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Review Article

Metastatic cancer stem cells: from the concept to therapeutics

Wen-Ting Liao[1], Ya-Ping Ye[1], Yong-Jian Deng[1], Xiu-Wu Bian[2], Yan-Qing Ding[1]

[1]Department of Pathology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, China; [2]Institute of Pathology and Southwest Cancer Center, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

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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 chemo- and/or radio-therapies, 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 immunodeficient 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-differentiate 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], ALDH (+) [48], and PKH26 dye-retaining [49]. Side population

(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], deltaEF1 [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 CSCa 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].

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, PlGF, 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

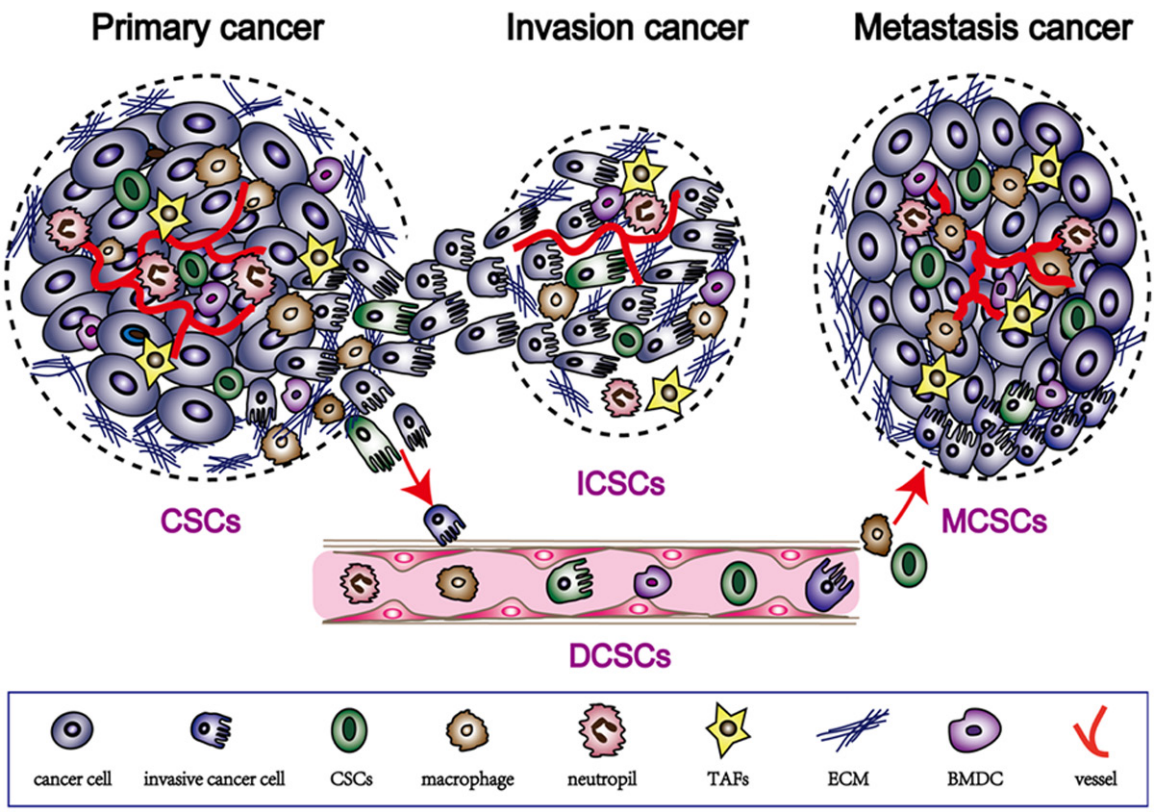

Figure 1. The evolving concept of MCSCs. ICSCs, invading into the ECMs and penetrating into blood vessels; DCSCs, surviving and disseminating in the circulation; MCSCs, undergoing extravasation and forming colonization at the distant organs.

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

Table 1. Biomarkers for MCSCs

Tumor type	Phenotype of MCSCs marker	References
Colorectal cancer	CD133 (+) CXCR4 (+), CD110 (+) CDCP1 (+), PrPc (+) CD44 (+), CD26 (+), CLIC4 (+), ERp29 (+) Smac/DIABLO (+), CD44v6 (+)	[104, 139-142]
Breast cancer	CD44 (+), TAZ (-), ANTXR1 (+), CD44 (+) CD24 (-/low), mCXCR4 (+)	[105, 143-146]
Pancreatic cancer	c-Met (+)	[148]
Head and neck cancer	c-Met (+)	[149]
Osteosarcoma	CD117 (+) Stro-1 (+)	[150]
Glioblastoma	MMP-13 (+)	[151]
Lung cancer	CXCR4 (+)	[151]

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 originations 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 (+)

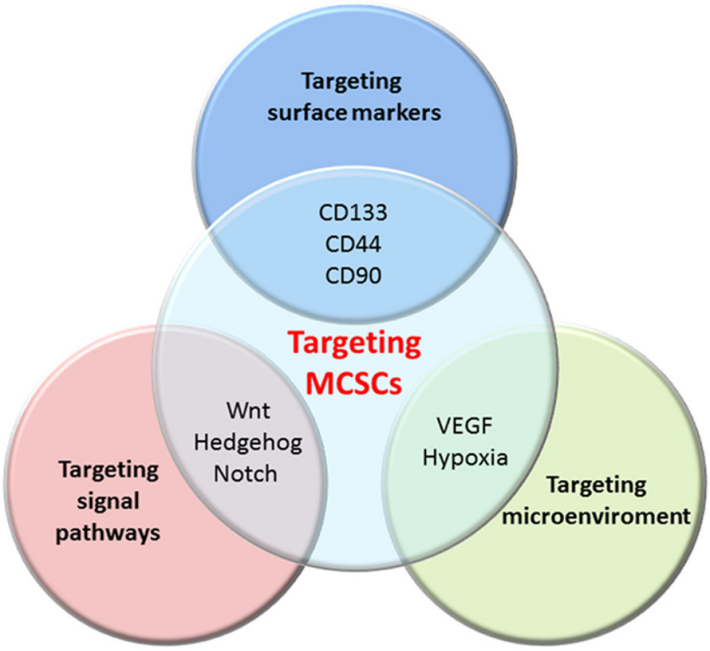

Figure 2. Proposed therapies targeting MCSCs. Three different strategies represent the current popular opinions mostly. Targeting distinctive surface biomarkers on CSCs in a variety of human cancers (blue area), such as CD133, CD44 CD90. Targeting aberrant crucial signaling pathways (red area), such as Wnt, Hedgehog, Notch signaling pathways. Targeting tumor microenvironment for MCSCs, such as inhibiting angiogenesis 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]. $CD44^{+}CD24^{-/low}$ 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.

Address correspondence to: Xiu-Wu Bian, Institute of Pathology and Southwest Cancer Center, Southwest Hospital, Third Military Medical University, Chongqing 400038, China; E-mail: bianxiuwu@263.net; Yan-Qing Ding, Department of Pathology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, China. E-mail: dyqgz@126.com

References

[1] Nguyen DX, Bos PD and Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer 2009; 9: 274-284.

[2] Valastyan S and Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011; 147: 275-292.

[3] Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF and Groom AC. Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am J Patho 1998; 153: 865-873.

[4] Reya T, Morrison SJ, Clarke MF and Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414: 105-111.

[5] Li F, Tiede B, Massague J and Kang Y. Beyond tumorigenesis: cancer stem cells in metastasis. Cell Res 2007; 17: 3-14.

[6] Dreesen O and Brivanlou AH. Signaling pathways in cancer and embryonic stem cells. Stem Cell Rev 2007; 3: 7-17.

[7] Baumann M, Krause M and Hill R. Exploring the role of cancer stem cells in radioresistance. Nat Rev Cancer 2008; 8: 545-554.

[8] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL and Wahl GM. Cancer stem cells-perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res 2006; 66: 9339-9344.

[9] Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA and Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994; 367: 645-648.

[10] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ and Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100: 3983-3988.

[11] Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD and Dirks PB. Identification of human brain tumour initiating cells. Nature 2004; 432: 396-401.

[12] O'Brien CA, Pollett A, Gallinger S and Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007; 445: 106-110.

[13] Huynh CK, Brodie AM and Njar VC. Inhibitory effects of retinoic acid metabolism blocking agents (RAMBAs) on the growth of human prostate cancer cells and LNCaP prostate tumour xenografts in SCID mice. Br J Cancer 2006; 94: 513-523.

[14] Collins AT, Berry PA, Hyde C, Stower MJ and Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 2005; 65: 10946-10951.

[15] Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF and Simeone DM. Identification of pancreatic cancer stem cells. Cancer Res 2007; 67: 1030-1037.

[16] Dalerba P, Cho RW and Clarke MF. Cancer stem cells: models and concepts. Annu Rev Med 2007; 58: 267-284.

[17] Medema JP. Cancer stem cells: the challenges ahead. Nat Cell Biol 2013; 15: 338-344.

[18] Stower H. Telomeres: stem cells, cancer and telomerase linked by WNT. Nat Rev Genet 2012; 13: 521.

[19] Moserle L, Ghisi M, Amadori A and Indraccolo S. Side population and cancer stem cells: therapeutic implications. Cancer Lett 2010; 288: 1-9.

[20] Baccelli I and Trumpp A. The evolving concept of cancer and metastasis stem cells. J Cell Biol 2012; 198: 281-293.

[21] Alison MR, Lim SM and Nicholson LJ. Cancer stem cells: problems for therapy? J Pathol 2011; 223: 147-161.

[22] Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E, Lio P, Macdonald HR and Trumpp A. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 2008; 135: 1118-1129.

[23] Essers MA, Offner S, Blanco-Bose WE, Waibler Z, Kalinke U, Duchosal MA and Trumpp A. IFNalpha activates dormant haematopoietic stem cells in vivo. Nature 2009; 458: 904-908.

[24] Fuchs E. The tortoise and the hair: slow-cycling cells in the stem cell race. Cell 2009; 137: 811-819.

[25] Ishii H, Iwatsuki M, Ieta K, Ohta D, Haraguchi N, Mimori K and Mori M. Cancer stem cells and chemoradiation resistance. Cancer Sci 2008; 99: 1871-1877.

[26] Tehranchi R, Woll PS, Anderson K, Buza-Vidas N, Mizukami T, Mead AJ, Astrand-Grundstrom I, Strombeck B, Horvat A, Ferry H, Dhanda RS, Hast R, Ryden T, Vyas P, Gohring G, Schlegelberger B, Johansson B, Hellstrom-Lindberg E, List A, Nilsson L and Jacobsen SE. Persistent malignant stem cells in del (5q) myelodysplasia in remission. N Engl J Med 2010; 363: 1025-1037.

[27] Bjerkvig R, Johansson M, Miletic H and Niclou SP. Cancer stem cells and angiogenesis. Semin Cancer Biol 2009; 19: 279-284.

[28] Ribatti D. Cancer stem cells and tumor angiogenesis. Cancer Lett 2012; 321: 13-17.

[29] Ping YF, Yao XH, Jiang JY, Zhao LT, Yu SC, Jiang T, Lin MC, Chen JH, Wang B, Zhang R, Cui YH, Qian C, Wang J and Bian XW. The chemokine CXCL12 and its receptor CXCR4 promote glioma stem cell-mediated VEGF production and tumour angiogenesis via PI3K/AKT signalling. J Pathol 2011; 224: 344-354.

[30] Ye XZ, Yu SC and Bian XW. Contribution of myeloid-derived suppressor cells to tumor-induced immune suppression, angiogenesis, invasion and metastasis. J Genet Genomics 2010; 37: 423-430.

[31] Yao XH, Ping YF and Bian XW. Contribution of cancer stem cells to tumor vasculogenic mimicry. Protein Cell 2011; 2: 266-272.

[32] De Maria R, Grignani F, Testa U, Valtieri M, Ziegler BL and Peschle C. Gene regulation in normal and leukaemic progenitor/stem cells. Haematologica 1999; 84 Suppl EHA-4: 8-10.

[33] Bonnet D and Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3: 730-737.

[34] Wang Y, Yang J, Zheng H, Tomasek GJ, Zhang P, McKeever PE, Lee EY and Zhu Y. Expression of mutant p53 proteins implicates a lineage relationship between neural stem cells and malignant astrocytic glioma in a murine model. Cancer Cell 2009; 15: 514-526.

[35] Li H, Collado M, Villasante A, Strati K, Ortega S, Canamero M, Blasco MA and Serrano M. The Ink4/Arf locus is a barrier for iPS cell reprogramming. Nature 2009; 460: 1136-1139.

[36] Utikal J, Polo JM, Stadtfeld M, Maherali N, Kulalert W, Walsh RM, Khalil A, Rheinwald JG and Hochedlinger K. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. Nature 2009; 460: 1145-1148.

[37] Kawamura T, Suzuki J, Wang YV, Menendez S, Morera LB, Raya A, Wahl GM and Izpisua Belmonte JC. Linking the p53 tumour suppressor pathway to somatic cell reprogramming. Nature 2009; 460: 1140-1144.

[38] Takahashi K and Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126: 663-676.

[39] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K and Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131: 861-872.

[40] Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007; 318: 1917-1920.

[41] Okita K, Ichisaka T and Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007; 448: 313-317.

[42] Kim JB, Zaehres H, Wu G, Gentile L, Ko K, Sebastiano V, Arauzo-Bravo MJ, Ruau D, Han DW, Zenke M and Scholer HR. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. Nature 2008; 454: 646-650.

[43] Aoi T, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, Chiba T and Yamanaka S. Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science 2008; 321: 699-702.

[44] Liang Y, Zhong Z, Huang Y, Deng W, Cao J, Tsao G, Liu Q, Pei D, Kang T and Zeng YX. Stem-like cancer cells are inducible by increasing genomic instability in cancer cells. J Biol Chem 2010; 285: 4931-4940.

[45] Yang MH, Hsu DS, Wang HW, Wang HJ, Lan HY, Yang WH, Huang CH, Kao SY, Tzeng CH, Tai SK, Chang SY, Lee OK and Wu KJ. Bmi1 is essential in Twist1-induced epithelial-mesenchymal transition. Nat Cell Biol 2010; 12: 982-992.

[46] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J and Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133: 704-715.

[47] Tang DG. Understanding cancer stem cell heterogeneity and plasticity. Cell Res 2012; 22: 457-472.

[48] Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS and Dontu G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1: 555-567.

[49] Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, Bernard L, Viale G, Pelicci PG and Di Fiore PP. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell 2010; 140: 62-73.

[50] Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA and Brenner MK. A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci U S A 2004; 101: 14228-14233.

[51] Hope KJ, Jin L and Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol 2004; 5: 738-743.

[52] Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS and Polyak K. Molecular definition of breast tumor heterogeneity. Cancer Cell 2007; 11: 259-273.

[53] Park SY, Gonen M, Kim HJ, Michor F and Polyak K. Cellular and genetic diversity in the progression of in situ human breast carcinomas to an invasive phenotype. J Clin Invest 2010; 120: 636-644.

[54] Lottaz C, Beier D, Meyer K, Kumar P, Hermann A, Schwarz J, Junker M, Oefner PJ, Bogdahn U, Wischhusen J, Spang R, Storch A and Beier CP. Transcriptional profiles of CD133+ and CD133- glioblastoma-derived cancer stem cell lines suggest different cells of origin. Cancer Res 2010; 70: 2030-2040.

[55] Horwitz EM. Stem cell plasticity: the growing potential of cellular therapy. Arch Med Res 2003; 34: 600-606.

[56] Martin-Rendon E and Watt SM. Stem cell plasticity. Br J Haematol 2003; 122: 877-891.

[57] Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A and Anversa P. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410: 701-705.

[58] Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A and Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci U S A 2001; 98: 10344-10349.

[59] Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK and Goodell MA. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001; 107: 1395-1402.

[60] Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G and Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998; 279: 1528-1530.

[61] Terskikh AV, Easterday MC, Li L, Hood L, Kornblum HI, Geschwind DH and Weissman IL. From hematopoiesis to neuropoiesis: evidence of overlapping genetic programs. Proc Natl Acad Sci U S A 2001; 98: 7934-7939.

[62] Hendrix MJ, Seftor EA, Hess AR and Seftor RE. Molecular plasticity of human melanoma cells. Oncogene 2003; 22: 3070-3075.

[63] Casal C, Torres-Collado AX, Plaza-Calonge Mdel C, Martino-Echarri E, Ramon YCS, Rojo F, Griffioen AW and Rodriguez-Manzaneque JC. ADAMTS1 contributes to the acquisition of an endothelial-like phenotype in plastic tumor cells. Cancer Res 2010; 70: 4676-4686.

[64] Wang R, Chadalavada K, Wilshire J, Kowalik U, Hovinga KE, Geber A, Fligelman B, Leversha M, Brennan C and Tabar V. Glioblastoma stem-like cells give rise to tumour endothelium. Nature 2010; 468: 829-833.

[65] Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, Maira G, Parati EA, Stassi G, Larocca LM and De Maria R. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature 2010; 468: 824-828.

[66] Valastyan S and Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011; 147: 275-292.

[67] Cameron MD, Schmidt EE, Kerkvliet N, Nadkarni KV, Morris VL, Groom AC, Chambers AF and MacDonald IC. Temporal progression of metastasis in lung: cell survival, dormancy, and location dependence of metastatic inefficiency. Cancer Res 2000; 60: 2541-2546.

[68] Hynes RO. Metastatic potential: generic predisposition of the primary tumor or rare, metastatic variants-or both? Cell 2003; 113: 821-823.

[69] Bernards R and Weinberg RA. A progression puzzle. Nature 2002; 418: 823.

[70] Thiery JP, Acloque H, Huang RY and Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009; 139: 871-890.

[71] Cano A, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F and Nieto MA. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol 2000; 2: 76-83.

[72] Bolos V, Peinado H, Perez-Moreno MA, Fraga MF, Esteller M and Cano A. The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. J Cell Sci 2003; 116: 499-511.

[73] Eger A, Aigner K, Sonderegger S, Dampier B, Oehler S, Schreiber M, Berx G, Cano A, Beug H and Foisner R. DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. Oncogene 2005; 24: 2375-2385.

[74] Liu Y, El-Naggar S, Darling DS, Higashi Y and Dean DC. Zeb1 links epithelial-mesenchymal transition and cellular senescence. Development 2008; 135: 579-588.
[75] Song LB, Li J, Liao WT, Feng Y, Yu CP, Hu LJ, Kong QL, Xu LH, Zhang X, Liu WL, Li MZ, Zhang L, Kang TB, Fu LW, Huang WL, Xia YF, Tsao SW, Li M, Band V, Band H, Shi QH, Zeng YX and Zeng MS. The polycomb group protein Bmi-1 represses the tumor suppressor PTEN and induces epithelial-mesenchymal transition in human nasopharyngeal epithelial cells. J Clin Invest 2009; 119: 3626-3636.
[76] Yilmaz M and Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 2009; 28: 15-33.
[77] Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H, Sprick MR, Kemper K, Richel DJ, Stassi G and Medema JP. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol 2010; 12: 468-476.
[78] Moustakas A and Heldin CH. Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. Cancer Sci 2007; 98: 1512-1520.
[79] Polyak K and Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 2009; 9: 265-273.
[80] Acloque H, Adams MS, Fishwick K, Bronner-Fraser M and Nieto MA. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. J Clin Invest 2009; 119: 1438-1449.
[81] Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, Diehn M, Liu H, Panula SP, Chiao E, Dirbas FM, Somlo G, Pera RA, Lao K and Clarke MF. Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell 2009; 138: 592-603.
[82] Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, Waldvogel B, Vannier C, Darling D, zur Hausen A, Brunton VG, Morton J, Sansom O, Schuler J, Stemmler MP, Herzberger C, Hopt U, Keck T, Brabletz S and Brabletz T. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 2009; 11: 1487-1495.
[83] Battula VL, Evans KW, Hollier BG, Shi Y, Marini FC, Ayyanan A, Wang RY, Brisken C, Guerra R, Andreeff M and Mani SA. Epithelial-mesenchymal transition-derived cells exhibit multilineage differentiation potential similar to mesenchymal stem cells. Stem Cells 2010; 28: 1435-1445.
[84] Asiedu MK, Ingle JN, Behrens MD, Radisky DC and Knutson KL. TGFbeta/TNF(alpha)-mediated epithelial-mesenchymal transition generates breast cancer stem cells with a claudin-low phenotype. Cancer Res 2011; 71: 4707-4719.
[85] Yang L, Ping YF, Yu X, Qian F, Guo ZJ, Qian C, Cui YH and Bian XW. Gastric cancer stem-like cells possess higher capability of invasion and metastasis in association with a mesenchymal transition phenotype. Cancer Lett 2011; 310: 46-52.
[86] Zlobec I and Lugli A. Epithelial mesenchymal transition and tumor budding in aggressive colorectal cancer: tumor budding as oncotarget. Oncotarget 2010; 1: 651-661.
[87] Markl B and Arnholdt HM. Prognostic significance of tumor budding in gastrointestinal tumors. Expert Rev Anticancer Ther 2011; 11: 1521-1533.
[88] Brabletz T, Jung A, Reu S, Porzner M, Hlubek F, Kunz-Schughart LA, Knuechel R and Kirchner T. Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. Proc Natl Acad Sci U S A 2001; 98: 10356-10361.
[89] Tsai JH, Donaher JL, Murphy DA, Chau S and Yang J. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. Cancer Cell 2012; 22: 725-736.
[90] Husemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, Forni G, Eils R, Fehm T, Riethmuller G and Klein CA. Systemic spread is an early step in breast cancer. Cancer Cell 2008; 13: 58-68.
[91] Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW and Hayes DF. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004; 351: 781-791.
[92] Fehm T, Braun S, Muller V, Janni W, Gebauer G, Marth C, Schindlbeck C, Wallwiener D, Borgen E, Naume B, Pantel K and Solomayer E. A concept for the standardized detection of disseminated tumor cells in bone marrow from patients with primary breast cancer and its clinical implementation. Cancer 2006; 107: 885-892.
[93] Baccelli I, Schneeweiss A, Riethdorf S, Stenzinger A, Schillert A, Vogel V, Klein C, Saini M, Bauerle T, Wallwiener M, Holland-Letz T, Hofner T, Sprick M, Scharpff M, Marme F, Sinn HP, Pantel K, Weichert W and Trumpp A. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. Nat Biotechnol 2013; 31: 539-544.
[94] Maheswaran S and Haber DA. Circulating tumor cells: a window into cancer biology and

metastasis. Curr Opin Genet Dev 2010; 20: 96-99.
[95] Balic M, Lin H, Young L, Hawes D, Giuliano A, McNamara G, Datar RH and Cote RJ. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. Clin Cancer Res 2006; 12: 5615-5621.
[96] Theodoropoulos PA, Polioudaki H, Agelaki S, Kallergi G, Saridaki Z, Mavroudis D and Georgoulias V. Circulating tumor cells with a putative stem cell phenotype in peripheral blood of patients with breast cancer. Cancer Lett 2010; 288: 99-106.
[97] Sun YF, Xu Y, Yang XR, Guo W, Zhang X, Qiu SJ, Shi RY, Hu B, Zhou J and Fan J. Circulating stem cell-like epithelial cell adhesion molecule-positive tumor cells indicate poor prognosis of hepatocellular carcinoma after curative resection. Hepatology 2013; 57: 1458-1468.
[98] Tinhofer I, Saki M, Niehr F, Keilholz U and Budach V. Cancer stem cell characteristics of circulating tumor cells. Int J Radiat Biol 2014; 90: 622-7.
[99] Yang ZF, Ngai P, Ho DW, Yu WC, Ng MN, Lau CK, Li ML, Tam KH, Lam CT, Poon RT and Fan ST. Identification of local and circulating cancer stem cells in human liver cancer. Hepatology 2008; 47: 919-928.
[100] Kasimir-Bauer S, Hoffmann O, Wallwiener D, Kimmig R and Fehm T. Expression of stem cell and epithelial-mesenchymal transition markers in primary breast cancer patients with circulating tumor cells. Breast Cancer Res 2012; 14: R15.
[101] Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R and Kasimir-Bauer S. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Res 2009; 11: R46.
[102] Ma J, Lin JY, Alloo A, Wilson BJ, Schatton T, Zhan Q, Murphy GF, Waaga-Gasser AM, Gasser M, Stephen Hodi F, Frank NY and Frank MH. Isolation of tumorigenic circulating melanoma cells. Biochem Biophys Res Commun 2010; 402: 711-717.
[103] Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev 1989; 8: 98-101.
[104] Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 2003; 3: 453-458.
[105] Polyak K and Hahn WC. Roots and stems: stem cells in cancer. Nat Med 2006; 12: 296-300.
[106] Wicha MS. Cancer stem cells and metastasis: lethal seeds. Clin Cancer Res 2006; 12: 5606-5607.
[107] Todaro M, Gaggianesi M, Catalano V, Benfante A, Iovino F, Biffoni M, Apuzzo T, Sperduti I, Volpe S, Cocorullo G, Gulotta G, Dieli F, De Maria R and Stassi G. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. Cell Stem Cell 2014; 14: 342-356.
[108] Liu H, Patel MR, Prescher JA, Patsialou A, Qian D, Lin J, Wen S, Chang YF, Bachmann MH, Shimono Y, Dalerba P, Adorno M, Lobo N, Bueno J, Dirbas FM, Goswami S, Somlo G, Condeelis J, Contag CH, Gambhir SS and Clarke MF. Cancer stem cells from human breast tumors are involved in spontaneous metastases in orthotopic mouse models. Proc Natl Acad Sci U S A 2010; 107: 18115-18120.
[109] Smith MC, Luker KE, Garbow JR, Prior JL, Jackson E, Piwnica-Worms D and Luker GD. CXCR4 regulates growth of both primary and metastatic breast cancer. Cancer Res 2004; 64: 8604-8612.
[110] Su L, Zhang J, Xu H, Wang Y, Chu Y, Liu R and Xiong S. Differential expression of CXCR4 is associated with the metastatic potential of human non-small cell lung cancer cells. Clin Cancer Res 2005; 11: 8273-8280.
[111] Zlotnik A. New insights on the role of CXCR4 in cancer metastasis. J Pathol 2008; 215: 211-213.
[112] Saur D, Seidler B, Schneider G, Algul H, Beck R, Senekowitsch-Schmidtke R, Schwaiger M and Schmid RM. CXCR4 expression increases liver and lung metastasis in a mouse model of pancreatic cancer. Gastroenterology 2005; 129: 1237-1250.
[113] Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ and Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 2007; 1: 313-323.
[114] Conley SJ, Gheordunescu E, Kakarala P, Newman B, Korkaya H, Heath AN, Clouthier SG and Wicha MS. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. Proc Natl Acad Sci U S A 2012; 109: 2784-2789.
[115] Keith B and Simon MC. Hypoxia-inducible factors, stem cells, and cancer. Cell 2007; 129: 465-472.
[116] Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, Shi Q, McLendon RE, Bigner DD and Rich JN. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res 2006; 66: 7843-7848.
[117] Folkins C, Shaked Y, Man S, Tang T, Lee CR, Zhu Z, Hoffman RM and Kerbel RS. Glioma tumor stem-like cells promote tumor angiogenesis and vasculogenesis via vascular endothelial growth factor and stromal-derived factor 1. Cancer Res 2009; 69: 7243-7251.

[118] Yao XH, Ping YF, Chen JH, Xu CP, Chen DL, Zhang R, Wang JM and Bian XW. Glioblastoma stem cells produce vascular endothelial growth factor by activation of a G-protein coupled formylpeptide receptor FPR. J Pathol 2008; 215: 369-376.
[119] Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S and Lyden D. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 2005; 438: 820-827.
[120] Sceneay J, Smyth MJ and Moller A. The pre-metastatic niche: finding common ground. Cancer Metastasis Rev 2013; 32: 449-464.
[121] Kaplan RN, Psaila B and Lyden D. Bone marrow cells in the 'pre-metastatic niche': within bone and beyond. Cancer Metastasis Rev 2006; 25: 521-529.
[122] Psaila B and Lyden D. The metastatic niche: adapting the foreign soil. Nat Rev Cancer 2009; 9: 285-293.
[123] Erler JT, Bennewith KL, Cox TR, Lang G, Bird D, Koong A, Le QT and Giaccia AJ. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the pre-metastatic niche. Cancer Cell 2009; 15: 35-44.
[124] Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci 2012; 33: 207-214.
[125] Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF and Huelsken J. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 2012; 481: 85-89.
[126] Lu X and Kang Y. Hypoxia and hypoxia-inducible factors: master regulators of metastasis. Clin Cancer Res 2010; 16: 5928-5935.
[127] Wang Y, Liu Y, Malek SN, Zheng P and Liu Y. Targeting HIF1alpha eliminates cancer stem cells in hematological malignancies. Cell Stem Cell 2011; 8: 399-411.
[128] Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, Teng SC and Wu KJ. Direct regulation of TWIST by HIF-1alpha promotes metastasis. Nat Cell Biol 2008; 10: 295-305.
[129] Fazilaty H, Gardaneh M, Bahrami T, Salmaninejad A and Behnam B. Crosstalk between breast cancer stem cells and metastatic niche: emerging molecular metastasis pathway? Tumour Biol 2013; 34: 2019-2030.
[130] Massague J. TGFbeta signalling in context. Nat Rev Mol Cell Biol 2012; 13: 616-630.
[131] Brabletz T, Jung A, Spaderna S, Hlubek F and Kirchner T. Opinion: migrating cancer stem cells an integrated concept of malignant tumour progression. Nat Rev Cancer 2005; 5: 744-749.
[132] Dieter SM, Ball CR, Hoffmann CM, Nowrouzi A, Herbst F, Zavidij O, Abel U, Arens A, Weichert W, Brand K, Koch M, Weitz J, Schmidt M, von Kalle C and Glimm H. Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. Cell Stem Cell 2011; 9: 357-365.
[133] Pang R, Law WL, Chu AC, Poon JT, Lam CS, Chow AK, Ng L, Cheung LW, Lan XR, Lan HY, Tan VP, Yau TC, Poon RT and Wong BC. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell 2010; 6: 603-615.
[134] Patel SA, Dave MA, Murthy RG, Helmy KY and Rameshwar P. Metastatic breast cancer cells in the bone marrow microenvironment: novel insights into oncoprotection. Oncol Rev 2011; 5: 93-102.
[135] Sun S and Wang Z. ALDH high adenoid cystic carcinoma cells display cancer stem cell properties and are responsible for mediating metastasis. Biochem Biophys Res Commun 2010; 396: 843-848.
[136] Ocana OH, Corcoles R, Fabra A, Moreno-Bueno G, Acloque H, Vega S, Barrallo-Gimeno A, Cano A and Nieto MA. Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. Cancer Cell 2012; 22: 709-724.
[137] Moriyama T, Ohuchida K, Mizumoto K, Cui L, Ikenaga N, Sato N and Tanaka M. Enhanced cell migration and invasion of CD133+ pancreatic cancer cells cocultured with pancreatic stromal cells. Cancer 2010; 116: 3357-3368.
[138] Bailey JM, Alsina J, Rasheed ZA, McAllister FM, Fu YY, Plentz R, Zhang H, Pasricha PJ, Bardeesy N, Matsui W, Maitra A and Leach SD. DCLK1 marks a morphologically distinct subpopulation of cells with stem cell properties in preinvasive pancreatic cancer. Gastroenterology 2014; 146: 245-256.
[139] Zhang SS, Han ZP, Jing YY, Tao SF, Li TJ, Wang H, Wang Y, Li R, Yang Y, Zhao X, Xu XD, Yu ED, Rui YC, Liu HJ, Zhang L and Wei LX. CD133 (+) CXCR4 (+) colon cancer cells exhibit metastatic potential and predict poor prognosis of patients. BMC Med 2012; 10: 85.
[140] Gao W, Chen L, Ma Z, Du Z, Zhao Z, Hu Z and Li Q. Isolation and phenotypic characterization of colorectal cancer stem cells with organ-specific metastatic potential. Gastroenterology 2013; 145: 636-646, e635.
[141] Du L, Rao G, Wang H, Li B, Tian W, Cui J, He L, Laffin B, Tian X, Hao C, Liu H, Sun X, Zhu Y, Tang DG, Mehrpour M, Lu Y and Chen Q. CD44-positive cancer stem cells expressing cellular

prion protein contribute to metastatic capacity in colorectal cancer. Cancer Res 2013; 73: 2682-2694.
[142] Deng YJ, Tang N, Liu C, Zhang JY, An S, Peng YL, Ma LL, Li GQ, Jiang Q, Hu CT, Wang YN, Liang YZ, Bian XW, Fang W and Ding Y. CLIC4, ERp29 and Smac/DIABLO derived from metastatic cancer stem-like cells stratifies prognostic risks of colorectal cancer. Clin Cancer Res 2014; 20: 3809-17.
[143] Bartucci M, Dattilo R, Moriconi C, Pagliuca A, Mottolese M, Federici G, Benedetto AD, Todaro M, Stassi G, Sperati F, Amabile MI, Pilozzi E, Patrizii M, Biffoni M, Maugeri-Sacca M, Piccolo S and De Maria R. TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells. Oncogene 2014; [Epub ahead of print].
[144] Chen D, Bhat-Nakshatri P, Goswami C, Badve S and Nakshatri H. ANTXR1, a stem cell-enriched functional biomarker, connects collagen signaling to cancer stem-like cells and metastasis in breast cancer. Cancer Res 2013; 73: 5821-5833.
[145] Wei W, Hu H, Tan H, Chow LW, Yip AY and Loo WT. Relationship of CD44+CD24-/low breast cancer stem cells and axillary lymph node metastasis. J Transl Med 2012; 10 Suppl 1: S6.
[146] Krohn A, Song YH, Muehlberg F, Droll L, Beckmann C and Alt E. CXCR4 receptor positive spheroid forming cells are responsible for tumor invasion in vitro. Cancer Lett 2009; 280: 65-71.
[147] Pilati P, Mocellin S, Bertazza L, Galdi F, Briarava M, Mammano E, Tessari E, Zavagno G and Nitti D. Prognostic value of putative circulating cancer stem cells in patients undergoing hepatic resection for colorectal liver metastasis. Ann Surg Oncol 2012; 19: 402-408.
[148] Li C, Wu JJ, Hynes M, Dosch J, Sarkar B, Welling TH, Pasca di Magliano M and Simeone DM. c-Met is a marker of pancreatic cancer stem cells and therapeutic target. Gastroenterology 2011; 141: 2218-2227, e5.
[149] Sun S and Wang Z. Head neck squamous cell carcinoma c-Met(+) cells display cancer stem cell properties and are responsible for cisplatin-resistance and metastasis. Int J Cancer 2011; 129: 2337-2348.
[150] Adhikari AS, Agarwal N, Wood BM, Porretta C, Ruiz B, Pochampally RR and Iwakuma T. CD117 and Stro-1 identify osteosarcoma tumor-initiating cells associated with metastasis and drug resistance. Cancer Res 2010; 70: 4602-4612.
[151] Inoue A, Takahashi H, Harada H, Kohno S, Ohue S, Kobayashi K, Yano H, Tanaka J and Ohnishi T. Cancer stem-like cells of glioblastoma characteristically express MMP-13 and display highly invasive activity. Int J Oncol 2010; 37: 1121-1131.
[152] Nian WQ, Chen FL, Ao XJ and Chen ZT. CXCR4 positive cells from Lewis lung carcinoma cell line have cancer metastatic stem cell characteristics. Mol Cell Biochem 2011; 355: 241-248.
[153] Pantel K, Brakenhoff RH and Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. Nat Rev Cancer 2008; 8: 329-340.
[154] Pantel K and Alix-Panabieres C. The clinical significance of circulating tumor cells. Nat Clin Pract Oncol 2007; 4: 62-63.
[155] Wu Y and Wu PY. CD133 as a marker for cancer stem cells: progresses and concerns. Stem Cells Dev 2009; 18: 1127-1134.
[156] Grosse-Gehling P, Fargeas CA, Dittfeld C, Garbe Y, Alison MR, Corbeil D and Kunz-Schughart LA. CD133 as a biomarker for putative cancer stem cells in solid tumours: limitations, problems and challenges. J Pathol 2013; 229: 355-378.
[157] Ma S, Lee TK, Zheng BJ, Chan KW and Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene 2008; 27: 1749-1758.
[158] Piao LS, Hur W, Kim TK, Hong SW, Kim SW, Choi JE, Sung PS, Song MJ, Lee BC, Hwang D and Yoon SK. CD133+ liver cancer stem cells modulate radioresistance in human hepatocellular carcinoma. Cancer Lett 2012; 315: 129-137.
[159] Rappa G, Fodstad O and Lorico A. The stem cell-associated antigen CD133 (Prominin-1) is a molecular therapeutic target for metastatic melanoma. Stem Cells 2008; 26: 3008-3017.
[160] Draffin JE, McFarlane S, Hill A, Johnston PG and Waugh DJ. CD44 potentiates the adherence of metastatic prostate and breast cancer cells to bone marrow endothelial cells. Cancer Res 2004; 64: 5702-5711.
[161] Riechelmann H, Sauter A, Golze W, Hanft G, Schroen C, Hoermann K, Erhardt T and Gronau S. Phase I trial with the CD44v6-targeting immunoconjugate bivatuzumab mertansine in head and neck squamous cell carcinoma. Oral Oncol 2008; 44: 823-829.
[162] Piotrowicz RS, Damaj BB, Hachicha M, Incardona F, Howell SB and Finlayson M. A6 peptide activates CD44 adhesive activity, induces FAK and MEK phosphorylation, and inhibits the migration and metastasis of CD44-expressing cells. Mol Cancer Ther 2011; 10: 2072-2082.
[163] Lee TK, Cheung VC and Ng IO. Liver tumor-initiating cells as a therapeutic target for hepatocellular carcinoma. Cancer Lett 2013; 338: 101-109.

[164] Takebe N, Harris PJ, Warren RQ and Ivy SP. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol 2011; 8: 97-106.
[165] McGowan PM, Simedrea C, Ribot EJ, Foster PJ, Palmieri D, Steeg PS, Allan AL and Chambers AF. Notch1 inhibition alters the CD44hi/CD-24lo population and reduces the formation of brain metastases from breast cancer. Mol Cancer Res 2011; 9: 834-844.
[166] Feldmann G, Fendrich V, McGovern K, Bedja D, Bisht S, Alvarez H, Koorstra JB, Habbe N, Karikari C, Mullendore M, Gabrielson KL, Sharma R, Matsui W and Maitra A. An orally bioavailable small-molecule inhibitor of Hedgehog signaling inhibits tumor initiation and metastasis in pancreatic cancer. Mol Cancer Ther 2008; 7: 2725-2735.
[167] Ye J, Wu D, Wu P, Chen Z and Huang J. The cancer stem cell niche: cross talk between cancer stem cells and their microenvironment. Tumour Biol 2014; 35: 3945-3951.
[168] Yi SY, Hao YB, Nan KJ and Fan TL. Cancer stem cells niche: a target for novel cancer therapeutics. Cancer Treat Rev 2013; 39: 290-296.
[169] Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, Mino M, Cohen KS, Scadden DT, Hartford AC, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Chen HX, Shellito PC, Lauwers GY and Jain RK. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004; 10: 145-147.
[170] Hill RP, Marie-Egyptienne DT and Hedley DW. Cancer stem cells, hypoxia and metastasis. Semin Radiat Oncol 2009; 19: 106-111.