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Review

Biomarkers and targeted therapy for cancer stem cells

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Cancer stem cells (CSCs) are a small subpopulation of cancer cells with capabilities of self-renewal, differentiation, and tumorigenicity, and play a critical role in driving tumor heterogeneity that evolves insensitivity to therapeutics. For these reasons, extensive efforts have been made to identify and target CSCs to potentially improve the antitumor efficacy of therapeutics. While progress has been made to uncover certain CSC-associated biomarkers, the identification of CSC-specific markers, especially the targetable ones, remains a significant challenge. Here we provide an overview of the unique signaling and metabolic pathways of CSCs, summarize existing CSC biomarkers and CSC-targeted therapies, and discuss strategies to further differentiate CSCs from non-stem cancer cells and healthy cells for the development of enhanced CSC-targeted therapies.

Highlights

Cancer stem cells (CSCs) are a small subpopulation of cancer cells with capabilities of self-renewal, differentiation, and tumorigenicity, and play a critical role in driving tumor heterogeneity drug resistance.

CSCs that survive chemotherapy or radiation therapy, even in small numbers, can rebuild a highly aggressive tumor resistant to existing therapeutics within days to months.

The identification and targeted treatment of CSCs, which albeit remains challenging, is critical in achieving a better therapeutic outcome.

CSCs exhibit distinct signaling and metabolic pathways from non-stem cancer cells and healthy cells, enabling the identification of cell-surface and intracellular biomarkers.

Metabolic glycan labeling provides an emerging method to install artificial chemical receptors on the membrane of CSCs, for subsequent conjugation of therapeutics.

CSCs and CSC-targeted cancer therapy

Cancer remains a leading cause of human death, with more than 10 million lives taken away by cancer annually in the world. While a variety of cancer therapies have been developed over the past decades, chemotherapy and radiation therapy remain the mainstream treatment for nearly all types of cancers [1,2]. However, the therapeutic efficacy of chemotherapy and radiation therapy is still limited in general and is often accompanied by severe side effects, largely due to the lack of effective cancer-targeted therapies and the resistance of tumors to drugs and immune attack [3,4]. Among the various factors contributing to the intractability of tumors, the presence of CSCs, a small subpopulation of cancer cells with capabilities of self-renewal, differentiation, and tumorigenicity (Figure 1A,B), has been notorious [5–8]. Historically, CSCs were first identified from the blood as a subpopulation of cancer cells that possess infinite proliferative ability and were initially named leukemia stem cells [6]. Further research uncovered the presence of CSCs in various types of solid tumors, including breast cancer, brain cancer, liver cancer, head and neck cancer, colorectal cancer, melanoma, and others [9–14]. These CSCs are characterized by their stem cell-like differentiation potential [7], often constitute less than 1% of tumor cells [8], and are able to maintain their high proliferative ability in the tumor microenvironment [15,16]. A small number of CSCs could often lead to the growth of a significant tumor mass [16,17], indicating the immense aggressiveness and toughness of CSCs [15,16].

While CSCs possess infinite proliferation and differentiation potential, they are often quiescent in the core region of tumor masses and are more resistant to treatments such as chemotherapy and radiation therapy than the non-CSC population [17–20]. The surviving CSCs, even small numbers, can rebuild a highly aggressive tumor resistant to existing therapeutics within days to months (Figure 1C,D) [18–20]. Failure to destroy CSCs has been one of the key factors responsible for the overall low efficacy of numerous therapeutics. CSCs also play a critical role in driving tumor heterogeneity and develop insensitivity to therapeutics, dramatically increasing the chance of tumor recurrence and metastases [19–21]. For these reasons, the identification and targeted treatment of CSCs, which albeit remains challenging, is critical to achieve a better therapeutic outcome.

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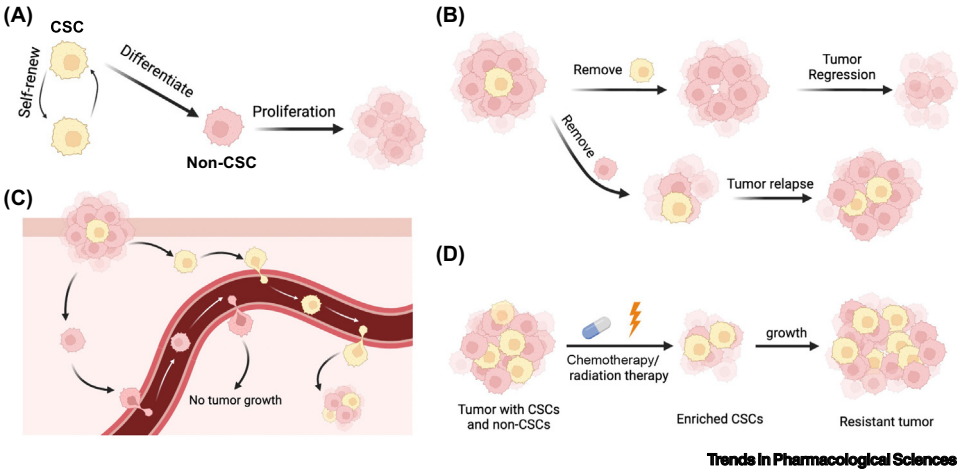

Figure 1. Characteristics of cancer stem cells (CSCs) and their relation to cancer. (A) Ability of CSCs to self-renew and differentiate into non-stem cancer cells. CSCs can self-renew to new CSCs while maintaining their stem likeness. They can also differentiate into non-CSCs and further proliferate into tumor. (B) CSCs in small numbers can re-form the tumor mass. Eradication of CSCs can effectively reduce the regression of tumors, while removing only non-CSCs will result in tumor relapse and enrichment of CSCs. (C) CSCs outperform non-stem cancer cells in the formation of metastatic tumors *in vivo*. CSCs that migrate into remote tissues (e.g., lung) via the bloodstream are more likely to result in the formation of a new tumor compared with non-stem cancer cells. (D) CSCs contribute to the resistance of tumors to conventional chemotherapy and radiation therapy. CSCs tend to be more resistant to conventional chemotherapy and radiation therapy due to their stem-like properties and can be enriched during these therapies, resulting in the relapse of tumors with drug resistance.

The development of CSC-targeted therapies inevitably lies in the ability to identify CSC-specific surface receptors or biomarkers [5,7,8] and necessitates a comprehensive understanding of the molecular and metabolic differences between CSCs and non-stem cancer cells or normal cells.

In this review, we provide an overview of the altered signaling and metabolic pathways of CSCs that enable the identification of CSC-associated biomarkers, summarize the overexpressed molecules in CSCs that could serve as potential CSC biomarkers, and discuss CSC-targeted therapies including chemotherapy and **immunotherapy** (see Glossary). As conventional endogenous markers are hardly specific to CSCs, efforts have also been made to introduce artificial biomarkers to the surface of CSCs for the subsequent development of CSC-targeted therapies, which are also discussed in this review. We also provide insights on future directions for the identification or metabolic tagging of CSCs and the further development of CSC-targeted cancer therapies with potent antitumor efficacy and minimal off-target effects.

Altered signaling and metabolic pathways in CSCs

Accompanied by the unique stemness of CSCs, various intracellular pathways of CSCs, including the Wnt pathway, Notch pathway, Hedgehog pathway, PI3K/Akt pathway, NF-κB pathway, and JAK/STAT pathway, are altered compared with non-stem cancer cells (Figure 2) [22–25]. These alterations in genetic and epigenetic regulations could be essential for the initiation and development of CSCs and confer infinite proliferative ability and differentiation potential on CSCs [7,8,15,16]. They were also shown to play a critical role in shaping the drug resistance and metabolic activities of CSCs [17–21]. As a special type of stem cell-like population residing in the tumor mass, CSCs also manage to adapt their metabolic programs for the tumor microenvironment compared with conventional stem cells [26–28]. For example, different from conventional stem cells that are biased towards aerobic glycolysis instead of oxidative phosphorylation (OXPHOS) to minimize the production of reactive oxygen species (ROS), which could impair the

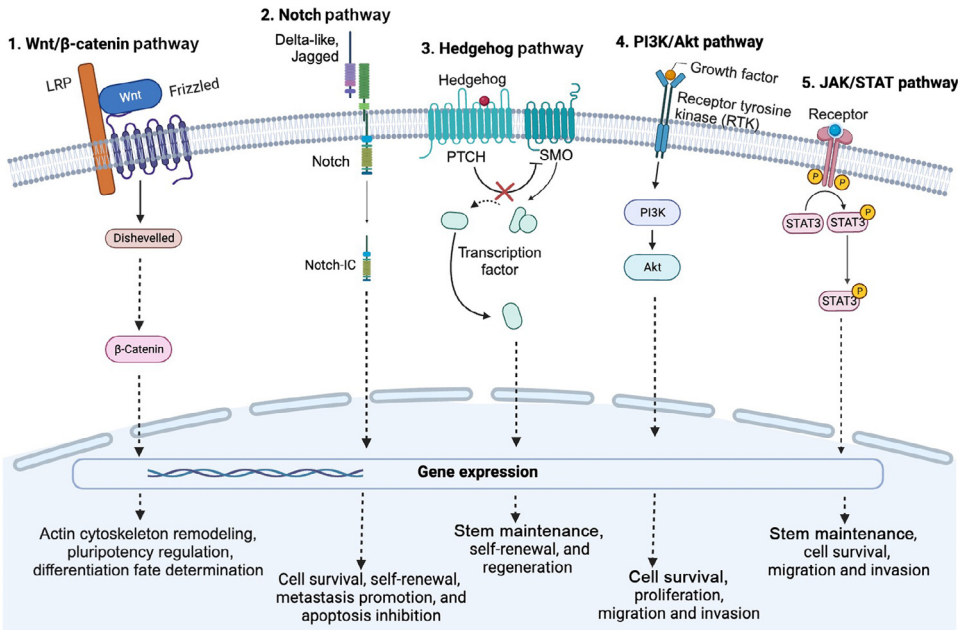

Figure 2. Representative upregulated signal pathways that contribute to the stemness, survival, self-renewal, and invasion of cancer stem cells (CSCs). (1) Wnt/β-catenin pathway: The Wnt protein binds to its receptors Frizzled and LRP to induce the activation of Dishevelled, which leads to the activation of β-catenin that regulates gene expression for actin cytoskeleton remodeling, pluripotency regulation, and cell differentiation. (2) Notch pathway: Binding of the Jagged delta-like ligand to the Notch receptor on the cell surface induces the cleavage of the two proteolytic sites of the receptor to release Notch-IC (intracellular domain). Notch-IC can then mediate gene expression related to cell survival, self-renewal, metastasis, and apoptosis. (3) Hedgehog pathway: Binding between ligand (red circle) and the transmembrane protein PTCH results in inhibition of the transmembrane protein SMO, resulting in the activation of transcription factors and downstream gene expression. (4) PI3K/Akt pathway: Binding of a ligand (e.g., growth factor) to receptor tyrosine kinase (RTK) can induce the activation of PI3K and thus the phosphorylation of Akt. The activated Akt can then regulate the expression of genes related to the survival, proliferation, and invasion of CSCs. (5) JAK/STAT pathway: Binding of a ligand (blue) to cytokine receptors can induce the phosphorylation of STAT3, for subsequent regulation of genes related to the stemness, survival, and invasion of CSCs.

state of stemness, CSCs heavily rely on glucose consumption for energy production to sustain the growth of tumors while maintaining their stemness (Figure 3A) [27–29]. CSCs also undergo active mitochondrial oxidative metabolism to match the upregulated oxygen consumption in the tumor microenvironment [29,30]. Compared with non-stem cancer cells, CSCs also exhibit altered lipid metabolic activities (Figure 3B). In particular, the lipid desaturation pathways were reported to be upregulated in CSCs to maintain their stem cell-like properties [31,32]. Iron metabolism and ferroptosis in CSCs are also upregulated to sustain the intracellular levels of iron and transferrin [33]. Also, ceramide signaling, which is important in regulating the immune status of the tumor microenvironment and programmed cell death, is upregulated in CSCs to facilitate the immune evasion and progression of tumors [34]. These aberrantly altered intracellular pathways of CSCs, compared with non-stem cancer cells and normal cells, provide molecular candidates for CSC biomarkers. However, when narrowed to specific molecules, the differences between CSCs and other subpopulations are often non-significant, posing difficulty in the identification of unique CSC markers for the development of CSC-targeted therapies.

Conventional CSC biomarkers and CSC-targeting strategies

The potential CSC biomarkers identified based on their aberrant signaling and metabolic pathways can be divided into two major categories: cell-surface markers and intracellular markers

Glossary

Antibody-toxin conjugate: the conjugate of a toxin molecule with an antibody that can bind to a specific ligand.
Cancer vaccine: a type of immunotherapy that functions by delivering tumor antigens and adjuvants to antigen-presenting cells such as dendritic cells.
Click chemistry: a type of quantitative and rapid conjugation reaction between two functional chemical groups.
Immunotherapy: therapy that boosts the body's immune responses against diseased cells such as cancer cells.

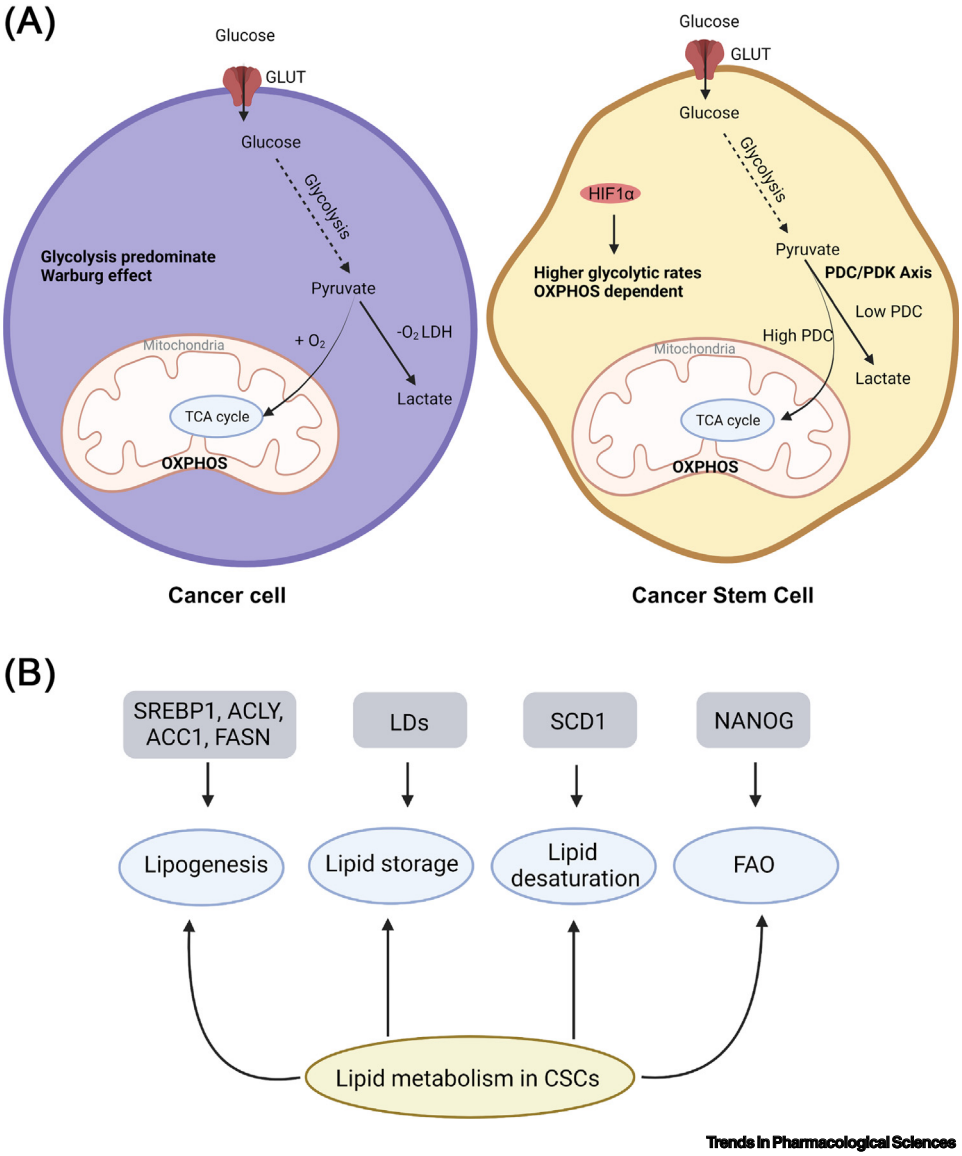

Figure 3. Cancer stem cells (CSCs) exhibit aberrant sugar and lipid metabolism. (A) Carbohydrate metabolism of CSCs and non-stem cancer cells. Non-stem cancer cells largely rely on glycolysis for ATP production even in the presence of sufficient oxygen, which refers to the Warburg effect. In addition to glycolysis, CSCs also leverage oxidative phosphorylation (OXPHOS), with the help of hypoxia inducible factor 1 subunit alpha (HIF1α), for energy production. The tendency towards the glycolysis or OXPHOS energy production pathway in CSCs is partially regulated by the pyruvate dehydrogenase complex (PDC)/pyruvate dehydrogenase kinase (PDK) axis. (B) CSCs show upregulated lipid desaturation, lipogenesis, lipid storage, and fatty acid oxidation (FAO) pathways compared with non-stem cancer cells. For lipogenesis, multiple genes encoding transporters and enzymes, including sterol regulatory element-binding protein 1 (SREBP1), ATP citrate lyase (ACLY), acetyl-coenzyme A carboxylase 1 (ACC1), and fatty acid synthase (FASN), are upregulated. Lipid droplets (LDs) are upregulated to facilitate lipid storage. Stearoyl-CoA desaturase1 (SCD1) is overexpressed to facilitate the lipid desaturation. The transcription factor NANOG is upregulated to enhance the FAO. Abbreviations: LDH, lactate dehydrogenase; TCA, tricarboxylic acid.

(Figure 4). Cell-surface markers, most of which are transporters and signaling receptors, are often a more attractive target as they can be directly utilized for targeted delivery of diagnostic and therapeutic agents to CSCs. For example, ABCB5, a cell-surface transporter in the ATP-binding

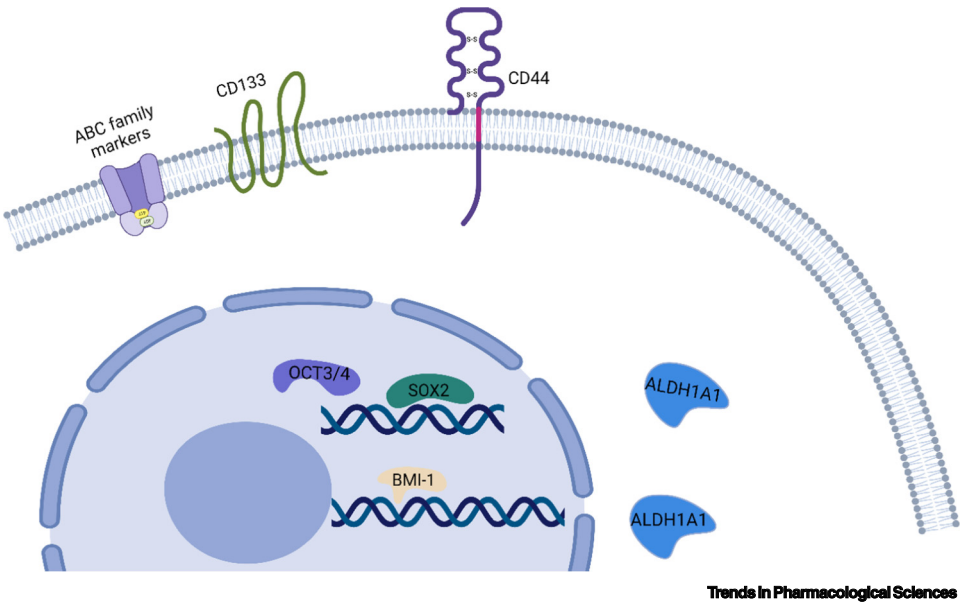

Figure 4. Common cancer stem cell (CSC)-associated biomarkers for targeting purposes. CSC-associated biomarkers, either surface or intracellular markers, are identified from their aberrant signaling and metabolic pathways. Cell-surface markers include CD133, CD44, and ABC family markers. Among them, CD133 is involved in the Wnt and PI3K–Akt signaling pathways. CD44 is a receptor for hyaluronic acid and other extracellular matrix (ECM) components and is often overexpressed by CSCs. ABC family markers are closely related to the drug transportation pathways and drug resistance of CSCs. Intracellular markers include aldehyde dehydrogenase 1 (ALDH1) and transcription factors such as OCT3/4, SOX2, and BMI-1. ALDH1, especially the isoform ALDH1A1, is overexpressed by CSCs to facilitate the oxidization of retinal and aliphatic aldehydes. OCT3/4, SOX2, and BMI-1 are transcription factors that maintain the stemness of CSCs.

cassette subfamily, is a commonly used marker for melanoma CSCs [35]. ABCG2, another ATP-binding cassette subfamily transporter, has been identified as a biomarker for lung cancer, pancreatic cancer, liver cancer, breast cancer, and ovarian cancer CSCs [35]. Some surface markers including ABCG2 are shared by different types of CSCs, while others are more specific to certain types of CSCs. For example, CD133, a type of cell-surface glycoprotein that can bind to cholesterol, is conserved across breast cancer, liver cancer, lung cancer, and ovarian cancer CSCs [36]. By contrast, CD34 is a reliable marker for the identification and targeting of leukemia CSCs instead of solid tumor CSCs [8,35]. Cell-surface markers commonly used to characterize conventional stem cells have also been utilized to identify CSCs. One notable example is CD44, a cell-surface adhesion receptor shared by nearly all types of stem cells or adult cells with some levels of stemness. CD44 is widely used as a biomarker for breast cancer, colon cancer, and glioblastoma CSCs [7,8,15,16]. These cell-surface markers have enabled the development of antibody- or aptamer-based targeting technologies for the diagnosis and targeted treatment of CSCs in various preclinical studies. Therapeutic agents, including small molecules, proteins, nucleic acids, and nanoparticles, can be functionalized with the corresponding antibodies or other targeting ligands for enhanced binding to CSCs [5,7–9,37]. One central challenge in leveraging the cell-surface markers of CSCs, however, is their poor specificity and low abundance. All of the identified surface markers are hardly specific to any type of CSCs, as they are often also expressed by non-stem cancer cells or healthy cells, albeit at a lower abundance.

While a large library of intracellular molecules might show varied concentrations between CSCs and other populations, intracellular enzymes that are overexpressed by CSCs have been the main type of molecules explored for CSC-targeting purposes. Various CSC-overexpressed

enzymes enable the design of prodrugs that can be activated in the presence of the specific enzyme and thus preferentially kill CSCs [9,38,39]. For example, aldehyde dehydrogenase 1 (ALDH1) is overexpressed by breast cancer, prostate cancer, colon cancer, lung cancer, and ovarian cancer CSCs and has been utilized to design CSC-activatable prodrugs [40]. ALDH1 has also been actively explored as an intracellular marker for the detection and sorting of CSCs. In addition to leveraging the enzymatic activity, various types of enzymes involved in essential intracellular metabolic pathways of CSCs have been the target of inhibitor design. Similarly, transcription factors that dictate the proliferation and differentiation processes of CSCs, including BMI-1 and c-MYC, have allowed the design of inhibitors that induce the apoptosis of CSCs [41,42]. Other CSC-associated transcription factors of interest include OCT3/4 and SOX2, which are essential in maintaining the tumorigenicity and stemness of CSCs, respectively [43,44]. One concern, however, is that intracellular transcription factors are not specific to CSCs, as most signaling and metabolic pathways are shared by CSCs, non-stem cancer cells, and healthy cells.

CSC-targeted chemotherapy and immunotherapy

Cancer therapies, including chemotherapy and immunotherapy, that aim to eradicate CSCs have been actively explored. CSC-targeted chemotherapies aim either to disrupt or orchestrate the essential intracellular pathways of CSCs or utilize surface receptors to deliver chemotherapeutic agents into CSCs, to effectively kill CSCs. As the immunosuppressive tumor microenvironment plays a critical role in maintaining the stem-like property of CSCs, a variety of immunotherapies that can reprogram the tumor microenvironment are also shown to effectively eliminate CSCs.

Chemotherapy remains the first-line treatment for the vast majority of cancer patients. Various types of chemotherapeutic drugs have been designed to preferentially kill CSCs, with a goal of improving the overall therapeutic benefit while reducing the off-target side effects. One common strategy is to design drugs to inhibit the intracellular proteins that play a critical role in the proliferation, differentiation, or metabolism of CSCs. For example, inhibitors of ALDH1 were able to suppress the expression of stemness-associated factors and induce the apoptosis of CSCs [40]. Several kinase inhibitors were developed to disrupt essential intracellular pathways of CSCs such as the Wnt/β-catenin signaling pathway [38,39,45–47]. Napabucasin, a STAT3 inhibitor, was able to induce the effective killing of lung cancer CSCs, reduce metastasis, and prolong the survival of patients in clinical trials [47]. GDC-0449, an inhibitor of the hedgehog pathway designed to target glioblastoma CSCs, is also being evaluated for the treatment of glioblastoma in a clinical trial [48]. In addition to the intracellular pathways, cell-surface receptors such as CD133 and CD44 have been utilized to deliver chemotherapeutic drugs into CSCs. For example, the CD133 **antibody-toxin conjugate** was developed for the treatment of ovarian cancer in a mouse model [37]. Gemcitabine-incorporating nanoparticles functionalized with CD44 antibody were able to target CD44-positive breast cancer CSCs and improve the overall therapeutic efficacy [49].

CSCs play a critical role in shaping the immunosuppressive tumor microenvironment, especially for poorly immunogenic solid tumors such as breast cancer, glioblastoma, lung cancer, liver cancer, and pancreatic cancer [50–52]. CSCs can suppress the activity of effector T cells, antigen-presenting cells, and natural killer cells by expressing high levels of inhibitory receptors (e.g., PD-L1), secreting non-inflammatory cytokines including IL-4, IL-10, IL-13, and TGF-β, expressing a low level of MHC I, and preventing the tumor infiltration of immune cells [53,54]. Existing immunotherapies such as immune checkpoint blockade, chimeric antigen receptor (CAR) T cell therapy, and therapeutic **cancer vaccines** were also able to reprogram CSCs as part of the mechanism to reprogram the immunosuppressive tumor microenvironment (Figure 5). For example, natural killer cell therapy was shown to effectively kill glioblastoma CSCs [55,56]. CSC-based therapeutic cancer

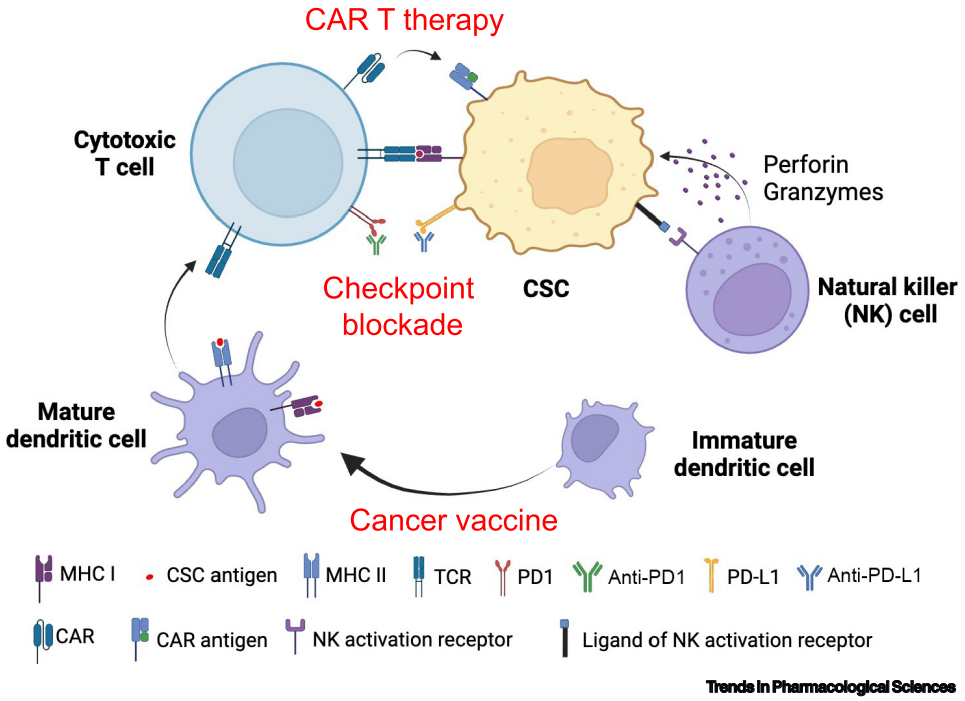

Figure 5. Cancer stem cell (CSC)-targeted immunotherapy. (1) CSCs overexpress PD-L1 as part of the mechanism to suppress T cells. Immune checkpoint blockades such as anti-PD-1 and anti-PD-L1 can reactivate effector T cells in the tumor microenvironment for improved killing of CSCs. (2) Chimeric antigen receptor (CAR) T cell therapy that targets surface markers of CSCs such as CD133 and CD44 can be designed. (3) CSC-based cancer vaccines can also induce the presentation of CSC antigens by dendritic cells, for subsequent priming of CSC-specific effector $CD8^+$ T cells. (4) As CSCs express high levels of ligands for natural killer (NK) activation receptors (NKG2D, DNAM1, and NKp30), NK cell therapy has also been explored for effective killing of CSCs. Abbreviation: TCR, T cell receptor.

vaccines were also able to induce a potent CSC-specific cytotoxic T lymphocyte (CTL) response and robust antitumor efficacy against murine D5 melanoma and SCC7 squamous cell tumors [57]. Silencing the Nanog gene of tumor cells, a characteristic gene of CSCs, further improved the CSC-specific CTL response and therapeutic efficacy [58]. In addition, similar to chemotherapeutics and radioisotopes, the surface receptors of CSCs have been utilized for targeted delivery of immunomodulatory agents [50,51].

Installation of artificial markers on CSCs

While endogenous surface receptors of CSCs are promising for the development of CSC-targeted therapies, none of the known surface markers are specific to CSCs. The difference in the expression levels of these biomarkers between CSCs, non-stem cancer cells, and healthy cells is typically small, limiting the achievable CSC-targeting efficiency [59–61]. In view of this, effort has been made to install artificial markers on the surface of CSCs for subsequent targeting applications. Metabolic glycan labeling of unnatural sugars provides a facile but powerful strategy to introduce unique chemical tags (e.g., azide, alkyne, alkene, ketone) onto the membrane of cells [62,63]. These unnatural sugars can be taken up by cells including CSCs, undergo metabolic glycoengineering processes, become conjugated to glycoproteins and glycolipids, and are eventually expressed on the cell membrane [64–66]. Recently, a controlled metabolic glycan labeling strategy (i.e., selective labeling of cells of interest in a trigger-responsive manner has also been developed (Figure 6A,B) [67,68]. Only in the presence of a specific trigger, which could be UV light, hydrogen peroxide, or enzymes, can the unnatural sugars be reactivated and metabolized

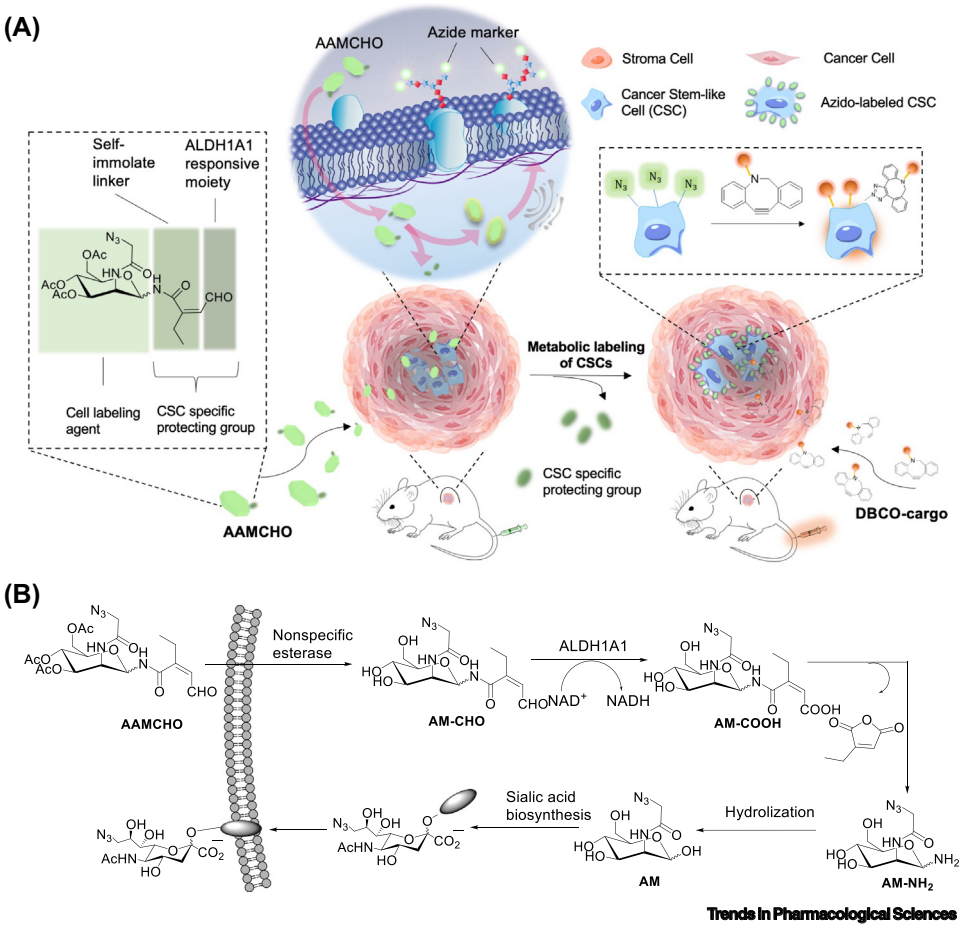

Figure 6. Aldehyde dehydrogenase 1 isoform A1 (ALDH1A1)-responsive AAMCHO can selectively label cancer stem cells (CSCs) with azido groups. (A) Schematic illustration of AAMCHO-mediated metabolic labeling of CSCs with azido groups and subsequent targeting of therapeutics via click chemistry. AAMCHO is designed to have an ALDH1A1-responsive moiety and a self-immolative linker. Only in the presence of ALDH1A1 can AAMCHO be reactivated and metabolized via the metabolic glycoengineering pathways. As CSCs overexpress ALDH1A1, AAMCHO can preferentially metabolically label CSCs with azido groups. The azido-labeled CSCs then enable targeted conjugation of therapeutics via efficient and bioorthogonal click chemistry. (B) Metabolic glycoengineering process of ALDH1A1-responsive AAMCHO. AAMCHO can be oxidated by ALDH1A1 to form AM-CHO, which is further converted to AM via self-immolative cleavage of the linker and hydrolysis. AM (*N*-azidoacetylmannosamine) can then participate in metabolic glycoengineering pathways and become expressed on the cell membrane in the form of glycoproteins. Adapted from [68].

by the cells. One such azido-sugar, histone deacetylase (HDAC)/cathepsin L (CTSL)-responsive acetylated azidomannose (DCL-AAM), could preferentially label HDAC/CTSL-overexpressing cancer cells with azido groups. The cell-surface azido groups can serve as artificial receptors to mediate targeted conjugation of dibenzocyclooctyne (DBCO)-bearing therapeutics via efficient and bioorthogonal **click chemistry** between the azide and DBCO [67]. Recently, an ALDH1A1-responsive sugar precursor named AAMCHO was developed for selective metabolic labeling of ALDH1A1-overexpressing CSCs (Figure 6A,B). It was demonstrated that AAMCHO can metabolically label colon cancer and lung cancer CSCs with azido groups *in vitro* and *in vivo*, enabling subsequent targeted conjugation of DBCO chemotherapeutics to CSCs via efficient click chemistry. Compared with the conventional CD44 marker, AAMCHO-mediated metabolic tagging could better distinguish CSCs from non-stem cancer cells, and provides an unprecedented approach to the targeting of CSCs [68].

Concluding remarks and future perspectives

In summary, the past decades have witnessed significant progress in the elucidation of aberrant intracellular signaling and metabolic pathways of CSCs and the identification of CSC biomarkers. These biomarkers include surface receptors, enzymes, transcription factors, and other molecules upregulated in CSCs [8,69,70]. Cell-surface markers of CSCs such as CD133 and CD44 enable the targeted delivery of therapeutics such as chemotherapeutics, radioisotopes, and immunomodulators [71,72]. CSC-overexpressed enzymes can be utilized to design enzyme-activatable prodrugs that preferentially kill CSCs and allow the design of enzyme inhibitors that disrupt the essential pathways and thus induce the apoptosis of CSCs [73,74]. Other intracellular molecules involved in key intracellular pathways of CSCs, including transcription factors, have also inspired the design of inhibitors as CSC-targeted chemotherapeutics [75–77]. However, none of the biomarkers is exclusively specific to CSCs, with most of them showing slight differences between CSCs and non-stem cancer cells or normal cells. These issues motivate the identification of new endogenous CSC markers or the development of novel strategies to differentiate CSCs from other cell populations (see Outstanding questions).

Outstanding questions

How can we identify new CSC-specific biomarkers, either cell surface or intracellular?

How can we design CSC-specific unnatural sugars that can selectively label CSCs with clickable chemical tags *in vivo*?

How can we make the best use of existing CSC-associated markers for cancer treatment?

How can CSC-targeted cancer therapies be better integrated with conventional chemotherapy, radiation therapy, and immunotherapy?

In addition to the endogenous biomarkers, strategies to introduce artificial biomarkers into CSCs have also been actively explored. Metabolic glycoengineering of unnatural sugars provides a facile tool to introduce artificial chemical receptors onto cell membranes for subsequent targeting purposes. However, the metabolic glycoengineering processes occur in both cancer cells and healthy cells. The recently developed controlled labeling strategy (i.e., modification of the carbon-1 site of azidomannoses with a trigger-cleavable protecting ether bond) enables metabolic labeling of cells in a trigger-dependent manner [67,68]. This trigger could be external (e.g., UV light) or internal (e.g., enzymes, hydrogen peroxide). One such azido-sugar, AAMCHO, can metabolically label cells in an ALDH1A1-activatable manner and thus can preferentially label ALDH1A1-overexpressing CSCs with azido groups [68]. This approach converts the intracellular ALDH1A1 to the cell-surface clickable tags, providing a new avenue to developing CSC-targeting technologies. In principle, unnatural sugars that can reactivated by other CSC-overexpressed enzymes can also be developed for CSC labeling and targeting. One concern, however, is the relatively low labeling efficiency of AAMCHO, as the installation of an ALDH1A1-responsive moiety is at the sacrifice of the overall metabolic labeling efficiency. A fine balance between labeling efficiency and selectivity needs to be reached. This issue can be partially addressed by deploying strategies to improve the delivery of enzyme-activatable unnatural sugars into tumors, such as the use of nanoparticles [78–80].

Metabolic lipid labeling could be an alternative approach to achieve chemical tagging of CSCs [81–83]. Considering the aberrant lipid metabolisms in CSCs, the rational design of unnatural lipids based on either structural lipids [e.g., dipalmitoylphosphatidylcholine (DOPC)] or signaling lipids (e.g., ceramide, phosphatidylinositol lipid) could potentially achieve preferential tagging of CSCs over non-stem cancer cells or healthy cells [84]. The chemical tags (e.g., azido groups) then enable targeted delivery of therapeutics to CSCs. Compared with metabolic glycoengineering pathways that involve numerous reactions, lipid metabolic pathways involve fewer steps and potentially lead to a higher labeling efficiency [85]. Similar to metabolic glycan labeling, strategies that can improve the delivery of unnatural lipids to tumors and the uptake of unnatural lipids by CSCs could further increase the labeling efficiency and subsequent CSC-targeting effects [86].

In addition, efforts to make the best use of existing CSC markers are also needed. While it remains challenging to differentiate between CSCs and non-stem cancer cells, the differences between these cancer cells and healthy cells are often more significant. This allows the preferential delivery of therapeutics to CSCs and non-stem cancer cells or the activation of prodrugs in tumor tissues.

The synergistic attack at CSCs and non-stem cancer cells could potentially lead to favorable antitumor efficacy [87–89]. Simultaneous targeting of CSCs and non-stem cancer cells could be essential in amplifying the immunomodulation of the tumor microenvironment, tumor antigen spreading, and systemic antitumor T cell responses [90–92].

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Declaration of interests

The authors declare no competing interests.

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