Expert Opinion

Cancer stem cell patents

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Background: Cancer stem cells (CSCs) are a small subpopulation of the tumour mass that are capable of sustaining its growth, are resistant to many cancer specific therapies, and can reinitiate disease relapse. CSCs are resistant to current cancer treatments due to specialised transporters, cell cycle changes, DNA repair and antiapoptotic mechanisms. *Objective*: This paper reviews CSC biology and diagnostic and therapeutic patents as well as those associated with the isolation of CSCs. *Methods*: Literature and patent searches using the NCBI PubMed, European Patent Office, Scopus, and PATENTSCOPE® websites were conducted to examine and link CSC biology and therapy development with current patent literature. *Conclusion*: Development in basic CSC biology research is increasing the patents filed in this area; however, therapeutic patents directly targeting CSCs are more limited, as research in this area remains in its infancy.

Keywords: cancer stem cell, haematopoiesis, leukaemia, patents, tumour

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1. Introduction

Advances in early screening and treatment have extended lifespans and improved quality of life for many cancer patients, yet current therapies are still limited in their ability to cure or prevent malignancies. Current cancer treatments are highly toxic and are directed at reducing the size of the tumour; however, they are also nonspecific and destroy other viable cells. Upon completion of therapy, there is often regrowth of the tumour and relapse of the disease. Current evidence suggests that rare populations of cells, termed cancer stem cells (CSCs), are at the core of tumour formation and are resistant to current cancer therapies. Advances in CSC biology may lead to the development of more effective therapies, and as research expands in the CSC field, so does the number of CSC patents published. This review examines the biology, mechanisms for resistance, and therapeutic potential of CSCs, as well as how the current patent literature is threading these together.

2. Cancer stem cell model

The cancer stem cell model proposes that the growth and metastasis of cancers is initiated by a small CSC subpopulation that gives rise to either daughter stem cells through self-renewal, or differentiated non-tumorigenic cells that form the bulk of the tumour and give each tumour its heterogeneity. The CSC model may explain why current cancer therapies result in initial reduction of the tumour mass; however, due to CSCs' resistance to these current therapies, disease relapse upon completion is a likely consequence.

CSCs were identified from acute myeloid leukaemic (AML) patients using fluorescence-activated cell sorting [1]. This technique uses fluorescent-labelled antibodies to separate populations of cells based on differences in the expression of proteins on the cell surface. These leukaemic stem cells (LSCs) were isolated into a mature population that expressed the cell surface markers CD34 and CD38 ($CD34^+/CD38^+$) and an immature population that only expressed CD34 ($CD34^+/CD38^-$). When transplanted into immunocompromised mice to

assay for the tumorigenicity of each population, the immature $CD34^+/CD38^-$ cells gave rise to leukaemia whereas the more mature $CD34^+/CD38^+$ cells did not [2], although a recent study has demonstrated that the $CD34^+/CD38^+$ population can also give rise to leukaemia if the inhibitory effects of anti-CD38 antibodies are prevented [3]. From these and other findings [4], a hierarchy of AML that closely resembled the hierarchy of normal haematopoiesis was proposed.

The AML stem cell has similar CSC counterparts in other solid tumours including breast [5], brain [6,7] prostate [8-10], colon [11,12], lung [13], and pancreatic [14] cancers. In a healthy state, mature cells are replenished by long-lived stem cells unique to the organ. Through a tightly regulated process, each tissue stem cell has the common ability to form another stem cell through self-renewal, or differentiate into progenitor cells that give rise to the mature cells. These daughter progenitor cells are more restricted in their lineage choice and divide frequently.

In the disease state the tightly regulated process of self-renewal is abnormal. Like the early xenotransplantation experiments of the LSCs, transplantation of CSCs from solid tumours into susceptible mice gives rise to a developmental hierarchy that is reminiscent of the tissues from which they were derived. The tumours comprise both tumorigenic stem cells and non-tumorigenic bulk cells that can also be isolated using surface marker expression and analysed in transplant assays. Identification and isolation of solid tumour CSCs, LSCs, and cell line-derived CSCs have been the subject of a number of patents [15-17], and their isolation has been aided by exploiting their qualitative and quantitative differences from non-tumorigenic cells.

3. Identification and isolation of CSCs

3.1 Blood cancer

Clonal stem cell disorders of the blood include AML, chronic myeloid leukaemia (CML), myelodysplastic syndromes, and acute lymphoblastic leukaemia (ALL). AML is characterised by proliferation of immature progenitor cells that are incapable of terminal differentiation. The disease is generated from a $CD34^+/CD38^-$ LSC [2] that represents 0.1 – 1% of the AML population and shares many similarities with its normal haematopoietic stem cell (HSC) counterpart [18]. The LSC can establish cellular diversity *in vivo* and can generate distinct stem cell classes, namely the long-term and short-term LSCs, that differ in their self-renewal potential [4]. The cellular diversity of AML suggests that the disease is arranged as a hierarchy and closely resembles normal HSC organisation.

Acute promyelocytic leukaemia (APL) is a subtype of AML, characterised by an overproduction of myeloid progenitor cells blocked at the promyelocytic stage. Progenitor proliferation results from a translocation of chromosomes 15 and 17 that gives rise to a promyelocytic leukaemia (PML) and retinoic acid receptor-α (RARα) fusion protein. APL originates from an early hematopoietic progenitor/stem cell that is CD34-positive [19] and is currently treated by the vitamin A derivative all-trans retinoic acid (ATRA). In combination with chemotherapy, ATRA, whose action is to bind RARα, can achieve complete remission in the majority of cases. In instances of relapsed APL, the retinoid Am80 (Amnolake®, Toko Pharmaceutical Ind. Co. Ltd, Adachi-ku Tokyo, Japan) or arsenic trioxide (Trisenox®, Cephalon, Inc., Frazer, USA) with autologous stem cell transplantation are effective to induce remission [20] and in cases of complete ATRA resistance, the ATRA derivative, TWB-8083 [21] may prove to be effective by binding to RARα and inducing differentiation and apoptosis of the ATRA-resistant cell.

Similar to AML, ALL and the myelodysplastic syndromes are derived from a $CD34^+CD38^-$ [22,23] and a $CD34^+CD38^-$ Thy-1^+ stem cell [24], respectively, and are characterised by a proliferation of immature lymphoid (ALL) or myeloid (myelodysplastic syndromes) progenitor cells that do not terminally differentiate. The ALL $CD34^+CD38^+$ subfraction has also been demonstrated to transfer the disease in xenotransplantations and is positive for the B-cell antigen CD19 [25]. A monoclonal antibody conjugate against this antigen has been investigated for its therapeutic potential [26].

The Philadelphia chromosome of CML is generated from a translocation of chromosomes 9 and 22, and can be detected in all blood lineages, suggesting that its cell of origin is a HSC. The translocation gives rise to a constitutively active tyrosine kinase, the BCR-ABL fusion protein that activates several signalling pathways to promote the survival and proliferation of differentiated granulocytes. Current treatment for CML is with imatinib mesilate (Gleevec®, Novartis, Basel, Switzerland). Patients generally respond successfully to imatinib, and in some cases BCR-ABL mRNA expression is absent, but patients can relapse quickly when removed from the drug [27]. Several studies have demonstrated that imatinib targets the CML progenitors and leaves the stem cells untouched [28,29], thus allowing the disease to progress. Additional therapies include IFN-α, allogeneic stem cell transplantation, analogues of sodium chlorogenate [30], and peptide nucleic acid conjugates [31].

Multiple myeloma results in an overexpansion of plasma cells and is another haematological malignancy believed to derive from a CSC, although its exact identity remains unknown. Evidence suggests that B-cell populations, negative for the membrane protein syndecan-1 (CD138) but positive for CD20 and CD27, are highly drug-resistant and able to establish the disease when transplanted into mice [32,33]. These CSC populations appear to rely on the Hedgehog signalling pathway to maintain their stem cell characteristics, and are sensitive to cyclopamine derivatives [34,35].

3.2 Brain tumours

CSCs have been found in several human brain tumours and can be enriched by selecting for the CD133 (prominin-1) antigen. *In vitro*, $CD133^+$ cells can form neurosphere-like clusters, express the neural precursor cell marker nestin, and

differentiate into neural lineages [6]; while *in vivo*, $CD133^+$ cells can generate disease in xenotransplantations [7]. Several studies, however, have since shown that $CD133^-$ cells of glioblastomata are also capable of *in vitro* growth and initiating disease [36,37]. Within the $CD133^+$ fraction, upregulation of several genes important for drug resistance, DNA repair, and inhibition of apoptosis increases their resistance to chemotherapy [38], while activation of DNA damage checkpoints and DNA repair mechanisms promotes their resistance to ionizing radiation [39].

3.3 Colon cancer

Until recently, a CSC for colon cancer had not been identified. Indeed, no assays existed nor surface markers identified that isolated these stem cells for *in vitro* study other than assays that studied stem cells within their histological and positional context of the epithelium in the crypt-villus unit [40]. The colon cancer-initiating population was initially isolated based on the CD133 antigen, and a patent [41] describing its potential as a diagnostic tool for digestive system cancers has been published. In xenotransplantation experiments, $CD133^+$ cells could re-establish the original tumour whereas $CD133^-$ cells could not [11,12]. However, $CD133^-$ cells that were also $CD44^+CD24^-$ could grow in culture [42], while colonic tumour cells expressing the epithelial cell adhesion molecule $(EpCAM)^{(high)}/CD44^+$ could recapitulate the tumour in xenotransplantations [43]. Taken together, these studies suggest that clearly defined markers are still required to isolate the colon's CSC population.

3.4 Lung cancer

Difficulties in the identification of a CSC target cell have arisen from differences in tumour subclasses between distinct regions of the lung, such as adenocarcinomas/bronchoalveolar carcinomas (bronchioalveolar duct junction; BADJ), small lung carcinomas (midlevel bronchioles), and squamous cell carcinomas (proximal airways) [44]. Regional tumour heterogeneity demonstrates that distinct cellular niches support tumour growth. Within these niches exist precursor cells capable of reconstituting the epithelial layers of the lung [45] such as the $Sca\text{-}1^+$ $CD34^+$ bronchioalveolar stem cells of the murine BADJ that are capable of differentiation or self-renewal *in vitro* [13]. Similarly, in human small cell and non-small cell lung tumours, a subfraction of $CD133^+$ cells has been isolated that is capable of both *in vitro* propagation and differentiation as well as generating tumours in xenotransplantations [46]; however, it is still unknown whether lung cancers can be identified with a common biomarker or if several exist.

3.5 Breast cancer

The mammary gland is an estrogen-dependent organ, and the prognosis and response to antiestrogen treatment is favourable in tumours expressing the estrogen receptor (ER). However, the prognosis is poor in cases where the tumour has arisen from an ER-negative (ER^-) cell. ER^- tumours are thought to be derived from ER^- stem or early progenitor cells that can either self-renew or differentiate into ER^+ or ER^- tumour cells [47]. Alternatively, it has been proposed that ER^- tumours can be derived from lineage-committed ER^- luminal progenitors [48]. The identity of the breast CSC was further elucidated by the discovery of a tumorigenic population expressing a $CD44^+CD24^{-/low}$lineage negative phenotype which, when transplanted *in vivo*, could successfully produce tumours [5,49]. This population has also been linked to BRCA1 and ER^- basal-like breast cancers [50].

3.6 Prostate cancer

The prostate is an androgen-dependent organ and the majority of tumour bulk cells are responsive to androgen therapy. These tumour cells are comprised primarily of the secretory luminal cells that express the androgen receptor and are fully differentiated, and the prostate basal cells that are undifferentiated and express low androgen receptor levels [51]. The identity of the prostate CSC remains unknown, although the candidate markers Sca-1 [8,52], Bcl-2 [52], $\alpha2\beta1$-integrin bright [53], $\alpha6$ integrin (CD49f) [52], and CD133 [54] have been suggested. The $CD44^+/\alpha2\beta1^{hi}/CD133^+$ phenotype was used to isolate a population from human tumours that was clonogenic and invasive in an *in vitro* Matrigel experiment [9,55], while $CD44^+$ cells were shown to be more tumorigenic than $CD44^-$ cells *in vivo* [56].

3.7 Melanoma, pancreatic and liver cancers

As with the other CSCs described, the $CD133^+$ cell surface marker can be used to isolate a tumorigenic population in liver, melanoma, and pancreatic cancers (**Table 1**). Liver CSCs can be enriched for CD133 [57] and aldehyde dehydrogenase (ALDH [58,59]) or $CD90^+CD44^+$ [60], with each population demonstrating tumorigenic capacity *in vivo*. In addition, tumorigenic $CD133^+$ subpopulations within melanoma tumours and cell lines have been demonstrated [61] with melanoma cells expressing a $CD133^+CD44^+CD24^+$ phenotype most resembling a CSC [62]. Similarly, pancreatic CSCs expressing a $CD44^+CD24^+$ phenotype along with epithelial-specific antigen (ESA^+) were highly tumorigenic in xenotransplantations [14].

4. Biology of CSC resistance

Ineffectively targeting the CSC population with surgery, chemo-, radio- and immunotherapies may allow for the recurrence of the initial tumour following therapy. Survival mechanisms that have allowed normal stem cells to survive may explain CSCs' resilience to current forms of cancer treatments. These mechanisms include quiescence, antiapoptosis, drug resistance through increased efflux and selective transporters, and DNA repair (**Figure 1**).

4.1 Quiescence

The hallmark of the normal stem cell is to survive throughout the lifespan of the organism; like its healthy counterpart, the

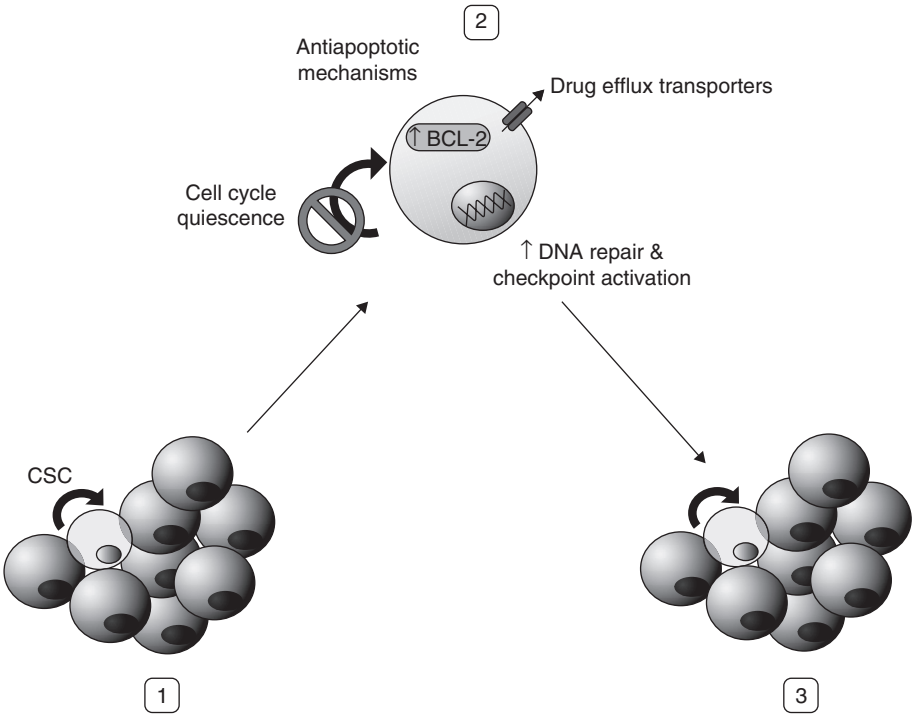

Figure 1. Biology of CSC resistance. Tumours contain cells with a variety of phenotypes, but only a small number of cells in the tumour bulk are cancer stem cells (1; lighter-coloured cell). CSCs are able to self-renew, can differentiate into different cell types, and can re-establish the original tumour in transplantation studies. Conventional treatments reduce the bulk of the tumour, but CSCs remain relatively unaffected (2). They are able to resist various chemotherapeutic agents by increased efflux of these drugs via ABC transporters, and have increased antiapoptotic protein expression such as Bcl-2 and Bcl-xL, as well as concomitant decreased expression of proapoptotic proteins such as Bax. In addition, CSCs are able to remain quiescent, thus circumventing drugs that target actively dividing cells. Finally CSCs have increased DNA repair and checkpoint activation. Therefore, eventually the CSC can re-establish the tumour (3); novel treatments that also target the CSC are being developed.

CSC is also able to remain quiescent for extended periods [63]. Current cancer therapies, such as cytosine arabinoside (Ara-C) used for the treatment of AML, and 5-flurouracil, only target dividing tumour cells that are in the S-phase of the cell cycle; they are ineffective against the CSC population. No therapies exist to actively induce stem cells to enter the cell cycle; however, the mechanism of a small metabolite, 8-quinolinol, whose patent has recently been published, may be able to inhibit mitotic proteins and is effective against breast CSCs [64].

4.2 Drug-resistant transporters

ABC (ATP-binding cassette) transporter proteins include several family members and contribute to the drug resistance of leukaemic [65], melanoma [66], brain [67], lung [68], breast [69], pancreas [70], and prostate [71] cancers. There is generally a high correlation of transporter proteins with fluorescence-activated cell sorting side population (SP) profiles as described by patent [72], and a distinct SP population was demonstrated in neuroblastoma cells and linked to the breast cancer resistance protein, BCRP1, and ABCA3 (intracellular) transporters [73]. These cells could asymmetrically divide, expressed high levels of BCRP1 and ABCA3 (intracellular) transporter genes, and could expel cytotoxic drugs [73]. In addition, other populations demonstrating a similar SP phenotype were found in lung cancer, breast cancer, and glioblastoma cell lines [73], and more recently in thyroid [74] and oesophageal cell lines [75].

4.3 Apoptosis

The resistance of the CSC is further aided by the ABCB1 transporter and its protection against caspase-dependent apoptosis [76,77]. Despite its role as a drug efflux pump, ABCB1 and the antiapoptotic proteins Bcl-2 and Bcl-xL have been associated with leukaemic $CD34^+$ cells [78]. These antiapoptotic proteins, including Mcl-1, were highly expressed within the $CD34^+$ population of patients and correlated with the primitive haematopoietic markers CD133 and CD117, whereas Bax (the proapoptotic) protein showed low levels [78].

4.4 DNA repair

Altered activation of DNA repair mechanisms and DNA damage checkpoint responses may also contribute to the resistance of CSCs. Glioblastomata derived from both patients and cell lines have demonstrated increased resistance

to ionizing radiation within the $CD133^+$ [79,80] and $CD133^+CD117^+CD71^+CD45^+$ [81] subpopulations. This increased radioresistance was shown to be caused by activation of DNA damage checkpoint responses and more efficient DNA repair mechanisms in the $CD133^+$ cells compared with the $CD133^-$ fraction [79]. In addition, the radioresistance of these cells could be inhibited with a checkpoint kinase inhibitor [79], and a recently published patent has described the development of compounds used to specifically inhibit the G2 checkpoint within cancer cells that will sensitise them to currently used cancer treatments [82].

5. Exploiting stem cell differences for therapeutic gain

Transformation of a normal stem cell into a CSC results in only a few subtle differences between the two populations, which makes targeting CSCs difficult. These unique differences may form the only means of targeting the CSC population while sparing the normal, healthy stem cells. Below, we describe how surface phenotype, apoptotic activation, niche requirements, self-renewal pathways, cell cycle, and gene signatures have been exploited for therapeutic gain.

5.1 Surface phenotype

Examples of the CSC surface markers for the cancers described in Section 3 provide potential targets unique to LSCs. Although CD33 is the target of the AML drug gemtuzumab ozogamicin (Mylotarg®, Wyeth Pharmaceuticals, Madison, USA), this antibody is only specific for differentiated cells within the tumour bulk. Despite the nonspecificity to target the CSC, a cell surface receptor unique to LSCs was finally shown by Jin and colleagues [83]. They demonstrated that mice transplanted with human AML stem cells and given subsequent injections of an antibody targeted against the transmembrane glycoprotein, CD44, inhibited the movement of the leukaemic cells to the bone marrow and spleen and induced their differentiation into a more mature state. This idea was not new, and CD44 was shown to have similar effects on AML stem cells *in vitro* [84,85]; however, the importance of this study was that it was demonstrated *in vivo*. In a similar series of experiments, Krause and others [86] also demonstrated that CD44 was important for CML stem cell homing and that antibody neutralisation of CD44 retarded the development of leukaemia, though not as effectively as in the AML mice of the Jin study [83]. CD44 is not only highly expressed on LSCs but is also present on breast, colon, liver, pancreas, melanoma, and prostate CSCs, and therapeutic reagents directed at this molecule, such as an anti-CD44 antibody described in patent [87], may have profound effects on how other cancers are treated.

5.2 Apoptosis

Resistance to apoptosis is one of the mechanisms used by CSCs to maintain survival. NF-κB is an antiapoptotic transcription factor that remains in an inactive state within the cytoplasm of normal HSCs, yet is constitutively activated within LSCs [88]. Upon degradation of the regulatory molecule IκBα, NF-κB becomes active and translocates to the nucleus. Proteasome inhibitors, such as MG-132, block the degradation of IκBα and are used to target NF-κB, but when used alone are only able to sensitise LSCs to a slow cell death [89]. In combination with idarubicin, an anthracycline drug whose action is to induce apoptosis through activation of p53-regulated genes, LSCs undergo a rapid and broad apoptotic death while normal HSCs are spared [89].

Parthenolide, a sesquiterpene lactone found in the herbal plant Feverfew, is less toxic than anthracyclines and has the same effects and mechanisms as the MG-132/idarubicin combination, but is further enhanced by its ability to change the oxidative state within the target cells [90]. Unfortunately, parthenolide has poor water solubility, and as such a method describing how chemical modifications of parthenolide can result in structural analogues with greater water solubility has been patented [91]. In addition, ABT-737, a BH3 mimetic whose action is to disrupt the Bcl-2/Bax protein association and induce apoptosis, has demonstrated a high specificity to LSCs [92], while more recently a method has described the induction of apoptosis in CSCs through the administration of 3-deazaneplanocin (DZNep) and a carrier compound in patent [93].

5.3 Niche

The stem cell niche is a specialised microenvironment that maintains stem cells, acts as a physical anchoring site, and provides extrinsic factors for a cell's proliferation and differentiation [94]. As described above, CD44 is a unique cell surface glycoprotein expressed on LSCs and required for stem cell homing to the bone marrow niche [83,86]. CD44-deficient stem cells transduced with the *BCR-ABL* oncogene resulted in defective homing, decreased engraftment, and impaired disease induction [86]. Similarly, anti-CD44 treatment of AML stem cells blocked the homing of these cells to the bone marrow and spleen, and abolished the adhesion of the CSCs to its ligand hyaluronan [83].

Extrinsic factors are equally important for providing a supportive environment for the CSC. Calabrese and others demonstrated that the brain niche for tumour CSCs is located beside tumour capillaries [95]. The authors showed that endothelial cells provide a supportive environment for the CSCs by secreting factors that promote self-renewal and non-differentiation *in vitro*, while *in vivo*, the CSCs self-renewed and accelerated tumour growth when placed in a vascular environment. As expected, treatment with antiangiogenic drugs resulted in a decrease in the CSC population and tumour growth, and suggested that multidrug combinations may be required for effective targeting not only of the CSC population but also of its supportive niche.

5.4 Self-renewal pathways

Cancer has been regarded as a disease of stem cells in which genetic alterations have transformed HSCs into CSCs; however,

leukaemogenic transforming events may not occur solely at the level of the HSC but also further down the hierarchy, at the level of the committed progenitor. These transforming events reconfer self-renewal properties and impair differentiation [96,97], and in combination may allow for the development of AML upon secondary cooperating mutations. Similarities in the molecular programs between normal and leukaemic HSCs would be expected, since both are capable of self-renewal, or if one of the transforming events within a progenitor cell was the reacquisition of self-renewal. As such, similar self-renewal pathways involving the *HOX* genes [98], BMI-1 [99,100], Wnt/beta-catenin [101], Hedgehog [100], and PTEN [102,103] have been shown in normal and carcinogenic stem cells.

Until recently, no targetable differences existed between normal and leukaemic self-renewal pathways that could be exploited for therapeutic purposes. Recently, two groups identified mice with a phosphatase and tensin (PTEN) homologue deletion that gave rise to a short-lived HSC expansion and subsequent development of leukaemia [102,103]. PTEN is a phosphatase that inhibits signaling through the phosphatidylinositol-3-OH kinase (PI3K; AKT) pathway and is upstream of the mammalian target of rapamycin (mTOR) protein. When rapamycin was applied to PTEN-deficient mice, mTOR was inhibited and leukaemia was prevented [102]. In addition, mTOR reduced the number of LSCs but increased the number of normal HSCs, resulting in an inhibition of further disease progression [102].

5.5 Cell cycle

In a related context, PTEN was shown to be regulated by the cell cycle; in PTEN-deficient mice, HSCs demonstrated a two- to threefold decrease in quiescent G_0 stem cells and an increase in cells in the S, G_2, and M phases [103]. PTEN-deficient mice expressed higher levels of cyclin D1, a G_1/S phase transition regulator. The authors suggested that PTEN regulates the switch between quiescent and active stem cells, and in its absence would readily induce a HSC to enter the cell cycle. Taken together with the results of Yilmaz and colleagues [102], this study provides a unique link between the cell cycle, LSCs, and PTEN, and may provide a target for therapy development.

5.6 Gene signatures

HSCs are composed of 'stemness' genes that confer the ability to self-renew. Leukaemic transformation of committed progenitors reactivates dormant self-renewal programs and other stemness genes to allow progenitors to self-renew. In such a study and its corresponding patent [104,105], RNA expression profiles of leukaemic MLL-AF9 granulocyte monocyte progenitors (GMPs) were compared with healthy GMP counterparts, HSCs, and other committed progenitors. The authors found that the expression profile of the leukaemic GMPs closely resembled that of normal GMPs; however, a subset of genes, originally activated in the HSCs but latent in the normal progenitors, were reactivated within the leukaemic GMPs. Upon closer examination, these genes comprised a self-renewal signature and included members of the *HOX* gene family. HOX transcription factors are involved in normal haematopoietic development and have been previously identified in MLL translocation leukaemogenesis [106].

Other unique CSC gene signatures have also been identified for the brain [107], prostate [108], and breast [109], where expression differences were used to construct an 'invasiveness' gene signature for breast cancer. Taken together, genetic profile differences between CSCs and normal stem cells that include identification of reactivated self-renewal programs in committed progenitors may provide complementary strategies for future therapies [110].

6. Summary of patents

Patents have been cited throughout the manuscript, and this section describes some of them in more detail and ties together the three main uses to which patents on CSCs have been put. Patents in this area of cancer research can be classified as those describing i) methods for obtaining or producing CSCs that can be used for research or screening purposes, ii) methods for the diagnosis or prognosis of cancer in patients based on CSC biology, and iii) methods for treating various types of cancer that specifically target the CSC population.

6.1 Research

Obtaining CSCs for research purposes typically involves isolating them from the rest of the tumour by using differential expression of cell surface markers. This has been demonstrated with a patent [111] for locating CSCs within the organs of the digestive system, based on the presence of the CD133 antigen. The patent describes how CD133-positive tissues are identified using fluorescence-activated cell sorting, microscopy, or chromatography and the amount of CD133 within the sample is determined through the strength of binding to its target. In another method, as described by patent [112], CSCs are directly produced by overexpressing the *JAB1* gene in transgenic animal models and the CSCs are used to screen for the efficacy of antileukaemic inhibitors. Alternatively, Takakura and colleagues describe a method for producing CSCs by fusing a tumour cell with a HSC *in vitro*, which can then be placed into an animal to create an animal model of the disease [113], while Sanchez-Garcia and Voces describe transgenic animal models expressing leukaemic fusion oncogenes [114]. In addition, patent [115] describes a defined medium that lacks serum and heregulin for culturing primary tumour cells *in vitro*, whereas patent [116] describes a method for isolating tumorigenic and non-tumorigenic cells from tumour tissue explants that have been plated onto a substrate-coated adhesive layer.

6.2 Diagnosis and prognosis

The majority of methods diagnose CSCs based on the expression of cell surface markers or genes. Mazar describes

Table 1. Cancer stem cell markers.

Tissue	Cancer	Markers	Ref.	Model
Blood	ALL	$CD34^+$, $CD19^+$, $CD38^{(+/-)}$	[22,23,25]	Human
	AML	$CD34^+$, $CD38^-$	[1,2]	Human
	CML	$CD34^+$, BCR-ABL	[28,29]	Human
	APL	PML-RAR-α, $CD34^+CD38^-$	[19]	Human
	Myelodysplastic syndromes	$CD34^+$, $CD38^-Thy\text{-}1^+$	[24]	Human
	Multiple myeloma	$CD138^-$, $CD20^+$, $CD27^+$	[32,33]	Human
Breast		$CD44^+CD24^{-/low}lineage^-$	[5]	Human
Brain	Pilocytic astrocytoma	$CD133^+$, nestin	[6]	Human
	Medullablastoma	$CD133^+$, nestin	[7]	Human
	Glioblastoma	$CD133^{(+/-)}$, nestin	[7,36-39]	Human
Colon		CD133	[11,12]	Human
		$CD133^-$, $CD44^+$, $CD24^-$	[42]	Human
		$EpCAM^{(high)}$, $CD44^+$	[43]	Human
Liver		$CD133^+$, ALDH	[58]	Human
		$CD90^+CD44^+$	[60]	Human
Lung	Adenocarcinoma	Sca-1^+, $CD34^+$	[13]	Mouse
	Small cell and non-small cell	$CD133^+$	[46]	Human
Pancreas		$CD44^+$, $CD24^+$, ESA^+	[14]	Human
Prostate		Sca-1^+, CD49f$^+$, Bcl-2^+	[52]	Mouse
		$CD44^+/\alpha2\beta1^{hi}/CD133^+$	[9]	Human
Skin	Melanoma	$CD133^+CD44^+CD24^+$	[62]	Mouse
		$CD133^+$, ABCG2	[61]	Human

ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; APL: Acute promyelocytic leukaemia; CML: Chronic myeloid leukaemia.

how fluorophore-conjugated antibodies against the urokinase-type plasminogen activator receptor would be beneficial for the detection of CSCs, and also for their eradication if the antibodies were conjugated to a drug moiety [117]. In prostate cancer, Reiter and Witte describe how antibodies to the prostate stem cell antigen may be useful for identifying cells expressing this marker and delaying or destroying its growth [118]. Donnenberg and Donnenberg describe how CSCs could be diagnosed in a patient based on the presence of an ABC transporter protein (see Section 4.2) [119], while Laird and Widschwendter describe a method for diagnosing patients based on DNA methylation of gene promoter regions for polycomb group (PcG) protein targets [120].

6.3 Treatments

The ultimate aim of CSC research to to provide effective therapies for patients suffering from cancer. Towards that end, several methods, such as a patent [121] describing the effectiveness of cantharidin and its analogues in specifically targeting the CSC population have recently been published. Cirrito and Bergstein describe a very general and broad approach that includes 'the stabilisation, reduction, or elimination of the cancer stem cell population... by administering a therapy for a longer period of time than currently used' and 'without being bound by a particular theory or mechanism' [122].

Stimulating an immune response to target antigens present on CSCs is a common approach. Yu describes a method for creating a neural cancer vaccine by exposing antigen-presenting cells (dendritic cells) with neural CSC antigens so that the dendritic cells 'present' the neural CSC antigens [123]. Gurney, Sato, and Fitch-Bruhns describe how antibodies against the glutamate ligand binding region of the Notch receptor could be used as a therapeutic agent to inhibit tumours [124] while Lewicki, Gurney, and Satyal describe a similar method for inhibiting tumours using antibodies against the discoidin/coagulation domain of the DDR2 protein [125]. Philip and Keller describe how a cytotoxic T-lymphocyte response could be used to eliminate CSCs from a patient through immunotherapy with cancer specific peptides [126], while Yu, Tan and Yang have patented an unnamed compound that is effective at inducing apoptosis and modulating pluripotency and/or self-renewing characteristics of a stem/progenitor cell [127].

7. Conclusion

The tumour mass is no longer viewed as a homogeneous entity, and current evidence suggests that a small group of

CSCs in blood and solid tumours are responsible for tumour growth and resistance to therapeutic agents. To date, CSCs have been identified in blood, brain, colon, lung, breast, prostate, pancreatic, melanoma, and liver cancers. In each of these cancers there has been identified a unique subpopulation of CSCs based on cell surface marker expression and tumour recapitulation in xenotransplantation assays. The durability of the CSC is dependent, in part, on its ability to expel cytotoxic drugs, resist apoptosis, repair DNA and quiesce. Unique differences in surface phenotype, apoptotic activation, niche requirements, self-renewal pathways, cell cycle activity, and gene expression signatures exist between CSCs and normal stem cells, and several studies have already taken steps in targeting these properties for therapeutic benefit. Although no CSC-specific drugs have reached clinical trials, it is only a matter of time until our understanding of this young field improves and ideas are translated into therapies.

8. Expert opinion

During the last decade there have been significant advancements in the identification and isolation of CSCs within haematological and solid malignancies. These advances have been made, in part, as a result of the increased availablility of commercial antibodies, technological improvements in fluorescence-activated cell sorters, and better xenotransplantation assays. To date, much of the patent literature has focused on methods for isolating a CSC population through unique surface and genetic biomarkers and on eliminating this population through assay systems that test the effects of chemical compounds.

Current therapies treat solid tumours as a homogeneous entity and result in targeting the bulk tumour without affecting the CSC core. Treatment is achieved primarily through the use of surgery, immuno-, chemo-, and radiotherapies where the end point is determined by the successful removal of proliferating cells. Current research is focusing on compounds and their derivatives to successfully target the CSC subpopulation. If these compounds are translated into therapies within the next decade, it will be important to determine their effect on the CSC population, not just on the tumour mass. This may require a different end point to determine whether the compound was successful; one that goes beyond antiproliferative and survival effects. It is probable that many therapies already in use are capable of targeting the CSC population; however, at present they would also need to have an effect on proliferation and survival to be deemed successful.

Differences in signalling pathway proteins as well as surface receptors have been identified between normal and cancerous stem cells; however, our current knowledge of these differences is limited, and it is necessary to discover further ways to distinguish these populations in order to maximise the number of druggable targets. Therapeutic agents such as small chemical compounds, RNA interference, or antibodies to inhibit CSC function will also need to avoid affecting normal tissues in order to be successfully translated into usable therapies.

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Declaration of interest

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