Cancer stem cells and Chinese herbs, isolates and other complementary medical botanicals

Daniel A Weber¹,²,⁵, Janelle M Wheat¹,³,⁴, Geoffrey M Currie¹,³,⁴
1 Faculty of Science, Charles Sturt University, Wagga Wagga, Australia.
2 School of TCM, Tianjin University. Tianjin, China
3 Centre for Research in Complex Systems, Charles Sturt University, Wagga Wagga, Australia.
4 Australian School of Advanced Medicine, Macquarie University, Sydney, Australia.
5 Oncology Section; World Federation of Chinese Medical Societies

ABSTRACT: To elucidate the connection between the origin of certain cancers and stem cells, cancer stem cells, the stem cell niche and the tumor microenvironment and to examine the ability of traditional Chinese medicines and isolates in treating these types of cancers, the existing literature was examined and eight studies regarding Chinese herbal medicines and the prevention of cancer recurrence were critically analyzed and evaluated. Tumor stem cells may be the final target of traditional Chinese medicine. Soy flavones, ginsenoside Rg3, parthenolide, berbamine and curcumin are several examples of Chinese herbal medicines, which have been shown to be effective in the treatment of cancer, and seem to act by targeting cancer stem cells and associated pathways resulting in tumorigenesis. The treatment approaches combined with an overall treatment protocol for the tumor microenvironment and chronic systemic inflammation, is likely to provide a more successful outcome than a single tactical approach. As shown in numerous studies in the literature, using complementary disciplines with orthodox treatments may enhance outcomes.

KEYWORDS: Stem cells (SCs), cancer stem cells (CSCs), stem cell niche, tumor microenvironment, DNA methylation, hypoxia, telomeres and telomerase, metastatic CSCs (mCSCs), Chinese herb isolates
1 General introduction of cancer stem cells

1.1 Stem cells and cancer stem cells  Stem cells (SCs) are unique cell populations with the ability to undergo both self-renewal and differentiation. SCs possess two unique characteristics: pluripotency, which allows mature cells to compose specific organs or tissue, and self-renewal, which supply an organ with an adequate number of cells to maintain the function of the organ\[1\]. A wide variety of adult mammalian tissues harbor SCs, yet ‘adult’ SCs may be capable of developing into only a limited number of cell types. In contrast, embryonic stem (ES) cells, which are derived from blastocyst-stage early mammalian embryos, have the ability to form any fully differentiated cell of the body\[2\].

SCs have the ability to choose between prolonged self-renewal and differentiation. The choice is regulated by intrinsic signals and the external microenvironment\[3\]. SCs play a critical role not only in the generation of complex multicellular organisms, but also in the development of tumors. Experimental findings support the notion that cells with properties of SCs are integral to the development and perpetuation of several forms of human cancer\[4\].

Cancer stem cells (CSCs) are cancer cells (found within tumors or hematological cancers) that possess characteristics associated with normal SCs, specifically the ability to give rise to all cell types found in a particular cancer sample\[5\]. CSCs are therefore tumorigenic, in contrast to other non-tumorigenic cancer cells. CSCs may generate tumors through the SC processes of self-renewal and differentiation into multiple cell types. Such cells are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. Increasing evidence suggests that only a subset of cancer cells have tumor-propagating ability\[6,7\]. In comparison with the bulk of tumor cells, a relatively small number of these tumor-propagating cells, often referred to as CSCs, can form tumors\[8\]. Therefore, development of specific therapies targeted at CSCs holds hope for the improvement of survival rates and quality of life of cancer patients, especially for sufferers of metastatic disease\[9\]. Thus, tumors act as caricatures of their corresponding normal tissues and are sustained in their growth by a pathological counterpart of normal adult SCs and CSCs\[9\]. Clarke (2007)\[10\] demonstrated that breast cancer cell lines and primary tumors contain a self-renewing, colony-forming population that can be enriched by cell surface markers such as CD44, alone or in combination with other markers and the cancer-initiating stem-like cell population to be highly regulated by the estrogen, epidermal growth factor (EGF) and Notch receptor signaling pathways.

CSCs may arise from normal SCs by mutation of genes that make the SCs cancerous\[11\]. SC populations are established in niches, the specific anatomic locations that regulate how they participate in tissue generation, maintenance and repair. The niche saves SCs from depletion, while protecting the host from over-exuberant SC proliferation\[12\]. The function of adult tissue-specific SCs declines with age, which may contribute to the physiological decline in tissue homeostasis and the increased risk of neoplasm during aging\[13\]. Studies in invertebrates have highlighted the changes in SC and niche during aging, and the mechanisms underlying some of these changes\[14\]. Decline in self-renewal factors contributes to aging of the SC niche in the Drosophila testis\[15,16\].

1.2 Genetic and epigenetic alteration in the cancer stem cell niche  The transformation from SC to CSC occurs in the SC niche. Epigenetic changes in SCs and somatic cells contribute significantly to carcinogenesis by disruption of cellular differentiation programs. Epigenetic interference and loss of cellular identity may be particularly relevant for the emergence of CSCs\[17\]. Epigenetic alterations may act via an entirely distinct route than genetic mechanisms by compromising cellular differentiation pathways and facilitating the emergence of tumorigenic cells\[17\].

The microenvironment seems to be of crucial importance for primary tumor growth as well as metastasis formation. Combined with its role in the protection of CSCs against genotoxic insults, these data strongly suggest the niche as an important target for novel therapies\[18\]. The insult by aging or other mechanisms to the SC niche is a factor by which SCs are transformed to CSCs. The long-known association between cancer and chronic tissue injury suggest that carcinogenesis proceeds by misappropriating homeostatic mechanisms that govern tissue repair and SC self-renewal\[19\].
1.3 Factors which alter niche function  Telomerase activity and telomere maintenance have been associated with immortality in tumor cells, embryonic SCs\[20\] and in adult SCs. In both embryonal SCs and CSCs the telomere shortening occurs during replicative aging\[21\], which may in turn reactivate telomerase activity for repair. Armanios & Greider (2005)\[22\] have suggested a SC origin for certain cancers, implying that the genetic alterations that lead to cancer accumulate in tissue-specific SCs and CSCs would already have telomerase so that it would not need to be reactivated in tumors. Thus, telomerase appears to be stringently repressed in normal human somatic tissues but activated in cancer, where immortal cells are likely required to maintain tumor growth\[23\]. It is hence proposed that CSCs may originate from SCs that have lost the ability to regulate proliferation, or alternatively, may arise from a more differentiated population of progenitor cells that have acquired abilities to self-renew due to telomerase activation\[24\]

Over 80% of human cancers show a re-activation of telomerase catalytic activity\[25\] but most human SCs show a constitutive expression of telomerase yet retain normal cell proliferation control, which argues against telomere-independent functions of telomerase during carcinogenesis\[26\]. There is also some evidence from hematological malignancies that the dual role of telomere shortening and telomerase re-activation during cancer initiation and progression also applies to CSCs\[27\].

1.4 Cancer cell origin  Solid cancers may derive from single SCs, committed progenitor cells, or differentiated cells. The identification of tumor-initiating cells in human breast and brain cancers provides growing evidence supporting a stem cell origin of solid cancers\[28,29\]. The reactivation of telomerase also occurs in cancer stem cells. On one hand, telomere shortening may be involved in the development of chromosomal instability and transformation of stem cells. On the other hand, tumor stem cells may require telomerase activation to achieve immortal self-renewal capacity\[27\].

1.5 Cancer stem cell and metastasis  The ability of a tumor to metastasize is an inherent property of a subset of CSCs, coined here as metastatic CSCs (mCSCs). This ability is modulated through the interactions of the mCSCs with the local microenvironment or “niche” \[24\]. Metastasis is a complex, multi-step process. Tissue tropisms associated with cancer metastasis indicate that specific and distinct cellular and molecular mechanisms are involved. These factors govern tissue tropism for a given cancer, a process that has been observed for over one hundred years by Virchow (1863), Paget (1889), and Warburg (1956), as cited by Balkwill and Mantovani (2001)\[30\]. Trafficking towards preferred tissues and organs of mCSCs is guided by cues such as oxygen gradients or other chemottractants derived from niche sites, which include hypoxia, the enhanced conversion of glucose to lactate by tumor cells, oncometabolites, hyperglycemia and/or acid pH\[31-35\]. Formation of organ-specific metastases may require both the CSCs property and the ability of either the mCSCs or their progeny to adapt to particular target tissue microenvironments\[24\].

Zhang, Li, Lewis, Chang, and Rosen (2007)\[36\] identified a tumor initiating subpopulation within a unique p53 null mouse mammary tumor model. The increased expression of genes involved in DNA damage response pathways may help explain the hypothesized resistance of CSCs to chemo- and radiation therapy, which leads to an increase in the relative proportions of CD44+/CD24-/low cells. P53 plays an important role in stem cell checkpoints induced by telomere shortening\[37\].

1.6 Mechanisms of cancer metabolism  Tumors are being increasingly perceived as abnormal organs that, in many respects, recapitulate the outgrowth and differentiation patterns of normal tissues. The microenvironment seems to be of crucial importance for primary tumor growth as well as metastasis formation\[18\]. During tumor development, the metabolism of cancer cells may be modified to meet the requirements of cellular proliferation, thus facilitating the uptake and conversion of nutrients into biomass\[38\].

1.6.1 Tumor microenvironment  The tumor microenvironment also plays a significant role in cancer stem cell regulation. In order for each organ to operate successfully within the context of the organism, all cells must be integrated into an architectural and signaling framework such that each cell functions to contribute to the tumor as a whole.

Tumors may originate from CSCs, which may be reactivated in specific sites to recreate the original tissue phenotype, and it has been hypothesized and tested (though not yet proven) that mutated mammary SCs are the origin of breast cancer\[6,93\]. The microenvironment regulates tissue specificity and contributes significantly to tumorigenesis in two specific ways. Firstly, genetically
damaged tissue-specific stem cells (TSSC) can be held in check for long periods of time, explaining the long delay between environmental exposure and/or germ line suppressor mutations and cancer onset. Secondly, ionizing radiation, or other physical and chemical insults, can result in changes to the microenvironmental composition that can then trigger mutations in stem cells and eventually result in cancer.\textsuperscript{[94,95]}

1.6.2 Hypoxia Reduced oxygenation (hypoxia) inhibits differentiation and facilitates SC maintenance. Hypoxia commonly occurs in solid tumors and promotes malignant progression. Hypoxic tumors are aggressive and exhibit SC–like characteristics.\textsuperscript{[39]}

1.6.3 Methylation DNA methylation is an important regulator of gene transcription and has a role in carcinogenesis. Both hypermethylation, which represses transcription of the promoter regions of tumor suppressor genes leading to gene silencing, and global hypomethylation have been recognized as causes of oncogenesis.\textsuperscript{[90]} Some of these methylation changes may initiate in subpopulations of normal cells as a function of age and progressively increase during carcinogenesis. Age-related methylation appears to be widespread and is one of the earliest changes marking the risk for neoplasia.\textsuperscript{[91]} Analysis of colon methylation patterns infers SCs live in niches containing multiple ‘stem’ cells and niche succession cycles may potentially accumulate multiple alterations because they resemble superficially the clonal succession of tumor progression.\textsuperscript{[92]}

Gene signatures derived from CSCs predict tumor recurrence for many forms of cancer. Methylation abnormalities may play a role in chromosome segregation processes in cancer cells.\textsuperscript{[40]} Data show that promoter methylation of Wnt target genes is a strong predictor for recurrence of cancer, and suggest that CSC gene signatures, rather than reflecting CSC numbers, may reflect the differentiation status of the malignant tissue. Several Wnt target genes, including achaete-scute complex homolog 2 (ASCL2) and leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), become silenced by cytosine-guanine (CpG) island methylation during progression of tumorigenesis, and their re-expression is associated with reduced tumor growth.\textsuperscript{[41]} The Wnt signaling pathway is a network of proteins known for their roles in embryogenesis and cancer.\textsuperscript{[42]}

Most cancer-associated genomic aberrations (deletions, amplifications, mutations, translocations, etc) are irreversible, but neoplastic epigenetic modifications are potentially reversible.\textsuperscript{[43]} To reverse transcriptionally repressive DNA methylation, numerous cytosine analogs that covalently and irreversibly bind to the active site of DNA methyltransferase (DNMTs), resulting in the stalling of the DNA replication form and the eventual cellular depletion of the methyltransferase,\textsuperscript{[44]} are now being investigated for their ability to clinically reverse CpG island methylation in cancer and de-repress epigenetically silenced genes.\textsuperscript{[45,46]}

Polycomb group proteins (PCGs) are involved in the repression of genes that are required for stem cell differentiation. It has recently been shown that promoters of PCG target genes (PCGTs) are 12-fold more likely to be methylated in cancer than non-PCGTs\textsuperscript{[47]} and that age may predispose to malignant transformation by irreversibly stabilizing SC features. The PCGs silence gene expression, allowing cells to both acquire and maintain identity. Stem and cancer cells commonly share gene expression patterns, regulatory mechanisms, and signaling pathways. Many microRNA species have oncogenic or tumor suppressor activities, and disruptions to these networks are commonly found in cancers.\textsuperscript{[48]}

CpG island methylation plays an important role in epigenetic gene control during mammalian development and is frequently altered in disease situations such as cancer.\textsuperscript{[49]} DNA hypermethylation is associated with gene silencing and is often observed in CpG islands. It has recently been suggested that aberrant CpG island methylation in tumors is directed by PCG proteins.\textsuperscript{[50]} Substantial hypermethylation and hypomethylation of CpG island shores are two mechanisms for epigenetic reprogramming in induced pluripotent stem (iPS) cells and cancer. Many differentially methylated regions (DMRs) are broadly involved in tissue differentiation, epigenetic reprogramming and cancer.\textsuperscript{[51]}

1.6.4 Inflammation The α-chemokine stromal-derived factor (SDF)-1 and the G-protein-coupled seven-span transmembrane receptor (CXCR4) axis regulates the trafficking of various cell types. SDF-1-CXCR4 axis is a master regulator of trafficking of both normal and cancer stem cells, because most (if not all) malignancies originate in the stem/progenitor cell compartment and CSCs
express CXCR4 on their surface. The responsiveness of CXCR4\(^{-}\) normal and malignant SCs to an SDF-1 gradient may be regulated positively/primed by several small molecules related to inflammation which enhance incorporation of CXCR4 into membrane lipid rafts, or may be inhibited/blocked by small CXCR4 antagonist peptides\(^{[52]}\).

Chronic inflammation-induced carcinogenesis is a commonly accepted entity, and the inflammatory microenvironment classically affects tumor promotion in its role as an altered stem cell niche and can also affect tumor initiation and tumor progression. The origin of the tumor cells is often attributed to SCs\(^{[53]}\).

1.6.5 Telomere shortening Telomere shortening has been implicated in replicative senescence, chromosomal instability and cell cycle arrest\(^{[82]}\). Because of the prolonged life of SCs due in part to the activation of telomerase, it is thought that telomerase may play an important role in CSC biology. This is also supported by the observations that the telomerase protein extends the life of somatic human cells\(^{[86]}\), telomerase mRNA expression is upregulated in tumor cells\(^{[87,88]}\) and telomerase is a key component in the CSC population\(^{[89]}\).

2 Chinese herbal medicines and cancer stem cells in the literature

2.1 Materials and methods A literature search was performed in both Chinese and English. Over 260 articles were examined on SCs, SC niche, CSCs, the cancer microenvironment, methylation, telomerase activity and systemic inflammation as well as Chinese herbs and isolates. Eight studies regarding Chinese herbal medicines and the prevention of cancer recurrence were critically analyzed and evaluated.

2.2 Results from the eight studies regarding Chinese herbal medicines and cancer stem cells Tumor SCs may be the final target of traditional Chinese medicine in preventing cancer recurrence and metastasis\(^{[54]}\).

In study 1, it shows the soy flavones have an obvious inhibition to liver cancer SMMC7721 cell line in 24 h, 48 h and 72 h, and the inhibition rates increased following the flavones concentration rise. After treatment of the soy flavones for 24 h, the numbers of CD133 tumor SCs showed a significant decrease. It was concluded that soy flavones not only inhibited the proliferation of tumor cells, but also inhibit CD133 brain tumor SCs\(^{[55]}\). Five concentrations were used: 100, 200, 300, 400 and 500 \(\mu\)g/mL. Interestingly, the human hepatic cell line HL-7702 and the human hepatoma cell line SMMC-7721 were successfully treated with 3-30 \(\mu\)mol/L selenium dioxide (SeO\(_2\)). SeO\(_2\) at 30 \(\mu\)mol/L markedly inhibited cell proliferation and viability, and prompted apoptosis of both normal hepatic and hepatoma cells after 48 h treatment. SeO\(_2\) could also down-regulate the Bcl-2 level, greatly in HL-7702 and slightly in SMMC-7721 cells, but up-regulate wild type p53 level slightly in HL-7702 and significantly in SMMC-7721 cells\(^{[56]}\).

Study 2 stated the inhibitory effect of ginsenoside Rg3 on the proliferation of C6 glioma CSCs was studied\(^{[57]}\). Cell immunofluorescence was used to determine the expression of nestin (the specific marker of brain tumor SCs expressing nerve SCs), Glial fibrillary acidic protein (GFAP) and neuron-specific enolase (NSE). The tetracole assay showed that compared with negative control group, the absorbance value or optical density (OD) in positive control group increased significantly; compared with positive control group, OD value expression in the ginsenoside Rg3 group decreased. In CSCs with nerve trunk characteristics in glioma, Chinese herb isolate ginsenoside Rg3 had an inhibitory effect on the proliferation of CSCs in brain glioma\(^{[57]}\). Rg3 (1-103 nmol/L) dose-dependently suppressed the capillary tube formation of human umbilical vein endothelial cells (HUVEC) on the matrigel in the presence or absence of 20 ng/mL vascular endothelial growth factor (VEGF). The VEGF-induced chemoinvasion of HUVEC and ex vivo microvascular sprouting in rat aortic ring assay were both significantly attenuated by Rg3. In addition, Rg3 (150 and 600 nmol/L) remarkably abolished the basic fibroblast growth factor (bFGF)-induced angiogenesis\(^{[58]}\).

The study 4 shows that the parthenolide (PTL), a sesquiterpene lactone derived from the leaves of \textit{Tanacetum parthenium}, is considered a main bioactive component of the herb. PTL also shows anticancer activities in a variety of cell lines. It contains an \(\alpha\)-methylene-\(\gamma\)-lactone ring and an epoxide moiety, both of which are able to interact with nucleophilic sites of biologically important...
molecules. PTL modulates multiple targets by inhibiting mammalian DNA methyltransferase; tRNA aspartic acid methyltransferase 1 (TRDMT1) (formally known as DNMT2) activity and induces global hypomethylation of DNA, which can restore the expressions of some suppressor genes, thereby contributing to its various in vitro and in vivo effects. It also seems to have the potential to target some CSCs.\(^{[59]}\) PTL can induce the death of human leukemia stem cells (LSCs) in vitro while sparing normal hematopoietic cells while an analog, dimethylamino-parthenolide (DMAPT), induces rapid death of primary human LSCs from both myeloid and lymphoid leukemia, and is also highly cytotoxic to bulk leukemic cell populations. DMAPT actions include induction of oxidative stress responses, inhibition of nuclear factor kappa B (NF-κB), and activation of p38.\(^{[60]}\)

According to study 5, berbamine, from Chinese herb Berberis amurensis, has been found to selectively induce apoptosis of imatinib (IM)-resistant-Bcr/Abl-expressing leukemia cells from the K562 cell line and chronic myeloid leukemia (CML) patients. Cell cycle analysis results showed that berbamine could reduce the proportion of G0/G1 cells. In particular, the compound displayed potent inhibition of the cytoplasm-to-nucleus translocation of NF-κB p65, which plays a critical role in the survival of LSCs. These results suggest that berbamine could be a good starting point for the development of novel lead compounds for leukemia.\(^{[61]}\) It pointed the view that CML is a pluripotent hematopoietic SC disorder, and berbamine can selectively induce cell death of both Gleevec-sensitive and-resistant Ph+ CML cells. Similarly, berbamine was also found to display a selective anti-proliferative activity of primary leukemia cells from CML patients, and its IC\(_{50}\) values were 4.20–10.50 µg/mL in primary CML cells, and 185.20 µg/mL in normal bone marrow cells. Importantly, the study showed that berbamine down-regulates p210 Bcr/Abl oncoprotein level, and induces apoptosis of Bcr/Abl+ cells through a caspase-3-dependent pathway.\(^{[62]}\) The dosage for berbamine is 30 to 60 mg/kg twice a day.\(^{[63,64]}\)

In study 6, the findings have shown that berbamine has potent anti-inflammatory properties, and it suppresses the growth, migration and invasion in highly-metastatic human breast cancer cells. This is potentially achieved by inhibiting Akt and NF-κB signalling with their upstream target c-Met and downstream targets Bcl-2/Bax, osteopontin, VEGF, matrix metalloproteinase (MMP)-9 and MMP-2.\(^{[65]}\)

In study 7, the CSC hypothesis asserts that malignancies arise in tissue stem and/or progenitor cells through the dysregulation or acquisition of self-renewal. Mammosphere formation assays were performed after treatment with curcumin, piperine and a control in unsorted normal breast epithelial cells and normal stem and early progenitor cells, selected by aldehyde dehydrogenase (ALDH) positivity. Wnt signalling was examined using a Topflash assay. Both curcumin and piperine were found to inhibit mammosphere formation, serial passaging and percent of ALDH+ cells, by 50% at 5 µmol/L and completely at 10 µmol/L concentrations in normal and malignant breast cells. There was no effect on cellular differentiation. Wnt signaling was inhibited by both curcumin and piperine by 50% at 5 µmol/L and completely at 10 µmol/L.\(^{[66]}\)

Study 8 has shown that 5-fluorouracil (5-FU) plus oxaliplatin (FOLFOX) remains the backbone of colorectal cancer chemotherapeutics but with limited success. This could partly be due to the enrichment of CSCs that are resistant to conventional chemotherapy. Only 0.04% of the total colon cancer HCT-116 cells are CD44-positive cells. CSC theory, which states that only a small proportion of cells within the tumor are responsible for initiating and maintaining the processes of carcinogenesis and CD44-positive cells are classified as CSCs. Treatment of FOLFOX-surviving colon cancer cells with either curcumin alone or together with FOLFOX resulted in a marked reduction in CSCs, as evidenced by the decreased expression of CD44 and CD166 as well as epidermal growth factor receptor and by their ability to form anchorage-dependent colonies. Data suggest that curcumin alone or together with conventional chemotherapy could be an effective treatment strategy for the prevention of the emergence of chemo-resistant colon cancer cells by reducing or eliminating CSCs.\(^{[67]}\)

### 2.3 Traditional Chinese medicines, cancer stem cells and associated pathways

Isolates from Chinese herbs were studied for this survey, as there are no studies available of whole herbs and/or herbal formulations on CSCs or SC niche. The isolates however do indicate some of the properties of
whole herbs and may be extrapolated for their use. An advantage for the use of isolates is their simplicity of action, which is more manageable in adjunctive treatment protocols.

It was demonstrated that sesquiterpenes extracted from a variety of Chinese herbs inhibited cell proliferation and/or telomerase activity in human ovarian cancer cell line HO-8910\textsuperscript{[96]}. It has also been shown that human prostate cancer cell lines contain a small population of CD44\textsuperscript{+}CD133\textsuperscript{+} CSCs and their self-renewal capacity is inhibited by epigallocatechin gallate (EGCG)\textsuperscript{[97]}. Furthermore, EGCG inhibits the self-renewal capacity of CD44\textsuperscript{+}a2β1\textsuperscript{+}CD133\textsuperscript{+} CSCs isolated from human primary prostate tumors.

Soy flavones, ginsenoside Rg3, PTL, berbamine, curcumin and pepper extract, piperine are examples of herbal isolates, which have been shown to be effective in the treatment of cancer, and, as in the above examples, seem to act by targeting CSCs, the SC niche and/or the tumor microenvironment. However, further work into the specific mechanisms and pathways by which these traditional medicines act is required. It is then necessary to further examine ways in which these pathways may be targeted using a combination of traditional and modern medicines.

2 Discussion

Several key points may be surmised through a culmination of the information garnered from the studies analyzed. A tissue SC must possess two qualities to perform its natural function: self-renewal (i.e., the ability to produce more SCs) and differentiation. The CSC hypothesis predicts that long-lived SCs are more likely to accumulate the initial mutations leading to cancer than their short-lived differentiated progeny. Transiently amplifying cells are immediate daughters of somatic SCs. Growing evidence shows these daughter cells inherit parental mutations and may serve as targets for the final transforming events that give rise to a tumor. Transplantable leukemia is not a result of acquired mutations selected for upon transplantation and subsequent proliferation but rather is a reflection of the genetic mutations inherent in the original LSC population. Multiple signaling pathways are crucial in the biology of CSCs as well as normal SCs.

Table 1. Sample of cancer stem cell markers identified for various cancers

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Stem cell marker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myeloid leukemia</td>
<td>CD34\textsuperscript{+} CD38\textsuperscript{−}</td>
<td>[68]</td>
</tr>
<tr>
<td>Brain tumor (Human glioblastomas)</td>
<td>CD133\textsuperscript{+} U87</td>
<td>[69-72] [73]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>CD44\textsuperscript{+}CD24\textsuperscript{−}/lowLineage\textsuperscript{−}</td>
<td>[6] [8]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>CD44\textsuperscript{+}α β hi CD133\textsuperscript{+}</td>
<td>[74]</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>ABCG2\textsuperscript{+}</td>
<td>[75]</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>SP-C\textsuperscript{+}CCA\textsuperscript{+}</td>
<td>[76]</td>
</tr>
<tr>
<td>Colorectal</td>
<td>CD133\textsuperscript{+} SW1222, LS180, CCK81</td>
<td>[77] [78]</td>
</tr>
</tbody>
</table>

3 Conclusion

CSCs arise from somatic SCs for a variety of reasons, largely implicated by aging\textsuperscript{[21]}. Whether CSCs arise due to activation/reactivation of telomerase, hypo/hypermethylation, cellular/systemic pH, hyperglycemia or chronic systemic inflammation or a combination of these factors still requires elucidation. Cancer results from unregulated expansion of a self-renewing CSC population, a property, which is shared with normal SCs. The observation that not all solid cancer cells can establish or maintain tumor growth may assist in defining critical pathways that drive the growth and spread of a tumor, and examine methods by which to target these pathways\textsuperscript{[79]}. Two important observations have led to the hypothesis that CSCs may be responsible for growing and maintaining tumors. Firstly, most tumors arise from a single cell\textsuperscript{[80]}. It is thought that the cellular heterogeneity found in tumors may be attributed to genomic instability and the selection for cells that can adapt to the tumor microenvironment\textsuperscript{[81,82]}. The second observation upon which the CSC theory
was built came from studies that demonstrated that a large number of cancer cells were required to grow a tumor\[^{83,84}\]. Under the assumption that all cancer cells have similar potential to grow tumors and that tumors are usually clonal in origin, one would expect that even a few cancer cells would be able to grow new tumors; however, this is not the case\[^{79}\]. The concept of a CSC within a more differentiated tumor mass, as an aberrant form of normal differentiation, is now gaining acceptance over the current stochastic model of oncogenesis, in which all tumor cells are equivalent both in growth and tumor-initiating capacity\[^{85}\].

Tumors are complex organisms of varied origin that arise from multiple factors. CSCs play a pivotal role in their initiation, progression and metastasis. Treatment of tumors requires a complex multi-level approach to deal with the various contributing conditions, which have given rise to cancer.

Treatment approaches described above combined with an overall treatment protocol for the tumor microenvironment and chronic systemic inflammation may provide a more successful outcome than a single tactical approach. As shown in numerous studies in the literature, the use of complementary disciplines with orthodox treatments may enhance outcomes.

4 Competing interests

The authors declare that they have no competing interests.

REFERENCES


8. Marcato P, Dean CA, Pan D, Araslanova R, Gillis M, J. Joshi. Aldehyde Dehydrogenase Activity of Breast Cancer Stem Cells is Primarily Due to Isoform ALDH1A3 and its Expression is Predictive


54. Zhang YH, Lin HS. Tumor Stem Cells May Be the Final Target of Traditional Chinese Medicine in Preventing Cancer Recurrence and Metastasis. Zhong Guo Zhong Xi Yi Jie He Za Zhi [Chinese


