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REVIEW

Stem Cells and Cancer: the Cancer Stem Cell (CSC) Model

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ABSTRACT

Cancer stem cells (CSCs) can be defined as cells within the tumour which are tumour-initiating and which possess the capacity to self renew and generate the heterogenous lineages of cancer cells that comprise the tumour. They are functionally characterized by their abilities to self-renew, differentiate and form tumours in immunocompromised mice. Only a certain subpopulation of cells within the tumour possesses these abilities. CSCs have been isolated from a variety of cancers and are believed to play pivotal roles in tumour initiation, tumour progression and metastasis as well as in tumour recurrence. CSCs may arise from normal stem cells in tissue, which undergo a loss of regulation of proliferation; alternatively, they can arise from normal somatic cells which acquire stem cell like characteristics. At the molecular level, intracellular signalling pathways involved in normal stem cell self-renewal and proliferation often show dysregulation or aberrant activation in CSCs. Inherent mechanisms present in CSCs appear to render them comparatively more resistant to conventional anti-cancer approaches like chemotherapy and radiotherapy. Thus, for maximal effectiveness, anti-cancer therapies would probably need to target critical molecular pathways essential for CSC self-renewal, proliferation and survival. This review provides an overview of CSCs, their roles in various cancers and discusses their probable origins, molecular pathways involved in their functions and some possible molecular methods for targeting CSCs which may hold promise for developing therapies for alleviation of cancer.

Key words: Neoplastic Stem Cells; Cancer Stem Cells; Metastasis; Antineoplastic Agents

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INTRODUCTION

Stem cells comprise a variety of unspecialized cells characterized by an extensive capacity for self-renewal and an ability to differentiate into a variety of cell types[1,2,3]. Stem cells differ from each other both in their intrinsic capability to self-renew and to differentiate. They are usually classified based on potency or origin. In mammals, stem cells are primarily divided into embryonic stem cells (ESCs) which are isolated from the inner cell mass (ICM) of blastocysts, adult (somatic) stem cells, which are found in various tissues and cord stem cells derived from the umbilical cord[3].

Cancer covers a plethora of conditions characterized by uncontrolled cellular proliferation. The causes of cancer are many and varied and include genetic predisposition, environmental influences, infectious agents and ageing. These can transform normal cells into cancerous cells by hampering a wide range of regulatory pathways[4]. Stem cells and cancerous cells appear to have a number of common characteristics including rapid proliferation, the ability to express telomerase and the possession of an indefinite replicative life span[2,5]. The possible involvement of stem cells in cancer generation and tumour growth and metastasis has been discussed by scientists for many years. However, only recently, following some success in isolation of malignant stem cells and a more comprehensive

understanding of stem cell behaviour, has serious attention been given to the role of stem cells in cancer and the consequences which arise from retention of stem cell behaviour in malignancy[1,6-9].

Cancer stem cells (CSCs) can be defined as cells within the tumour which are tumour-initiating and which possess the capacity to self renew and generate the heterogenous lineages of cancer cells that comprise the tumour[7,10]. CSCs are functionally defined by their abilities to self-renew, differentiate (i.e. produce cells with non-stem cell characteristics) and form tumours in immunocompromised mice[6]. A number of observations have indicated that only a limited subset of cells within the tumour, the CSCs, can initiate and promote tumour formation. Only 1-4% of lymphoma cells were found to form colonies *in vitro* or initiate carcinomas in mouse spleen; thus, not all cells within the tumour were tumour initiating[11]. The cells from various types of acute myeloid leukaemia (AML) which could lead to initiation of haematopoietic malignancies upon injection into severe combined immunodeficiency (SCID) mice were found to be $CD34^{++}CD38^{-}$ regardless of the heterogeneity in maturation of blasts. This subpopulation of cells also possessed the abilities of self-renewal and proliferation[12]. Experiments demonstrating that $CD44^{+}CD24^{-/low}Lin^{-}$ cells from breast cancer patients had significantly greater tumour forming ability when injected into SCID mice first indicated the presence of CSCs in solid tumours. This tumorigenic subpopulation could be serially passaged and would generate new tumours containing $CD44^{+}CD24^{-/low}Lin^{-}$ tumorigenic cells as well as the phenotypically diverse populations of non-tumorigenic cells present in the initial tumour[13]. CSCs have subsequently been isolated from a number of other solid tumours including cancers of the colon[14], brain[15], ovary[16], prostate[17], lung[18] and melanomas[19]. A fraction of cells with stem cell properties have been shown to be present in established cell lines like HeLa (cervical cancer), C-6 (glioma), MCF-7 (breast cancer) and A549 (non-small cell lung cancer)[2,20].

This review seeks to provide an overview of CSCs and their roles in various cancers and discusses their probable origins, molecular pathways involved in their functions and some possible methods for targeting and removal of CSCs which may hold promise for developing therapies for alleviation of cancer.

THE CANCER STEM CELL (CSC) HYPOTHESIS

Two models have been proposed to explain why only a limited number of cells within a tumour are capable of initiating and propagating tumour growth. The clonal evolution model (CE model) postulates that the cellular heterogeneity within a tumour is primarily caused by subclonal differences that result from genetic and epigenetic changes during cancer development. All tumour cells can contribute to tumour maintenance and any cell, having accumulated sufficient genetic changes, can potentially become invasive and cause metastasis or become resistant to therapies and cause recurrence. Stem or differentiated cell characteristics are thus phenotypes and may change over time[7,21,22].

The cancer stem cell model (CSC model) states that a specific subset of tumour cells, the CSCs, play a pivotal role in tumour initiation, progression and recurrence (Figure 1). These CSCs can self-renew indefinitely and also differentiate, leading to the production of the various heterogeneous cell types which make up a tumour. However, the majority of these cell types lack the capacity for unlimited self-renewal and the ability to produce the varied cell populations present in the tumour. Thus, the heterogeneity of the cells within a tumour result from asymmetric division of CSCs and tumours are highly hierarchical with a self-renewing population of CSCs at the top of the hierarchy[7,9,21,23]. During this process, metastasis and malignancy may occur. It has been suggested that the lower the degree of CSC differentiation, the more malignant is the tumour initiated by CSCs[24,25].

Although much of the currently available evidence appears to support the CSC model, certain findings seems to indicate that the CE model cannot be completely rejected. In metastatic sites, cell subpopulations with the capacity for self-renewal appear to display a cell surface marker profile different from the CSC that caused the origin of the primary tumour[26]. Studies on BCR-ABL1 lymphoblastic leukaemia indicate that many clinical samples contain a number of genetically distinct leukaemia-initiating subclones, linking clonal diversity with leukaemia-initiating-cell function[27]. On the other hand, in addition to the previously mentioned evidence, clinical studies of breast cancer indicate that, despite patients having thousands of single disseminated cancer cells circulating in their bloodstream, only a small percentage of cells ultimately form macroscopic metastases and secondary tumours produced by metastasis often possess heterogeneity similar to the primary tumour. Such heterogeneity could arise as a result of patterns of regeneration of CSCs[21,28]. A possible explanation, which would combine the hypothesis of both models, could be that while CSCs play important roles in tumour initiation and progression, they might undergo clonal evolution over time (Figure 2).

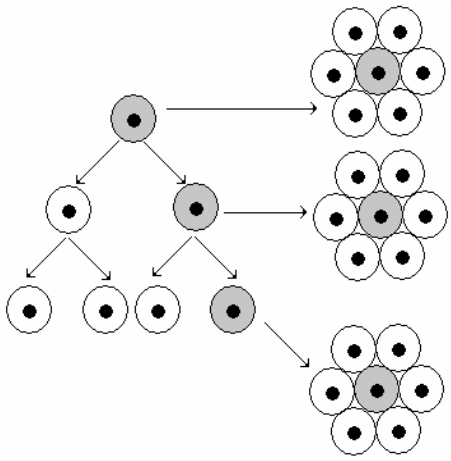

Figure 1 The Cancer Stem Cell (CSC) model. CSCs (shown in grey) are a specific subset of tumour cells which play pivotal roles in tumour initiation and tumour development. CSCs can self-renew indefinitely as well as differentiate, leading to the production of the various heterogeneous cell types (shown in white) which make up the tumour; these cell types however, lack the capacity for unlimited self-renewal.

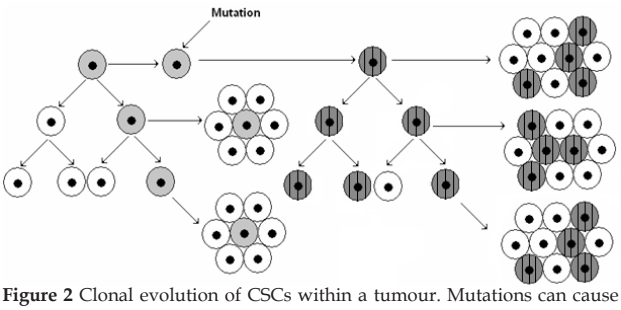

Figure 2 Clonal evolution of CSCs within a tumour. Mutations can cause the formation of a separate subclone of CSCs (shown in dark grey with vertical stripes) which are phenotypically different from the CSC which originally led to tumour initiation (shown in grey). These mutated CSC subclones are more aggressive in promoting tumour growth, metastasis and formation of secondary tumours. The various heterogeneous cell types which make up the tumour but lack the capacity for unlimited self-renewal are shown in white.

THE ORIGIN OF CSCS

CSCs can presumably arise from a number of potential pathways. CSCs may arise from normal stem or progenitor cells present within the tissue which generate tumours due to genetic mutations or environmental alterations. Alternatively, CSCs may arise from normal somatic cells which manage to acquire stem cell like characteristics and malignant behaviour through genetic or other alterations.

(a) Origin from Stem Cells Present in Tissue

Many CSCs show similarities to normal stem/progenitor cells in phenotype, function and cell surface markers; for example, $CD44^{+}CD24^{-/low}$ mammary gland progenitor cells resemble $CD44^{+}CD24^{-/low}Lin^{-}$ CSC cells in breast cancer[29]. With an increase in age, most adult stem cells appear to increase the expression of gate-keeping tumour suppressors like $p16^{Ink4a}$, $p19^{ARF}$ and p53[4,30]. These suppressors negatively regulate cell survival and regeneration. This may reduce incidence of cancer in aging tissues while simultaneously downregulating proliferative capacity. Transformation of normal cells into cancer cells often requires a series of mutations in oncogenes and tumour suppressor genes; these may accumulate over a period of years[4]. While somatic cells are periodically replaced by cellular turnover, stem cells may persist in tissues long enough to accumulate the multiple mutations required for cancer initiation. Inactivation of the *retinoblastoma* (Rb) gene in retinoblasts can cause their transformation into CSCs that ignore growth regulatory signalling cascades which would normally have caused proliferation to cease. As the pRb protein is crucial in maintaining quiescence in cells (including adult stem cells), loss of pRb function in CSCs may cause cancer initiation[2,4,31]. Other childhood cancers such as Wilm's tumour and some forms of leukaemia are presumed to arise from stem cells in the kidney and haematopoietic system respectively and appear to require relatively few genetic modifications for neoplastic transformation[2,4]. Chronic tissue damage, as may occur after long-term tobacco usage or prolonged UV irradiation, could lead to an increased proliferation of stem cells within the affected tissues as the body seeks to repair the damage. These constitutively proliferating stem cells could be targets for further carcinogen induced mutations leading to tumorigenesis[2,32]. Loss of *ten-eleven-translocation-2* (*Tet2*) gene has been reported to increase self-renewal in stem cells, contributing to progressive defects in haematopoiesis and myeloid transformation *in vivo*[33].

(b) Origin from Somatic (Non-Stem) Cells

The ability of a differentiated cell to acquire the property of self renewal and become a CSC has been shown by transfection studies using oncogenes[2]. Reports indicate that *Src*, an inducible oncogene, can cause transformation of MCF10A cells and generate CSC-like cells within 16-24 hours of its activation[6,34]. Epithelial-to-mesenchymal transition (EMT) occurs during normal morphogenesis and development and is also involved in metastasis of cancer cells[4]. Induction of EMT in normal human mammary epithelial (HMLE) cells by expression of Snail, Twist or treatment with transforming growth factor beta1 (TGFβ1) caused a number of cells to exhibit the $CD44^{+}CD24^{-/low}$ expression profile of CSCs. These cells also exhibited an increased ability to form mammospheres; EMT thus appears to result in the enrichment of CSCs in HMLE cells[6,29,35,36]. The mechanism by which EMT induces CSC formation may involve transcription factors like Forkhead box protein C2 (FOXC2). FOXC2 was upregulated in immortalized HMLE cells in response to EMT inducing stimuli and its suppression led to downregulation of CSC characteristics[6,37].

MOLECULAR PATHWAYS INVOLVED IN CSC FUNCTION

Intracellular signalling pathways essential for normal stem cell self-renewal and proliferation often show dysregulation or aberrant activation in CSCs. Molecular pathways involved in modulating stem cell self-renewal, like Wnt, Notch and phosphatase and tensin homolog (PTEN) are deregulated in a number of tumours. Other intracellular signalling pathways involving phosphatidylinositol-3-kinase (PI3K)/ Akt, nuclear factor kappa beta (NF-kβ), mitogen activated protein kinase (MAPK) and phospholipase C (PLC)/ protein kinase C (PKC) may also be involved[10,38-40].

The Wnt/ β-catenin pathway has been reported to be involved in modulation of CSC self-renewal in a number of cancers including leukaemia, melanoma, and breast, lung, and liver cancers[41-44]. Signalling through the Wnt pathway involves mediation by β-catenin which translocates to the nucleus and coordinates with lymphoid enhancer-binding factor (LEF) resulting in the activation of genes such as *CCND1* (cyclin D1), *c-Jun* and *c-Myc*[41,45]. Notch signalling also plays an important role in activation of *c-Myc*, *CCND1* and *NF-κB* genes. Cross-talk between Notch and Wnt signalling pathways has been reported[41,46]. Signalling through the Hedgehog (Hh) pathway plays a crucial role in mammalian embryonic development and appears to be essential for maintenance of normal stem cells as well as CSCs in various human cancers including breast cancer[10,47]. NF-κB may also be involved in modulation of signalling through the Hh pathway by activation of sonic hedgehog[41,48]. Experiments indicate that CSCs from various cell lines including C-6 (glioma), MCF-7 (breast cancer) and A549 (non-small cell lung cancer) appear to proliferate efficiently via epidermal growth factor receptor (EGFR) mediated signalling cascades even in the absence of growth factors[20]. Tumour microenvironment may also play a role in regulation of CSC self renewal and proliferation[49] with CSCs being possibly able to reciprocally modulate their microenvironment via secretion of paracrine factors or via cell-cell contact. In human brain cancers, $CD133^{+}Nestin^{+}$ CSCs in medulloblastomas, glioblastomas and oligodendrogliomas have been found to interact closely with endothelial cells promoting angiogenesis[50].

DETECTION OF CSCS USING SPECIFIC MARKERS

The most widely used method for identifying CSCs is based on specific cellular markers, especially cell surface markers. These include CD133, CD24, CD44, epithelial-specific antigen (ESA) and aldehyde dehydrogenase1 (ALDH1)[10,13,29,51]. ALDH activity has been shown to enrich haematopoetic stem cells and cells with increased stem-like properties in solid malignancies[29,52]. Stem cells including hESCs and CSCs also express the octamer-4 (Oct-4) transcription factor while normal differentiated adult cells do not express Oct-4[53]. However, the expression of many specific-CSC markers have been found to vary in a tissue specific and even in a tumour subtype-specific manner[10,29]. For instance, the lung carcinoma cell marker SP-C has been reported to show variable expression in lung cancer spheres, possibly reflecting the phenotypic variability in human CSCs[54]. A number of CSC markers which have been reported in human cancers have been enumerated in Table 1.

Table 1 Cancer Stem Cell (CSC) markers in human cancers. Some of the principal CSC markers which have been reported in a variety of human cancers are listed in the table. These markers can be of use in identification of CSCs.

Human Cancer Type	Observed Phenotype/ Markers in CSCs
Breast cancer	$CD44^+/CD24^{-/low}/Lin^-$ [13,55], $ALDH1^+$ [41,55]
Pancreatic cancer	$CD44^+/CD24^+/ESA^+$ [13,29,56], $CD133^+$ [29,57], $ALDH1^+$ [29,58]
Brain cancer	$CD133^+$ [29,59,60], SSEA-1 [29,61]
Colon cancer	$CD133^+$ [29,41,62], $ALDH1^+$ [41,63]
Lung cancer (small cell/ non-small cell lung cancer)	$CD133^+$ [29,54], $ALDH1^+$ [41,64]
Head and neck cancers	$CD44^+$ [29,65], Cytokeratin 5/14 [29,65]
Leukemia (AML)	$CD34^+/CD38^-$ [12,29,41,66]
Melanoma	$ABCB5^+$ [41,67]

CANCER STEM CELL THERAPEUTICS

As tumour cells tend to proliferate rapidly, most conventional cancer treatment strategies, including radiotherapy and chemotherapy, are targeted at relevant molecules which direct them to inhibit rapidly dividing cells[4]. Evidence indicates that inherent mechanisms present in CSCs render them more resistant to chemotherapy and radiotherapy; thus, conventional anti-cancer approaches might fail to eradicate the CSC subset that initiates and perpetuates tumorigenesis[2,50,68]. Stem cells possess a wide variety of transporters, including ATP-binding cassette (ABC) transporters such as ABCG2, ABCB1/ multi-drug resistance-1 (MDR-1), ABCC1 and ABCA2, as a possible defense against xenobiotic toxins[2,69,70]. Several of these transporters play important roles in drug efflux and resistance to chemotherapeutic drugs. For example, leukaemic progenitor stem cells can efflux mitoxantrone and daunorubicin, two agents commonly used in treatment of AML[71]. Drug-resistant variants of CSCs can also produce a population of DNA-repairing tumour cells. $CD133^+$ CSCs in gliomas express 30-fold higher levels of the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) than $CD133^-$ cells and, due to increased DNA repair capacity, are more resistant to radiotherapy[41,59]. Overexpression of certain enzymes involved in catalyzing xenobiotic substrates, e.g. ALDH1 (which catalyses oxidation of acetaldehydes produced from ethanol) may provide some resistance against chemotherapeutic drugs like cyclophosphamide[41,72]. Cell cycle kinetics may also explain why CSCs are comparatively resistant. As mentioned previously, rapidly dividing cells are more sensitive to cytotoxic therapies. Experiments indicate that a particular subset of leukaemia CSCs remain quiescent. Similar phenomena may also occur in CSCs in other cancers[68,73]. Thus, CSCs appear to employ a combination of mechanisms to increase their resistance to cancer therapy. These mechanisms might vary between tumour types.

A number of methods have been proposed for dealing with CSC proliferation and self-renewal. Support for the potential therapeutic utility of targeting CSCs has been provided by the observation that selective killing of CSCs identified by ABCB5 expression in human melanoma inhibits experimental tumour growth[67]. Some of these proposed methods are discussed in the following sections. However, these are still mainly at an experimental or pre-clinal stage and further research is necessary to fully develop and explore the therapeutic potential of such methods in the treatment of human cancers.

(a) Targeting CSCs using surface markers

Surface markers used to identify or isolate CSCs may serve as attractive targets for monoclonal antibody based or siRNA based therapies. In human glioma cells and breast cancer, CSCs exhibit reduced 26S proteasome activity and increased resistance to ionizing radiation compared with other cell subpopulations within the tumour. Targeted killing of CSCs via a proteasome-dependent thymidine kinase suicide gene was reported to cause tumour regression[74]. Conjugating an anti-human CD133 antibody to mono-methyl auristatin F, a potent cytotoxic drug, inhibited growth and induced apoptosis in Hep3B hepatocellular and KATO III gastric cancer cells *in vitro*[10,75]. *In vitro* apoptosis of $CD133^+$ glioma CSCs has been reported to be induced by shRNA mediated knockdown of L1 cell adhesion molecule (L1CAM) which is preferentially expressed on these $CD133^+$ cells[76]. Thus, conjugating drugs to antibodies directed against specific cell surface markers might be a potential method for targeted therapy of CSCs. Salinomycin, a potassium ionophore has been reported to induce breast CSC specific toxicity and downregulate expression of certain CSC associated genes[77]. However, although such approaches appear promising, one potential shortcoming of such targeting is that many CSC surface antigens may also be overexpressed by stem cells in normal tissue[68] and targeting approaches using such markers can have toxic side effects[7,10]. Also, as CSCs generally comprise only a small minority of cells within cancer cell populations, most high-throughput cell viability assays, when applied to populations of cancer cells *in vitro*, often fail to identify agents with CSC specific toxicity[77].

Instead of direct killing, another approach could involve increasing sensitivity of CSCs to chemotherapy or radiotherapy. ABCB5 mediates resistance to the chemotherapeutic drug doxorubicin in malignant melanoma. Such drug resistance can be overcome using targeted monoclonal antibodies to inhibit ABCB5-dependent drug efflux[50,78]. siRNA-mediated *ABCB5* gene silencing also overcomes doxorubicin resistance and increases the sensitivity of melanoma cells to the chemotherapeutic drugs 5-fluorouracil (5-FU) and camptothecin[50,79]. Enhanced resistance of HNSCC CSCs to radiotherapy can be reduced by knockdown of the transcriptional repressor Bmi-1[52]. Inhibiting Chk1 and Chk2 checkpoint kinases has been reported to reduce resistance of $CD133^+$ glioma CSCs to ionizing radiations[50]. Curcumin (diferuloyl methane), a major component of the rhizome of *Curcuma longa* L., has also been reported to exert its anti-cancer activity by targeting CSCs in colorectal, pancreatic, breast, brain and head and neck cancers[80].

(b) Targetting molecular pathways involved in CSC self-renewal and proliferation

Potential signalling pathways that may serve as therapeutic targets for controlling CSC self-renewal and proliferation include Wnt/ β-catenin, Hh, Notch, NF-κB, PTEN and bone morphogenetic protein (BMP) mediated signalling cascades[41,68,70,81]. Inhibition of the Notch pathway with γ-secretase inhibitors can potentially be of use in downregulating CSC self-renewal; however, clinical use could be restricted by high hydrophobicity and side effects including possibly goblet cell metaplasia[41,70]. Cyclopamine, a Hh signalling inhibitor is also under study as a therapeutic against CSCs but again, clinical use could be limited by high hydrophobicity and systemic toxicity[41,82]. Silencing H-Ras in a tumour initiating cell (i.e. CSC) enriched breast

cancer cell line has been reported to downregulate self renewal without affecting cell differentiation[83]. Treatment with miR145 incorporated with polyurethane-short branch polyethylenimine (PU-PEI) has been found to block key signal transduction pathways and effectively downregulate Oct-4 and Sox2, transcription factors which control pluripotency in stem cells including CSCs[84].

(c) Differentiation therapy

As promoting differentiation of CSCs within a tumour would lead to tumour degeneration and might also increase susceptibility to conventional chemotherapies, potential therapeutic strategies could include modulation of specific signalling cascades and alteration of specific gene expression[50]. Modulation of cellular signalling cascades by Notch pathway inhibitors in medulloblastoma and modulation of BMP signalling in experimental models of human glioblastoma have been shown to promote CSC differentiation[15,85]. Enforced expression of let-7 miRNA in breast cancer has been reported to induce differentiation of $CD44^{+}CD24^{-/low}$ CSCs[81]. Administration of a monoclonal antibody directed against the cell adhesion molecule CD44 to nonobese diabetic SCID mice transplanted with human AML has been reported to induce differentiation and appreciably reduce leukaemic repopulation[86].

(d) Use of nanomedicine in targeting CSCs and drug delivery

Some recent therapeutic approaches for targeting CSCs involve the use of nanomedicine. Imetelstat decreases telomerase activity, suppresses breast CSC self-renewal potential and inhibits tumorigenicity of PANC1 and MDA-MB-231 cells *in vivo*. Delivery of such inhibitors to CSCs by nanotechniques may allow efficient targeting for therapeutic purposes[70,87]. Even conventional chemotherapeutic drugs can be promising for CSC therapy if efficient targeting methods can be developed. Doxorubicin-tethered gold nanoparticles have been reported to mediate potent drug delivery to breast CSCs and reduce cancer initiation and tumour growth in murine models[88].

CONCLUSION

CSCs appear to be play pivotal roles in tumour initiation, development, metastasis and development of therapeutic resistance. Thus, modern cancer treatments would need to target self-renewal or other critical molecular pathways in CSCs as their eradication could possibly lead to tumour regression and a better prognosis in cancer afflicted patients. The challenge, for effective alleviation of the disease cancer, lies in devising therapies which can cause destruction of CSCs while causing minimal damage to normal cells.

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CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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