

## Open Review

# Understanding and targeting cancer stem cells: therapeutic implications and challenges

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Cancer stem cells (CSCs) have been identified as rare cell populations in many cancers, including leukemia and solid tumors. Accumulating evidence has suggested that CSCs are capable of self-renewal and differentiation into various types of cancer cells. Aberrant regulation of gene expression and some signaling pathways has been observed in CSCs compared to other tumor cells. CSCs are thought to be responsible for cancer initiation, progression, metastasis, recurrence and drug resistance. The CSC hypothesis has recently attracted much attention due to the potential for discovery and development of CSC-related therapies and the identification of key molecules involved in controlling the unique properties of CSC populations. Over the past several years, a tremendous amount of effort has been invested in the development of new drugs, such as nanomedicines, that can take advantage of the “Achilles’ heel” of CSCs by targeting cell-surface molecular markers or various signaling pathways. Novel compounds and therapeutic strategies that selectively target CSCs have been identified, some of which have been evaluated in preclinical and clinical studies. In this article, we review new findings related to the investigation of the CSC hypothesis, and discuss the crucial pathways involved in regulating the development of CSC populations and the advances in studies of drug resistance. In addition, we review new CSC-targeted therapeutic strategies aiming to eradicate malignancies.

**Keywords:** cancer; stem cell; leukemia; biomarker; ATP-binding cassette transporter; signaling pathway; tumor microenvironment

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## Introduction

What are cancer stem cells (CSCs)? Recent studies have suggested that CSCs are immortal tumor-initiating cells that can self-renew and have pluripotent capacity<sup>[1]</sup>. CSCs have been identified in multiple malignancies, including leukemia and various solid cancers. Due to their extraordinary characteristics, CSCs are thought to be the basis for tumor initiation, development, metastasis and recurrence. In 1963, Bruce *et al* observed that only 1%–4% of lymphoma cells (not all cancer cells) can form colonies *in vitro* or initiate carcinoma in mouse spleen<sup>[2]</sup>. However, the first compelling evidence proving the existence of CSCs is generally acknowledged to have been provided by Bonnet and Dick in 1997<sup>[3]</sup>. In their reports, only the CD34<sup>+</sup>CD38<sup>-</sup> cells from acute myeloid leukemia (AML) patients could initiate hematopoietic malignancy in NOD/SCID mice. Importantly, this cell population possessed the ability to self-renew, proliferate and differentiate<sup>[3]</sup>. The first

report of CSCs in solid cancer came in 2003 from Al-Hajj, who demonstrated the presence of CSCs in breast cancer<sup>[4]</sup>. To date, CSCs have been discovered in a broad spectrum of solid tumors, including lung cancer<sup>[5]</sup>, colon cancer<sup>[6]</sup>, prostate cancer<sup>[7]</sup>, ovarian cancer<sup>[8]</sup>, brain cancer<sup>[9]</sup>, and melanoma<sup>[10]</sup>, among others.

## The controversial cancer stem cell models

Most tumors are thought to arise from one single cell that develops into a heterogeneous population. However, two distinct models have been proposed to account for tumor growth and the heterogeneity within tumors. In the cancer stem cell model, the modalities of cancer, such as initiation, progression, metastasis and recurrence, depend primarily on rare stem cells. The heterogeneity and hierarchy between all of the cells within a tumor result from asymmetric division of CSCs. This model suggests that tumors are highly hierarchical with a unique self-renewing population of cells at the top of the hierarchy. All other cells comprising the tumor bulk are derived from differentiated CSCs<sup>[11]</sup>.

However, an alternative view also exists, called the clonal

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evolution model (CE model)<sup>[12]</sup>. This model posits that all tumor cells contribute to tumor maintenance with differing capacities. The intercellular variation is primarily attributed to subclonal differences that result from genetic and/or epigenetic changes during cancer development.

Generally speaking, the CSC model emphasizes the concept of functional heterogeneity without considering the existence of intracellular genetic variation or genetically diverse subclones. In contrast, the CE model focuses on genetic heterogeneity but ignores the functional variation within individual genetic subclones.

Although these two models seem to be mutually exclusive<sup>[13]</sup>, accumulating evidence has suggested that neither the CSC model nor the CE model should be rejected. Recently, several groups have combined functional assays with genetic analysis to examine the genetic diversity of tumor propagating cells or tumor initiating cells in both leukemia and solid cancer<sup>[14-16]</sup>. Anderson and co-workers first directly proved the existence of genetic diversity of cancer propagating cells within individual ETV6-RUNX1-positive acute lymphoblastic leukemia (ALL) patients<sup>[14]</sup>. In addition, the genetic diversity and relative dominance of subclones vary with the development of disease<sup>[14]</sup>. Similarly, Notta *et al* have established that individual Bcr-Abl<sup>+</sup> ALL samples are composed of genetically distinct subclones that are related by a complex evolutionary process<sup>[15]</sup>. These authors also determined that genetically diverse subclones already possess variably aggressive properties at the time of diagnosis<sup>[15]</sup>.

These studies have indicated that cancer stem cells exist but might evolve over time. An ancestral clone gives rise to at least two clonal lineages that evolve independently, with each clone acquiring diverse genetic aberrations; one clone emerges as the dominant diagnostic clone, while the other clone gives rise to the predominant clone containing additional mutations at relapse<sup>[15]</sup>.

### Characteristics of cancer stem cells

CSCs are distinct populations of tumor cells. CSCs have multiple unique features that cause them to be vital for tumor formation. CSCs can self-renew<sup>[17]</sup> and are immortal and

necessary to maintain the population of tumor cells. CSCs are pluripotent and can generate tumor cells with different phenotypes, which results in the growth of the primary tumor and emergence of new tumors.

### CSC biomarkers

Due to the close relationship between CSCs and tumor initiation, progression, metastasis and drug resistance, the isolation of these cells from the total cancer cell population is essential for detailed studies. Distinct and specific surface biomarker phenotypes can be used to distinguish CSCs from other tumor cells and normal stem cells. Currently, the most common method used to identify CSCs is fluorescence-activated cell sorting (FACS) based on cell surface markers or intracellular molecules.

The leukemia stem cell (LSC), which specifically displays a CD34<sup>+</sup>CD38<sup>-</sup> surface marker phenotype, was the first studied and best understood type of CSC<sup>[3]</sup>. The loss of CD38 distinguishes LSCs from normal hematopoietic stem cells (HSCs), although both LSCs and HSCs are CD34<sup>+</sup><sup>[3]</sup>. Subsequently, more selective surface markers of LSCs have been discovered, and combinations of these markers have been used. For example, the combination of CD34<sup>+</sup>CD38<sup>-</sup>HLA-DR<sup>-</sup>CD71<sup>-</sup>CD90<sup>-</sup>CD117<sup>-</sup>CD123<sup>+</sup> is uniquely present on the surface of LSCs but not on normal HSCs<sup>[18]</sup>.

Regarding solid cancers, Al-Hajj *et al* have identified ESA<sup>+</sup>CD44<sup>+</sup>CD24<sup>-/low</sup>Lineage<sup>-</sup> cells as breast cancer stem cells. The authors found that compared to other cell populations, this population has a greater capacity for tumor formation in NOD/SCID mice<sup>[4]</sup>. In addition, Singh *et al* have found that transplantation of a very small number of CD133<sup>+</sup> brain cancer cells can generate tumors in NOD/SCID mice, suggesting that CD133 might be an important CSC marker<sup>[19]</sup>.

Although great progress has been made in understanding CSC surface molecules, many issues still remain to be addressed. Here, we summarize partial phenotypes of CSC markers according to the cancer types (Table 1). However, we must realize that CSC markers are still not perfect; not all CSCs express the markers, and some non-CSC cancer cells may also express the markers. For this reason, the markers

**Table 1.** Cell surface phenotypes of CSCs.

Tumor type	Phenotype of CSCs markers	Reference
Leukemia	CD34 <sup>+</sup> CD38 <sup>-</sup> HLA-DR-CD71 <sup>-</sup> CD90 <sup>-</sup> CD117 <sup>-</sup> CD123 <sup>+</sup>	[18]
Breast cancer	ESA <sup>+</sup> CD44 <sup>+</sup> CD24 <sup>-/low</sup> Lineage <sup>-</sup> , ALDH-1 <sup>high</sup>	[4, 20]
Liver cancer	CD133 <sup>+</sup> , CD49f <sup>+</sup> , CD90 <sup>+</sup>	[21, 22]
Brain cancer	CD133 <sup>+</sup> , BCRP1 <sup>+</sup> , A2B5 <sup>+</sup> , SSEA-1 <sup>+</sup>	[19, 23]
Lung cancer	CD133 <sup>+</sup> , ABCG2 <sup>high</sup>	[24, 25]
Colon cancer	CD133 <sup>+</sup> , CD44 <sup>+</sup> , CD166 <sup>+</sup> , EpCAM <sup>+</sup> , CD24 <sup>+</sup>	[26-28]
Multiple myeloma	CD138 <sup>-</sup>	[29, 30]
Prostate cancer	CD44 <sup>+</sup> , α2β1 <sup>high</sup> , CD133 <sup>+</sup>	[7]
Pancreatic	CD133 <sup>+</sup> , CD44 <sup>+</sup> , EpCAM <sup>+</sup> , CD24 <sup>+</sup>	[31, 32]
Melanoma	CD20 <sup>+</sup>	[10]
Head and neck cancer	CD44 <sup>+</sup>	[33]

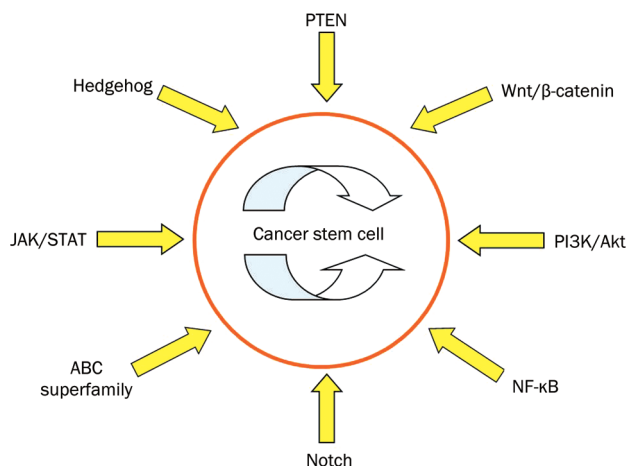
can be used to identify CSC-rich subpopulations but might not be able to unambiguously isolate all of the CSCs.

### ATP-binding cassette

ATP-binding cassette (ABC) transporters are membrane transporters that can pump various distinct and structurally unrelated small molecules (such as cytotoxic drugs and dyes) out of cells at the expense of ATP hydrolysis. Normal stem cells and cancer stem cells appear to express high levels of ABC transporters. This phenomenon could contribute to multidrug resistance (MDR) because many anti-tumor drugs can be pumped out, thereby resulting in low intracellular drug concentrations<sup>[25, 30, 34]</sup>. Thus, the elevated levels of ABC transporters enable cancer stem cells to resist current cancer therapies<sup>[25, 35]</sup>. The well-known “shining stars” of the ABC superfamily include multidrug resistance proteins (MRPs/ABCC), breast cancer resistance protein (BCRP/ABCG2) and P-glycoprotein (P-gp/ABCB1)<sup>[35]</sup>. High expression levels of ABC transporters in malignant stem cells can be determined by treatment of cells with Hoechst 33342 dye. Rare cells containing high levels of the ABC transporters expulse Hoechst; this fraction is designated as side population (SP) cells<sup>[35]</sup>. Accumulated data have shown that numerous cell lines and tumors contain SP cells and that this cell population possesses a greater capacity for tumorigenesis than non-SP cells.

### Key signaling pathways

Dysregulation of signaling pathway networks plays an important role in enabling CSCs to retain stem cell properties. As shown in Figure 1, the pathways and elements that are involved in the control of self-renewal and differentiation of cancer stem cells and normal stem cells include PI3K/



**Figure 1.** Signal pathways related with cancer stem cells. Dysregulation of signal pathway network plays an important role in retaining the stemness of CSCs. Pathways and elements involved in the control of self-renewing and differentiation of cancer stem cells as well as normal stem cells include PI3K/Akt, JAK/STAT, Wnt/ $\beta$ -catenin, hedgehog, Notch, NF- $\kappa$ B, ABC superfamily and so on. Depending on these aberrant pathways, cancer stem cells acquire its unique ability to initiate carcinoma and promote recurrence after surgery.

Akt, PTEN<sup>[36]</sup>, JAK/STAT, Wnt/ $\beta$ -catenin, hedgehog, Notch, NF- $\kappa$ B, Bcl-2, and others<sup>[37–43]</sup>.

The PI3K/AKT signaling pathway is involved in numerous cancers, including acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). AKT activation is very important for cellular transformation and tumorigenesis. We have observed the role of AKT1 containing an E17K mutation in v-Abl-mediated cellular transformation<sup>[38]</sup>. The AKT1 (E17K) mutant can cause increases in the protein levels of Bcl-2 and the phosphorylation of the pro-apoptotic protein BAD, resulting in enhanced resistance to apoptosis. In addition, other members of the PI3K pathway (such as PTEN and mTOR) also function in the maintenance of LSCs. Silencing or defective mutations of PTEN have been observed in various cancers, including T-cell acute lymphoblastic leukemia, prostate cancer, melanoma, glioblastoma and endometrial carcinoma<sup>[44]</sup>.

The JAK/STAT signaling pathway is also involved in tumor initiation. Aberrations in the JAK/STAT pathway have been recognized in many cancers, especially leukemia. v-Abl is a strong non-receptor tyrosine kinase that can induce the malignant transformation of pre-B cells by affecting the JAK/STAT pathway. Pim-1 and Pim-2 kinases have been shown to be induced by v-Abl-dependent JAK/STAT signaling in pre-B cells and play important roles in cellular transformation<sup>[45]</sup>. In addition, the JAK2-V617F mutation has been shown to be a critical factor that contributes to the malignant transformation of hematopoietic cells<sup>[46]</sup>. An important mechanism for negative regulation of the JAK/STAT signaling pathway is mediated by members of the suppressor of cytokine signaling (SOCS) family. Because activation of JAK/STAT signaling is required for transformation by several oncogenes, overcoming the regulatory effects of SOCS-1 and SOCS-3 has been proposed to be necessary for tumor formation. Indeed, signaling activated by Bcr-Abl, a tyrosine kinase that causes CML, has been shown to lead to tyrosine phosphorylation of SOCS-1 and SOCS-3, thereby impairing the ability of these proteins to inhibit JAK/STAT signaling pathway activation<sup>[47]</sup>.

Nuclear factor kappa B (NF- $\kappa$ B), a transcription factor that regulates the expression of multiple genes, participates in numerous cellular responses to stimuli such as cytokines, microbial antigens, free radicals and ultraviolet irradiation<sup>[39]</sup>. An increasing body of evidence has shown that NF- $\kappa$ B affects the expression of several apoptosis-related proteins, such as Bcl-xL, Bcl-2, survivin, cellular inhibitors of apoptosis (cIAPs), TRAF and cell cycle regulatory components. Aberrant NF- $\kappa$ B activation causes cancer development and progression, chemoresistance, chronic inflammation and autoimmune diseases<sup>[48, 49]</sup>.

The well-known Notch, Hedgehog and Wnt signaling pathways play fundamental roles in maintaining CSC populations. Notch signaling affects self-renewal and lineage-specific differentiation of normal human breast stem cells<sup>[50]</sup>. Moreover, Notch4 activity is elevated in breast CSCs, and inhibiting Notch4 activity can reduce the breast CSC population, thereby suppressing tumor initiation<sup>[42]</sup>.

Aberrant regulation of the Hedgehog pathway is associated

with numerous human malignancies and is a critical factor affecting the outcome of treating the disease<sup>[51]</sup>. Several agents targeting the Hedgehog pathway have shown promising pre-clinical results and are currently under investigation in phase I and II clinical trials<sup>[51]</sup>.

The maintenance of CSC stem cell properties has been shown to be associated with the Wnt signaling pathway. Abnormal Wnt/ $\beta$ -catenin signaling has been identified in various malignancies, such as leukemia, colon, epidermal, breast and cutaneous carcinoma<sup>[52, 53]</sup>. Human colon carcinoma, one of the best studied tumors, is caused by defective mutations in the adenomatous polyposis coli (APC) gene<sup>[54]</sup>. Defective mutations in APC result in the inappropriate stabilization of  $\beta$ -catenin, thus activating the Wnt cascade and inducing epithelial cell transformation<sup>[54]</sup>.

### Therapies targeting cancer stem cells

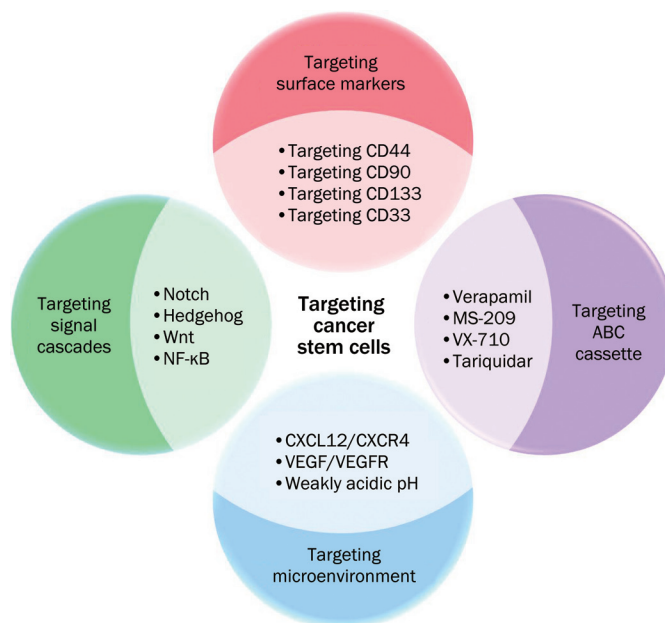
Current therapeutic strategies against cancer have severe limitations that frequently lead to treatment failure. A common cause of treatment failure in multiple malignancies is resistance to chemotherapy and radiotherapy. In addition, many strategies that are not sufficiently selective against CSCs can be toxic to healthy tissues, and patients usually face the risk of recurrence and metastasis because most therapies cannot eliminate CSCs<sup>[55]</sup>. Accumulated evidence has established that CSC populations are more resistant to conventional cancer therapies than non-CSC populations. Therefore, the elimination of CSCs is crucial in treating malignant diseases<sup>[56-58]</sup>.

Recently, multiple novel therapeutic systems have been designed with the aim of killing CSCs and altering the microenvironment (niches) supporting these cells. Both subtle surface marker differences and alterations in signaling pathways are alluring therapeutic targets. Scientists have identified multiple potential CSC therapeutic targets, including the ABC superfamily, anti-apoptotic factors, detoxifying enzymes, DNA repair enzymes and distinct oncogenic cascades (such as the Wnt/ $\beta$ -catenin, hedgehog, EGFR and Notch pathways)<sup>[58, 59]</sup>. Currently, some therapeutic strategies can successfully kill CSCs, while others are still under preclinical and clinical evaluation. A summary of new CSC-targeted therapeutic strategies is shown in Figure 2.

#### Targeting cellular surface markers

Researchers often choose ligands or antibodies against tumor surface markers to enhance the specificity of therapeutic strategies. Important interest has been generated in the development of monoclonal antibodies to target CSCs. For example, CD33 is highly expressed in most AML cells. Thus, gemtuzumab ozogamicin, a humanized anti-CD33 mouse monoclonal antibody conjugated to the cytotoxic agent calicheamicin, has been developed and widely used to treat AML<sup>[60]</sup>.

Markers differentially expressed between normal stem cells and CSCs, including CD44<sup>[61]</sup>, IL-3R<sup>[62]</sup>, and the immunoglobulin mucin TIM-3<sup>[63]</sup>, have been utilized to specifically target leukemia stem cells in human AML. In each case, treatment with antibodies against these cell surface molecules dramati-



**Figure 2.** Therapies targeting cancer stem cells. Numerous therapies aiming at eradicating cancer stem cells have been developed during these years. Four different areas could recapitulate the current popular ideas mostly. By selectively targeting surface markers of CSCs (red area), more accurate and less side effects could be achieved. With the help of modern molecular biology techniques, more and more crucial signal elements and pathways have been unearthed (green area). By intervening aberrant pathways, specific characteristics of CSCs are suppressed and promising outcome has been reported. Molecular drugs inhibiting ABC cassette (purple area) have reached the third generation (tariquidar) and some of them are undergoing clinical trials. In addition, tumor microenvironment nursing CSCs also draws much attention (blue area). Either cutting off the growth of blood vessel or exploiting the special PH environment, has showed alluring prospects.

cally decreases leukemogenicity and eradicates CSCs in mice. Antibodies against CD47, which is expressed at much higher levels in ALL than in normal cells, may also effectively kill leukemia stem cells<sup>[64]</sup>. In treating MCF-7 breast cancer, an anti-CD44 antibody-conjugated gold nanorod has been used to target and photo-ablate CD44<sup>+</sup> cells, which display significant cancer stem cell characteristics. Using this approach, targeted cells absorb near infrared light, which results in increased local temperature at the designated location<sup>[65]</sup>.

An anti-CD90 antibody has also been reported to protect bone marrow-derived multipotent stromal cells from differentiation into chondro-, osteogenic, or adipo-lineages<sup>[66]</sup>. In addition, CD133 is highly expressed in many types of CSCs, including lung cancer, breast cancer, and glioma. Patients with high levels of CD133 show poor clinical outcomes<sup>[67]</sup>. For example, increased CD133 mRNA expression correlates with a high risk of death in colon, prostate and head and neck cancer<sup>[68]</sup>. Thus, therapy against CD133 might represent a promising strategy for cancer treatment. CD133<sup>+</sup> cells in glioblastoma (GBM) display cancer stem cell-like properties<sup>[69]</sup>. Interestingly, a recent study has shown that treatment with carbon nanotubes con-

jugated to an anti-CD133 monoclonal antibody followed by irradiation with near infrared laser light can selectively target CD133<sup>+</sup> GBM cells, and the photothermolysis caused by the nanotubes can kill the targeted cells<sup>[69]</sup>. In addition, disruption of CD133 expression by short hairpin RNA in human glioblastoma neurospheres has been shown to impair the self-renewal and tumorigenic capacity of the neurosphere cells<sup>[70]</sup>.

#### Targeting ATP-driven efflux transporters

Antitumor drug efflux caused by ATP-driven pumps is the primary reason for chemoresistance<sup>[55, 71, 72]</sup>. Investigators have designed numerous methods to evade, neutralize or even exploit drug efflux pumps to overcome drug resistance. Several pharmacological agents that can interact with ABC transporters have been developed to inhibit multidrug resistance (MDR)<sup>[73, 74]</sup>.

The first P-gp efflux pump inhibitor to be identified was verapamil, which is often used in SP analysis to block the exclusion of Hoechst dye<sup>[75]</sup>. Simultaneous treatment with verapamil and antitumor drugs, such as Dox, paclitaxel or vincristine, has displayed promising therapeutic effects.

Khdair *et al* have delivered methylene blue (a type of P-gp inhibitor) and Dox simultaneously into BALB/c mice bearing syngeneic JC adenocarcinoma tumors. Markedly increased accumulation of drugs within the lesion, enhanced tumor cell apoptosis, suppressed cancer cell proliferation, impaired tumor growth and significantly improved animal survival were observed<sup>[76]</sup>.

Some investigations have identified a number of novel ABC transporter inhibitors that suppress both P-gp and MRP1, such as MS-209, VX-710, and tariquidar<sup>[73, 77, 78]</sup>. Recent trials with MS-209 have shown promising results in overcoming drug resistance in breast cancer<sup>[77]</sup> and other solid cancers (ClinicalTrials.gov Identifier: NCT00004886). Tariquidar is another inspiring inhibitor. The combination of tariquidar and docetaxel is under investigation (sponsored by the National Cancer Institute) for the treatment of recurrent or metastatic ovarian, cervical, lung and kidney malignancies (ClinicalTrials.gov identifier: NCT00072202). Similarly, the combination of tariquidar with Dox, etoposide, mitotane and vincristine is being tested in patients with primary, recurrent, and metastatic adrenocortical cancer (Clinical-Trials.gov identifier: NCT00073996)<sup>[73]</sup>.

An alternative strategy targeting ABC transporters involves regulating the protein expression levels of these transporters. Sims-Mourtada *et al* have indicated that Hedgehog signaling can regulate the expression of MDR1 and ABCG2. The expression levels of ABCG2 and MDR1 are downregulated upon treatment of PC3 cells with cyclopamine (a SMO signaling element inhibitor), and targeted knockdown of ABCG2 and MDR1 expression by siRNA partially reverses chemoresistance<sup>[79]</sup>.

#### Targeting key signaling cascades

Active anti-apoptotic pathways and parallel inactive pro-apoptotic pathways are also hot spots attracting researchers.

Monoclonal antibodies targeting Notch signaling have shown alluring prospects<sup>[80, 81]</sup>. Inhibition of Notch1 can significantly reduce the CD44<sup>+</sup>CD24<sup>-/low</sup> subpopulation and lower the incidence of brain metastases from a breast cancer cell line<sup>[82]</sup>.

High levels of  $\beta$ -catenin have been described to correlate with CSC tumorigenicity in colon cancer<sup>[83]</sup>. Several pharmaceuticals are under investigation as inhibitors of Wnt signaling<sup>[84, 85]</sup>. Monoclonal antibodies against the Wnt cascade have been tested with promising anti-tumor activity<sup>[86]</sup>.

Small-molecule Hedgehog antagonists have shown alluring potential to inhibit systemic metastases in mice bearing orthotopic xenografts from human pancreatic cancer cell lines. A significant reduction in the population of ALDH-positive cells (the tumor-initiating population in pancreatic cancer) has also been observed<sup>[87]</sup>. Several groups have exploited cyclopamine, a SMO signaling element inhibitor, to inhibit the hedgehog cascade, and cyclopamine has been shown to inhibit the growth, invasion and metastasis of breast, prostatic, pancreatic and brain malignancies both *in vitro* and *in vivo*<sup>[88-91]</sup>. Cyclopamine can act synergistically with gemcitabine to reduce the population of ALDH<sup>high</sup> pancreatic CSCs<sup>[92]</sup>. Similarly, Clement *et al* have demonstrated that the combination of cyclopamine and temozolomide (TMZ) can also reduce the cell mass of glioma CSCs *in vivo*<sup>[93]</sup>.

Several groups have found that inhibiting NF- $\kappa$ B can greatly suppress chemoresistance, and inhibitors targeting NF- $\kappa$ B can mediate antitumor responses and enhance the sensitivity of tumor cells to anticancer drugs. Simultaneous delivery of DOX and the NF- $\kappa$ B inhibitor PDTG successfully overcomes multidrug resistance<sup>[94]</sup>. Ganta and Amiji have also observed enhanced cell apoptosis upon treatment with paclitaxel and curcumin, another NF- $\kappa$ B inhibitor<sup>[95]</sup>. Curcumin can dampen the protein levels of MDR1<sup>[96]</sup> and activate apoptosis pathways, hence impairing the capacity for multidrug resistance.

Ceramide, a secondary lipid messenger implicated in the regulation of cell proliferation, apoptosis and differentiation, has drawn much attention as a pro-apoptotic molecule that can activate apoptotic pathways via receptor independent mechanisms<sup>[97]</sup>. Devalapally *et al* have increased the level of ceramide in drug resistant cancer cells to lower the threshold for apoptotic signaling. Tamoxifen can inhibit the activity of ceramide-metabolizing enzymes and thus increase intracellular ceramide levels. Drug resistance in P-gp-overexpressing SKOV3(TR) cells is significantly suppressed upon simultaneous delivery of paclitaxel and tamoxifen<sup>[97]</sup>.

In addition, IL-4 can protect the tumorigenic CD133<sup>+</sup> CSCs in human colon carcinoma from apoptosis. Thus, an anti-IL-4 antibody or IL-4Ra antagonist can cause CSCs to become apoptotic and markedly sensitize CSCs to chemotherapeutic drugs<sup>[41]</sup>.

#### Targeting the tumor microenvironment

The tumor microenvironment can create a niche to nurse and protect CSCs from drug-induced apoptosis. For example, most mature B-cell malignancies are incurable. Compelling evidence has suggested that accessory stromal cells in the

tissue microenvironments of bone marrow and secondary lymphoid organs favor disease progression by promoting malignant B-cell growth, proliferation and drug resistance<sup>[98]</sup>. One example is the interaction between stromal cell-derived factor-1 (SDF-1/CXCL12), secreted by bone marrow stromal cells, and its receptor CXCR4<sup>[40]</sup>. CXCR4 can guide leukemia cell trafficking and homing to the bone marrow microenvironment, where secreted CXCL12 ensures that leukemia cells closely contact the marrow stromal cells, consequently activating cell growth and chemoresistance signals. CXCR4 antagonists, such as Plerixafor (AMD3100) and T14003 analogs, can damage adhesive tumor-stroma interactions and induce leukemia cell mobilization away from the bone marrow stromal microenvironment, causing the cells to become more vulnerable to cytotoxic drugs. The novel approach of targeting the CXCR4-CXCL12 axis is being explored in clinical trials for leukemia<sup>[40, 98]</sup>.

Tumor angiogenesis has also been reported to be related to CSC survival and drug resistance. VEGF has been recognized to correlate with microvasculature formation and tumor growth. Targeting VEGF with bevacizumab leads to the normalization of tumor vasculature, resulting in a disruption of the CSC niche. Previous studies have demonstrated that treatment of mouse glioblastoma with bevacizumab causes a dramatic reduction in the number of glioblastoma stem cells<sup>[99]</sup>. Calabrese *et al* have treated mice bearing U87 glioma cell xenografts with bevacizumab and observed decreased microvasculature density and tumor growth. The authors also observed a reduction in the number of CD133<sup>+</sup>/nestin<sup>+</sup> tumor-initiating cells<sup>[100]</sup>. The combination of the VEGFR2 antibody DC101 and the cytotoxic agent cyclophosphamide is also more effective against C6 glioma xenografts *in vivo* than individual agents alone<sup>[101]</sup>.

Weakly acidic pH is a well-known characteristic of the microenvironment of solid tumors. TAT peptide is an arginine-rich peptide (YGRKKRRQRRR) that currently serves to rapidly transport various types of attached molecules, such as small molecule drugs, into mammalian cells both *in vitro* and *in vivo*. Lee *et al* have introduced TAT to a pH-sensitive cargo that is also conjugated to biotin. This cargo exposes biotin on its surface under slightly acidic pH values (6.5 < pH < 7.2). Thus, the pH environment together with exposed biotin can greatly increase the specificity of TAT. The latter quickly elevates the concentration of Dox in the cytosol. These processes have been reported to significantly increase the potency of Dox. This novel cargo has been evaluated in tumor xenografts of non-small cell lung cancer A549 cells, A2780/AD-resistant breast cancer MCF-7 cells and nasopharyngeal carcinoma KB cells; an obvious reduction was observed in the size of all tumors<sup>[102]</sup>.

## Conclusions

In summary, compelling evidence has shown that CSCs indeed exist in various malignancies and display capacities for self-renewal and differentiation that are critical for tumor initiation, progression, metastasis and recurrence. Currently,

the identification and isolation of CSCs are primarily based on CSC surface markers. However, CSC markers are not universal, and CSC populations have repeatedly been refined due to the identification of new markers. Thus, to what extent will a suitable number of markers be identified to define the real population of CSCs? Would these biomarkers change during cancer progression? These questions still remain to be addressed.

Designing novel approaches to target CSCs has received much attention over the past several years. Increasing evidence has suggested that a comprehensive strategy might improve cancer treatments. For example, a nanoparticle conjugated to the following four crucial elements has been designed: 1) a molecule (*ie*, ligand) targeting a specific CSC; 2) a cytotoxic anticancer drug to eliminate CSCs; 3) a chemosensitizer to overcome drug resistance (such as an ABC transporter inhibitor); and 4) an imaging agent to facilitate tumor diagnostics. Such a combination would exert the anti-tumor effect more specifically and powerfully with fewer side-effects. Furthermore, this approach would enable accurate identification of the localization of the primary tumor and its metastases. The rise of the cancer stem cell hypothesis broadens our horizons and provides a new approach to eradicate malignancies. However, the identification of strategies that exploit the unique characteristics of CSCs requires further study and the cooperation of multidisciplinary areas.

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