

EMT may serve as a critical step to the metastatic processes of BCSCs [61,64]. Vimentin, one of the key genes regulating EMT and tumor aggressiveness in breast cancer [65,66] is shown to be indicative as a poor prognostic factor for triple-negative breast cancer [67] and upregulated in normal human breast epithelial cells overexpressing HER2 [64,68]. We found that HER2 was able to activate Stat 3 signaling pathway [3] and EMT-associated genes, such as vimentin (Fig. 2A). These results, together with enhanced EMT-associated genes, suggest that HER2-associated activation of Stat3 signaling can induce EMT of normal mammary epithelial cells such as MCF-10A cells, and contributes to metastatic BCSCs, for which a signaling pathway is proposed here shown in Fig. 2B. However, the occurrence of EMT is not acquired spontaneously but regulated by microenvironment signaling. Regulation of EMT plasticity is most likely dependent on normal cells in the tumor microenvironment, including cancer-associated stromal cells, such as infiltrating immune cells, fibroblasts, and endothelial cells [69]. During tumorigenesis, changes in immunological signaling networks occur not only in epithelial tumor cells but also in the proximal tumor-associated stromal cells. Interestingly, several cytokines, chemokines, or growth factors were identified in mammary tumor stroma compared with normal mammary stroma [70]. Further investigation is needed to clarify our understanding of how BCSCs and each of above tumor-associated factors influences cancer cell development, EMT plasticity and tumor aggressiveness.

CSCs, tumor microenvironment, and metastatic lesions

Here we would like to illustrate the mechanism of interplay of tumor–tumor microenvironment by showing the case of bone marrow vascular and endosteal niches for hematopoietic stem cells (HSCs) which is known to contribute to the tumor microenvironment of CSCs [71–73]. In the vascular niche, HSCs monitor hematopoietic function and promote rapid responses to hematopoietic demand. Endosteal HSCs regulates other cells, including mesenchymal stem cells, CXCL12-abundant reticular cells, and chimeric antigen receptor (CAR) cells. The endosteal niche is primarily controlled by osteoblasts expressing cytokines, growth factors, and many adhesion molecules, which are critical for maintenance of