Tracing and targeting cancer stem cells: New venture for personalized molecular cancer therapy

Ramin Radpour

Tumor Immunology and Cancer Stem Cells, Inselspital, Bern University Hospital, Department for BioMedical Research, University of Bern, 3008 Bern, Switzerland

ORCID number: Ramin Radpour (0000-0002-5632-7833).

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Correspondence to: Ramin Radpour, MSc, PhD, Tumor Immunology and Cancer Stem Cells, Inselspital, Bern University Hospital, Department for BioMedical Research, University of Bern, Murtenstrasse 35, 3008 Bern, Switzerland. ramin.radpour@dbmr.unibe.ch
Telephone: +41-31-6320956
Fax: +41-31-6323297

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Abstract

Tumors consist of a mixture of heterogeneous cell types. Cancer stem cells (CSCs) are a minor sub-population within the bulk cancer fraction which has been found to reconstitute and propagate the disease and to be frequently resistant to chemotherapy, irradiation, cytotoxic drugs and probably also against immune attack. CSCs are considered as the seeds of tumor recurrence, driving force of tumorigenesis and metastases. This underlines the urgent need for innovative methods to identify and target CSCs. However, the role and existence of CSCs in therapy resistance and cancer recurrence remains a topic of intense debate. The underlying biological properties of the tumor stem cells are extremely dependent on numerous signals, and the targeted inhibition of these stem cell signaling pathways is one of the promising approaches of the new antitumor therapy approaches. This perspective review article summarizes the novel methods of tracing CSCs and discusses the hallmarks of CSC identification influenced by the microenvironment or by having imperfect detection markers. In addition, explains the known molecular mechanisms of therapy resistance in CSCs as reliable and clinically predictive markers that could enable the use of new targeted antitumor therapy in the sense of personalized medicine.

Key words: Cancer stem cells; Cancer recurrence; Cancer therapy; Combination therapy; Chemotherapy; Radiation therapy; Immunotherapy

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Core tip: Cancer stem cells (CSCs) are small subpopulation of the tumor that can survive from conventional treatment, escape from the immune system and can cause recurrence of cancer disease. Therefore, any attempt in detection and selective therapeutic targeting of CSCs will ultimately lead to better cancer treatments and can play an important role in reducing the cancer related mortalities. This review highlights the trends and approaches in CSC tracing, isolating, characterizing and targeting, which are key strategies for a novel personalized molecular cancer therapy.
INTRODUCTION

Cancer originates from deregulation of growth and resistance to apoptosis of transformed cells that acquire proliferative and metastatic capacity. While only a few genetic and epigenetic alterations can initiate the malignant transformation of healthy cells, clinically visible tumors are extraordinarily complex structures with cancer cells displaying a large number of mutations and altered gene expression[1]. The hierarchical model of tumor organization represents a similar, albeit distorted, arrangement of the tumor cells, as are their tissues of origin. The stem cell population is positioned at the top of the cell hierarchy and has the ability to self-renew and multilineage differentiate to progenitors or differentiated cell types whose proliferation capacity is restricted[2,3].

The theory of the cancer stem cell (CSC) was postulated in the 1970s and was confirmed experimentally by the isolation of tumor-initiating cells using cellular/molecular biomarkers that allowed the isolation of CSCs in acute myeloid leukemia[4]. Further, CSC has been demonstrated in a variety of solid tumors such as tumors in brain, colorectal, head and neck, liver, lung, mammary glands, pancreatic prostate carcinomas, melanoma and hematopoietic malignancies (e.g., myeloid or lymphoid leukemia)[5-7]. Cell lines derived from these tumors also contain CSCs and tumor precursor cells, which represent a promising model for cancer stem cell research[1]. The functional characterization of CSCs revealed that these cells represent a small subpopulation of the tumor that can survive from conventional treatment, scape from the immune system and therefore can cause recurrence of cancer disease. Therefore, CSCs are driving force of tumorigenesis and metastases (Figure 1). According to the concept of a stem cell, it is assumed that even a few surviving CSCs after tumor therapy, is sufficient to form a new tumor[8].

In each cancer cell clone, which is characterized by harboring different combinations of mutations or genetic alterations, the processes of self-renewal, and differentiation occur differently based on the type of genetic lesions[9]. Nevertheless, significant similarities between normal and tumorigenic, experimentally identified stem cells could be expected. Both stem cell types (normal or cancerous) are rarely active, dependent on a specific microenvironment (so-called “stem cell-niche”) and have a number of self-protection mechanisms[2]. This niche enables a dynamic interaction between stem cells and surrounding cells including immune cells (“immune-niche”), cytokines and chemokines that regulates maintenance, quiescence, self-renewal and differentiation of stem cells to provide an optimal stem cell-supporting setting. What contributes to formation of the niche for tumor stem cells is the subject of intensive research[10]. Normal stem cells are more microenvironment dependent in order to get dynamic input to balance between activation and differentiation or self-renewal and quiescence “extrinsic factors”[11,12]. Although CSCs can represent more autonomous regulatory characterization “intrinsic factors”, similar concept of stem cell niche support could also hold for them[13]. The majority of studies using the isolated CSCs, shows the dominant effect of intrinsic factors on CSC regulation. While, other studies propose a role for the CSC niche[12]. This model suggests that less malignant tumors may have more demand on the stem cell niche but upon cancer progress this dynamic interplay might be weaken or even diminished[14].

An inductor of the stem cell phenotype is hypoxia[15-17]. The self-protection mechanisms are due to the expression of numerous proteins, which reduce the effects of genotoxic xenobiotics. These include the members of efflux pumps, such as ABCB1-MDR1, ABCG2-MRP1 and ABCG2-BCRP, other specific detoxification systems, such as aldehyde dehydrogenase and increased DNA repair capacity. The symmetric cell division and asymmetric distribution of the DNA can also be regarded as part of stem cell self-protection mechanisms[15]. For the tumor stem cells, the existence of the same mechanisms is a crucial cause of their therapeutic resistance.

In addition to hypoxia as a triggering factor, growth factors play an important role, leading to epithelial-mesenchymal transition (EMT) in cells. It is shown a high-level regulation of stem cell markers after the induction of EMT in normal epithelial cells of the breast gland tissue and in mammary carcinoma cells[18]. One of the EMT effects can be the induction of the stem cell phenotype[18].

Numerous findings could show that routine tumor therapy approaches (classical chemotherapy or radiation therapy) and even the majority of currently used targeted antitumor drugs, so-called biological therapy, have little effect on the tumor stem cells even in chemoradio-sensitive tumors[19]. While, the stationary tumor stem cells largely retain their epithelial character and are therefore responsible for the primary tumor growth or recurrence, the migrating tumor stem cells exhibit ability for invasion and distance metastasis. This highlights the above-mentioned plasticity of the tumor stem cells (Figure 1).

THERAPY RESISTANCE IN CSCs

A small number of immortal cells within the bulk tumor with a character of CSC causes the chemoradiotherapy resistance. Such cells with stem cell characteristics, seem to grow aggressively and metastasize easily. It is not yet clear how CSCs are formed, whether they develop from tissue stem cells or are formed from differentiated cells by recovering embryonic properties. Chemotherapeutic agents and radiotherapy mainly destroy dividing cells[20].
Since CSCs are particularly dormant, in one hand they are not detected by the routine screening measures, and in the other hand, they are positively selected upon the routine therapy approaches.

**MOLECULAR MECHANISMS OF THE THERAPY RESISTANCE OF CSCs**

Central regulators of the cellular response to DNA damage are checkpoint kinases 1 and 2 (Chk1/2), which are activated after genotoxic stress and stop cell proliferation to allow DNA repair. Activation of Chk1 as a response to DNA damage by ionizing radiation or chemotherapy agents can be detected preferentially in CD133$^+$ glioblastoma precursor cells\(^{[21]}\). By pharmacological inhibition of Chk1, it was possible to increase the sensitivity of CD133$^+$ glioblastoma precursor cells against therapy\(^{[21]}\).

An efficient inactivation of reactive oxygen species (ROS) is another feature of CSCs. The excessive production of ROS under chemo/radiotherapy leads to a cell damage because of its interaction with DNA and proteins and triggering the cell death. In some tumors, including mammary carcinoma and gastrointestinal carcinoma, fewer amounts of ROS were detected in CSCs with a simultaneously increased amount of free-radical scavenger compared to the cell populations without CSC phenotype\(^{[22]}\). In addition, the expression of stem cell marker CD44 in tumor cells was associated with an increased expression of the glutathione as a free-radical scavenger\(^{[23,24]}\). Pharmacologically induced reduction in the concentration of free-radical scavenger in tumor cells can significantly increase their sensitivity to the chemo/radiotherapy\(^{[25]}\). It remains unclear whether the increased CD44 expression as a biomarker is suitable for the detection of ROS-resistant CSCs and thus can identify patients who can benefit from therapy with inhibitors of free-radical scavengers in combination with the chemo/radiotherapy.

Another factor contributing to the chemo/radiotherapy resistance of CSCs is hypoxia. Among other factors, hypoxia is the most common cause of therapy-resistant CSCs, which activates the hypoxia inducible factor signaling pathway and triggers cellular processes that can lead to a better survival and expansion of CSCs\(^{[26]}\). The presence of hypoxia in the tumor tissue or its decrease by reoxygenation in the course of chemo/radiotherapy could be correlated with an accelerated repopulation of CSCs with therapy-resistance phenotype\(^{[27]}\).

There are several critical proliferation-promoting and survival-inducing pathways triggering the maintenance and survival of CSCs. The canonical Wnt pathway, which is central signaling pathway for stem cell maintenance and development, is constitutively active in breast cancer, colorectal cancer, myeloid leukemia, lung cancer and skin cancer\(^{[28,29]}\). Hedgehog Signaling (HH), which has three different homologues desert Hedgehog, Indian
Hedgehog and Sonic Hedgehog is essential in a variety of molecular and cellular processes during tissue homeostasis, development or embryogenesis. Aberrant HH activation which regulates the CSC's maintenance and potential proliferation, is reported in different cancers including acute myeloid leukemia (AML), breast cancer, chronic myeloid leukemia (CML), glioblastoma, lung carcinoma, myeloma, pancreatic adenocarcinoma, pancreatic cancer, and solid tumors. Canonical Notch signaling is the other conserved signaling pathway in tissue homeostasis and development. Activation of Notch signaling upon binding of the extracellular ligands, regulates the expression of target genes involving in CSC self-renewal and maintenance and potential proliferation like EGF/VEGF signaling is illustrated in the Figure 2.

The predicted crosstalk among Wnt signaling, Notch pathway, Hedgehog signaling and other pathways like EGF/VEGF signaling in CSCs and cancer. Gene networks and canonical pathways were assessed using the Ariadne Genomics Pathway Studio® program and database (Elsevier). EGF: Epidermal growth factor; VEGF: Vascular endothelial growth factor; WNT: Wnt signaling pathways; PI3K: Phosphoinositide-3-kinase; PIP3: Phosphatidylinositol 3,4,5 trisphosphate; CSC: Cancer stem cell; SHH: Sonic Hedgehog.

Figure 2  Crosstalk between cancer and cancer stem cell-related pathways. Predicted crosstalk among Wnt signaling, Notch pathway, Hedgehog signaling and other cancer-related pathways like EGF/VEGF signaling in CSCs and cancer. Gene networks and canonical pathways were assessed using the Ariadne Genomics Pathway Studio® program and database (Elsevier). EGF: Epidermal growth factor; VEGF: Vascular endothelial growth factor; WNT: Wnt signaling pathways; PI3K: Phosphoinositide 3-kinase; PIP3: Phosphatidylinositol 3,4,5 trisphosphate; CSC: Cancer stem cell; SHH: Sonic Hedgehog.

Methods for Screening of CSCs
Over the past decade, different CSC markers were identified in a wide range of hematopoietic malignancies and solid tumors. A widely used method for characterizing CSC-related markers is multiparameter flow cytometry. This method, which is originally developed for the analysis of blood cells and hematopoietic stem cells, offers the possibility to detect CSCs by means of specific surface markers that are stained with fluorescence-coupled antibodies. Frequently, the expression of CD133 or CD44 alone or in combination with further markers such as CD20, CD24, CD90 or CD44 alone or in combination with further markers such as CD20, CD24, CD90 or a-2-3-integrin is used as a CSC-specific marker (Figure 3). Functional detection of CSC is also possible and is based on the increased expression of detoxification enzyme aldehyde dehydrogenase 1 (ALDH1) or the high activity of multidrug resistance transport proteins. These CSC-specific staining methods allow the isolation of single CSCs for further molecular characterization using single cell based molecular approaches (Figure 3). However, identified markers are not always reliable and none of the reported markers solely identify CSCs, therefore need to be used with caution (Table 1).

For example, inter- or intra-tumor heterogeneity
may completely render CSC markers inapt. Such tumor heterogeneity can be the result of different genetically distinct clones within the tumor due to having various genetic lesions or dysregulation of markers. Inactivation of specific markers due to any scape mechanism in a particular clone may completely render CSC markers inapt. Such tumor genetic carcinoma cell lines by inducing the EMT. After treatment with inhibitors, the survival of the enriched and may render these CSCs undetectable in the absence of other distinct markers.

While high-throughput genetic screening studies provide essential information about genes which are associated with a particular phenotype, molecular pharmacology can play an important role in development of a specific molecular therapy. Low molecular weight substances ("small molecules") show a higher penetrance in cell-based screening methods. Therefore, small molecules are one of the most frequently used therapeutic agents. The screening of large substance banks has identified many valuable compounds that can be used to modulate biological systems in cancer cells[45]. In order to systematically identify the genes that regulate the death and differentiation of CSCs, high-throughput screenings of RNA interference (RNAi) or chemical substance libraries are carried out using different approaches. The readout of such screen approaches can be survival analysis, reporter assays, luminescence or fluorescence-based analyzes of particular genes or pathways and imaging methods, in which several cellular properties can be examined on a particular genes or pathways and imaging methods, in which several cellular properties can be examined on a particular genes or pathways and imaging methods, in which several cellular properties can be examined on a particular genes or pathways and imaging methods, in which several cellular properties can be examined on a particular genes or pathways and imaging methods, in which several cellular properties can be examined on a particular genes or pathways and imaging methods, in which several cellular properties can be examined on a particular genes or pathways and imaging methods, in which several cellular properties can be examined on a single cell level.

Since CSCs only make up a small fraction in the entire tumor cell pool (Figure 1), appropriate enrichment methods must be applied. Gupta et al[46] enriched CD44+/CD24− cells within the CSC population of mammary carcinoma cell lines by inducing the EMT. After treatment with inhibitors, the survival of the enriched and

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**Figure 3** Tracing and targeting cancer stem cells. A: The complex process of distant metastasis including invasion of the tumor microenvironment, EMT, shedding of CSCs into the blood stream (intravasation), MET and invasion of circulating CTCs to the other tissues (extravasation). Only circulating CSCs are able to survive in the circulating blood, escape from immune-surveillance and home to secondary organs; B: The list of known compilation CSC-related molecular markers for different solid tumors and hematopoietic malignancies. The level of specificity of these markers differs per each type of tumor. Markers are ordered alphabetically and not according to their sensitivity or specificity; C: Four important approaches of CSC-targeted therapy: CSC: Cancer stem cell; CC: Cancer cell; NC: Normal cell; EMT: Epithelial-mesenchymal transition; MET: Mesenchymal-epithelial transition; PI3K: Phosphoinositide 3-kinase; MAPK: Mitogen-activated protein kinase; TGF: Transforming growth factor; mTOR: Mechanistic target of rapamycin; RAS: Ras-activated signaling; PD-1: Programmed death 1; PD-L1: Programmed death-ligand 1; EMT: Epithelial cell adhesion molecule.
the non-selected cell population was investigated using a luminescence-based reporter assay. This study was able to identify salinomycin as a selective inhibitor of the CSC population in breast carcinoma.\cite{46}

Recent advances in computer-based image analysis have enabled rapid achievements in the development of image-based high-throughput analysis approaches. The direct visualization of cellular features and biological processes allows a more comprehensive measurement of responses to interferences. Xia et al.\cite{47} have developed a novel fluorescence imaging method to identify cancer cells with CSC properties through their increased ability to deliver fluorescent dyes via dedicated molecular transporters. Based on this method, a library of active substances was examined for their effect in CSCs. It was possible to identify substances that selectively inhibit the molecular transporters.\cite{48}

A further high-throughput method has recently been developed to characterize the biochemical and biophysical environmental conditions of CSCs. Microarray glass slides with over 2000 test chambers can be used to cultivate stem cells in different cell densities in a hydrogel of polyethylene glycol, to which different biological molecules have been coupled by robot technology.\cite{49} Using the microscopic imaging, cell proliferation, morphology and differentiation can be monitored at a single cell level. This method as a platform for the investigation of individual stem cells in a microfluid culture system with simultaneous live-cell microscopy, represents an important step towards the miniaturization of the cellular processes as a high-throughput screening approach.\cite{50}

| Problems                                                                 | Potential solutions                                                                 |
|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| CSC-related markers may not be specific by their own for a certain type of tumor | Combined used of different markers may be the solution                              |
| Some of CSC-related markers may be down-regulated or suppressed in a given tumor due to different genetic or epigenetic regulatory mechanisms | Using of distinct markers or a combination them                                      |
| Splice variants of some CSC-related markers may render detection difficult | The exact splice variant should be considered for the detection                       |
| Markers can be detected using one method (e.g., FACS), but not with other methods (e.g., immunohistochemistry) | Stringent selection of related markers might be required                             |
| Different tumors have clonal variation and heterogeneous cell population. Less malignant clones may harbor CSCs that express different markers. Therefore, CSC-related markers may be differentially regulated within different clone or be completely missed | Using more specific and sensitive methods, isolate more enriched CSC populations     |
| Many of reported CSC-related markers are not validated, since they derived from cell-line or mouse model studies | Markers should be validated in xenotransplants or primary human materials           |

CSC: Cancer stem cell; FACS: Fluorescence-activated cell sorting.

**Targeting efflux transporters**

Membrane efflux transporters, which are mainly located in blood-brain barrier, hepatocytes, intestinal cells or kidney proximal tubules, play important roles in drug metabolism, availability, and toxicity of drugs in human body.\cite{51} Several studies indicate that transporter-mediated drug disposition plays an important role in mediating chemo-sensitivity and -resistance of cancer cells and CSCs.\cite{52} The interaction between efflux transporters and chemotherapeutic drugs on cancer cells is significantly linked to the efficacy of cancer therapy. Two major superfamilies of efflux transporters are the ATP-binding cassette (ABC) transporters [ABCB1 (MDR1), ABCC1 (MRP1), ABCC2 (MRP2) and ABCG2 (BCRP)] and the solute carrier (SLC) transporters [SLC19A1 (RFC1) and SLCO1B1 (SLC21A6)]. Therefore, targeting efflux transporters within cancer therapy combined with routine therapies could significantly increase the eradication rate of resistant cancer cells.\cite{53}

**Targeting key signaling pathways**

The CSC phenotype depends on various cellular signals, which are triggered by the underlying genetic lesions and by the support of the stem cell niche. Some of
these signals have already been identified; the most
disease cussed signaling pathways are the classic Wnt-
ß-catenin, Notch and Sonic Hedgehog signaling57-59. For
these three pathways, pharmacological inhibitors have
been developed which are now undergoing clinical trials
in many independent studies60. However, the clinical
effect is largely depending on the tumor type and not
all three pathways are equally important in all types of
tumors. It has been shown that, although some signaling
pathways are highly tumor-promoting in a certain type
of cancers (which makes it a suitable therapeutic target),
they might react as tumor suppressive in another tumor
type; therefore, their inhibition may become dangerous
(e.g., Notch-1 has been identified as a tumor suppressor
in urinary bladder carcinoma)61. High Wnt pathway
activity marks colon or leukemia CSCs and is required
for stemness signature as a prognostic marker6,7,62.
In addition, Wnt activity is associated with the CSC
markers CD133, CD44 and LGR5 in colon cancer63
whereas Hedgehog activity is linked to ABC transporter
expression in esophageal and prostate cancer17,64
TGF-ß signaling via the family members Nodal and
Activin is attributed to pancreatic CSCs65. The effect of
Hedgehog inhibitors is actually the most evident in the
basal cell carcinoma. In addition, inhibition of Hedgehog
pathway blocks stemness in breast CSCs, whereas its
activation enhances self-renewal66. It is also necessary
to distinguish whether those signaling pathway has
been activated within CSCs only because of harbored
genetic lesions67. If only the CSCs are targeted, it is
hardly possible to expect a dramatic tumor shrinkage
as in classical successful chemo/radiotherapy; rather,
this would be a disease stabilization and a slowing of
the progression (Figure 1).

Disulfiram was developed as an inhibitor of aldehyde
dehydrogenase for the treatment of alcoholism. This
inhibition leads to the accumulation of acetaldehyde
after alcohol consumption, resulting in a marked nausea
that should reduce the probability of further alcohol
consumption68. The same enzymatic activity - aldehyde
dehydrogenase - is, however, a component of the self-
protection of the CSCs, and thus disulfiram was used
for the elimination of CSCs. Thoridazine is an inhibitor
of dopamine receptors, and is a standard medication
for mental disorders such as schizophrenia. Its rational
use in tumor therapy is based on the finding that CSCs
in several types of tumors (e.g., AML, breast carcinoma,
glioblastoma), in contrast to the corresponding normal
tissue stem cells, upregulate the expression of
dopamine receptors69.

Niclosamide was identified that specifically targeted
Wnt-ß-catenin signaling pathways70. Interestingly,
niclosamide is known as an antiparasitic and inhibitor
of oxidative phosphorylation, which has been used in
human medicine for almost 50 years71. However, what
has emerged is that these two antiparasites are inhibitors
of numerous other signaling pathways. Niclosamide
inhibits not only the Wnt-ß-catenin signaling pathway,
but also the Notch, PI3′K-Akt - mTOR, STAT-3 and
NFkB signaling pathways, which are essential for tumor
stem cells72. Salinomycin was similarly described as
an inhibitor of ABC efflux pumps and the Wnt-ß-catenin
signaling pathway73. Not enough, analogous effects
have been discovered for disulfiram and thioridazine.
Disulfiram has not only proved to be an effective inhibitor
of aldehyde dehydrogenase, but also polo-like kinase
1 and O6-methylguanine methyltransferase as well as
NFkB74,75.

The advantages of identifying such new indications
for old drugs are obvious. These drugs have long been
out of patent protection and their use should therefore
be much cheaper than for newly developed drugs, which
is an important aspect in the current discussion on costs
of tumor treatment. In addition, they have already
undergone clinical trials, their potential toxicity, side
effects, pharmacokinetics, contraindications, and possible
drug-drug interactions are known. Therefore, their use in
tumor therapy should be relatively easy. Perhaps the best
opportunity to see how the effects of tumor stem cell-
targeted therapy can be demonstrated is in the area of
combination therapy (Figure 1). Tumor stem cell-directed
drugs should be able to prolong the efficacy of cytotoxic
therapy and reduce recurrence risk76,77. On the other
hand, combined administration has significantly greater
chances of total elimination of all tumor cells. Taken
together, there are many possibilities for therapeutic
treatment for the elimination of tumor stem cells, both
from the group of newly developed inhibitors of some
stem cell-specific signaling pathways as well as for
some old drugs that can find a new application in tumor
therapy.

Targeting cellular surface markers (tumor
immunotherapy and cancer vaccination)
Many types of normal cells like immune cells infiltrate
tumors. Over the last years, immune infiltration has
become a central focus in cancer research50. It is
increasingly recognized that cancer cells and CSCs need
to escape immune recognition. IL-6/JAK/STAT3 signaling
an important pathway in many solid tumors. Anti-IL-6
mAb siltux-imab was tested on various cancer types,
which was not able to provide promising outcome to
improve overall survival of patients with multiple myeloma
according to a recent Phase II clinical trial on patients78.
While, checkpoint blockade antibodies such as cytotoxic
T-lymphocyte antigen 4 (CTLA-4) or programmed death-
ligand 1 (PD-1/PD-L1) like ipilimumab or nivolumab could
provide marked clinical benefits for lung adenocarcinoma,
melanoma or Hodgkin lymphomas79,80. These agents can
boost the immune system and display clinical benefits for
a fraction of patients58.

Many tumors cells including CSCs, alter the expression
of their genes or down-modulate of antigen
processing and presentation to build an immuno-sup-
pressive microenvironment that creates physical or
chemical barriers against immune cells81. Indeed,
CSCs by low express of MHC-I, and over expressing
of IL-4 are escaping from cytotoxic T lymphocytes82.
Boosting T-cell response can be a promising approach to eradicate CSCs. This can be achieved by boosting neo-antigens within CSCs, considered as tumor vaccination. Adaptive transfer of CSC-specific T-cells into tumor-bearing mice could show a success\(^\text{(83)}\). In addition, genetically modified T cells to express chimeric antigen receptors (CAR T-cells) upon adaptive transfer could provide remarkable benefit for patients suffering from different solid tumors or leukemia\(^\text{(84)}\). Therefore, the major goal of immunotherapy is to thwart these barriers in order to enhance pre-existing or elicit a new immune response against cancer.

**CONCLUSION**

Because of the CSCs’ ability to therapy resistance and initiate a recurrence after therapy, cancer stem cell is an important therapeutic target. Future research is essential to elucidate how CSCs dictate metastasis, therapy-resistance or immune-scape signature. However, without having reliable markers it will be a challenging pursuit. An exact molecular characterization of this small subpopulation in the tumor tissue requires the development of specific CSC markers and suitable enrichment methods. Particularly from innovative high-throughput screening technologies, we can expect valuable insights regarding suitable CSC-associated biomarkers and new therapeutic approaches to target CSCs. This could be an important step towards individualized cancer therapy.

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