Review Article

Metastatic cancer stem cells: from the concept to therapeutics

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Received July 21, 2014; Accepted August 7, 2014; Epub September 5, 2014; Published September 15, 2014

Abstract: Metastatic cancer stem cells (MCSCs) refer to a subpopulation of cancer cells with both stem cell properties and invasion capabilities that contribute to cancer metastasis. MCSCs have capability of self-renewal, potentials of multiple differentiation and development and/or reconstruction of cancer tissues. As compared with stationary cancer stem cells, MCSCs are capable of invasion to normal tissues such as vasculatures, resistance to chemotherapies, escape from immune surveillance, survival in circulation and formation of metastasis. MCSCs are derived from invasive cancer stem cells (iCSCs) due to the plasticity of cancer stem cells, which is one of the characteristics of cancer cell heterogeneity. Both stages of iCSCs and MSCSs are the potential therapeutic targets for cancer metastasis in the future strategies of personalized cancer therapy.

Keywords: Cancer stem cells, invasion and metastasis, therapeutics

Introduction

Tumor progression towards metastasis is a complex, multistage process, which is classically simplified as local invasion, intravasation, and survival in the circulation, extravasation, and colonization [1, 2]. Indeed, only a small percentage of tumor cells left from a primary tumor successfully form distant metastatic lesions. Approximately 90% of released tumor cells can complete one or more early steps and about 2% of the tumor cells can form micro-metastasis. However, merely about 0.2% of the tumor cells can effectively induce angiogenesis and eventually form metastases in distant organs [3].

Cancer stem cells (CSCs), also known as tumor initiating cells (TICs), are a small subset of tumor cells with the biological characteristics that are similar to normal stem cell: self-renewal and differentiation [4]. CSCs are proposed to be the fundamental driving force of tumor development, initiation of invasion and metastasis as well as recurrence [5]. CSCs can differentiate and generate tumor cells with a variety of phenotypes and are under the regulation of various signaling pathways that are critical in key development process, including Notch, Hedgehog, NF-kB, Wnt and TGF-beta pathways [6]. CSCs are also involved in chemoresistance and radioresistance [7]. Such properties of CSCs suggest that they are the fundamental driving force for not only tumor development, but also initiation of metastatic progression as well as recurrence. However, the exact role of CSCs in multistage cancer progression, especially in metastasis, has not been well-clarified. This review will focuses on the current knowledge of metastatic cancer stem cells (MCSCs).

CSCs and their plasticity

Cancer stem cell, also called tumor stem cell (TSC) or tumor initiating cell (TIC) is a newly theory referred to a small subgroup of tumor cells with self-renewal capacity and differentiation potential. CSC is precisely defined by AACR in 2006: ‘a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor [8]’. Over a century ago, the pathologist Rudolph Virchow and his student, Julius Cohnheim, proposed that cancer might arise from embryonic-
like cells. The hypothesis of CSCs was subsequently raised. Tumor cells with properties of CSCs were first identified in 1994 by John Dick and his colleagues [9]. They found that a small group of acute myeloid leukemia (AML) cells, recognized by CD34 (+) CD38 (-), could engraft SCID mice to produce large numbers of colony-forming progenitors. In 2003, Al-Hajj et al. identified and isolated the tumorigenic cells as CD44 (+) CD24 (-/low) Lineage (-) in breast cancer, which proved the existence of CSCs in solid tumors for the first time [10]. Presently, emerging evidence for existence of CSCs has been proved in several tumors, including breast cancer, glioma, colorectal cancer, prostate cancer, as well as pancreatic cancer [10-15].

**Properties of CSCs**

There are three main properties of CSCs [16]. First, CSCs share similar biological characteristics with physiological stem cells: self-renewal and differentiation. CSCs have the capacity to maintain the stem cell pool, sustain the heterogeneous growth of cancer lesions and generate all the cell types observed in the parent tumor.

CSC expresses a unique repertoire of surface biomarkers, which allows its isolation from non-tumorigenic cells in a reproducible manner. CSCs can be isolated by the expression of distinctive and well-characterized cell surface biomarkers, including CD Molecules (CD133, CD44, CD24, CD166, etc.), ATP-Binding Cassette Transporters (ABCG2, ABCB5), EpCAM, ALDH1, CXCR4, Nestin and LRCs [17]. Telomerase and SP cells are also applied for identification of CSCs [18, 19].

CSCs have high tumorigenic capacity [20] and are able to generate tumors with high efficiency when injected in limiting dilutions into immuno-deficient mice [21]. Other CSC functional properties include dormant or slow-cycling states [22-24], increased resistance to conventional therapies such as chemotherapy and radiotherapy [25]. Dormancy or slow-cycling behavior might contribute to the therapy resistance or tumor relapse after therapy [26]. In addition, CSCs are able to promote angiogenesis and lymphangiogenesis [27-31].

**Possible resources of CSCs**

The resource of CSCs remains in debate. It has been suspected that CSCs might origin from developing stem cells [32]. For instance, the leukemic stem cell possesses differentiation and self-renewal capabilities, and cell-surface phenotype of CD34 (+) CD38 (-), suggesting that normal primitive cells are the target for leukemic transformation [33].

Nevertheless, CSCs can also be originated from adult stem cells/progenitor cells, or differentiated cells that possess stem-like properties by accumulation of mutations in certain oncogenes and tumor suppressors [32]. For example, accumulation of p53 mutation first occurs in neural stem cells, which subsequent expand to progenitor-like cells and initiates glioma formation [34]. The silence of Ink4/Arf locus and p53 are rate-limiting for this reprogramming process [35-37]. These cells acquire self-renewal potential without de-differentiation after loss of tumor suppressors. Moreover, The researches on induced pluripotent stem cells (iPS) has demonstrated that the acquisition of self-renewal and pluripotency initiation from any somatic cells can be achieved by activation of four or few transcription factors [38-43]. Recently, it has been proposed that genomic instability can be an essential driver to generation of stem-like cancer cells from the “common” cancer cells [44]. These findings have provided evidence that the accumulation of genetics or epigenetic alterations in certain oncogenes and tumor suppressors may lead to reprogramming process for the acquisition of properties of CSC activity.

Alternatively, CSCs may arise from transformed epithelial cells through an Epithelial-mesenchymal transition (EMT) process to acquire migratory and metastatic properties [45, 46]. The molecular link between EMT and stemness was first described by Mani et al. in 2008 [46], and subsequent study demonstrated that Twist1 induced EMT and tumor initiation in cancer cells by directly targeting Bmi-1 [45].

**Heterogeneity of CSCs**

CSCs display phenotypic and functional heterogeneity in the same type of human cancer [47]. Phenotypically, CSCs have distinct cell surface markers in different tumors or in an individual tumor. For example, human breast CSCs have been characterized by distinct markers, including CD44 (+) CD24 (-/lo) [10], ALDH1 (+) [48], and PKH26 dye-retaining [49]. Side population
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(SP) cells are also used for enrichment of CSCs [50]. Functionally, in a hierarchical model, CSCs would develop into tumor progenitors with different tumorigenic capacity. In analyzing AML leukemia stem cells, divergent tumorigenic subsets were dissected by means of serial transplantations coupled with clonal tracking [51]. On the other hand, co-existence of independent CSC subsets which have different origins may present in a tumor. In some breast cancers, although the CD44 (+) CD24 (−) cells are enriched in progenitor cells and the CD24 (+) subset is luminal-differentiated, no lineage relationship exists between CD24 (−) and CD24 (+) cells [52, 53]. Likewise, CD133 (+) and CD133 (−) glioma may have different cells-of-origin and harbor different genetic alterations [54]. These observations indicate that CSCs are phenotypically and functionally heterogeneity.

Stem cell plasticity and CSC plasticity

Stem cell plasticity refers that a stem cell differentiates into a cell other than its committed or expected one [55, 56]. For example, bone marrow stem cells are able to give rise to cardiac myocytes and blood vessels [57-59]. Other studies have shown that bone marrow stem cells are able to generate skeletal muscle and neurons [60, 61]. However, most cell plasticity occurs in response to injuries or upon experimental manipulations. Such significant stem cell plasticity may not be generally manifested in normal tissues. Nevertheless, CSCs themselves are highly heterogeneous and their progenies may also possess plasticity, further contributing to heterogeneity of both cancer cells and CSCs. It has been documented that poorly differentiated cancer cells (presumed to be CSCs) in various human tumors can transdifferentiate into cells with endothelial-like phenotype [62-65].

CSCs in invasion and metastasis

Metastasis is a complex process of multi steps that encompasses several fundamental biological processes: tumorigenesis in the primary site, epithelial-mesenchymal transition (EMT), detachment from the primary tumor mass and invasion into the extracellular matrix, intravasation, survival and dissemination in the circulation, extravasation, mesenchymal-epithelial transition (MET), colonization and formation of micrometastases, and outgrowth of secondary cancer [66]. It is well known that metastasis is an inefficient process. Less than 1% of disseminated tumor cells are capable of forming macrometastases in distant organs [3]. The metastatic inefficiency primarily depends on the initiation of growth of a subset of extravasated cells under the regulation of environment in secondary sites [67]. It has been proposed that the initial mutation in a small subset of tumor cells forces them to possess highly metastatic potentials after additional mutations [68, 69]. Thus, it has been assumed that metastasis-initiating cells might exist, which have not yet been prospectively identified to date. This resumption of metastasis-initiating cells fits that of the origination of CSCs to some degree. In combination with the biology properties of CSCs, the assumption may have important implications not only in tumorigenesis, but also in cancer metastasis.

CSCs and epithelial-mesenchymal transition (EMT)

Epithelial-mesenchymal transition (EMT) is considered as an essential procedure in early steps of metastasis [70]. Pleiotropic transcriptional factors, such as Snail [71], Slug [72], deltalEF1 [73], Zeb1 [74] and Bmi-1 [75], are able to induce EMT through disruption of epithelial adhesion and junction. EMT endows the tumor cells the capacity to leave the primary tumor mass and acquire migratory and invasive abilities, which facilitates them penetrate into the microenvironment and enter into the circulation [76].

EMT has been linked to CSCs in recent years [46]. Various factors responsible for mediating EMT, including Hepatocyte Growth Factor (HGF), Epidermal Growth Factor (EGF), Transforming Growth Factor β (TGF-β), Wnt/β-catenin, Notch and Hedgehog signaling pathways, are also associated with stem cell maintenance [77, 78]. Activation of EMT programs is thought to endow neoplastic epithelial cells with both mesenchymal attributes and stemness traits [46, 79]. Epithelial stem cells express several sets of mesenchymal markers and EMT-inducing transcription factors or microRNAs [45, 70, 80-82], and cells undergoing EMT acquire stem cell-like properties. Induction of EMT by EMT inducers in differentiated epithelial cells can upregulates CD44, downregulates CD24 and alters other stem cell phenotypic markers [46, 83, 84]. Decreased
epithelial-marker E-cadherin and increased MMP-2 have been found to be associated with the capacity of glioma CSCs to metastasize [85]. Moreover, the histological clues of EMT have been observed by pathologists in invasive tumors as tumor buddings, which is described as the occurrence of single tumor cells or small clusters (< 5) of dedifferentiated cells at the invasive front of gastrointestinal carcinomas and is linked to poor prognosis [86, 87].

EMT is often transient and reversible. Colonization of disseminated cells in the secondary sites often needs a re-differentiation process called mesenchymal-epithelial (re-)transition (MET). Thus, the EMT-MET transition processes are considered as a driving force of metastasis [88, 89].

Disseminating tumor cells, circulating tumor cells and CSCs

Disseminating tumor cells present in the bone marrow (Disseminating Tumor Cells, DTCs) and peripheral blood (circulating tumor cells, CTCs) are highly relevant to the biology of early metastasis and poor prognosis of patients [90-94]. Recent researches have shed light on the relationship of DTCs and CTCs to CSCs and metastasis. A large proportion of DTCs detected in bone marrow of breast cancer patients display a cancer stem cell marker phenotype (CD44+/CD24−) [95]. CTCs isolated from peripheral blood of patients frequently have EMT features and putative stem cell phenotypes [96-98]. A subgroup of CSCs cells (CD45−CD90+) is detectable in 90% of blood samples from liver cancer patients. Similarly, the CD45−CD90+ subgroup CSCs cells isolated from the primary tumors and circulation of liver cancer patients generate tumors in a second and subsequently third batch of immunodeficient mice [99]. A recent study has showed that a major proportion of CTCs isolated from primary or metastatic breast cancer patients display a putative stem cell/progenitor phenotype and EMT characteristics [96, 100, 101]. CTCs isolated from patients with melanomas contain ABCB5-positive subpopulations and are capable of causing metastatic tumor progression in xenotransplantation models [102].

CSCs and metastasis

Beside the invasion step, CSCs are also implicated of critical importance in late steps of metastasis. The “seed and soil” hypothesis introduced by Paget in 1889 has suggested that both the cancer cell (‘seed’) and the environment (‘soil’) in distant sites contribute to the organ-specific pattern of metastasis [103, 104]. In 2006, Balic et al. linked metastasis to CSCs by demonstrating that most early disseminated cancer cells in bone marrow from early breast cancer patients possess putative stem cell phenotype [95], suggesting that CSCs stem cells might be the ‘lethal seeds’ which are capable of re-initiating growth to form metastases [105, 106].

Several properties of CSCs make them likely candidates for metastasis and might be of essential importance for colonization and formation of secondary tumors in distant organs. Genetic signatures in CSCs are frequently thought to predict tumor recurrence and metastases. For example, CD44v6, a marker of constitutive and reprogrammed CSCs in colorectal cancer, is required for cell migration and metastasis [107]. In fact, CD44+ breast CSCs from both primary breast tumors and lung metastases are highly metastatic in xenograft experiments [108]. CXCR4 is another example, which has a fundamental role in metastatic spread of a variety of cancers [109-112], has also been known as an important marker for metastatic potential of CSCs. It has been demonstrated that a distinct subpopulation of CD133+CXCR4+ CSCs in the invasive front of pancreatic tumors is essential for tumor metastasis, while CD133+CXCR4− cells are not [113].

CSCs and angiogenesis

CSCs also participate in both angiogenesis and lymphangiogenesis [27, 31], which are significant pathological changes in metastasis. CSCs express highly angiogenic and lymphangiogenic factors under hypoxia, suggesting that CSCs promote angiogenesis and lymphangiogenesis indirectly [114, 115]. Additionally, CSCs can give rise to tumor vasculogenic stem/progenitor cells or generate a tumoral microcirculation by developing vasculogenic mimicry, indicating that CSCs can directly initiate angiogenesis and lymphangiogenesis [31, 116, 117]. Evidence indicates that CSCs produce higher levels of VEGF [118]. Brain CSCs are able to promote glioma growth and angiogenesis by secreting high levels of VEGF under the regulation of CXCL12 and its receptor CXCR4 [29].
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CSCs and pre-metastatic niche

Emerging evidence raises a novel issue that the primary tumor-derived pre-metastatic niches in secondary organs facilitate to form a microenvironment suitable for homing or recruitment of tumor cells, and colonization by DTCs [119, 120]. In 2005, Kaplan and colleagues described the existence of pre-metastatic niche formed by bone marrow-derived cells (BMDCs) for the first time [119]. The mechanisms and timeline of pre-metastatic niche formation during primary tumor progression is well summarized and described by Andreas Moller et al [120]. As the primary tumor grows, tumor cells secrete diverse TDSFs to influence various pre-metastatic organs such as lungs and liver. In response to TDSFs, BMDCs are recruited to pre-metastatic organs and help to create pre-metastatic niches by altering the microenvironment. Early DTCs begin to arrive at pre-metastatic sites in secondary organs. Upon arrival, some DTCs survive and enter dormancy until a suitable microenvironment is established, and then DTC-containing pre-metastatic niches facilitate microenvironment to allow tertiary tumor spread [120].

Tumor-derived secreted factors (TDSFs) and BMDCs are crucial to the formation of pre-metastatic niche [119-122]. A group of TDSFs has been shown to promote pre-metastatic niche formation in tumor models, including VEGF, PIgF, TNF-α, TGF-β, Lysyl oxidase (LOX), versican, and G-CSF. In addition, hypoxia of primary tumor has been demonstrated to be one of the main sources of pre-metastatic niche-promoting factors [123]. Most of the hypoxic response target genes, including VEGF, LOX, LOXL2, LOXL4, TGF-β, MMP2, MMP9, CXCR4, and SDF-1, are directly or indirectly associated with the formation of pre-metastatic niche [124]. A recent research has reported that stromal POSTN expression induced by infiltrating tumor cells in the secondary target organ is necessary to initiate metastatic colonization, indicating that the crosstalk between CSCs and their niche can administrate metastatic colonization [125]. Master regulators, such as hypoxia inducible factors and TGF-beta superfamily members, may not only determine CSCs activity, but also regulate the elements of metastatic niche [124, 126-130].

Concept of MCSCs

Theoretically, metastatic cancer stem cells (MCSCs) are a subgroup of CSCs display stem cell properties, mediate metastasis, and contribute to treatment resistance. Although there is no final definition for MCSCs up to now, the hypothesis that MCSCs directly contribute to the initiation of dissemination and metastases has been gaining acceptance.

The ‘migrating cancer stem (MCS)-cell’ concept was first introduced by Thomas Brabletz et al. in 2005, based on their observations in human colorectal cancer [131]. The heterogeneous activation of Wnt/β-catenin signaling in the invasive front of primary or metastatic tumors has suggested that there exist two forms of CSCs stem cells in tumor progression-stationary CSCs and mobile CSCs. In this model, stationary CSCs exist in the epithelial tissue and are already active in benign precursor lesions. They maintain in differentiated areas throughout tumor progression, but they cannot disseminate. The stationary CSCs and other tumor cells can be transited into MCS-cells in primary or metastatic tumor mass. MCS-cells are highly mobile and lead to the rapidly invasive growth and dissemination of tumor cells. One part of MCS-cells divides asymmetrically and generates differentiating daughter cells to start new proliferation and differentiation locally. Another part of MCS-cells migrate a short distance and then undergoing a new asymmetric division to enlarge the primary tumor. The remaining MCS-cells disseminate through blood or lymphatic vessels and generate a metastasis mass at their new location [131]. In 2012, Andreas Trumpp proposed an advanced model about MCSCs: The ‘metastasis initiating cell’ (MIC) concept. In this model, MICs are functionally distinguished from CSC clones by their metastatic capacity in vivo. Metastatic niche is essential for its expansion in the secondary site [20].

Emerging evidence supports the contributions of CSCs to metastasis and the existence of MCSCs. The most direct evidence comes from the demonstration of this heterogeneity within the colorectal CSCs in a human colon cancer animal model. They proposes that there are three types of distinct CSCs: (1) extensively self-renewing long-term TiCs (LT-TiCs), which possess extensively self-renewal ability and
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By integration of the established concepts of “migrating cancer stem (MCS) -cell” and “metastasis initiating cell” (MIC) with emerging novel identifications of CSCs in early invasion and distant metastasis, we hypothesize that metastatic associated CSCs are formed during the primary tumorigenesis (called invasive cancer stem cells, ICSCs) (Figure 1). The ICSCs have the ability to invade into the ECMs and subsequently penetrate into blood vessels. During these processes, ICSCs can turn into cancer stem cells with the capacity to survive and disseminate in the circulation (called disseminating cancer stem cells, DCSCs). The

can drive metastasis; (2) tumor transient amplifying cells (T-TACs), which predominantly contribute to tumor formation but lack self-renewal and metastasis-forming potential; (3) rare delayed contributing TICs (DC-TICs), which have no contribution to tumor formation but are exclusively active in secondary or tertiary mice [132]. Other supporting evidence mainly comes from studies to demonstrate overlapping profiles of molecules and signaling pathways that regulate both stem cell behaviors and cancer metastasis. CD44+ and CD24−/low breast cancer cells from both primary tumors and lung metastases are highly enriched for CSCs and are able to generate primary tumors and subsequently produce lung metastases in orthotopic models [108]. A distinctive subpopulation of migrating CD133 (+) CXCR4 (+) CSCs identified in the invasive front of pancreatic tumors is essential for tumor metastasis [113]. A subpopulation of CD26 (+) CSCs isolated from both primary and metastatic tumors in colorectal cancer patients with liver metastasis is able to promote distant metastasis in xenograft mice model [133]. It has also been found that ALDH+ breast CSCs are responsible for bone metastasis [134]. Sun and Wang found that ALDH (high) adenoid cystic carcinoma (AdCC) cells possess highly invasive capability and are responsible for mediating metastasis, suggesting ALDH+ CSCs are responsible for mediating AdCC metastasis [135].

The evolving concept of metastatic cancer stem cells

By integration of the established concepts of “migrating cancer stem (MCS) -cell” and “metastasis initiating cell” (MIC) with emerging novel identifications of CSCs in early invasion and distant metastasis, we hypothesize that metastatic associated CSCs are formed during the primary tumorigenesis (called invasive cancer stem cells, ICSCs) (Figure 1). The ICSCs have the ability to invade into the ECMs and subsequently penetrate into blood vessels. During these processes, ICSCs can turn into cancer stem cells with the capacity to survive and disseminate in the circulation (called disseminating cancer stem cells, DCSCs). The
transition from ICSCs to DCSCs is also under the complex regulation of mutations and alterations of signaling activities. Only a subgroup of DCSCs can undergo extravasation and form colonization at the distant organs (called metastatic cancer stem cells, MCSCs).

**Possible origins of MCSCs**

One possible origination of generation of MCSCs is EMT. A direct molecular link between EMT and CSCs is that EMT activators, such as Twist1, can induce EMT and endow stemness properties of CSCs simultaneously [46]. A recent research has demonstrated that activation of Twist1 in tumor cells at the primary site induces EMT and promotes them to disseminate into circulation. Turning off Twist1 in distant sites to allow reversion of EMT is essential for disseminated tumor cells to proliferate and form metastases [89]. However, unlike classic EMT activators, paired-related homeobox transcription factor 1 (Prrx1) suppresses stemness properties in the EMT and dissemination state [136]. Thus, putative CSCs can not only be embedded in the epithelial mass of benign precursors, primary tumors or metastases, but also be linked to EMT/motility in invading and disseminating.

MCSCs may be induced by mesenchymal factors or the cross-talk between CSCs and their microenvironment. CD133+ pancreatic cancer cells exhibit enhanced migration and invasion in the presence of stromal cells [137]. A recent research indicates that infiltrating tumor cells can educate stromal cells to help promoting CSC self-renewal and metastatic formation in metastatic colonization. In this context, infiltrating tumor cells induce stromal POSTN to create a metastatic niche to allow cancer stem cell maintenance and initiate metastatic colonization [125]. Another example is that the CD44v6-colorectal CSCs acquire metastatic capacity after induction of CD44v6 expression by mesenchymal cytokines such as hepatocyte growth factor (HGF), osteopontin (OPN), and stromal-derived factor 1alpha (SDF-1) [107]. Jennifer M. Bailey et al. found that the pre-invasive pancreatic cancer contains a subpopulation of cells with distinct morphologies and CSC-like properties, suggesting that CSCs with invasive ability could emerge from primary sites during early stages of tumorigenesis [138]. Primary tumor-derived pre-metastatic niches in secondary organs will help to create microenvironments suitable for homing or recruitment and colonization by MCSCs, which will be promoted by tumor-derived secreted factors (TDSFs) and bone marrow-derived cells (BMDCs) [119-122].

**Biomarkers for MCSCs**

Several studies have defined MCSCs by using distinctive biomarkers in a variety of human cancers (Table 1). In colorectal cancer, CD133 (+) CXCR4 (+) cells exhibited high potential of invasion and metastasis [139]. Gao et al. identified a distinct pattern of CD110 and CDCP1 in primary CRC tumor samples, and found that both CD110 and CDCP1 were markers of colon CSCs and CD110 (+)/CDCP1 (+) subpopulations mediated organ-specific metastasis [140].

Du et al. reported that PrPc (+) CD44 (+) colorectal CSCs possess high liver metastatic capability [141]. Pang et al. found that CD26 (+)
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Figure 2. Proposed therapies targeting MCSCs. Three different strategies represent the current popular opinions mostly. Targeting distinctive surface biomarkers on CSCs in a variety of human cancers (blue area), such as CD133, CD44 CD90. Targeting aberrant crucial signaling pathways (red area), such as Wnt, Hedgehog, Notch signaling pathways. Targeting tumor microenvironment for MCSCs, such as inhibiting angio-genesis and hypoxia (green area).

colorectal CSCs was identified in both the primary and metastatic lesions in colorectal cancer patients with liver metastasis. In addition, they demonstrated that CD26 (+) cells, but not CD26 (-) cells, gave rise to metastasis in an orthotopic mouse model and was associated with chemoresistance [133]. Our recent findings revealed that CLIC4, ERp29 and Smac/DIABLO isolated from cancer stem-like cells with high metastatic potential stratified the clinical outcomes of patients [142]. Most recently, Todaro et al. showed that CD44v6 was expressed in all colorectal CSCs, and CD44v6 (-) colorectal progenitor cells had no potential to metastasis, but re-expressed CD44v6 in the metastases initiated by these colorectal CSCs [107].

CD44 (+) breast CSCs were defined as breast MCSCs since they were capable of developing spontaneous metastases in orthotopic mouse models [108]. Bartucci et al. identified that TAZ was a mediator of BCSCs-initiated metastasis. Loss of TAZ in breast CSCs significantly impaired metastatic potential and chemoresistance [143]. Chen et al. showed that ANTXR1, a stem cell-enriched functional biomarker, enhanced self-renewal capacity of breast CSCs and metastasis ability of breast cancer cells [144]. CD44+CD24low breast CSCs might be associated with lymph node metastases in breast cancer patients [145], and CXCR4 expression is essential for invasiveness of breast CSCs [146].

Relatively few evidence for marker of MCSCs in other cancers has been identified. CD133-positive CTCs were isolated from CRC patients with liver metastasis [147]. c-Met was considered a novel marker for pancreatic CSCs and its was required for metastasis of pancreatic tumors [148]. c-Met could also serve as a marker for CSCs of human head neck squamous cell carcinoma in HNSCC and was responsible for metastasis [149]. CD117 (+) Stro-1 (+) osteosarcoma CSCs were associated with high metastatic of this cancer [150]. MMP-13 (+) CSCs-like cells of glioblastoma showed highly invasive activity [151]. Nian et al. showed that CXCR4 (+) cells from a lung cancer cell line exhibited cancer metastatic stem cell properties [152].

**Diagnostic and therapeutic significances of MCSCs**

The properties of MCSCs indicate that MCSCs may exist and occur at the early stage of tumor formation. Thus, detection of MCSCs may be a valuable method to make diagnosis or prediction for distant metastasis. Theoretically, MCSCs can be detected in primary tumors, circulations and distant metastatic organs. Disseminating tumor cells in the bone marrow and circulating tumour cells (CTCs) in the peripheral blood of cancer patients can be detected and analyzed at the single cell level. These cells are thought to have highly diagnostic and therapeutic relevance for metastasis [153, 154]. Main approaches for the detection of DTCs and/or CTCs is the immunological assay using antibodies against specific surface markers and PCR-based assay [153]. Further verifications need experiments that aim at
observation of CSC phenotypes and evaluation of tumorigenesis and metastatic abilities.

The hypothesis of MCSCs has fundamental implications for therapies of metastasis. As we propose in Figure 2, targeting MCSCs via their specific surface markers provides a valuable method in therapy for cancer metastasis. Additionally, targeting self-renewal and differentiation pathways, or interrupting the metastatic niche and the quiescent state also represent novel approaches to eliminate MCSCs and reduce cancer recurrence and metastasis.

**Directly targeting CSCs via surface markers**

CD133 is a well characterized marker for putative cancer stem cells [155, 156]. Consistent with its role as a CSC marker, CD133 expression is closely associated with increased chemoradiation resistance and poor prognosis in various cancers [157, 158]. Blockage of CD133 reduced the capacity of the melanoma to metastasize [159], suggesting that CD133 might be an potential therapeutic target for MCSCs in melanoma and other cancer types [155]. CD44 is a marker of CSCs and also an adhesion receptor involved in metastasis and drug-resistance. Several studies indicated that blocking CD44 may represent a novel strategy for targeting CSCs and inhibiting metastatic disease. Inhibition of CD44 using an siRNA decreases cancer cell adhesion to bone marrow endothelial cells in prostate and breast cancer cell lines [160]. A CD44v6-targeting immuno-conjugate, bivatuzumab mertansine, has been evaluated in phase I clinical trial in the case of head and neck squamous cell carcinoma [161]. Piotrowicz et al. demonstrated that targeting CD44 by an A6 peptide (acetyl-KPSSPPEE-amino) blocked the migration and metastatic of CD44-positive cells [162]. Neutralizing CD44 can also inhibit CD90+ cell-mediated tumor formation and metastasis in vivo, suggesting an therapeutic strategy against CD90+ liver CSCs by targeting CD44 [163].

The CSC surface markers are often heterogeneously expressed in different patients with the same cancer, or the tumor cells of different stages in the same patient. Thus, it is important to assess CSCs in biopsies from the primary and/or metastatic tumors in order to select specific targets.

**Targeting self-renewal and differentiation pathways**

Signaling pathways, such as Wnt, Notch, and Hedgehog (Hh), are essential for both regulation of EMT/metastasis and self-renewal of CSCs in several cancers. Development of agents that target critical steps in these pathways will be complicated due to signaling cross-talk, which may, however, provide effective strategies to suppress tumor growth/re-growth and metastasis [164]. Several novel agents targeting Wnt/β-catenin have been developed. Some of these agents have been shown to selectively target the cancer stem cell subpopulation in vivo, inhibit tumor growth and inhibit metastasis [164]. Inhibition of Notch1 can significantly decrease the CD44+CD24−/low subpopulation and inhibited the development of brain metastases from breast cancer [165]. Pharmacologic blockage of aberrant Hedgehog signaling might be an effective therapeutic strategy for inhibiting metastases in human cancers through targeting CSCs. A small-molecule Hedgehog inhibitor, IPI-269609, has been proved to profoundly inhibit systemic metastases in orthotopic xenografts derived from human pancreatic cancer cell lines, accompanied with a significant reduction in the population of ALDH-positive cells (the CSCs in pancreatic cancer) [166].

**Interrupting both CSC niche and pre-metastatic niche**

CSC niche provides appropriate microenvironment for self-renewal and dedifferentiation of CSCs. CSC niche has a complex anatomical unit and functions in determining CSC fate, which is composed of homeostatic processes such as inflammation, EMT, hypoxia and angiogenesis [167]. Targeting CSCs by disturbing their niches may help to inhibit stem cell growth and maintenance, as well as migration [168]. Vascular endothelial cells are critical components of CSC niche. The VEGF-specific antibody bevacizumab have direct and rapid anti-vascular effects and seem to be useful in targeting CSCs by disturbing niche [169]. Hypoxia produces a hypoxic tumor microenvironment, which promotes tumor progression, regulates CSCs and increases their metastatic potential [170]. Interestingly, inhibition of hypoxia eliminates metastasis in mice without effect on the primary tumor, suggesting that hypoxia is an important process in the formation of pre-metastatic niche [120].
Conclusions

In conclusion, the hypothesis of MCSCs in tumor dissemination and the formation of distant metastases have opened a broad view for designing novel diagnostic and therapeutic strategies in order to detect and specifically target MCSCs.

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