Characteristics of the cancer stem cell niche and therapeutic strategies

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Abstract
Distinct regions harboring cancer stem cells (CSCs) have been identified within the microenvironment of various tumors, and as in the case of their healthy counterparts, these anatomical regions are termed "niche." Thus far, a large volume of studies have shown that CSC niches take part in the maintenance, regulation of renewal, differentiation and plasticity of CSCs. In this review, we summarize and discuss the latest findings regarding CSC niche morphology, physical terrain, main signaling pathways and interactions within them. The cellular and molecular components of CSCs also involve genetic and epigenetic modulations that mediate and support their maintenance, ultimately leading to cancer progression. It suggests that the crosstalk between CSCs and their niche plays an important role regarding therapy resistance and recurrence. In addition, we updated diverse therapeutic strategies in different cancers in basic research and clinical trials in this review. Understanding the complex heterogeneity of CSC niches is a necessary pre-requisite for designing superior therapeutic strategies to target CSC-specific factors and/or components of the CSC niche.

Keywords: Cancer stem cells, Chemokines, Hypoxia, Metastasis, Niche/microenvironment

Definitions and background
Stem cells are undifferentiated cells that have the capacity to self-renew and proliferate for longer than non-stem cells as well as having the ability to generate multiple types of cells in the body [1, 2]. Regardless of their proliferation potential, stem cells are usually quiescent, remaining in an inactive dormant state (G1, G0) and are protected from cellular damage or mutations [3, 4].

In general, stem cells exhibit various levels of differentiation potential, starting with totipotency (greatest differentiation potential), pluripotency, multipotency, oligopotency and finally unipotency/monopotency (stem cells can generate only one cell type) [4]. The survival of stem cells is ensured at two levels: cellular asymmetrical division and population asymmetrical renewal. With asymmetrical division, one stem cell gives rise to a stem cell and one differentiating cell. With population asymmetry, one stem cell produces two stem cells or two differentiating cells. As the frequency of these two processes are similar, both result in a comparable amount of stem cells and differentiating cells [5].

Some types of tissues have higher turnover and renewal rates than others (such as gastric and intestinal tissues and bone marrow as compared to brain or liver tissues), making the capability of stem cell self-renewal essential for their replenishment and providing an explanation for their broad presence in such tissues [6]. Many studies have explored whether the stemness of these cells is a result of an independent mechanism or the interaction with other cells and their surrounding environment and...
it has been established that these interactions are vital for the survival of stem cells [7].

Due to the inherent nature of stem cells, their use in regenerative medicine and stem cell-based therapy has been widely explored. However, to date, clinical applications of such therapeutic approaches are only available in hematopoietic malignancies and limited immune deficiencies [4].

Similar to normal stem cells, Cancer stem cells (CSCs) are also able to self-renew and differentiate into tumor cells [8]. With the multipotent capacity of CSCs, heterogeneous lineages of different kinds of cancer cells can be generated [9]. The ability of CSCs to initiate and constitute tumor lesions together with features such as differentiation and chemo/radiotherapy resistance has been widely investigated [10]. Although many features of CSCs are similar to normal stem cells, an important difference is that normal stem cells are usually dormant during adulthood until their regeneration ability is required, whereas this ability of CSCs is active. This leads to the possibility of detecting CSC based on markers found specifically in active stem cells but not dormant ones, such as PSF1 which has been identified in upregulated hematopoietic stem cells (HSCs) [7]. Many technologies and assays have been proposed for the identification of CSCs including microsphere assay, serial dilution assay, side population assay and aldehyde dehydrogenase activity assay, the gold standard for the CSC identification is the sphere colony formation assay and in vivo xenotransplantation of tumor cells into immunodeficient mouse models. Although each of these methods has limitations, they assist in identifying CSCs in several tumors such as breast cancer, brain cancer, liver cancer, stomach and colon cancer [10].

The fundamental mechanism by which CSCs function in cancer progression is unclear. A number of proposals have been made with regards to this, such as CSCs derived from normal somatic cells regain stemness due to cancerous changes and acquired genetic/epigenetic mutations [11]. For this, two models prevail, namely the hierarchical and stochastic models. In the hierarchical model, tumor initiation begins with stem cells that escape normal growth control and regulation. Therefore, they can transform into CSCs which give rise to a distinct population of cells forming the biological basis of tumors. This model has been validated clinically and would explain why only a full elimination of CSCs can prevent the relapse of cancers. Nevertheless, this model is unable to explain the interaction between CSCs and other differentiated cancer cells. In contrast, the stochastic model suggests that under suitable conditions all tumor cells (differentiated or not) can initiate further lesions. However, this model has limitations in explaining the relation between tumor heterogeneity and the capacity of initiating tumors. These findings have led to the concept of cellular plasticity, where both models are merged. Based on the genetic/epigenetic and microenvironmental signals, it is suggested that cancer cells have the ability to shift between stemness and differentiation states [12].

The microenvironment of CSCs is essential for their function and due to their complex nature and interactions with other components and factors, is referred to as the CSC niche. The CSC niche usually includes niche cells, such as cancer cells, stromal and endothelial cells extracellular matrix (ECM), signaling molecules, intrinsic factors, blood vessels and other cellular and acellular components such as exosomes. The components and structure of the niche can vary among organisms and different types of tissues in order to provide distinct functions in response to the needs of the tumor (Fig. 1) [13–16]. CSC niches have been identified in many locations including intestinal, tissues and neural tissues, aiding the regulation and maintenance of stem cell renewal and differentiation [6, 17].

Due to the rarity of stem cells (compared to non-stem cells), it is challenging to clearly elucidate the mechanisms in the CSC niche [1]; however, some advances have been made with regards to this. The regulation mechanisms in the niche includes intrinsic mechanisms (associated with transcription factors expressed by cells), and extrinsic mechanisms (based on the signaling of the microenvironment and the connection to ECM). Disruption or interference in these mechanisms can lead to phenotypic changes that alter homeostasis of the niche [18]. Since the niche can contain more than one type of stem cells, competition between different stem cells within the niche is considered to control its function by factors such as E-cadherin [19], where the strength of the stem cell-niche connection can directly affect the fate of stem cells [14]. Even when only one type of stem cells are present in a niche, they still may lose their connection and, therefore, be replaced due to limited space or competition for occupancy [5] and this concept is crucial with regards to CSC niches.

Another important CSC feature is their ability to change and modify nearby stroma by CSC secreted proteins and molecular components, such as ECM proteins. The mechanism of ECM regulation has already been identified in different mammalian stem cells. In return, the niche can effectively regulate the biochemical status of keeping CSC in dormant. This is essential for the fate and plasticity of CSC and plays a role in their resistance to conventional therapies [15]. In order to better understand the characteristics of the CSC niche, we summarized the molecular features and mechanisms of the niche and reviewed the different therapeutic strategies
targeting the CSC niche in various cancers from both basic and clinical aspects.

**Conditions of the CSC niche**

**Hypoxia and its role in CSCs**

Hypoxia is known for its role in the maintenance of the undifferentiated status of CSCs. The location of CSCs in hypoxic niches allows them to easily keep their slow cell cycling status with reduced proliferation, compared to a cycle with normal levels of oxygen. It is suggested that the rapid expansion of tumor mass and its vascular supply system might be the reason for tumor hypoxia that is responsible for the activation of several signaling pathways required in CSC functions. In solid tumors, the rapid growth of tumor tissues leads to a decrease in the quality of vascularization which affects the perfusion of blood and diffusion of substances it carries. That can be a cause for the poor outcome and the resistance of hypoxic niches against chemotherapy [20]. The hypoxic state of tumors is also linked to therapeutic resistance and local invasion [21, 22]. It has also been shown that HSCs under hypoxic conditions can enhance the stemness of HSCs and shift cells from a quiescence to renewal phase [23]. Low levels of oxygen can also protect cells from DNA damage caused by oxidative stress which are common in aerobic metabolism. Studies have shown that rates of cellular damage are significantly higher at 20% O₂ relative to 3% O₂. Low oxygen tension of 1% has also been shown to maintain the pluripotency of stem cells; therefore, hypoxia is essential for maintaining CSC characteristics [16].

In contrast to aerobic environments, hypoxic tissues use anaerobic glycolysis to metabolize glucose into lactate which produces much less ATP than in normal metabolism. This explains why cancer cells use higher rates of glucose compared to normal cells in aerobic conditions. However, reduced mitochondrial function is required, which is one way in which metabolic needs are down regulated in cancer cells and hypoxic niches. Such a difference between cancer cells and normal cells is usually controlled by the changes of oncogenes and tumor suppressor mutation profiles [20].

Many elements and factors participate in the control of hypoxic niches and related pathways such as mammalian target of rapamycin (mTOR) and the endoplasmic reticulum (ER) stress response. Examples of important transcription factors involved in the niche mechanisms are hypoxia inducible transcription factors (HIFs). Belonging to bHLH–PAS family of transcriptional factors, HIFs regulate many genes and play a role in oxygen homeostasis, glucose and iron metabolism and erythropoiesis [24–26]. These factors are also considered to be involved in several mechanisms such as the survival of cells under hypoxic conditions, cellular transcriptional responses, and activation of several signaling pathways. For example, expression of HIF-1α, CSC can promote tumor progression and metastasis. This transcription factor also affects genes involved in the regulation of the Notch and...
Oct4 pathways, as well as genes responsible for angiogenesis such as VEGF and GLUT-1 [1] by binding to HIF-1β and translocating to the nucleus where it can activate gene transcription. HIF signaling pathway can activate the angiogenic switch during tumor progression which is needed for the maintenance of oxygen homeostasis during the growth of tumor mass [27] (Fig. 2).

HIFs also play a role in regulating CSC proliferation, self-renewal, and tumorigenicity. The surface markers of CSC such as CD133 and CD44 show increased expression in hypoxic conditions [28, 29]. With regards to therapy response, radiation has been found to double HIF-1 activity within 24–48 h following exposure, imposing further limitations on the outcome of such therapeutic approaches in cancer patients. Even after HIF activity is lost (usually occurring within minutes of activation), the targets may still show certain activities [20].

**Metastasis of the CSC**

Metastasis of CSCs was originally studied in hematopoietic malignancies followed by solid tumors which exhibited a similar phenomenon in brain, colon, breast,
skin and pancreas cancers [30–34]. Most of these cancers are associated with specific metastasis-related genes as well as epigenetic amplification of cellular survival and renewal mechanisms than depend on driver mutations. Although migration occurs at an early stage of tumor progression, metastasis appears to take longer, due to the fact that not every migrated cell can initiate a tumor in distant locations and form a metastatic colony. Many conditions and mechanisms are required in both the migrated cells and the novel home tissues for this to occur, for example migrated cells must overcome many obstacles including lethal signals from reactive stroma that can downregulate the anti-apoptotic pathways in CSCs during the journey to the new environment [35]. CSCs must also survive the lack of growth factors and other environmental factors that are used to support their stemness and regulate their proliferation in the original niche [12, 36, 37]. Finally, after reaching the new site, the survivor CSCs need to induce specific niche factors to modulate the new niche according to their requirements.

It has also been found that one of the important stromal factors is POSTN (periostin) that normally plays a role in extracellular matrix formation and the development and function of teeth, bones, and heart tissues. In primary lesions, fibroblasts are responsible for POSTN expression; therefore, migrated tumor cells have to induce this factor in secondary lesions in order to successfully infiltrate into the new organs. POSTN is therefore a potential target for anti-metastasis therapies as it is considered to play a role in cancer stem cell maintenance. However, its deficiency only affects metastasis of tumors, but not normal cells nor primary lesions. Therefore, combination therapies may be necessary, but targeting POSTN alone may still prevent metastasis [38].

Following invasion of a new site, CSCs form a new niche to ensure their survival in the new environment where they face an aggressive immune response and a lack of survival signals and supportive stroma. Although most organs and tissues fend off CSC metastasis, some of these target tissues may be more susceptible than others. This creates an opportunity for CSCs to modulate the surrounding environment and build the metastatic niche (a novel niche in the new site that contains CSCs, niche components produced by CSCs themselves, and components produced by surrounding stromal cells) [12, 35].

Epithelial to mesenchymal transition
During the development of tissues, some epithelial cells exhibit changes in their characteristics (such as a loss of adhesion contact with other cells and cellular polarity) and transform into a mesenchymal type with improved ability to relocate into different tissues. This phenomenon is termed the epithelial to mesenchymal transition (EMT) [1]. In cancer niches, transcription factors (e.g., Snail, Twist, Six1, Slug, Cripto, Zeb 1–2, E12-46, and others) can affect the connection of cells by activating EMT and downregulating important molecules such as E-cadherin and catenins. These molecules play an essential role in cellular adherens junctions (AJs) [9]. EMT is important during the development of organisms due to its impact on tissue morphogenesis; however, it can become a cause of concern in cancer tissues due to the risk of metastasis. In addition, cells having undergone EMT are associated with stronger invasiveness and resistance against apoptosis. They usually acquire features similar to CSCs and recent studies to suggest that the CSC itself may be a result of EMT (since all signals and conditions needed to initiate EMT can come from tumor microenvironment) [15]. It has also been reported that the de-differentiation of cancer cells into CSCs occurs after those cells have gone through EMT and migrate to other locations, thus indicating the important role of EMT in this process [39]. The interactions within the niche between microenvironment cells and components can also initiate EMT, with participation of cytokines and growth factors. Such interactions were also found to play a role in maintaining stemness of CSCs in several cancers such as breast and oral squamous cancers [15] (Fig. 3).

Additional studies found that EMT indeed strongly correlates with hypoxia, with HIF-1α activating several EMT-related transcription factors such as Snail and Twist. Hypoxia can also elevate the CSC self-renewal genes expression levels and activate related signaling pathways such as Wnt and Notch [40, 41]. By activating EMT, hypoxic niches begin a chain of steps that eventually lead to metastasis, which also includes extracellular matrix modulation, intravasation, circulation, extravasation, homing, pre-metastatic niche formation, and mesenchymal epithelial transition (MET) [20].

Molecular mechanisms in the CSC niche

Essential molecules in the niche
The homeostasis of the CSC niche is based on the interaction between different cells and components of the microenvironment. In order to maintain this homeostasis, many complicated mechanisms and molecules are required. Some of these molecules have been mentioned already in this review. In this section, we discuss other important molecules in the CSC niche modulation such as chemokines, cytokines and adhesion molecules.

Chemokines are common components in niche interactions, with one of the important chemokines discovered being SDF-1α and its receptor CXCR4. Studies in HSC found CXCR4 as essential for the retention, maintenance, and homing of tumor cells. The receptor is also
important for the regulation of the CSCs’ renewal capacity. Mesenchymal stem cells (MSCs) are considered as a major source of the SDF-1 production whose expression is linked to metastasis in solid tumors (e.g., breast, liver, and lung cancers). Therefore, this chemokine has been suggested to play a contributing role in the changes that migrated CSCs apply on the surrounding environment during the initiation of metastasis colonization [1, 6, 15]. Another important component is reactive oxygen species (ROS) which usually affects the hypoxic conditions of the cancer niche in a dynamic manner. This can lead to DNA damage and changes in protein expression which are required in DNA repair [20]. Altering ROS levels in the cell also affects cellular stress responses which may favor tumor cells over normal ones. By increasing the levels of several factors (e.g., HIF-1α, HGF, EGF, TGF-β, TNF-α, TPA, MMP-3, and even micro-RNA), ROS can enhance and help the EMT and promote the metastasis of tumor cells [42].

Another family of molecules required for CSC metastasis is the cytokine family. CSCs, together with other tumor cells, can secrete different cytokines in the niche. Such cytokines and chemokines can further recruit several types of immune cells to create immunosuppression (e.g., tumor-associated neutrophils (TAN), tumor-associated macrophages (TAM), and myeloid-derived suppressor cells (MDSC)). Other cells involved in the niche region and are affected by CSCs include cancer-associated fibroblasts (CAF), which are known to increase proliferation, enhance ECM production and secret essential factors such as CXCL12, VEGF, PDGF, and HGF [12].

A crucial ability of stem cell niches is the self-renewal of cells and cell–cell adhesion. Such adhesion relies on several molecular mechanisms and factors expressed in both the niche cells and stem cells. E-cadherin is a well-known example of such a cell adhesion molecule. In general, cadherins can modulate cell adhesion by interacting with intra- and extracellular domains, linking cells to cytoskeleton-associated proteins and nearby cells [43]. In doing so, cadherins can form strong AJs between cells. Cadherins have been found in stem cells in several types of tissues including brain stem cells and HSCs. Some cadherins can even regulate gene expression and signaling pathways by interacting with certain proteins and receptors. For example, N-cadherin can regulate the signaling by interacting with FGF receptors and also affect HSCs into quiescence by a similar mechanism.

In addition to cadherins, stem cells can also express integrins which are transmembrane molecules that modulate ECM interactions. Integrins can bind to ECM through certain proteins (e.g., collagen and laminin) and bind to surface adhesion molecules (e.g., CD54) and vascular cell adhesion molecules (e.g., CD106). Due to their ability to modulate cell adhesions, both cadherins and integrins are essential for spindle orientation of certain
tissues (e.g., skin and brain) where the spindle of stem cells determines the type of division (symmetric or asymmetric division). It has been shown that the removal of such molecules can easily translate into randomized orientations. Other cellular mechanisms such as cell polarity, maintenance and signaling pathways can also be affected by adhesion molecules; therefore, it is difficult to draw a clear link between cellular adhesion and other cellular events. Other molecules that play a role in stem cell adhesion include Connexin 43 (a junction protein that support HSCs in stroma and affect CXCL12 secretion) and Vcam 1 [14].

Cancer-induced inflammation is a hallmark in solid tumor progression and considered to be essential in modifying their microenvironment. Although chemokines and cytokines, tumor surrounding cells (e.g., endothelial, mesenchymal, pericytes and fibroblast cells) can communicate directly with immune cells such as macrophages, neutrophiles, NK cells, T and B lymphocytes and other immune cells. Such interactions and the level of activity of each type of cell determine the resulting immune responses (pro or anti-tumor). For example, difference with tumor-induced inflammation is that it takes the profile of chronic inflammatory responses and uses cytokines and chemokines not only to communicate but also to recruit immune elements in order to resemble an unhealed wound. Consequently, the niche would remain in tumor favoring conditions such as hypoxia and EMT to enhance the survival and functioning of cells [9].

A complicated network of molecules is therefore responsible for the inflammatory response in the niche, and some of these are also involved in other pathways and functions. IL-6, IL-8, TGF-β, NFκ and TNF-α are some of the elements shown to play a role in the tumor-induced inflammatory environment. IL-6 is an important cytokine linked to proliferation, differentiation, and maturity of cells, making this molecule a promising target for many anti-tumor therapies. In CSCs, IL-6 has been found to play a role in Notch-3 regulation that leads to the malignant behavior of cells in human ductal breast carcinoma. The role of IL-6 in regulating CSCs was also studied in other types of human cancer (e.g., glioblastoma), where targeting it led to cellular apoptosis and reduction in tumor growth. NFκB pathway has also been found to be activated by inflammatory cytokines in the CSC niche. This pathway plays a role in modulating EMT factors Twist, Snail, and Slug. Therefore, NFκB is important for CSC migration and invasion in human cancers (e.g., pancreas, skin, and ovarian cancer) [1].

Current CSC-associated studies focus on their molecular features and differences and discovered novel markers including CD44, CD133, and CD24 [28, 29, 44]. However, markers specificity is still a challenge, since many are shared with normal stem cells and progenitors [3]. Several markers have already been linked to certain types of CSCs (breast cancer: CD44+ /CD24−/ALDH+; leukemia: CD34+/CD8−; liver: CD90+; pancreas: CD44+/CD24+/ ESA+; epidermal surface antigen) [11]. Therefore, more efforts are required to identify CSC markers as there are contradicting reports regarding the exact role of markers in CSCs [6].

**Genetics and epigenetic modulations in the CSC niche**

The genetic diversity and heterogeneity of cancers has led to exploration of their genetic background and importance in CSC niches. Differences in genetic features or mutations among CSC clones can determine their phenotype (such as the level of activeness or dormancy, which can directly affect the survival of CSCs against chemotherapies) [45]. With the bidirectional relation between CSCs and their niches, genetic alterations on both sides can affect or determine the fate of CSCs or their link to the niche. This shows the advantage of the slow cycling of CSCs which helps avoid genetic mutations (which might not fit with the niche requirements) [3, 46]. So far, two models prevail in explaining the intratumor heterogeneity, namely the hierarchical model which proposes that CSCs are originally organized in their niches in a cellular hierarchy similar to normal niches and the clonal evolution model which suggests that different somatic mutations and epigenetic alterations are acquired by different sub-clones of CSCs during tumor evolution, leading to molecular and biological differences among clones [8]. CSC niche conditions also influence the DNA profile and stability; for example, severe hypoxia leads to DNA replication arrests, which introduces different biological actions within the cells and adds to the intratumor heterogeneity [47, 48]. As mentioned earlier, several transcriptional factors and signaling pathways can also affect the genetic profile of CSCs [49].

DNA methylation is a type of common epigenetic modification, and evidence is accumulating, suggesting that DNA methylation is a critical epigenetic reprogramming mechanism that may play a crucial role in CSCs biology [50]. BEX1, under the regulation of DNA methyltransferases 1 (DNMT1), was shown to have differential expression levels in Hepatoblastoma, CSC-hepatocellular carcinoma (HCC), and non-CSC HCC patients. This is essential for the self-renewal and maintenance of liver CSCs through activation of Wnt/β-catenin signaling [51]. Huang W et al. performed whole-genome bisulfite sequencing on tumor-repopulating cells (TRC), which are cancer stem cell (CSC)-like cells with highly tumorigenic and self-renewing abilities, their results showed that CSCs markers were biased toward altering their methylation in non-CG methylation and enriched in the
involved in the adhesion of CSCs in the niche, making it difficult to draw a clear line between the role of Notch in signaling and adhesion, especially in niches such as those in skin tissues [61, 62]. All these functions of the Notch pathway make it a promising target of anti-tumor therapeutic approaches, regardless of its non-specific CSC nature. For example, blocking Notch-4 in breast cancer affected the ability of new cells formation [15].

The Wnt is another pathway and is similar to Notch as it is involved in the development and a functioning regulation of normal stem cells and therefore strongly correlates with CSCs. The hyper activation of Wnt has been shown to initiate cancer in the pituitary gland in animal models and increases the CSCs’ symmetrical division [63–65]. In HCC in particular, the Wnt/β-catenin pathway activation increases CSC activity and cells proliferation. Such an activation is controlled by PTEN, which is a tumor suppressor gene. Studies have shown that controlling or inhibiting the Wnt/β-catenin pathway can elevate the efficiency of drugs and other therapeutic approaches [66–68]. This pathway is also a potential target for future molecular therapies, since it depends on the concentration of β-catenin within the cytoplasm [69]. Together with Notch, Wnt is also involved in stemness stimulation in CSCs, which is essential for the identity of these cells [37, 70].

The Hedgehog (Hh) pathway also plays a pivotal role in the CSC niche. In addition to its involvement in the development of cancer, it plays a role in determining the invasiveness and histological differentiation in tumors. Studies found that the simultaneous activation of the Hh pathway and EMT may be a reason for the chemoresistance that HCC cells usually exhibit [59]. Many drugs have been found to target Hh pathway (e.g., vismodegib, itraconazole, cyclopamine, and PTCN antibodies) to regulate CSC functions and inhibit tumor growth [11, 15].

Another CSC controlling pathway is TGF-β. A recent study showed that tumor-initiating cells play a crucial role in creating a CSC niche microenvironment, which is required for cancer progression and therapy resistance [37]. Tumor cells release the interleukin-33 cytokine, which promotes the differentiation of the myeloid cells into macrophages. In turn, these macrophages further send TGF-β signals to CSCs, promoting their malignant progression and therapy resistance [71]. Other important pathways include TF pathway [72], Hippo pathway [73], and cell cycle pathways [74].

**The CSC niche in different cancers**

CSC niches have already been identified in several types of human cancers including brain, liver, breast, lung, head and neck, prostate, melanoma, gastric, pancreatic, renal, ovarian, esophageal and colorectal cancers [75–87]. Various studies have focused on the similarities...
and differences among the niches in different cancers and how cancer specific microenvironments can affect the formation and the development of CSC niches [4, 6, 88–90]. Here, we review some of the important examples of CSC niches in major cancers in order to further understand the interaction between tumors and CSC niches, and how the previously mentioned molecules and mechanisms participate in different tumors.

In general, the complexity of hepatic cancers posed a challenge for researchers since different tissues showed different sets of CSC markers and expression patterns [91, 92]. To initiate a tumor in the liver, a CSC needs to survive the journey to the liver through the hepatic microvesiculation where it faces several intravascular death risks. CSCs therefore have to inhibit the liver immune responses to successfully complete clinical metastasis. In order to succeed, CSCs require several mechanisms and changes in the liver microenvironment, such as the inhibition of proteases by TIMP-1 which enables the formation of pre-metastatic niches and the expansion of metastasis. Hypoxia and HIF-1 can also activate HGF and its receptor Met which play a role in the development of liver metastasis. It has also been found that Kupffer cells and neutrophils in the niche increase the production of TNF-α, IL-1, and MMP-9 [68]. EGFR, VEGF, PDGF, TNF, SDF1, and other angiogenic factors enhance the growth and survival of hepatic cancer stem cells (HCSCs) and their resistance to radio- and chemotherapies [93–96].
So far, several HCSCs markers have been proposed (e.g., CD133, CD90, CD24, CD13, EpCAM, ALDH, and OV6) and current research efforts aim to link different combinations of these markers in HCSCs to the differences in phenotypes among CSC populations. For example, CD90+ cells have been found to upregulate genes related to inflammation and drug resistance while CD133+ cells have Akt/PKB pathway activation which translate into suppression of apoptosis and resistance to radiotherapy [59].

Studies showed that post surgery, any remaining HCSCs may be re-activated and even invade the vascular system leading to recurrence and metastasis of the primary tumor [97]. As for chemotherapy, HCSCs are more resistant to anti-tumor drugs including doxorubicin and 5-flouracil (5-FU). Such drugs can also alter the expression of certain genes (e.g., ABCG2) which increases the proliferation rate of HCSCs. Many agents are being developed to target certain molecules and markers of HCSCs, including AKT1 inhibitors, CD44 neutralizing antibodies, exogenous BMP4, pimozide, cylopamine, and other agents. Markers including CD133 and EpCAM also show great potential in the creation of specific targeted therapies as well as novel strategies targeting the cross-talk between the stroma and the tumor [59].

Gastric cancer is another type of malignancy with increasing incidence and mortality. Gastric cancer stem cells (GCSCs) have already been identified in different cell lines with markers including CD44, CD54, CD24, and CD71. Interestingly, many reports indicate the interaction between Helicobacter Pylori (HP) infection and GCSCs, such infection leads to modulation of stem cells into GCSCs in animal models [88, 98]. The chronic inflammation caused by such an infection is also helpful for the GCSC niche. Since treatments such as surgery, chemotherapy, radiation, and immunotherapy are often challenged with the problem of tumor relapse, GCSCs have been proposed as potential targets for drugs that inhibit their stemness and functions. For example, Trastuzumab targets CD90+ GCSC and decreases the growth of gastric cancer tissues (when used with chemotherapy) [99]; Salinomycin targets ALDH+ GCSC (a cell population which is resistant to chemotherapy drugs such as 5-FU and CDDP) [100].

In the case of breast cancer, it has been found that different populations of breast CSCs (BCSCs) can generate different lines of tumor cells. Yet, the genotype of the new cells may not completely resemble the original CSC genetic profile, which may indicate a number of mutations along the way [8]. It has been shown that BRCA1 expression (well-known tumor suppressor gene in breast cancer) is decreased by transcription factors such as Slug, while at the same time Slug stability is reduced by BRCA1 gene expression. This negative feedback-based balance is a key element in maintaining a normal growth of tumor and determining the level of stemness of niche cells [101, 102]. Pluripotency factors including SOX are also over expressed in BCSC niches and other than their role in EMT and metastasis, the SOX family upregulates the expression of EZH2 which is important for histone methylation for several genes and can activate Ral1-β-catenin pathway [103, 104]. With the identification of drugs that can specifically target BCSCs, essential pathways including Wnt can be inhibited. BCSCs resistance to radiotherapy can also be solved by advanced approaches including gold Nano-shell-mediated hyperthermia therapy [105].

In hematopoietic malignancies, HCSCs (also known as leukemia stem cells, LSCs) have been identified in acute myeloid leukemia (AML) in the 1990s, with specific markers such as CD34+/CD38− [45, 106]. Although the interaction and communication mechanism between LSCs and their niche is not as clear as in solid tumors, targeting the niche may still be useful as it can affect the mobility of LSCs before escaping the original niche. This theory is supported by the expression of certain chemokines and receptors in the niche such as CXCR4 and CXCL12 [107]. In order to prevent relapse of primary cancer, targeting the niche might be of major therapeutic importance, as it provides a sanctuary for LSCs against conventional therapies [108].

The chemotherapeutic drugs cytarabine and daunorubicin have been the standard choices for many years, despite the risks of relapse or therapeutic resistance. As a result, there is an urgent need for novel therapies addressing the potential role of LSCs. In animal models, G-CSF treatment has shown promising results as it stimulates dormant LSCs and activated their cell cycle, leading to increased sensitivity to chemotherapy. Such treatments still require more development, but they certainly create new hope in this field [107].

In other examples of cancer, malignant bone tumors usually include osteosarcoma, multiple myeloma, and solid tumor metastasis. Bone metastasis pose a significant challenge as it is in general incurable and is associated with resistance to chemotherapy. Other than migrated tumor cells, the bones are also the source of HSCs. It has been shown that these two different populations of cells compete for the same niches in the bones, suggesting that stem cell niches may hold a great potential in the future as a target of treating metastatic tumors [109]. Although tumor stem cells can alter the microenvironment of the bone and the existing hematopoietic niches, the niche can still distinguish a normal stem cell from a CSC. Consequently, modulating the niche in favor of normal SC compared to CSC can prevent the invasion of migrated cells [110].
Therapeutic strategies targeting the CSC niche

Therapy resistance and tumor relapse are two major obstacles in the clinical management of solid cancers [111]. There is evidence that many residual tumors are enriched with CSCs after therapy [69, 112]. Thus far, this phenomenon has been explained by the theory that even after a radical resection of tumor lesions (R0) by surgery and applying chemotherapy and radiotherapy, some CSCs still manage to escape and survive due to their therapy resistance [11, 113]. CSCs are usually quiescent and slow cycling and have an active anti-apoptotic machinery [114], efficient DNA repairing systems (DNA checkpoint kinases) and stabilized stemness features, all of which may contribute to their resistance [115]. To date, many chemoresistance mechanisms have been found across distinct cancer types such as hypoxia, EMT and the signaling pathways which regulate CSC function, for instance Notch, Wnt and Hedgehog signaling. The recent clinical trials targeting CSCs or CSC-associated pathways are shown in Table 1.

As described before, EMT is closely associated with the biological features of the CSC, including stemness, immune escape and resistance to chemotherapy [116]. Recent studies have shown that EMT plays a major role in therapy resistance [117–119]. Diverse factors and cytokines are involved in regulation of EMT, complicating targeted therapy approaches. For instance, responses to chemotherapy in a large group of breast cancer patients revealed a close association between chemoresistance and increased stromal gene expression, and this transcriptional upregulation appeared to be caused by activation of the EMT, which suggested anti-stromal agents may offer a new strategy to overcome therapy resistance [120]. A Phase I/II clinic trial showed that Plerixafor, as an anti-stromal agent which is an important inhibitor of CRCX4, combined with bortezomib

| Pathway | Agent | Tumor type | Phase | Study number |
|---------|-------|------------|-------|-------------|
| EMT     | Plerixafor | Pancreatic cancer | Phase 2 | NCT04177810 |
|         |         | Multiple myeloma | Phase 4 | NCT05087212 |
| Eribulin |      | Breast cancer | Phase 1 | NCT02120469 |
|         |      |             | Phase 2 | NCT04517292 |
| GI-6301 |       | Chordoma | Phase 2 | NCT02383498 |
| Vorinostat |   | T-cell lymphoma | Phase 3 | NCT01728805 |
| Fresolimumab | | Breast cancer | Phase 2 | NCT01401062 |
| Hypoxia | Temsirolimus | Renal cell carcinoma | Phase 1 | NCT00700258 |
|         | CRLX101 | Prostate cancer | Phase 2 | NCT03531827 |
|         |         | Renal cell carcinoma | Phase 2 | NCT02187302 |
| Notch   | PF-03084014 | Desmoid tumors | Phase 2 | NCT04195399 |
|         |         | Breast cancer | Phase 1 | NCT01876251 |
|         | LY3039478 | Solid tumors | Phase 1 | NCT02836600 |
|         | MK-0752 | Breast cancer | Phase 4 | NCT00756717 |
|         | Tarextumab | Solid tumors | Phase 1 | NCT01277146 |
|         | CB-103 | Advanced cancers | Phase 1/2 | NCT03422679 |
| Wnt     | PRI-724 | PDAC | Phase 1 | NCT01764477 |
|         | DKN-01 | Biliary tract cancer | Phase 1 | NCT04057365 |
|         |         | Gastric cancer | Phase 2 | NCT04363801 |
|         |         | Liver cancer | Phase 1/2 | NCT03645980 |
|         | Vantictumab | Breast cancer | Phase 1b | NCT01973309 |
|         | CWP232291 | Acute myeloid leukemia | Phase 1/2 | NCT03055286 |
| Hedgehog | Vismodegib | Basal cell carcinoma | Phase 2 | NCT02667574 |
|         | Chondrosarcoma | Phase 2 | NCT01267955 |
|         | Meningiomas | Phase 2 | NCT02523014 |
|         | Medulloblastoma | Phase 2 | NCT01878617 |
|         | Itraconazole | Esophageal cancer | Phase 2 | NCT04448100 |
|         |         | Prostate cancer | Phase 1/2 | NCT03513211 |
|         | Taladegib | Solid tumors | Phase 2 | NCT05199584 |
restored the chemosensitivity in relapsed multiple myeloma, which suggested that the novel therapy may target the tumor microenvironment and overcome resistance to therapy [121]. In addition, Kari et al. established an EMT lineage-tracking system in breast-to-lung metastasis mice models, using a mesenchymal-specific Cre-mediated fluorescent marker switch system. They found out that EMT cells significantly contribute to recurrent lung metastasis formation after chemotherapy [122]. According to these capacities of the EMT, creating more effective treatment strategies to directly target the EMT program is promising and urgently needed.

Moreover, TGFβ signaling is one of the characterized pathways involved in EMT induction, as enhanced TGFβ signaling in cancer accelerates EMT program and maintains a highly proliferative phenotype [123]. Accumulating research studies have demonstrated that TGFβ-mediated EMT may initiate and facilitate therapy resistance, which is inhibited by blockade of TGFβ [124–126]. Fresolimumab, a TGFβ blocking antibody, showed an effective clinical application which was demonstrated in many clinical trials [127–129]. Furthermore, two doses of fresolimumab were explored in metastatic breast cancer in a prospective randomized trial, patients receiving the higher fresolimumab dose had a better systemic immune response and longer median overall survival compared to the lower dose group [130]. Therefore, TGFβ blockade might be a feasible and promising strategy for a CSC targeted therapy.

Additionally, another potential therapeutic target for preventing the induction of EMT is hepatocyte growth factor (HGF)-HGR receptor (HGF, MET) signaling, which contributes to cancer pathogenesis, as exemplified by its frequent activation, often by point mutation or amplification of the MET, in many cancer types [131]. Therefore, targeting HGR or HGR-MET signaling is a promising therapeutic method which may also regulate CSCs. Recently, many studies show that anti-MET antibodies prevent HGF binding to MET and, subsequently, inhibit cancer progression and restore the chemoresistance, as well as improve the therapeutic efficiency [132–134].

In addition, hypoxia contributes to therapy resistance via a number of mechanisms, including maintaining CSC stemness, function of HIFs, drug transporters and affecting EMT [135]. Targeting these key factors is considered as a promising strategy for anti-CSC therapy. Acriflavin, a potent inhibitor of HIF-1α dimerization, could disturb glucose metabolism in melanoma regardless of the hypoxic condition [136]. Chetomin, another inhibitor of HIF-1α, could suppress the transcriptional activity via targeting p300 recruitment [137]. Furthermore, other agents, such as wortmannin, temsirolimus and camptothecin, have been proved to inhibit HIF-1α protein translation by targeting corresponding genes [138]. In addition, hypoxia can also mediate chemoresistance of CSCs by drug efflux through drug transporters [ATP-binding cassette (ABC) family membrane transporters]. ABCB1 and ABCG2 (BCRP), as the well-known ABC family transporters, have been reported to be induced in hypoxia [139, 140]. A recent study showed that vatalanib, a multitargeted tyrosine kinase inhibitor for all isoforms of VEGFR, PDGFr and c-Kit, makes ABCB1 and ABCG2-overexpressing multidrug-resistant colon cancer cells sensitive to chemotherapy in hypoxic microenvironment. Utilizing the combination of vatalanib with conventional anti-tumor drugs might be a promising tool to restore the chemosensitivity in colon cancer treatment [141]. Similar mechanism is found in ovarian cancer stem cells: Hypoxia-induced HIF-2α overexpression endows ovarian cancer stem cells with resistance to adriamycin by promoting BCRP expression and adriamycin efflux [139].

We have described the Notch, Wnt and Hedgehog signaling pathways in CSC regulations before, which are widely explored in current CSC target therapies (Fig. 4). γ-secretase inhibitor (GSI), as a major clinical method used to inhibit Notch signaling, has been proved in many clinical trials. Combining trastuzumab plus a GSI has been reported to reduce recurrence rate for ErbB-2-positive breast tumors [142]. Another clinical trial shows that pharmacologic inhibition of the Notch pathway can reduce CSCs in breast cancer models [143]. A phase I study shows that ipafricept (OMP-54F28, a Decoy Receptor for Wnt Ligands) can be safely administered with manageable toxicities [144]. In addition, HH ligands, SMO and GLI transcription factors are three main targets of Hh pathway antagonists [60], whose effectiveness in basal cell carcinoma and prostate cancer was already shown in many trials [145–147].

In the past few years, CSC-directed immunotherapy has emerged. Besides the antibodies targeting CSC-related signaling pathways mentioned above, novel anti-CSC immunotherapeutic approaches, such as cancer vaccines, checkpoint inhibitors and chimeric antigen receptor T-cell (CAR-T cell) therapies, have been developed [148, 149]. One personalized neoantigen dendritic cell vaccine was chosen to treat metastatic lung cancer (NCT02956551). In this trial, the disease control rate was 75% and the median overall survival was 7.9 months; the combination therapy of immune checkpoint inhibitors and the vaccines had a better progression free survival (2.2 vs 12.2 months) and overall survival (7.6 vs 11.2 months) trend; this study provided new evidence for cancer vaccine therapy and promising therapeutic opportunities for lung cancer treatment [150]. In addition, as a
novel therapeutic strategy, CAR-T cell therapy is widely used in many types of cancer, especially against hematologic malignancies. CD19/CD22/CD33, been detected in leukemia patients, is a noteworthy target for immune- cellular therapy against AML [151–154]. The ongoing clinical trials of CAR-T cell therapy are listed in Table 2.

The current studies have confirmed that CSCs are considered as the root of cancer relapse, metastasis and therapy resistance, while further research is required for the development of more effective strategies to eradicate CSCs.

Conclusion
Recent studies have expanded our knowledge regarding CSCs and their niche. The novel markers and mechanisms in CSC niches as greatly improved our understanding of cancer and its microenvironment. It also changed the proposition that metastasis is a late-stage complication of cancer and explains the long-standing dilemma of why a tumor may relapse even after a complete surgical removal, chemotherapy or radiotherapy. Regardless to the improvements in this field, we still need more efforts to annotate CSC niches regarding molecular interactions and mechanisms controlling their stemness capacity and relationship with dormancy and chemo-immuno resistance. More challenges are ahead before we can move forward from basic to translational research and further clinical applications; nonetheless, such findings may bring a great benefit for millions of patients and guide future anti-cancer therapeutic strategies.

Table 2 CAR-T cell therapy in ongoing clinical trials

| Target          | Tumor type                  | Phase | Study number     |
|-----------------|-----------------------------|-------|------------------|
| CD19            | Relapsed/refractory B-ALL   | Phase 2 | NCT05334823     |
|                 | Relapsed/refractory leukemia/lymphoma | Phase 1 | NCT03984968     |
| CD19/CD22       | Non-Hodgkin lymphoma        | Phase 1 | NCT05096613     |
| CD30            | Relapsed/refractory Hodgkin lymphoma | Phase 2 | NCT04268706     |
| CD33            | Acute myeloid leukemia      | Phase 1/2 | NCT03971799      |
| CD38            | Acute myeloid leukemia      | Phase 1 | NCT05239689     |
| CD70            | Pancreatic/renal/breast cancer | Phase 1/2 | NCT02830724      |
| CD123           | Acute myeloid leukemia      | Phase 1 | NCT04230265     |
| CD171           | Neuroblastoma               | Phase 1 | NCT02311621     |
| c-Met/PD-L1     | Hepatocellular carcinoma    | Phase 1 | NCT03672305     |
| EpCAM           | Pancreatic/gastric/colorectal cancer | Phase 1 | NCT05028933     |
| GD2             | Neuroblastoma               | Phase 1 | NCT01822652     |
| GPC3            | Liver cancer                | Phase 1 | NCT02932956     |
| Hepatocellular carcinoma |                 | Phase 1 | NCT02905188     |
| MOv19-BBz       | Ovarian/fallopian tube/peritoneal cancer | Phase 1 | NCT03585764     |
| P-MUC1C-ALLO1   | Advanced or metastatic solid tumors | Phase 1 | NCT05239143     |
| IL13Ra2         | Brain tumor                 | Phase 1 | NCT04661384     |

Abbreviations
ABCG2: ATP-binding cassette sub-family G member 2; ADAM10: A disintegrin and metalloproteinase domain-containing protein 10; AJs: Adherens junctions; AKT1: RAC-alpha serine/threonine-protein kinase; ALDH: Aldehyde dehydrogenate; AML: Acute myeloid leukemia; APC: Adenomatous polyposis coli; ATP: Adenosine triphosphate; Axis: Axis inhibition protein; B-ALL: B-cell acute lymphoblastic leukemia; BCSGs: Breast cancer stem cells; bHLH–PAS: Basic helix loop helix Per Arnt Sim; BMP: Bone morphogenetic proteins; BRCA1: Breast-Cancer susceptibility gene 1; CAFs: Cancer-associated fibroblasts; CBP: CREB-binding protein; CD: Cluster of differentiation; CSCs: Cancer stem cells; CSF-1: Colony stimulating factor 1; CXCL12: C-X-C motif chemokine 12 also known as SDF 1; CXCR4: C-X-C chemokine receptor type 4; DVL: Dishevelled protein; ECM: Extracellular matrix; EGF: Epidermal growth factor; EMT: Epithelial to mesenchymal transition; EpCAM: Epithelial cell adhesion molecule; ER: Endoplasmic reticulum; ESA: Epidermal surface antigen; EZH2: Enhancer of zeste homolog 2; FGFR: Fibroblast growth factor receptor; F-FL: F-Flouracil; GSCs: Glioblastoma stem cells; GLUT-1: Glucose transporter 1; GSK-3β: Glycogen synthase kinase 3 beta; HCC: Hepatocellular carcinoma; HCSCs: Hepatic cancer stem cell; HGF: Hepatocyte growth factor; Hh: Hedgehog pathway; HIF: Hypoxia inducible transcription factor; HP: Helicobacter pylori; HSCs: Hematopoietic stem cells; IGF-1: Insulin-like growth factor-1; IL-6: Interleukin-6; JAK: Janus kinases; LSCs: Leukemia stem cells; MDSC: Myeloid-derived suppressor cell; MET: Mesenchymal epithelial transition; HGF: Hepatocyte growth factor receptor; miRNA: Micro RNA; MMP-3: Matrix metalloproteinase 3; MSC: Mesenchymal stem cell; mTOR: Mammalian target of rapamycin; NF-kB: Nuclear factor Kappa B; NIDC: Notch intracellular domain; NK: Natural killer cell; Oct4: Octamer-binding transcription factor 4; PDGF: Platelet-derived growth factor; PI3K: Phosphoinositide 3-kinase; PTCH: Protein patched homolog; PSF: Partner of SLD five 1; PTEN: Phosphatase and tensin homolog; ROS: Reactive oxygen species; RUNX3: Runt-related transcription factor 1; SDF-1: Stromal cell-derived factor-1; SDF-1: Stromal cell-derived factor-1; Six1: Sineoculis homeobox homolog 1; SMO: Smoothened; SNAI1: Signal transducer and activator of transcription proteins; TACE: Tumor necrosis factor-a converting enzyme; TAM: Tumor-associated macrophages; TAN: Tumor-associated neutrophils; TCR: T-cell receptor; TGF-β: Transforming growth factor-beta; TIMP-1: Tissue inhibitor of metalloproteinases 1; TNP: Tissue polypeptide antigen; Vcam 1: Vascular cell adhesion molecule 1; VEGF: Vascular endothelial growth factor; Zeb: Zinc finger E-box binding homeobox.
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Ms. FJ contributed to the writing and figure of the manuscript. Dr. MM. A participated in the reviewing of the literature and contributed to the writing and editing of the manuscript. Ms. NH participated in the reviewing and editing of the manuscript. Dr. SG and Prof. RV participated in the reviewing and editing of the manuscript. Prof. Dr. CJ Burns contributed to the reviewing and editing of the manuscript. Prof. Q-ZD, Dr. YZ and Prof. NR contributed to the construction of the manuscript and supervised the completion of the review. All authors read and approved the final manuscript.

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