

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# The Dark Side of Pluripotency – Cancer Stem Cell

---

Patricia Ng and Wang Cheng-I

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54369>

---

## 1. Introduction

Cancer stem cells are defined as cancer cells that show the two properties of stemness: unlimited self-renewal and, pluripotency or multipotency. These properties make cancer stem cell tumorigenic i.e. the ability to induce and sustain cancer.

The definition of cancer stem cell has been a topic of debate and has changed with time. Cancer stem cells were proposed in 1994 by John Dick and coworkers as the cells that initiated leukemia [1]. It was thought that this leukemic cell was derived from the mutation of a hematopoietic stem cell. Importantly, the term was used to distinguish a small subpopulation of leukemic cells that could initiate and maintain cancer from the rest of the leukemic cells that could not. Subsequently, it was also observed in other types of cancer that only a very small subpopulation of cancer cells had the ability to initiate cancer when transplanted into a new host [2–12]. This subpopulation of cancer cells was considered as cancer stem cells. The rest of the cancer cells, which ranged from progenitor to fully differentiated cancer cells, that formed the bulk of the cancer had limited proliferative capacity and hence could not initiate cancer when transplanted. Since cancer comprise a heterogeneous collection of cells, a unique set of cell surface markers that were expressed on cancer stem cells were used to define them.

The definition underwent revision when new experimental methods showed that tumorigenicity had been underassigned to a small group of cells due to limitations of the detection technique used. When different experimental approaches were undertaken, tumorigenicity was found to be widespread amongst phenotypically diverse cancer cells, resulting in a paradigm shift in the definition of cancer stem cells. Hence in 2006, the American Association of Cancer Research (AACR) defined a cancer stem cell as any cancer cell that possessed stem cell-like properties of unlimited self-renewal and multi/pluripotency. AACR specifically highlighted that the definition of a cancer stem cell does not imply that such cells are de-

rived from the stem cells of the corresponding tissue. Also, a cancer stem cell does not have to be that initial cell in the body that caused cancer. For example, a differentiated cell that reacquires immortality through genetic mutations is considered a cancer stem cell. Thus any cancer cell that possesses or acquires stemness which results in unlimited tumorigenic potential is considered a cancer stem cell.

More recently, interesting data has emerged demonstrating that partially differentiated cancer cells, when exposed to a specific set of microenvironmental factors, can reacquire stemness [13]. Induction of stemness through this mechanism is reversible and could also result in epigenetic modifications, which then becomes heritable. This finding would again modify our understanding of the nature of cancer stem cell, suggesting that the cancer stem cell can be a dynamic and reversible entity.

In this section, experimental data shaping the identification and definition of cancer stem cells are presented in three parts. It begins with a description of early studies demonstrating that cancer stem cells were found to be a small and distinct subpopulation of cancer. This is followed by evidence suggesting that cancer stem cells can also be a highly common and heterogeneous population of cancer cells. Finally, evidence that cancer stem cell is a dynamic and reversible entity in cancer is discussed.

### **1.1. Cancer stem cells: A distinct subpopulation of cancer cells**

The concept of cancer stem cell is not new. The first experimental evidence for the existence of cancer stem cell was in 1937 when Furth and Kahn injected a single leukemic cell from a mouse into an inbred mouse and transmitted leukemia [14]. At that time, it was unclear if every cancer cell or only a subpopulation of cancer cells possessed this ability to transmit. In 1994, a landmark experiment showed that only a subpopulation of cancer cells could transmit cancer [1]. John Dick and his group isolated cancer cells from patients with acute myeloid leukemia (AML) and separated these cells based on their expression of CD34 and CD38. In this study, transplanting half a million of CD34+CD38<sup>-</sup> cells into severe combined immunodeficiency (SCID) mice induced AML in mice within thirty days, while the same number of CD34+CD38<sup>+</sup> cells did not induce any AML in mice. The subpopulation of cancer cells that could transmit cancer was termed SCID-leukemia initiating cells and was thought to be amongst CD34+CD38<sup>-</sup> cells.

To determine the amount of the SCID-leukemia initiating cells within the CD34+CD38<sup>-</sup> cell population, a quantitative transplantation approach was used [15]. The cancer cells were serially diluted and transplanted into NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice. The minimum dose required to cause leukemia was then determined. Based on this experiment, it was found that there was about one cancer stem cell per 5,000 CD34+CD38<sup>-</sup> cells. This would mean that within a population of a million cancer cells, there was about one cancer stem cell. The ability of cancer stem cell to self-renew was provided by experiments that used the same transplantation approach described above, i.e. using human leukemic cells and NOD/SCID mice [15]. In one of these experiments, the number of human cancer stem cells in mice was initially found to be about three in 16 million leukemia cells. However, after six weeks, human cancer stem cells had increased to about 100 cancer stem

cells within a population of 20 million leukemia cells in the bone marrow of these mice. This indicated that SCID-leukemia initiating cells in the mice had multiplied from three to a 100 and therefore behaved like stem cells, possessing unlimited self-renewal ability.

Evidence that cancer stem cells could differentiate into the rest of the cancer cell population was provided by characterizing the CD34+CD38- cells after transplantation into NOD/SCID mice [15]. Flow cytometry analysis of human cells isolated from the bone marrow of mice showed that transplanting CD34+CD38- cells resulted in an increase in cancer cell population, of which 98% were positive for both CD34 and CD38. This differentiative capacity, together with self-renewal ability, led to the conclusions that cancer stem cells existed and formed a distinct subpopulation of cancer cells.

Following the identification of cancer stem cells in leukemia, a series of in vivo studies documenting the presence of cancer stem cells in other cancers came to light. These studies, summarized in Table 1, characterized human cancer-initiating cells by their surface markers and were based mostly on the NOD/SCID mouse xenotransplantation assay. The table highlights information regarding the frequency of expression of cancer-stem cell-associated markers in the cancer cells, and the estimated frequency of the cancer stem cells residing in the cell population that bears the cancer stem cell-associated markers. Based on these studies, it was estimated that cancer stem cell existed in, at most, one in ten thousand cancer cells. Importantly, it was shown that this small population of cancer stem cells had a distinct CD-phenotype, which when fully defined would serve as the address for accurate delivery of cytotoxic drugs.

In addition to the use of NOD/SCID mice, in vitro techniques that were previously used for the isolation of normal stem cells were also used to isolate cancer stem cells. These techniques included the formation of non-adherent spheroids in tissue culture method [10] and the exclusion of the fluorescent Hoechst dye method [16]. Both methods led to the identification of a subpopulation of cells that, when transplanted into mice, resulted in tumorigenicity. Hence both in vivo and in vitro studies suggested that tumorigenic cancer cells were stem-like in phenotype.

## **1.2. Cancer stem cells are not ALWAYS a distinct subpopulation of cancer cells**

After a decade of using human cancer cells with NOD/SCID mouse as a model for cancer stem cell detection, there were concerns of incompatibility issues between the two species with regards to the cytokines and receptors involved in cancer stem cell research. Cytokines and receptors from different species could prevent critical interactions that were required for cancer cells to survive. Furthermore, the NOD/SCID mouse immune system, even though rendered compromised, could still mount some level of response to reject the human cells, thereby potentially resulting in erroneously lower count estimation of cancer stem cell population.

Markers for enrichment of cancer stem cells	Cancer type	Cancer stem cell detection assay	Ref.
CD34 <sup>+</sup> CD38 <sup>-</sup>	Leukemia (0.02-2% of mononuclear cells) <sup>a</sup>	2 of 2 NOD/SCID mice (5,000 cells) <sup>b</sup>	[1,15]
CD44 <sup>+</sup> Lin <sup>-</sup>	Head and Neck cancer (0.1-42%)	5 of 7 NOD/SCID mice (5,000 cells)	[20]
CD44 <sup>+</sup> ESA <sup>+</sup> CD24 <sup>-</sup> or low Lin <sup>-</sup>	Breast cancer (2%)	4 of 4 NOD/SCID mice (200 cells)	[2,4]
CD44 <sup>+</sup> CD24 <sup>-</sup> ALDH1 <sup>+</sup> Lin <sup>-</sup>	Breast cancer (0.1-1.2%)	NOD/SCID mice (20 cells)	[21]
CD44 <sup>+</sup> ESA <sup>+</sup> CD24 <sup>+</sup>	Pancreatic cancer (0.2-0.8%)	6 of 12 NOD/SCID mice (100 cells)	[12]
CD44 <sup>+</sup> ESA <sup>high</sup> CD166 <sup>+</sup>	Colon cancer (1.2-16%)	1 of 2 NOD/SCID mice (150 cells)	[3]
CD44 <sup>+</sup> CD117 <sup>+</sup>	Ovarian cancer (0.2%)	9 of 10 nude mice (100 cells)	[11]
CD133 <sup>+</sup>	Brain cancer (6-29%)	4 of 4 NOD/SCID mice (100 cells)	[10]
	Colon cancer (1.8-24.5%)	5 of 6 NOD/SCID mice (500 cells)	[6]
		15 of 30 NOD/SCID mice (3,000 cells)	[8]
	Lung cancer (0.32-22%)	4 of 4 SCID mice (10,000 cells)	[5]
	Pancreatic cancer (1.1-3.2%)	Nude mice (500 cells)	[22]
ABC5 <sup>+</sup>	Melanoma (1.6-20.4%)	11 of 11 NOD/SCID mice (1,000,000 cells)	[9]

**Table 1.** Experiments using markers for the enrichment of human cancer stem cells and xenotransplantation assay for the detection and quantification of human cancer stem cells. An estimation of the population of cancer stem cell in a tumor is given based. Epithelial-specific antigen (ESA) and ATP-binding cassette B5 (ABC5) are surface markers. Aldehyde dehydrogenase 1 (ALDH1) is an enzyme inside the cell. Lin refers to a collection of lineage markers CD2, CD3, CD10, CD16, CD18, CD31, CD64 and CD140b. Mice were condition by irradiation prior to receiving the transplantation. <sup>a</sup>Percentages of tumor cells expressing the selected markers. <sup>b</sup>Minimum number of surface-marker expressing cells required to induce cancer in at least 50% of the mice.

To address these concerns, alternative experimental models were used. The first model used mouse cancer cells instead of human cancer cells to circumvent the issue of cross species barrier. One of these experiments involved transplanting mouse leukemic cells from trans-

genic mice bearing the oncogene *Myc* with the immunoglobulin heavy chain enhancer. Just ten mouse leukemic cells were sufficient to induce cancer. Indeed, this experiment recapitulates the very first experiment in 1937 in which a single cancer cell from a chemically-induced cancer mouse was able to cause cancer in an inbred mouse [14,17]. This suggested that cancer stem cells were not necessarily a small population of cancer cells but rather could be more common than previously thought.

In the second model, human cancer cells continued to be used. However, they were transplanted into mice that were rendered even more severely immunocompromised than NOD/SCID mice [18]. In one such study where NOD/SCID ILR2 $\gamma$ null mice were used, 27% (and hence more than a quarter) of single cell transplantation of human melanoma cells into the mice resulted in cancer [18,19]. Importantly, this experiment showed that these cancer stem cells were not associated with any of the surface markers that were previously characterized (See Table 1). A total of 85 cell surface markers from these cancer stem cells were studied. Of these, 22 cell surface markers showed heterogeneous expression within the cancer cell population of which none had an association with the capacity for tumor initiation. For example, both CD133+ and CD133- cells were able to induce cancer [19]. In addition, the cancers that resulted from both CD133+ and CD133- cancer cells created a population of cancer cells that was heterogeneous in their expression of CD133. These findings implied that cancer stem cells were both common and heterogeneous. Hence, these 2 models showed that cancer stem cells were not a small, distinct subpopulation of cancer cells but rather a common and heterogeneous population of cancer cells in cancers such as melanoma.

Amidst the new findings that challenged the concept of cancer stem cells as a small, distinct subpopulation of cancer cells, there were experiments which still showed that the cancer stem cell subpopulation was indeed low, and not common and heterogeneous, even when syngenic mice were used [23]. Interestingly, human experiments (which could not have been conducted currently due to ethical reasons) provided evidence that when human cancer cells were transplanted back into the human subject from whom the cancer cells originated, the likelihood of cancer-initiation in the autologous human host is rare [24–26].

Taking all the evidence together, cancer stem cells are indeed a small and distinct subpopulation of cancer cells in some cancers, whereas in other cancers, cancer stem cells are common and heterogeneous. An alternative explanation that could account for these varied observations about cancer stem cells is that a cancer stem cell is not a static entity but rather a state that cancer cells can transform into.

### **1.3. Cancer stem cells are dynamic**

Even more recently, it has been shown that a partially differentiated cancer cell, under the “right” microenvironmental influence, can reacquire stemness [13]. This finding is crucial in furthering our understanding of the cancer stem cell as a dynamic and reversible entity, rather than a static one.

In a study on colorectal cancer, differentiated colorectal cancer cells were able to dedifferentiate back into cancer cells with cancer stem cell phenotype after being exposed to hepato-

cyte growth factor (HGF) [27]. Upon exposure, these cells showed increased colony-forming ability (clonogenicity) and increased tumorigenicity. Biochemically, the cells exhibited an increase in the Wnt signaling pathway leading to the expression of  $\beta$ -catenin dependent genes. This finding is important as HGF is present within the natural microenvironment of colorectal cancer as it is normally produced by myofibroblasts which are prominent in the colorectal stroma. Hence, given the right microenvironment, non-tumorigenic cancer cells can become cancer stem cells.

Similarly, in a separate study using PDGF-induced glioma in mice, exposure to nitric oxide caused differentiated glioma cancer cells to transform into glioma cancer stem cells [25]. Again, nitric oxide is normally present in the natural microenvironment of gliomas as nitric oxide is produced by blood vessels. Hence glioma cancer cells in close proximity to brain blood vessels were able to re-acquire stem-like properties. These two recent studies presented reiterate the concept that cancer stem cells are dynamic - cancer cells are able to transform back into cancer stem cells given the right microenvironmental conditions.

#### 1.4. Conclusions

Cancer stem cells are cancer cells that have self-renewable and multi or pluripotent abilities. Our current understanding is that cancer stem cells can be a distinct subpopulation of cancer cells in certain cancers while in other cancers, they can be relatively common and heterogeneous. They are also dynamic in nature.

Understanding the defining characteristics of cancer stem cells is important as these have important therapeutic implications. In cancers in which the cancer stem cells form a distinct subpopulation, eliminating this subpopulation of cancer stem cells can potentially lead to a cure. In contrast, targeting one specific group of cancer stem cells in cancers in which the cancer stem cells are common and heterogeneous would be futile. In addition, learning more about the microenvironmental factors that promote the cancer stem cell state provides another interesting approach in finding a cure for cancer.

## 2. Cancer stem cell: The survivor

Chemotherapeutic agents against cancer are able to reduce tumor mass significantly but often a cure may not be achievable. In such cases, a cure is not possible due to a subpopulation of cells that are resistant to cancer drugs. The cancer stem cells amongst this resistant population then self-renew, proliferate and metastasize to cause relapse after treatment. In addition to understanding the defining characteristics of cancer stem cells for therapeutic purposes, a working knowledge of the molecular mechanisms of drug-resistance in cancer stem cells will empower researchers to better design new therapeutic agents that can overcome drug resistance. We will also explore the mechanism for metastasis in cancer stem cells, which serves as another potential therapeutic target.

## 2.1. Drug resistance

Normal stem cells have traits that confer high survival capacity under harsh environments. These include (1) cell quiescent, (2) active DNA-repair system, (3) expression of transporters that keeps toxic substances out, (4) high metabolism in detoxification, and (5) resistance to apoptosis. These mechanisms are thought to be employed by cancer stem cells, in addition to genetic mutations, to evade anti-cancer drugs.

### 2.1.1. Cell quiescence

Chemotherapeutic cancer drugs such as vincristine, vinblastine, paclitaxel and docitaxel works by arresting cancer cells in mitosis, thus leading to apoptosis [28]. One hypothesis to explain resistance of cancer stem cells to these drugs is that cancer stem cells are in a quiescent state. Indeed, quiescent cancer stem cells have been shown to exist in some cancers [29]. Moreover, drug resistance in slow-cycling cancer stem cell population has also been reported [30,31]. In addition to cell quiescence, cancer stem cells are likely to have other mechanisms for drug resistance as discussed below.

### 2.1.2. DNA-repair

Ionizing radiation and anti-cancer drugs that disrupt the genome kill cancer cells by targeting their DNA. Cancer stem cells have efficient DNA-repair systems that confer resistance to these anti-cancer agents. A study on glioblastoma demonstrated that cancer stem cells, identified by their expression of CD133, showed preferential activation of the DNA damage check point response resulting in an increase in their DNA repair capacity [32]. The study also shows that both in vitro as well as mouse brain samples of cancer cells have increased the proportion of CD133-positive cells to CD133-negative cells following radiotherapy. This suggested that the subpopulation of CD133-positive, i.e. cancer stem cells, had developed resistance to radiotherapy and were the cause of cancer relapse in the mouse.

### 2.1.3. Drug transporters

A third mechanism of drug resistance is the expression of transporters of the ATP-binding cassette (ABC) family. ABC transporters are efflux pumps that can actively expel a wide range of chemotherapeutic drugs from the cell. ABC transporters are expressed in both normal stem cells and cancer stem cells. Three members of this family of ABC transporters, ABCB1, ABCC1 and ABCG2 have been identified as the culprits of multidrug resistance in many cancers.

A study on neuroblastoma patients illustrates how cancer stem cells use the efflux transporter, ABCG2 to protect themselves from anti-cancer drugs. In this study, cells expressing ABCG2 were identified by the fluorescent Hoechst dye 33342, in flow cytometry, as a “side population” (SP) of cells that did not take up this dye. A previous study had shown that cancer stem cells reside in the SP fraction of neuroblastoma [10]. SP cells from neuroblastoma patients showed increased efflux of mitoxantrone when compared to non-SP cells. Also, treatment of neuroblastoma cell lines with mitoxantrone led to an increase in the proportion



of SP cells to non-SP cells, suggesting that ABCG2 conferred a survival advantage to cancer stem cells [33]. Similarly, in acute myeloid leukemia, SP cells derived from mononuclear cells in bone marrow of patients showed an increase efflux of daunorubicin and mitoxantrone when compared to non-SP cells [34]. Taken together, these findings suggest that cancer stem cell uses efflux transporters to guard against anti-cancer drugs.

#### *2.1.4. High detoxification activity*

Aldehyde dehydrogenase I (ALDH1) is a detoxifying enzyme that oxidizes intracellular aldehydes and is a marker of normal stem cell. Cancer stem cells from acute myeloid leukemia and breast carcinoma are known to have high levels of ALDH1. [21]. In breast cancer patients undergoing chemotherapy with paclitaxel and epirubicin, it was found that the proportion of ALDH1-positive cancer cells increased significantly post treatment, resulting in treatment failure [35]. A high ALDH1 level is thus associated with poor clinical outcomes. This finding indicates that new therapeutic agents must be able to overcome the detoxification prowess of cancer stem cells in order to be effective.

#### *2.1.5. Blockage of apoptosis*

Blockage of apoptosis is a major mechanism for drug resistance as it offers protection against any therapy that results in cell destruction. This ability to prevent apoptosis from occurring in cancer stem cell is mediated by both inherent cellular factors and extrinsic microenvironmental factors.

Inherent cellular factors are important in blocking the apoptotic process. In a study on cancer stem cells (isolated via CD133) from glioblastoma, exposure of cancer cells to conventional chemotherapeutics such as temozolomide, carboplatin, paclitaxel and etoposide, showed that CD133-positive cells had higher viability compared to CD133-negative cells [36]. In contrast to CD133-negative cells, CD133-positive cells had higher mRNA levels of the anti-apoptotic proteins, such as B-cell lymphoma (Bcl) -2 and -XL proteins, inhibitors of apoptosis proteins (IAPs), FLICE-like inhibitory protein (FLIP) and Sirtuin 1 (SIRT1). In addition, CD133-positive cells had lower mRNA level of the pro-apoptotic protein, including Bcl-2 associated X protein (BAX). In a separate study on colon cancer, autocrine production of interleukin-4 (IL4) by CD133-positive colon cancer cells was found to prevent apoptosis of cancer stem cells from occurring when conventional chemotherapeutics and a recombinant protein called TNF-related apoptosis-inducing ligand (TRAIL) were used [37]. These findings show that cancer stem cells have an armament of proteins to protect themselves from undergoing apoptosis.

In some cancers, resistance to apoptosis is highly dependent on extrinsic microenvironmental factors. For example, culturing ovarian cancer cells under stem cell culture conditions led to formation of spheroid cultures of cells that were self-renewing and resistant to cisplatin and paclitaxel [38]. However, this resistance was lost once the cells were cultured under a different set of conditions. One extrinsic factor that has been recognized as the main cause for resistance to cancer therapeutics is hypoxia. The normal stem cell niche has been associ-

ated with a hypoxic microenvironment. Expression of hypoxia-inducible factors (HIF) are important as these factors regulate stem cell self-renewal and pluripotency [39]. Although not well studied in cancer stem cells per se, the role of HIF in regulating apoptosis has been shown in cancer cell cultures. HIF directly regulates the transcription of anti-apoptotic genes such as myeloid cell leukemia 1 (MCL-1) and B-cell lymphoma extra-large (BCL-XL) [40,41]. Hypoxia also results in a lower level of reactive oxygen species (ROS) in the cell. A lower level of ROS leads to a decrease in activation of caspase-8, a decrease in expression of pro-apoptotic receptor TRAIL-R2 and an increase expression of pro-survival proteins like cFLIP and BCL-2 [42,43].

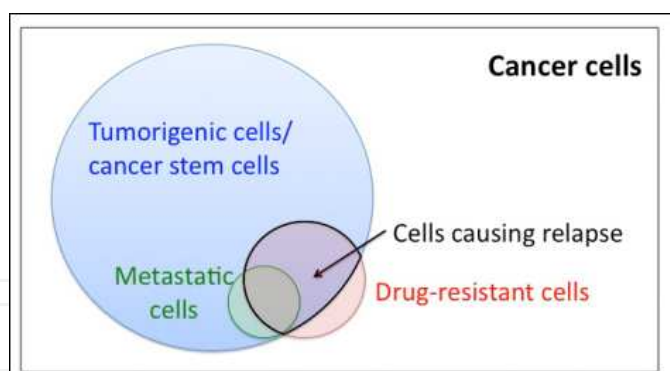
Besides the extrinsic chemical factors described above, another type of extrinsic factor that blocks apoptosis is the stimulating ligands produced by neighboring cells. The hematopoietic niche has been found to confer resistance to leukemic cells via adhesion molecules such as integrins and soluble molecules of the Wnt pathway [44]. Wnt, Notch and Hedgehog are developmental regulatory molecules that are increasingly shown to be involved in cancer stem cell self-renewal, growth and differentiation [27,45–50]. Therapeutics targeting factors of these pathways are currently undergoing clinical trials and have shown promising results in eliminating cancer stem cells that are resistant to existing therapies.

## 2.2. Metastasis

Tumorigenicity is an essential characteristic that metastatic cancer cells must possess in order to initiate tumor formation after metastasizing to a distant site. Hence, it can be assumed that cancer stem cells are responsible for metastasis as they have tumorigenic properties by definition. Recent findings suggest that not all cancer stem cells have the capacity to metastasize. Rather, this capacity is confined to a subset of cancer stem cells. Cancer stem cells (identified by their CD 133 marker) isolated from pancreatic cancer patients were found to contain a subset of cells that expressed CXCR4, the receptor for stromal-cell derived factor 1 (SDF-1). These cancer stem cells that expressed CXCR4 were able to induce tumors in mice, spread via the blood circulation and cause liver metastasis. On the other hand, cancer stem cells that were CXCR4-negative were only able to induce tumors in mice, failing to spread and cause metastasis [22]. In light of this finding, therapies targeting this subset of cancer stem cells could prevent metastasis.

## 2.3. Conclusions

The multitude of research in cancer stem cell has deepened our understanding in this field. We present a schema (figure 1) that summarizes the literature in cancer stem cell research from a therapeutic perspective. This schema illustrates that targeting cancer cells with tumorigenic abilities, i.e. cancer stem cells, is not enough. It is the problematic subset of resistant cancer stem cells (outlined black in figure 1) that accounts for failure of current cancer therapies. Overcoming resistance in cancer stem cells is crucial and we have described several mechanisms that cancer stem cells use to stem our efforts for a cure. One innovative approach to eliminate resistant cancer stem cells is differentiation therapy, where cancer stem cells are made to differentiate, thereby losing their resistant capabilities [51].



**Figure 1.** Properties of cancer cells. The subset of tumorigenic cells that are also drug-resistant should be targeted to ensure elimination of cancer.

### 3. Molecular targets for cancer therapy

#### 3.1. Current clinical drug trials targeting cancer stem cells

Translating research from bench to bedside is perhaps the most challenging and rewarding part of science. The development of drugs against cancer stem cell is an exciting field with many different, innovative approaches. In this section, a review of the drugs that have already reached clinical trials is presented (Table 2).

One approach is the targeting of the cancer stem cell machinery. An example of this approach is the telomerase inhibitors. Telomeric inhibitors block replication and a clinical candidate Imetelstat have shown efficacy in cancer stem cells [52]. As a bonus, telomerase inhibitors are expected to also target the bulk of the tumor. Importantly, unlike normal stem cells, cancer stem cells express higher levels of telomerase [53]. Hence this could potentially be a drug that targets cancer stem cells without hurting normal stem cells.

In a second approach the targeting of the cancer stem cell phenotype, the immunogenic-response that ironically had been a problem to researchers in the detection of cancer stem cells using the mouse model, has become a solution against cancer stem cells. In a study in which cancer stem cells were injected into immunocompetent syngenic mice, cancer stem cells induced antitumor response more effectively than unselected cancer cells [54]. This finding is important and has led to the development of various clinical candidates by three different pharmaceutical companies. These candidates, all of which are currently in clinical trials, were developed based on cancer stem cell-associated proteins. These proteins serve as antigens to evoke an immune response against cancer stem cells. In essence, these proteins act as vaccines against cancer stem cells. Immunocellular Therapeutics has developed a dendritic-based vaccine comprising dendritic cells that were obtained from patients and primed *in vitro* by two CD133-peptides. This vaccine has just recently been approved for phase I clinical trials. Using the same approach, other clinical candidates were developed by two pharmaceutical companies. Instead of obtaining dendritic cells from patients, peptides of cancer

stem cell antigens were injected directly into patients to prime the immune system against cancer stem cells. The peptides used in these vaccinations are found in both cancer stem cells and non-stem cancer cells. One of these antigens is Wilms' Tumor 1 (WT1). WT1 is a transcription factor that is expressed in leukemia. Although a direct association between WT1 and the leukemic stem cell has never been shown, WT1 however, has been associated with the CD34+CD38- cell population which is thought to harbor the hematopoietic stem cell [55] and also the leukemia stem cell [15].

Drug target	Drug name	Cancer	Stage	Company
Undisclosed	Cancer stem cell inhibitor BBI608	Colorectal cancer	Entering Phase III	Boston Biomedicals, Inc
Telomerase (inhibitor)	Imetelstat	Broad range	Phase II	Geron Corporation
CD-133	Dendritic cell-based vaccine ICT-121	Glioblastoma	Entering phase I	ImmunoCellular Therapeutics Ltd.
Focal adhesion kinase (inhibitor)	VS6063	Advance solid tumors	Phase I completed	Verastem and Pfizer
Wilms Tumor 1	Peptides from Wilms Tumor 1 (FPI-01)	Leukemia and mesothelioma	Phase II	Formula Pharmaceuticals
EphA3	Human monoclonal antibody (KB004) binds EphA3	Leukemia	Phase I	KaloBios Pharmaceuticals, Inc.
Notch pathway	Anti-DLL4 (demcizumab)	Solid Tumors	Phase I	OncoMed
	Anti-Notch2/3 (OPM-59R5)	Solid tumors	Phase I	
Wnt pathway	Anti-Fzd7 (OMP-18R5, binds 5 Frizzled receptors)	Solid Tumors	Phase I	
	Truncated Frizzled 8-Fc fusion protein (OMP-54F28)	Advance solid tumor cancers	Phase I	
Undisclosed cancer stem cell antigen	Peptides vaccine (SL401 and SL701)	Advance leukemia and advance brain cancer	Phase I/II completed	Stemline Therapeutics

**Table 2.** Current clinical drug trials in cancer stem cell therapy

A third approach is the targeting of the cancer stem cell and its microenvironment. Anti-EphA3 antibody is a clinical candidate against cancer stem cell that has been developed by KaloBios. This antibody treatment is now in phase I trial. EphA3 expression is found in preB leukemia cell line and in a subset of samples from leukemia patients [56]. There is no direct evidence that links EphA3 expression to the leukemic stem cells, however, in an in-house study by KaloBios, incubating anti-EphA3 with cancer cells leads to the cancer cells losing their ability to form colonies in vitro, suggesting that the antibody was active against cancer

stem cells. In addition, the antibody was also found to bind to EphA3 that was expressed on cancer vasculature cells as well as cancer stromal cells. The binding was reported to cause cell-cell repulsion, resulting in the destruction of new vessels and failure to establish a cancer stromal environment [57]. This strategy, which targets a protein that is found in cancer stem cell, cancer stromal cells and cancer vasculature cells, would be “killing-three-birds-with-one-stone”. Other clinical candidates that are based on a similar approach have also been developed via targeting the Wnt pathway and the Notch Pathway instead [47–50].

### 3.2. Potential new targets – Insights from Pluripotent Stem cells

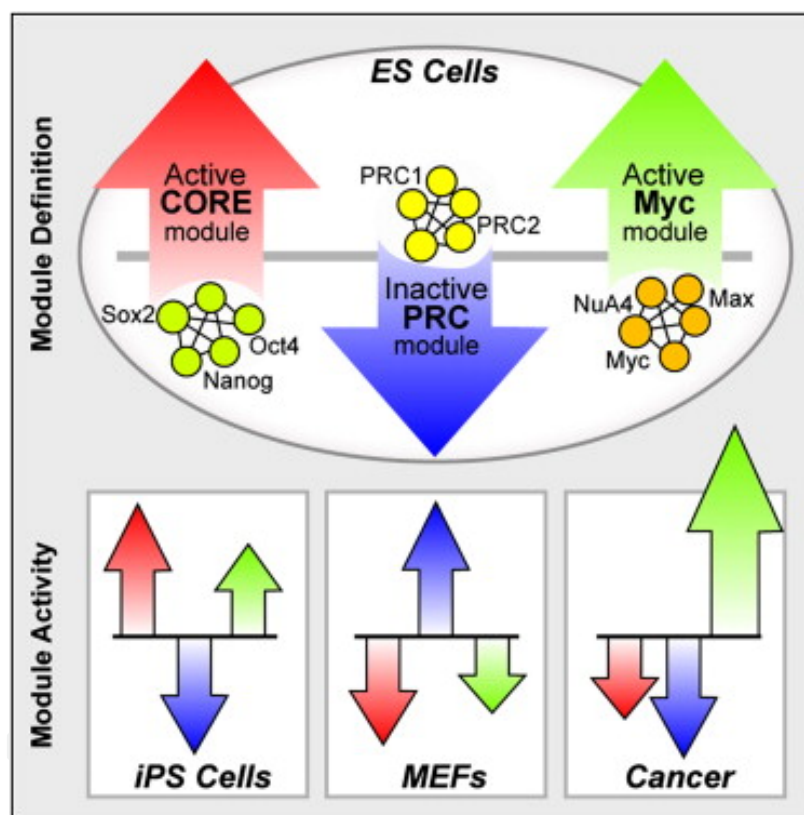
The discovery that transcription factors, namely, Oct4, Sox2, Klf4 and c-Myc, can induce pluripotency in a differentiated adult cell [58], accelerated the understanding of the molecular machinery driving pluripotent stem cells. Systems biology approaches based on these transcription factors generated genome-wide regulatory networks that are thought to be the supporting framework for an embryonic stem cell state. These data serves as a rich resource in furthering our understanding of the cancer stem cell.

In a recent study of regulatory networks in embryonic stem cell, analysis of the protein-protein interactions of key transcription factors and the downstream targeted genes revealed that the embryonic stem cell regulatory network can be divided into three independent modules (Figure 2). The three modules are the core module, the c-Myc module and the Polycomb Repressive Complex (PRC) module. The core module comprises genes that are regulated by the embryonic stem cell-specific transcription factor Oct4 and Oct4-associated proteins while the PRC module comprises genes that are repressed by the PRC. The Myc-module comprises genes that are regulated by c-Myc and its associated proteins. Proper functioning of all three modules are essential for having a normal pluripotent stem cell [59].

Using the 3-module model to study the genes expressed in bladder cancer and breast cancer samples, it was found that the Myc module was more active in cancers while that of the core module was more repressed, when compared to normal urothelium obtained from a distant site of the cancer [61,62]. This suggested that in cancer cells, the Myc module is re-activated but is not balanced by a core module. It should be noted that this comparison was done with the heterogeneous cancer cell population and not the cancer stem cell population. Repeating the same characterization analysis on cancer stem cell samples will likely highlight the key differences between the regulatory network of cancer stem cells and that of normal pluripotent stem cells. These differences could become potential targets for anti-cancer therapy.

Factors that are crucial for the maintenance of pluripotent stem cell have been found to be involved in cancer. Hypoxia-inducible factors (HIFs) have been found to be important in pluripotent stem cell [39,63]. Studies now show that HIFs could be the key factor in switching on the pluripotency machinery in cancer cells to form cancer stem cells [64]. In an experiment where glioma cells and cervical cells were exposed to HIFs, activation of the embryonic stem cell marker, Oct4, was observed [65]. Subjecting glioma cells to hypoxia resulted in an increase in the level of CD133 mRNA [65]. In samples from glioma patients, subjecting the CD133-positive fraction to hypoxia resulted in increased mRNA levels of OCT4, NANOG and cMYC. Interestingly, when CD133-positive and negative fractions were

cultured under hypoxic conditions, embryonic stem cell-association gene expression and formation of neurosphere were seen in both fractions [65]. Thus, low levels of oxygen promotes the transformation of cancer cells into cancer stem cells by activating the pluripotency machinery in cancer cells with expression and repression of modules that are similar in profile to embryonic cells. This should be taken into consideration when targeting cancer cells. Studies on HIF have shown that both HIF-1 $\alpha$  and HIF-2 $\alpha$  are associated with cancer. HIF-1 $\alpha$  has been shown to play a role in angiogenesis [66] and anti-angiogenesis therapies targeting HIF-1 $\alpha$  have been undertaken [67,68]. In contrast, recent findings suggest that HIF-2 $\alpha$  is involved in the triggering of stemness in cancer which in turn promotes cancer growth and aggressiveness [69]. Hence a potential pathway to target cancer stem cell will be the HIF-2 $\alpha$ -mediated pathway.



**Figure 2.** The three sets of genes that are activated or repressed by distinct sets of transcriptional regulators. Induce pluripotent stem (iPS) cells show similar gene expression profile as embryonic stem (ES) cells. Mouse embryonic fibroblast (MEF) represents a set of differentiated cells and shows a profile that is opposite to that of ES cells. Analysis of the profile in cancer highlights a pluripotency machinery in which the core module has been suppressed and the Myc module overactivated. Figure is a reprint of the graphical abstract provided by Kim et al [60] (Reprinted with permission from Cell Press)

Knowledge garnered from studies on pluripotent stem cell provides a rich resource for cancer stem cell research and paves the way in identifying novel key targets for cancer therapy. Targeting molecules or pathways specific to embryonic stem cells gives us the opportunity to kill cancer cells without harming innocent bystander cells.

## 4. Summary

Research in cancer is immense and complex as cancer is a diverse disease with a myriad of genetic mutations. A pressing practical concern in cancer therapeutics is the development of resistance of cancer cells to current treatment, resulting in failure of therapy and eventual death. In the last two decades, cancer stem cell hypothesis has emerged as the likely reason for this resistance in cancer. We now understand that cancer stem cells are present in different cancers. They can be a small, distinct population characterized by certain phenotypes in some cancers while heterogeneous and with no defining phenotypes in others. Cancer stem cells can also result from cancer cells under the influence of environmental factors such as hypoxia. They are also highly resistant to cancer drugs with several mechanisms employed for enhanced survival. Research into stem cells and pluripotency regulatory networks will provide further characterization and understanding of cancer stem cells. The information on cancer stem cells has pieced together a therapeutic framework to address cancer resistance with several potential therapies in clinical trials currently. So much more needs to be done in this field in our quest to conquer cancer totally.

## Acknowledgements

The authors acknowledge Dr Jansen Koh for active discussion and review of the manuscript. Patricia Ng is supported by the Agency for Science, Technology and Research (A\*STAR) Research Fellowship and the UNESCO-L'Oréal International Fellowship.

## Author details

Patricia Ng and Wang Cheng-I

Singapore Immunology Network, Agency for Science, Technology and Research, Singapore

## References

- [1] Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994;367:645–8.
- [2] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100(7):3983–8.

- [3] Dalerba P, Dylla SJ, Park I-K, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104(24):10158–63.
- [4] Dick JE. Breast cancer stem cells revealed. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100(7):3547–9.
- [5] Eramo a, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio a, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell death and differentiation* 2008;15(3):504–14.
- [6] O'Brien C a, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445(7123):106–10.
- [7] Prince ME, Sivanandan R, Kaczorowski a, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104(3):973–8.
- [8] Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007;445(7123):111–5.
- [9] Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, et al. Identification of cells initiating human melanomas. *Nature* 2008;451(7176):345–9.
- [10] Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. *Nature* 2004;432(November):396–401.
- [11] Zhang S, Balch C, Chan MW, Lai H-C, Matei D, Schilder JM, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer research* 2008;68(11):4311–20.
- [12] Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. *Cancer research* 2007;67(3):1030–7.
- [13] Li Y, Lathera J. Cancer stem cells: distinct entities or dynamically regulated phenotypes? *Cancer research* 2012;72(3):576–80.
- [14] Furth J, Kahn MC. The Transmission of Leukemia of Mice with a Single Cell. *American journal of cancer* 1937;31:276–82.
- [15] Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature medicine* 1997;3(7):730–7.
- [16] Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. *Cancer research* 2007;67(10):4827–33.
- [17] Prince ME, Sivanandan R, Kaczorowski a, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and



- neck squamous cell carcinoma. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104(3):973–8.
- [18] Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell stem cell* 2007;1(5):555–67.
- [19] Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell stem cell* 2007;1(3):313–23.
- [20] Barnes WA, Furth J. A transmissible leukemia in mice with atypical cells.
- [21] Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature* 2008;456(7222):593–8.
- [22] Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS, Johnson TM, et al. Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. *Cancer cell* 2010;18(5):510–23.
- [23] Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* 2006;441(7092):475–82.
- [24] Southam CM, Brunschwig A. Quantitative studies of autotransplantation of human cancer. *Cancer* 1961;14:971–8.
- [25] Koike a, Moore GE, Mendoza CB, Watne a L. Heterologous, Homologous, and Autologous Transplantation of Human Tumors. *Cancer* 1963;16:1065–71.
- [26] Nadler SH, Moore GE. Autotransplantation of Human Cancer. *JAMA®: the journal of the American Medical Association* 1965;191:105–6.
- [27] Vermeulen L, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nature cell biology* 2010;12(5):468–76.
- [28] Ribatti D. Cancer stem cells and tumor angiogenesis. *Cancer letters* 2012;321(1):13–7.
- [29] Moore N, Lyle S. Quiescent, slow-cycling stem cell populations in cancer: a review of the evidence and discussion of significance. *Journal of oncology* 2011;2011.
- [30] Naumov GN, Townson JL, MacDonald IC, Wilson SM, Bramwell VH, Groom AC, et al. Ineffectiveness of Doxorubicin Treatment on Solitary Dormant Mammary Carcinoma Cells or Late-developing Metastases. *Breast Cancer Research and Treatment* 2003;82:199–206.
- [31] Dembinski JL, Krauss S. Characterization and functional analysis of a slow cycling stem cell-like subpopulation in pancreas adenocarcinoma. *Clinical & experimental metastasis* 2009;26(7):611–23.

- [32] Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006;444(7120):756–60.
- [33] Hirschmann-Jax C, Foster a E, Wulf GG, Nuchtern JG, Jax TW, Gobel U, et al. A distinct “side population” of cells with high drug efflux capacity in human tumor cells. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101(39):14228–33.
- [34] Wulf GG, Wang R-Y, Kuehnle I, Weidner D, Marini F, Brenner MK, et al. A leukemic stem cell with intrinsic drug efflux capacity in acute myeloid leukemia. *Blood* 2001;98(4):1166–73.
- [35] Tanei T, Morimoto K, Shimazu K, Kim SJ, Tanji Y, Taguchi T, et al. Association of breast cancer stem cells identified by aldehyde dehydrogenase 1 expression with resistance to sequential Paclitaxel and epirubicin-based chemotherapy for breast cancers. *Clinical cancer research®: an official journal of the American Association for Cancer Research* 2009;15(12):4234–41.
- [36] Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Molecular cancer* 2006;5:67.
- [37] Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F, et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell stem cell* 2007;1(4):389–402.
- [38] Zhang S, Balch C, Chan MW, Lai H-C, Matei D, Schilder JM, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer research* 2008;68(11):4311–20.
- [39] Ji L, Liu Y, Yang C, Yue W, Shi S, Bai C, et al. Self-renewal and pluripotency is maintained in human embryonic stem cells by co-culture with human fetal liver stromal cells expressing hypoxia inducible factor 1alpha. *Journal of cellular physiology* 2009;221(1):54–66.
- [40] Shroff EH, Snyder C, Chandel NS. Role of Bcl-2 Family Members in Anoxia Induced Cell Death. *Cell Cycle* 2007;6(April):807–9.
- [41] Chen N, Chen X, Huang R, Zeng H, Gong J, Meng W, et al. BCL-xL is a target gene regulated by hypoxia-inducible factor-1{alpha}. *The Journal of biological chemistry* 2009;284(15):10004–12.
- [42] Jie W, Yi J. Cancer cell killing via ROS. *Cancer Biology & Therapy* 2008;7(December): 1875–84.
- [43] Reinehr R, Becker S, Eberle A, Grether-beck S, Häussinger D. Involvement of NADPH oxidase isoforms and SRC family kinases in CD95-dependent hepatocyte apoptosis. *Journal of biological chemistry* 2005;280:27179–94.

- [44] De Toni F, Racaud-Sultan C, Chicanne G, Mas VM-D, Cariven C, Mesange F, et al. A crosstalk between the Wnt and the adhesion-dependent signaling pathways governs the chemosensitivity of acute myeloid leukemia. *Oncogene* 2006;25(22):3113–22.
- [45] Xu Q, Yuan X, Liu G, Black KL, Yu JS. Hedgehog signaling regulates brain tumor-initiating cell proliferation and portends shorter survival for patients with PTEN-co-expressing glioblastomas. *Stem cells (Dayton, Ohio)* 2008;26(12):3018–26.
- [46] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414(6859):105–11.
- [47] Gurney A, Axelrod F, Bond CJ, Cain J, Chartier C, Donigan L, et al. Wnt pathway inhibition via the targeting of Frizzled receptors results in decreased growth and tumorigenicity of human tumors. *Proceedings of the National Academy of Sciences of the United States of America* 2012;109(29):11717–22.
- [48] Gurney A, Hoey T. Anti-DLL4, a cancer therapeutic with multiple mechanisms of action. *Vascular cell* 2011;3(1):18.
- [49] Fischer M, Yen W-C, Kapoun AM, Wang M, O'Young G, Lewicki J, et al. Anti-DLL4 inhibits growth and reduces tumor-initiating cell frequency in colorectal tumors with oncogenic KRAS mutations. *Cancer research* 2011;71(5):1520–5.
- [50] Hoey T, Yen W-C, Axelrod F, Basi J, Donigan L, Dylla S, et al. DLL4 blockade inhibits tumor growth and reduces tumor-initiating cell frequency. *Cell stem cell* 2009;5(2):168–77.
- [51] Leszczyniecka M, Roberts T, Dent P, Grant S, Fisher PB. Differentiation therapy of human cancer: basic science and clinical applications. *Pharmacology & therapeutics* 2001;90(2-3):105–56.
- [52] Marian CO, Cho SK, McEllin BM, Maher E a, Hatanpaa KJ, Madden CJ, et al. The telomerase antagonist, imetelstat, efficiently targets glioblastoma tumor-initiating cells leading to decreased proliferation and tumor growth. *Clinical cancer research®: an official journal of the American Association for Cancer Research* 2010;16(1):154–63.
- [53] Shay JW, Wright WE. Telomeres and telomerase in normal and cancer stem cells. *FEBS letters* 2012;584(17):3819–25.
- [54] Ning N, Pan Q, Zheng F, Teitz-Tennenbaum S, Egenti M, Yet J, et al. Cancer stem cell vaccination confers significant antitumor immunity. *Cancer research* 2012;72(7):1853–64.
- [55] Baird PN, Simmons PJ. Expression of the Wilms' tumor gene (WT1) in normal hemopoiesis. *Experimental hematology* 1997;25(4):312–20.
- [56] Dottori M, Down M, Hüttmann A, Fitzpatrick DR, Boyd AW, Dottori BM, et al. Methylation Regulates Expression in Hematopoietic Tumor Cells. 2012;3:2477–86.

- [57] Garber K. Of Ephs and Ephrins®: Companies Target Guidance Molecules in Cancer. *Journal of the national cancer institute* 2010;102(22):1692–4.
- [58] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;131(5):861–72.
- [59] Fazio TG, Huff JT, Panning B. An RNAi screen of chromatin proteins identifies Tip60-p400 as a regulator of embryonic stem cell identity. *Cell* 2008;134(1):162–74.
- [60] Kim J, Woo AJ, Chu J, Snow JW, Fujiwara Y, Kim CG, et al. A Myc Network Accounts for Similarities between Embryonic Stem and Cancer Cell Transcription Programs. 2010;:313–24.
- [61] Sanchez-Carbayo M, Socci ND, Lozano J, Saint F, Cordon-Cardo C. Defining molecular profiles of poor outcome in patients with invasive bladder cancer using oligonucleotide microarrays. *Journal of clinical oncology®: official journal of the American Society of Clinical Oncology* 2006;24(5):778–89.
- [62] Kim J, Orkin SH. Embryonic stem cell-specific signatures in cancer: insights into genomic regulatory networks and implications for medicine. *Genome medicine* 2011;3(11):75.
- [63] Forristal CE, Wright KL, Hanley N a, Oreffo ROC, Houghton FD. Hypoxia inducible factors regulate pluripotency and proliferation in human embryonic stem cells cultured at reduced oxygen tensions. *Reproduction Research* 2010;139(1):85–97.
- [64] Keith B, Simon MC. Hypoxia-inducible factors, stem cells, and cancer. *Cell* 2007;129(3):465–72.
- [65] Mathieu J, Zhang Z, Zhou W, Wang AJ, Heddleston JM, Pinna CM a, et al. HIF induces human embryonic stem cell markers in cancer cells. *Cancer research* 2011;71(13):4640–52.
- [66] Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics. *Trends in molecular medicine* 2002;8(4 Suppl):S62–7.
- [67] Ferrara N, Hillan KJ, Gerber H-P, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nature reviews. Drug discovery* 2004;3(5):391–400.
- [68] Tan C, Noronha RGD, Roecker AJ, Pyrzynska B, Khwaja F, Zhang Z, et al. Identification of a Novel Small-Molecule Inhibitor of the Hypoxia-Inducible Factor 1 Pathway Hypoxia-Inducible Factor 1 Pathway. 2005;:605–12.
- [69] Koh MY, Lemos R, Liu X, Powis G. The hypoxia-associated factor switches cells from HIF-1 $\alpha$ - to HIF-2 $\alpha$ -dependent signaling promoting stem cell characteristics, aggressive tumor growth and invasion. *Cancer research* 2011;71(11):4015–27.

