The Use of Normal Stem Cells and Cancer Stem Cells for Potential Anti-Cancer Therapeutic Strategy

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Abstract

BACKGROUND: Despite recent advance in conventional cancer therapies including surgery, radiotherapy, chemotherapy, and immunotherapy to reduce tumor size, unfortunately cancer mortality and metastatic cancer incidence remain high. Along with a deeper understanding of stem cell biology, cancer stem cell (CSC) is important in targeted cancer therapy. Herein, we review representative patents using not only normal stem cells as therapeutics themselves or delivery vehicles, but also CSCs as targets for anti-cancer strategy.

METHODS: Relevant patent literatures published between 2005 and 2017 are discussed to present developmental status and experimental results on using normal stem cells and CSCs for cancer therapy and explore potential future directions in this field.

RESULTS: Stem cells have been considered as important element of regenerative therapy by promoting tissue regeneration. Particularly, there is a growing trend to use stem cells as a target drug-delivery system to reduce undesirable side effects in non-target tissues. Noteworthy, studies on CSC-specific markers for distinguishing CSCs from normal stem cells and mature cancer cells have been conducted as a selective anti-cancer therapy with few side effects. Many researchers have also reported the development of various substances with anticancer effects by targeting CSCs from cancer tissues.

CONCLUSION: There has been a continuing increase in the number of studies on therapeutic stem cells and CSC-specific markers for selective diagnosis and therapy of cancer. This review focuses on the current status in the use of normal stem cells and CSCs for targeted cancer therapy. Future direction is also proposed.

Keywords Cancer · Stem cell · Cancer stem cell · Targeted cancer therapy · Marker

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1 Introduction

Cancer, a major threat to public health around the world, is the second leading cause of mortality after ischemic heart disease and stroke [1]. It is characterized by highly complex pathogenesis associated with proliferation, apoptotic resistance, inflammation, neovascularization, invasion, and metastasis under complex circumstances involving tumor cells themselves and host-tumor cell interactions [2–4]. Despite tremendous impact on health worldwide, progress in cancer treatment has been slow. Only 50% of newly diagnosed cancers could be cured [5]. For this reason, there is an increasing interest in potential anti-cancer strategies including surgery, radiation, and drugs. Tremendous progress has recently been made in understanding the basic biology and therapeutic potential of using stem cells against a variety of disease states. Thus, the concept of using stem cells for treating various diseases is of considerable importance in cancer research.

Stem cells are found in multi-cellular organisms. They are characterized by a specific function with abilities to renew themselves through mitotic cell division while maintaining undifferentiated state to differentiate into multilineages [6, 7]. Different stem cell types have been investigated in both pre-clinical and clinical applications, including embryonic stem cells (ESCs) isolated from the inner cell mass of human blastocysts [8], mesenchymal stem cells (MSCs) isolated from adult tissues, and induced pluripotent stem (iPS) cells that are reprogrammed from adult somatic cells for pluripotency [9]. ESCs have self-renewal capacity, pluripotency, and differentiation into various cell lineages for cellular therapy [7, 10, 11]. As a type of multipotent stem cell, MSCs can be isolated from different tissues including adipose tissue, skeletal muscle, umbilical cord blood, and bone marrow [12]. It has been shown that MSCs possess high differentiation ability to various cell types such as adipocytes, osteocytes, and chondrocytes [13, 14]. Currently, MSCs are the most commonly used cells in regenerative medicine in preclinical and clinical applications with several advantages, including easy isolation, high plasticity, safety, and exemption from ethical controversy [15–17]. Meanwhile, iPS cells are produced through somatic cell reprogramming by introduction of transcription factors (OCT4, SOX2, KLF4, and C-MYC) associated with pluripotency to reverse its condition to embryonic stage [18, 19]. iPSCs are able to self-renew and maintain differentiation potential into any cell type. These characteristics of iPSCs are almost similar to ESCs [20]. Due to lack of ethical issues and safety concerns on transplantation rejection [18, 21], iPS cells could become a prominent subject in modern regenerative medicine research era.

Patent law for protection of intellectual property is very important in promoting technical innovation and supporting dissemination of creative products. For this, an invention should satisfy adequate requirements for novelty, inventiveness, industrial applicability, and adequate disclosure. After an inventor receives exclusive rights to gain private profit from his invention for a limited time period, he/she can disclose public disclosure of his invention so that anyone can make this new knowledge and develop the technology [22]. In this review, we examined representative patents, which are important for the analysis of recent stem cell research from academic science to industrial innovation, of using cancer stem cells (CSCs) as targets for anti-cancer strategy and using normal stem cells as therapeutic approaches.

2 Normal stem cells as therapeutic agents against various diseases

Stem cell therapy makes it possible to treat human diseases by replacing damaged cells and alleviating related symptoms. For this, stem cells are able to exert beneficial effects via differentiation of immature stem cells into the target tissue or paracrine mechanisms that promote tissue repair through secretion of numerous factors such as cytokines, chemokines, and growth factors [23–28]. These beneficial properties of stem cells can vary greatly depending on tissue source and methods of isolation, cultivation, and differentiation. In a patent published in 2013, Phan and Lim have reported the isolation and cultivation methods of stem cells from the amniotic membrane of umbilical cord (EP2597149 [29]) (Table 1). Buehring et al. have identified the use of functional fragments of antibody for identifying stem cells having adipocytic, chondrocytic and pancreatic differentiation potential (WO201000415 [30]). Before stem cell therapy can progress for treating various diseases, the differentiation potential of stem cells into any desired cell type needs to be defined. In the invention of Malcuit et al. (WO2011063005 [31]), methods of producing human retinal pigmented epithelial cells known to be useful for treating retinal degenerative conditions from stem cells, including ESCs, iPS, and adult stem cells, have been demonstrated.

As shown in Table 1, potential target diseases for stem cell-based therapy include neurodegenerative disorders, diabetes, and diseases of the liver and heart [32, 33]. Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis result from massive loss of several types of neural cells, leading to nervous system dysfunction [34]. Despite decades of research, unfortunately effective therapies do not exist for them, resulting in growing interest in stem cell

Table 1 Summary of patents on stem cell-based therapy for various diseases

Patent (year)	Inventor(s)	References	Description of the invention
<i>Isolation, cultivation, differentiation</i>			
EP2597149 (2013)	Phan TT, Lim JJ.	[29]	Isolation and cultivation methods for stem cells isolated from the amniotic membrane of umbilical cord
WO201000415 (2010)	Buehring HJ, Trembl S, Lammers R	[30]	Isolation and identification of stem cells having adipocytic, chondrocytic and pancreatic differentiation potential
WO2011063005 (2011)	Malcuit C, Lemieux L, Holmes W, Huertas P, Vilner L	[31]	Methods of producing human retinal pigmented epithelial cells, which are useful for treating retinal degenerative conditions, from stem cells including ESCs, iPS, and adult stem cells
<i>Stem cell-based therapy</i>			
US20090214484 (2009)	Mironov N	[36]	MSC therapy for the treatment of central nervous system disorders including Parkinson's disease, Alzheimer's disease, and ischemic injury
US20070253931 (2007)	Varney T, Mills C, Danilkovitch A	[37]	Use of MSCs for treating amyotrophic lateral sclerosis
US20080260703 (2008)	Riordan N, Ichim T	[39]	Treatment of insulin resistance and diabetes through administration of stem cells possessing anti-inflammatory activity and capable of regenerating insulin producing cells
US20070031384 (2007)	Atala A, Milanesi A, Soker S	[40]	Regeneration of pancreatic islets by amniotic fluid stem cell therapy
EP3020405 (2017)	Lee EJ, Kim HS	[41]	MSCs for preventing and treating liver fibrosis or liver cirrhosis
US20160074437 (2016)	Wagner SC, KIM AJ, Ma H, Theofilopoulos D	[42]	Treatment of liver failure through administration of immunocytes and MSCs
US20050008626 (2005)	Fraser J, Hedrick M, Zhu M, Strem B, Daniels E, Wulur I	[43]	AT-MSCs that promote or support a therapeutic cardiovascular benefit
US20050214260 (2005)	Franco WP	[44]	Combination adult stem cells and growth factor for treatment of acute and chronic cardiomyopathy

therapies as attractive options for differentiation into functional neurons and/or symptom relief [35]. The use of MSC therapy for the treatment of central nervous system disorders including Parkinson's disease, Alzheimer's disease, and ischemic injury has been given by Mironov in a patent published in 2009 (US20090214484 [36]). In another patent, the role of MSCs for treating amyotrophic lateral sclerosis was provided (US20070253931 [37]). Stem cells are also considered as promising therapeutic options for advanced diabetes mellitus causing substantial morbidity and mortality [38]. The invention presented by Riordan and Ichim (US20080260703 [39]) showed that stem cells could be used for treating insulin resistance and diabetes through anti-inflammatory activity and regeneration of insulin producing cells. Atala et al. have also published the regeneration of pancreatic islets by amniotic fluid stem cell therapy (US20070031384 [40]). In addition, a patent published by Lee and Kim (EP3020405 [41]) in 2017 is related to prevention of liver fibrosis or liver cirrhosis with MSCs. Another study has exploited MSCs and immunocytes as effective therapeutics for treatment of liver failure (US20160074437 [42]). Cardiovascular disease as another target disease of stem cells is also undergoing intense research. Fraser et al. have discovered that

adipose tissue-derived MSCs (AT-MSCs) may promote or support a therapeutic cardiovascular benefit (US20050008626 [43]). The invention disclosure by Franco (US20050214260 [44]) has provided the role of combination adult stem cells and growth factor for treatment of acute and chronic cardiomyopathy.

3 Cancer and anti-cancer strategy

Cancer statistics estimated that the number of new cancer cases was 1,735,350 with cancer deaths of 609,640 in 2018 in the United States [45]. In addition to genetic factors for cancer risk, environmental (nongenetic) factors including smoking, poor diet, and physical inactivity also increase cancer burden [46]. Cancer can invade surrounding normal tissue throughout the body along with increased expression of several genes involved in cell motility [47–49]. There have been some therapeutic methods such as surgery, radiotherapy, chemotherapy, and immunotherapy for cancer. Along with surgical treatment including local excision of the tumor or involved organs [50], various forms of chemotherapy and radiotherapy have been used primarily for cancer treatment over the last several decades [51].

Chemotherapeutic agents used in anticancer strategy have various mechanisms, including antioxidation, carcinogen inactivation, antiproliferation, cell cycle arrest, apoptosis induction, and angiogenesis inhibition [52]. However, conventional chemotherapy can exert severe toxicity due to high-dose of anticancer agents, resulting in dose-reduction and subsequent therapeutic resistance [53]. Radiotherapy, also called radiation therapy, uses ionizing radiation with electrically charged particles that deposits energy to destroy cells in the target tissue by causing genetic changes in cancer cells, making them impossible to grow [54]. Cancer immunotherapy has also been used as a successful therapeutic anti-cancer strategy for a broad spectrum of tumors by modifying the host's immune system during initial transformation with the use of vaccines, viral vectors, immune check-point inhibitors, and chimeric antigen receptor-modified T cells [55, 56]. Although all these conventional therapies are still useful in lowering a significant proportion of tumor mass, unfortunately the tumor may recur [53, 57, 58]. Despite progress in understanding cancer biology for early detection and prevention, cancer mortality remained high for the past four decades [45, 59, 60]. For this reason, stem cell research is attracting attention as an anti-cancer strategy.

4 Use of normal stem cells as anti-cancer therapy

4.1 Normal stem cells as anti-cancer therapeutic agents

Stem cells can be attractive therapeutics for many types of cancer (Table 2) since they possess inherent tropism to tumor with the presence of a wide variety of chemokine and cytokine receptors on the stem cell surface that respond to many molecules produced by tumors [61–65]. In fact, many *in vivo* studies have shown that stem cells preferentially migrate to tumor sites and incorporate into tumors

after intravenous [66–71], intraperitoneal [72], and intracerebral delivery [67]. At first, it has been reported that menstrual blood-derived MSCs have anti-tumor effect for treatment of pancreatic carcinoma both *in vivo* and *in vitro*. Moreover, central involvement of dickkopf-related protein 3 (Dkk3) in this anti-tumor effect has been observed (US20170143764 [73]). Dkk3 is an antagonist of Wnt signaling pathway involved in major biological processes such as cell fate determination and tumorigenesis. It is known to impact tumor growth, providing a new therapy option for inhibiting cancer proliferation [74, 75]. Transplantation of placental stem cells expressing elevated level of one or more genes such as aminopeptidase regulator of type 1 tumor necrosis factor, leukocyte-derived arginine aminopeptidase, interleukin-6, and transforming growth factor (TGF) beta 2 has been identified as an anti-cancer therapy that can suppress the proliferation and growth of bone-related cancers such as multiple myeloma or chondrosarcoma cells (US9121007 [76]). Treating a metastatic cancer of the liver by MSCs transplantation has also been reported to be able to restore liver function (WO2017036925 [77]). In addition, it has been demonstrated that factors secreted by MSCs such as hepatocyte growth factor and keratinocyte growth factor can stimulate or support liver regeneration and inhibit metastatic liver tumor growth or migration [78]. Secretory products of human AT-MSCs such as cytokines, amino acids, and growth factors (transforming growth factor, fibroblast growth factor, insulin-like growth factor) can also prevent or treat diverse type of cancers when AT-MSCs are transplanted (US20120263685 [79]). MSCs can enhance anti-tumor activity of immunotherapy preferably chimeric antigen receptor T cells that bind specifically to a tumor-associated antigen by modulating the tumor microenvironment which leads to stimulation of immune cells (WO2017167959 [80]).

Successful development of stem cell-mediated anti-cancer therapy depends on many risk factors, including

Table 2 Summary of patents on normal stem cells for anti-cancer therapy

Patent (year)	Inventor(s)	References	Description of the invention
US20170143764 (2017)	Alcayaga F, Khoury M	[73]	Menstrual blood-derived MSCs secreting Dkk3, an inhibitor of Wnt pathway, for pancreatic cancer treatment
US9121007 (2015)	Zhang X, Yaccoby S, Abramson S, Robert JH	[76]	Placental stem cells expressing ARTS-1, LPAP, IL6, and TGFβ2 for suppression of proliferation and growth of bone-related cancers
WO2017036925 (2017)	Günther C, Peter S	[77]	MSCs as a medicament in restoring liver function in subjects with metastatic cancer of the liver
US20120263685 (2012)	Ra JC, Kang SK, Woo SK, Youn HY, Lee HW, Seo KW	[79]	AT-MSCs exhibiting an anti-tumor effect by activating immune systems via cytokines
WO2017167959 (2017)	Hermann F, Günther C, Geumann U	[80]	MSCs elevating anti-tumor immunotherapy with CAR-T cells

Table 3 Summary of patents using normal stem cells as delivery system

Patent (year)	Inventor(s)	References	Description of the invention
CA2406664 (2011)	Snyder EY, Aboody KS, Brown AB, Breakefield XO	[98]	NSCs as a vehicle for a gene of cytosine deaminase, a prodrug activator of non-toxic 5-fluorocytosine to cytotoxic agent 5-fluorouracil
US20120107282 (2012)	Kim SU	[99]	NSCs genetically engineered to express a suicide gene (cytosine deaminase) and a cytokine gene (IFN- β) to treat cancers
WO2009052394 (2009)	Cady C, Mcasey M	[109]	BMSCs labeled with monocrySTALLINE iron oxide nanoparticle and loaded with nucleic acid sequence encoding a cytosine deaminase
US20130171115 (2013)	Li G, Lee YW, Lau PY	[111]	MSCs expressing thymidine kinase which convert a prodrug, ganciclovir, to a drug for cancer therapy
EP2811023 (2015)	Sung YC, Kim SW, Kim SJ, Park SH	[114]	MSCs co-expressing dodecameric TRAIL and HSV-TK for cancer therapy
US20170020958 (2017)	Yong TK	[119]	MSCs expressing a VEGF, an antiangiogenic protein, to inhibit angiogenesis for treatment of cancer
US20170000886 (2017)	Chang A, Nolte J	[117]	MSCs delivering CCL5 promoter for inducing an immune response and inhibiting the growth of a tumor
WO2016166600 (2016)	Epenetos A, Prokopi M	[128]	Using MSCs for delivery of exogenous microRNA which affect cancer growth, metastasis, neovascularization to treat cancers

tumor formation, undesirable biodistribution, and differentiation into unwanted cell type. In spite of stem cell-based medicinal benefits related to anti-cancer therapy, stem cell can also be potential factor contributing to malignant transformation [81]. In animal models, promotion of angiogenesis, tumor growth, and metastasis in colon cancer or osteosarcoma have been reported following injection of human bone marrow mesenchymal stem cells (BM-MSCs) [82, 83]. Similar results have been observed for human MSCs by Tsai et al. [84]. It has been recognized that vascular support, immunosuppressive effects, and metastatic support are major mechanisms of stem cell-related tumor promotion [85–87]. Thus, when injecting these cells to human, potential risk should be carefully considered, including the possibility of stem cell-modulated tumor formation. As mentioned earlier, stem cells possess inherent tropism to tumor with the presence of a wide variety of chemokine and cytokine receptors on the stem cell surface that respond to many molecules produced by tumors [61–65]. Despite tumor tropism property of stem cells, another risk factor of stem cells is undesirable biodistribution of administered stem cells, resulting in higher risk associated with long-term survival of cells in undesirable tissue [88]. Thus, comprehensive analysis should be conducted to identify unwanted distribution of implanted cells to undesired tissues before clinical development of stem cell-based therapeutics [89].

4.2 Normal stem cells as delivery vehicles

To develop potential agents that can inhibit tumor growth, delivering anti-cancer agents to the site of the tumor is important [22]. Due to their tumor tropism property, stem

cells are attractive as delivery vehicles for anti-tumor biological agents [61–65]. In other words, stem cell-based delivery of anticancer therapeutics can have therapeutic advantages with enhanced tumor specificity involved in tumor homing ability of stem cells without unexpected systemic toxicity of anticancer agents in non-target healthy tissues [22]. Additionally, this can be a good way to maximize therapeutic efficacy of stem cells since many beneficial roles in tissue repair attributed to stem cells may be due to paracrine effects rather than cell differentiation [90]. Due to proven tumor-tropic characteristics of stem cells [62, 70, 91–96], tailoring bioengineered stem cells as carriers of anti-cancer agents are widely used (Table 3 and Fig. 1). Thymidine kinase/ganciclovir and cytosine deaminase/5-fluorocytosine suicide gene therapy-induced cell apoptosis is the most widely used approach for anti-cancer therapy [97]. In the absence (CA2406664 [98]) or presence (US20120107282 [99]) of interferon-beta, a potent cytokine that activates immune cells and responds to several diseases [100, 101], neural stem cells genetically modified to express cytosine deaminase can be used as a cellular and molecular therapy for cancer. As an enzyme found in bacteria and fungi, cytosine deaminase can convert non-toxic prodrug 5-fluorocytosine to cytotoxic agent 5-fluorouracil that is used to treat many types of cancer [102–104]. Along with remarkable progress of nanotechnology for molecular imaging associated with cancer detection and screening [105–108], nanoparticle labeling is widely accepted as a tracking method for BM-MSCs in preclinical and clinical models by MRI. It has been reported that BM-MSCs labeled with monocrySTALLINE iron oxide nanoparticle loaded with nucleic acid sequence encoding a cytosine deaminase can be used for treating and

Fig. 1 Use of normal stem cells as delivery vehicles for anti-cancer therapeutic agents

detecting cancer such as ovarian cancer (WO2009052394 [109]). Herpes simplex virus thymidine kinase also plays a role as an activator of some prodrug to show cytotoxic characteristics. It converts nontoxic prodrug ganciclovir monophosphate into a toxic ganciclovir triphosphate that can inhibit DNA replication, leading to cell apoptosis [109, 110]. Using MSCs expressing a suicide gene such as thymidine kinase can convert ganciclovir to a drug that is lethal to tumor cells (US20130171115 [111]). TNF-related apoptosis inducing ligand (TRAIL), a homotrimeric type II transmembrane protein expressed in immune cells that induces apoptosis, could interact with different cancer cell lines [112]. Thus, TRAIL-engineered stem cells are emerging trends for anti-cancer therapy [113]. MSCs co-expressing TRAIL have excellent anticancer effects (US20140369979 [114]). A combination of herpes simplex virus thymidine kinase with dodecameric TRAIL has also been studied as anti-cancer strategy [115].

Chemokine (C–C motif) ligand 5 (CCL5) acts to promote immune cell (T cells, macrophages, eosinophils, and basophils) recruitment into inflammatory sites [116]. MSCs that can deliver isolated polynucleotide comprising a promoter element that drives CCL5 expression have been demonstrated as a targeted cancer therapy (US20170000886 [117]). Vascular endothelial growth factor (VEGF) family of growth factors and its receptors can be fundamental mediators of tumor angiogenesis [118]. It has been shown that MSCs expressing VEGF receptor polypeptide that binds to VEGF and renders it non-functional can inhibit angiogenesis for treatment of cancer (US20170020958 [119]). Recently, exosomes (packaged vesicles released from cells) containing proteins, lipids, mRNA and miRNA are also considered as promising therapeutic tools for safe and targeted delivery of candidate molecules [120, 121]. Shtam et al. [122] have demonstrated that exosome-delivered siRNA against RAD51 involved in

the development, progression and drug resistance of cancer cells [123] can cause cell death of recipient cancer cells. Particularly, exosomes targeted to tumors may be used for treatment of tumor via systemic administration of miRNA. Ohno et al. [124] have shown that exosomes can efficiently deliver let-7a miRNA (a possible tumor suppressor associated with downregulation of RAS/HMGA2) to epidermal growth factor receptor (EGFR)-expressing breast cancer tissue and markedly suppress tumor growth. MicroRNAs play important roles in the regulation of many biological processes, including tissue differentiation and maintenance [11]. They could become a relevant therapeutic target for cancer [125–127]. Delivery of exogenous microRNAs such as miR-34, miR-192, miR-145, and miR-143 that down-regulate cancer-associated proteins by MSCs has been granted a patent as treatment for cancer (WO2016166600 [128]).

5 CSCs for targeted cancer therapy

5.1 Targeting CSCs via specific markers

It is known that stem cell biology is tightly involved in mechanisms of tumorigenesis. As the role of stem cells in cancer etiology becomes more apparent recently, it has been suggested that oncogenic mutation of normal stem cells may promote the generation of tumor-initiating stem cells, also known as CSCs, ultimately leading to tumor formation [6, 129–132]. CSCs exhibit self-renewal and multi-lineage differentiation abilities to generate tumors, persist in tumors, and eventually cause relapse or metastasis in secondary organs [133]. Understanding of these characteristics of CSCs has fundamental implications for the development of new anti-cancer therapeutic agents as well as carcinogenesis [134]. Based on CSC theory,

eradication of cancers, especially most advanced stage cancers, may require targeting of CSCs which have the capacity of extensive proliferation, relapse or metastasis, rather than tumor bulk [135]. As shown in Fig. 2, this anti-cancer strategy can selectively kill CSCs, induce differentiation of CSCs, and inhibit the maintenance of stem cell state in order to converse malignant cancers into benign tumors with higher cure rates [130, 136]. Although anti-cancer treatments can shrink tumors, the remaining tumorigenic cells may drive tumor recurrence if therapy fails to deplete CSCs [53, 57, 58].

According to this aspect, many studies have determined various markers for CSCs for selective diagnosis or therapy of cancer (Table 4). Programmed death-1 (PD-1) is known to be highly expressed in tumor antigen-specific T cells of circulating and tumor-infiltrating lymphocytes in patients with various types of epithelial, hematologic, and other malignancies [137–141]. It has been reported that siRNA or shRNA that can inhibit the expression of PD-1 (a CSC marker) can be used to kill CSCs in melanoma (EP3130923 [142]). Several monoclonal antibodies (mAbs) directed against tumor cell-surface antigens are currently used in the therapy of different cancers [143]. CD43, a mucin-like sialoglycoprotein and a leukocyte marker, is present in tumor tissue [144]. The expression of CD44, a transmembrane glycoprotein, has been identified in different types of cancer such as breast, cervical, colon, lung, esophageal, liver, and gastric cancers. CD44 enables communication between CSCs and microenvironment that is important for the invasiveness and metastasis of tumor cells, indicating that it can be an anti-cancer therapeutic target [145, 146]. CD47 has also been found in multiple human tumors [147]. Antibodies targeting CD44 (US8715945 [148]), CD47 (WO2016057980 [149]), CD43 (WO2017065493 [150]) have been studied as therapeutic molecules for selective killing of CSCs. As a marker of cells capable of initiating tumors [151], disialoganglioside GD2, a sialic acid-containing glycosphingolipid expressed primarily on the cell surface, is highly expressed in a variety of tumors, including neuroblastoma, melanoma, small cell lung

cancer, and brain tumors [152–154]. Since GD2 is expressed in CSCs of epithelial cancer or breast cancer, GD2-binding antibody is reported to be a selective killing agent of CSCs (WO2013130603 [155]). Epidermal growth factor like domain multiple 6 (EGFL6) is a secreted protein that belongs to the EGF repeat superfamily. The expression of EGFL6 during early fetal development stages of lung, skin, umbilical cord, liver, spleen, and placenta has been reported [156, 157]. It is also highly expressed in some tumors [158, 159]. It has been reported that EGFL6 blocking antibody can restrict CSC growth and delay disease recurrence (WO2014150720 [160]). Platelet-derived growth factor receptor (PDGFR) is a receptor tyrosine kinase overexpressed in a variety of solid tumors [161]. As a biomarker of CSCs, PDGFR- β can be used to eliminate CSCs (US20140275201 [162]). Cells expressing leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) have been considered as CSCs in intestinal cancers [163, 164]. A chimeric antibody that can selectively bind to Lgr5 in cancer stem cells and then show cytotoxic activity has been shown to have capacity for selective killing of CSCs (EP2772268 [165]). As an intestinal stem cell marker, doublecortin and CaM kinase-like-1 (DCAMKL-1) is expressed primarily in quiescent cells in the lower two-thirds of intestinal crypt epithelium [166–168]. DCAMKL-1 has been used as a biomarker of CSCs. It can also inhibit the growth of CSCs (WO2012112156 [169]). Claudins are a family of tight junction transmembrane proteins [170]. An antibody with ability to bind to claudin-6 can effectively treat or prevent cancer by inhibiting or eliminating CSCs (US20160159901 [171]). Several studies have demonstrated that aldehyde dehydrogenase 1 (ALDH1), an enzyme responsible for oxidizing aldehydes to carboxylic acids, is a CSC marker in adult cancers [172–175]. By determining ALDH1 expression, CSCs can be selectively targeted and treated (US8435746 [176]). Urokinase-type plasminogen activator (uPAR) is overexpressed in several types of human cancer, resulting in enhanced invasion, migration, and metastasis [177–179]. uPAR is expressed in CSCs, indicating the importance of uPAR ligands as

Fig. 2 Development of new anti-cancer strategy targeting of cancer stem cells (CSCs)

Table 4 Summary of patents targeting cancer stem cells (CSCs) via specific markers

Patent (year)	Inventor(s)	Reference	Description of the invention
EP3130923 (2017)	Frank MH, Frank NY	[142]	A siRNA or shRNA targeting PD-1, a cell surface gene, for killing of CSC in melanoma
US8715945 (2014)	Bergstein I	[148]	Antibodies targeting CD44 for selective killing of CSC by interrupting communication between CSCs
EP3204420 (2017)	Roberts DR, Kaur S, Liu C	[149]	An agent that affect CD47 signaling to induce CSC differentiation
WO2017065493 (2017)	Hong KP, Yoon S, Koukoulas I, Cristiano B, Wilson DS, Kopsidas G	[150]	An anti-CD43 antibody binding an extracellular region of CD43 for CSC inhibiting
US20150044233 (2015)	Battula V, Andreeff M, Mani SA, Sarkar TR	[155]	Determining the presence of CSCs by detecting GD2 expression
US9850300 (2017)	Buckanovich R	[160]	EGFL6 blocking antibody for restricting CSC growth and cancer recurrence
US20140275201 (2014)	Mani SA, Hollier BG, Evans KW, Werden SJ, Sarkar TR, Tinnirello A	[162]	Using PDGFR- β inhibitors to eliminate CSC selectively
US20160159904 (2016)	Yamazaki T, Okabe H, Kobayashi S, Watanabe T, Matsubara K, Natori O, Kato A, Suzuki M	[165]	A chimeric antibody which binds Lgr5 for selective killing of CSC
US8936941 (2015)	Anant S, Houchen C, Ramalingam S, Ramanujam R, Subramaniam D	[169]	Using DCAMKL-1 as a biomarker of CSC
US20160159901 (2016)	Sahin U, Tureci O, Walter K, Wagner M, Kreuzberg M, Hacker S, Jacobs S	[171]	Diagnosis and treatment of cancer involving CSC by targeting CLDN6
US8435746 (2013)	Wicha MS, Dontu G, Ginestier C, Charafe-Jauffret E, Liu S	[176]	A novel CSC marker ALDH1 for diagnosis and treatment of cancers
WO2007134274 (2008)	Mazar AP	[180]	Ligands of uPAR for detecting and inactivating uPAR-expressing CSC
US8703712 (2014)	Buchsbaum DJ, LoBuglio AF, Zhou T, Foreman K	[183]	Detecting IAP for killing CSC
US20150017677 (2015)	Chu P, Peach R	[188]	ABCG2 expressed in CSC
US20170240902 (2017)	Ochiya T	[191]	RPN2, which is associated with tumor metastasis, identified in CSC
US9617545 (2017)	Lee YM	[192]	EXT1 as a target for the detection and treatment of CSC

therapeutically active compounds for CSCs (WO2007134274 [180]). Inhibitors of apoptosis (IAP) proteins are often overexpressed in cancers. They are associated with cell death, immunity, inflammation, cell cycle, and migration [181, 182]. Since high-level expression of IAP has been reported in CSCs, IAP inhibitors such as AT-406 and SiRNA can be potentially useful molecules to effectively kill CSCs (WO201116344 [183]). ATP-binding cassette, subfamily G, member 2 (ABCG2) is expressed in several human cancers and stem cell populations [184–187]. CSC populations characterized by expression of ABCG2 and methods of isolating have been reported (US20130244268 [188]). High expression of ribophorin II (RPN2), part of the *N*-oligosaccharyltransferase complex, in breast CSCs is associated with tumor metastasis [189]. An endoplasmic reticulum-residing type II transmembrane glycoprotein exostoxin 1 (EXT1) is known to regulate cancer cell stemness in breast cancer cells [190]. These RPN2 (WO2012023345 [191]) and

EXT1 (US20160060634 [192]) genes can be targeted for the detection and treatment of CSCs. These CSC markers are also useful for developing potential anti-cancer therapeutic strategies using anti-sense oligonucleotides, small molecule inhibitors, and antibodies.

Although there are many reports on CSC markers for targeted cancer therapy, further study is needed to investigate the specificity of developed CSC markers since many CSC markers such as CD29, CD90, CD117, and Oct-4 have also been observed in normal stem cells [193]. Kim and Ryu [194] have also found that several surface markers for sorting CSCs are present in normal tissue cells as well as hESCs, indicating that accurate identification of CSC markers for distinguishing CSCs from normal stem cells is very important to reduce side effects when they are used as therapeutic targets for CSC-specific elimination, not normal stem cells. In addition to identification of CSC-specific markers, multiple surface markers in combination are also helpful for CSC-targeted therapy.

Table 5 Summary of patents treating cancers by targeting cancer stem cells (CSCs)

Patent (year)	Inventor(s)	Reference	Description of the invention
EP2544692 (2013)	Schimmer AD, Skrtic M	[197]	Tigecycline, a tetracycline derivative, exhibiting anti-neoplastic activity against CSC
US20140294994 (2014)	Huang CY	[199]	Use of trifluoperazine and an anti-cancer drug such as gefitinib or cisplatin for eliminating CSC
EP3146978 (2017)	Kurisaki A, Wang YY, Takada H, Ekimoto H	[204]	A retinoid agonist tamibarotene as a CSC proliferation inhibitor and apoptosis inducer
EP2119434 (2009)	Inserm	[206]	Heterosidic flavonoid for preventing cancer through the induction of apoptosis to CSC
US20160374944 (2016)	Srivastava R	[210]	A various combination of sulforaphane and resveratrol for inhibiting the growth of CSC
US20130089627 (2013)	Huang CC, Chen CC, Chen LG	[213]	<i>Antrodia camphorate</i> extract for restricting CSC
EP2475362 (2012)	Burger A	[215]	Sodium meta arsenite reducing or eliminating drug-resistant CSC
US9370554 (2016)	Chen WY, Lee EH	[217]	Human growth hormone prolactin for identification of CSC in breast cancer
US9745278 (2017)	Li CJ, Rogoff H, Li Y, Liu J, Li W	[224]	Use of a novel naphtho class for suppression of the aberrant STAT3 activity of CSC
US9623015 (2017)	Prost S, Kirszenbaum M, Le Dantec M, Rousselot P, LeBoulch P	[226]	A STAT5 antagonist thiazolidinedione with tyrosine kinase inhibitor for eliminating CSC
US9545417 (2017)	Smith-Resar LM, Huso D, Cope L	[228]	Inhibiting agents for HMGA1 oncogene in CSC from various cancers
US20170312333 (2017)	Cheong JH, Park ES, Park KC	[232]	Combinations of glucose uptake inhibitor and calcium pump inhibitor as a CSC inhibitor
US8846633 (2014)	Chiou SH, Hsu HS, Cherng JY	[235]	Inhibition of CSC-like properties and chemoradio-resistant properties by delivering miR145 to CSC
US8703120 (2014)	Martuza R, Rabkin S, Wakimoto H, Kanai R	[237]	Selective killing of CSC by oncolytic Herpes virus
US20140065694 (2014)	Buchholz C, Bach P, Abel T	[238]	An oncolytic virus comprising a recombinant binding domain specific for CSC marker

5.2 Treating cancers by targeting CSCs

As CSC population seems to be an important portion of cancer tissue, targeting CSCs to have a low proliferation rate and a high tumorigenic potential can be an effective strategy to treat cancer [195]. Various compounds have been reported to exert anti-cancer effects by targeting CSCs from cancer tissues (Table 5). Tigecycline, a gly-cycline antibiotic, could inhibit mitochondrial synthesis of a protein in hematological malignancies [196]. Tigecycline has anti-neoplastic activity against CSCs by decreasing levels of proteins essential for their proliferation (US20120329761 [197]). Yeh et al. [198] have indicated that a combination of trifluoperazine with either gefitinib or cisplatin can inhibit tumor growth and overcome drug resistance in lung CSCs. A pharmaceutical composition including trifluoperazine and an anti-cancer drug such as gefitinib or cisplatin can eliminate CSCs (US20140294994 [199]). As one of retinoid agonists with various functions in proliferation, differentiation, immune function, and early embryonic development [200–203], a retinoid agonist

tamibarotene can act as a CSC proliferation inhibitor and apoptosis inducer (EP3146978 [204]). Flavonoids as a group of bioactive compounds naturally produced from plants can also reduce the risk of cancer by modulating various mechanisms [205]. Heterosidic flavonoid derivatives can target GSK3 β , Akt, FOXO3a, and MnSOD signaling pathways and induce apoptosis of CSC of acute myeloid leukaemia (EP2119434 [206]). It has been shown that several plant phytochemicals (e.g., sulforaphane and resveratrol) can modulate carcinogenesis such as metastasis and angiogenesis [207–209]. A combination of sulforaphane and resveratrol can also inhibit CSCs (US20160374944 [210]). Ethylacetate extract of fruiting bodies of *Antrodia camphorata* (EAC) also exhibits anti-cancer effects in liver cancer cell lines through induction of apoptosis and inhibition of angiogenesis [211, 212]. This EAC extract is able to restrict CSCs (US20130089627 [213]). Arsenic compounds have been considered as effective traditional medicine. Among them, sodium meta arsenite has been demonstrated to be useful for cancer treatment [214]. It can eliminate drug-resistant CSCs and

mature cancer cells (US20110059186 [215]). The anti-cancer role of prolactin, a pituitary hormone regulating several physiological functions [216], in both breast CSCs and differentiated breast cancer cells has been reported (US8759289 [217]).

Several studies have shown that signal transducer and activator of transcription 3 (STAT3), an oncogene, can promote malignant stem cells in hematopoietic and solid tumors [218–223]. A novel naphtho class of compound for suppressing aberrant STAT3 activity of CSCs has been reported (WO2009036059 [224]). In the presence of tyrosine kinase inhibitor as an anti-cancer agent [225], a STAT5 antagonist thiazolidinedione can eliminate hematologic CSCs *in vivo* (WO2014068397 [226]). High mobility group A1 (HMGA1) oncogene is enriched in normal stem cells and poorly differentiated tumors [227]. Inhibiting agent of HMGA1 (US9545417 [228]) is a selective killer of CSCs in ovarian cancer, pancreatic cancer, breast cancer, and colorectal cancer. Cancer cells rely heavily on glycolysis to meet glucose demand as an energy source through upregulation of glucose transporters [229]. Disruption of normal Ca^{2+} signaling which plays a fundamental role in cellular physiology such as cell cycle control, autophagy, cell motility, and apoptosis has also been implicated in the development of malignant phenotypes [230, 231]. Similarly, a combination of glucose uptake inhibitor (2-deoxy glucose) and calcium pump inhibitor (caloxin or ni fedipine) has been found to have potential to inhibit CSC (WO2016068600 [232]). Micro-RNAs are single stranded molecules of about 22 nucleotides that can regulate gene expression by targeting mRNA for degradation [125, 233]. Among them, microRNA-145 (miR145) has been demonstrated to be able to induce CSC differentiation through down-regulation of transcription factors essential for maintaining pluripotency [234]. In addition, inhibition of CSC-like properties and chemoradio-resistant properties has been observed after delivering miR145 to cancer cells (US8846633 [235]). Oncolytic virus has been recognized as a therapeutic reagent for killing cancer cells without harming normal tissues [236]. An oncolytic herpes virus (US8703120 [237]) and an oncolytic virus having a recombinant binding domain specific for tumor stem cell marker CD133 (US20140065694 [238]) for treating a subject having CSC have been demonstrated.

6 Conclusion

All conventional cancer therapies including surgery, radiotherapy, chemotherapy, and immunotherapy are widely used in many hospitals. They are still useful for reducing the size of primary tumor and preventing

metastasis. Unfortunately, cancer mortality is still high despite efforts and progress in understanding of cancer biology. Under these circumstances, stem cell-based technology is an exciting and rapidly developing field. Regenerative cell therapy is considered an effective anti-cancer treatment by promoting organ repair and regeneration via paracrine mechanisms or differentiation into native tissues [239], although there are current challenges and potential risks involved in stem cell-modulated tumor formation and bio-distribution of stem cells in undesired tissues. In addition to stem cell transplantation as a therapeutic option, stem cell-mediated targeted drug-delivery systems have also been proposed in an effort to reduce undesirable side effects in non-target healthy tissues. A thorough understanding of the CSC concept as a potential target for anti-cancer therapy is very important to obtain improved clinical outcome through successful targeting of cancer. It has led us to a change in thinking about effective CSC-directed anti-cancer strategy since CSCs are closely related to the development of cancer. In recent years, a lot of researchers are in agreement that CSC-targeted approach is a promising tool in cancer treatments, leading them to study CSC-specific markers for identification and selective eradication of CSCs. Many studies concerning different uses of stem cells for the struggle against cancer have been conducted. This review is useful to understand the current status of normal stem cells as therapeutic candidates and CSCs as anti-cancer targets. It also suggests future direction for better anti-cancer strategy without major side effects despite current challenges associated with identification of more specific CSC marker distinguishing CSCs from normal stem cells and mature cancer cells since they share many common features.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement There are no animal experiments carried out for this article.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66:7–30.
2. Jiang L, Nick AM, Sood AK. Fundamental principles of cancer biology: Does it have relevance to the perioperative period? *Curr Anesthesiol Rep*. 2015;5:250–6.

3. Xiu LJ, Sun DZ, Jiao JP, Yan B, Qin ZF, Liu X, et al. Anticancer effects of traditional Chinese herbs with phlegm-eliminating properties - An overview. *J Ethnopharmacol*. 2015;172:155–61.
4. Wolchok JD, Chan TA. Cancer: Antitumour immunity gets a boost. *Nature*. 2014;515:496–8.
5. Arends J. Metabolism in cancer patients. *Anticancer Res*. 2010;30:1863–8.
6. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature*. 2001;414:105–11.
7. Wei X, Yang X, Han ZP, Qu FF, Shao L, Shi YF. Mesenchymal stem cells: a new trend for cell therapy. *Acta Pharmacol Sin*. 2013;34:747–54.
8. Keefer CL, Pant D, Blomberg L, Talbot NC. Challenges and prospects for the establishment of embryonic stem cell lines of domesticated ungulates. *Anim Reprod Sci*. 2007;98:147–68.
9. Harding J, Roberts RM, Mirochnitchenko O. Large animal models for stem cell therapy. *Stem Cell Res Ther*. 2013;4:23.
10. Yoon SW, Kim DK, Kim KP, Park KS. Rad51 regulates cell cycle progression by preserving G2/M transition in mouse embryonic stem cells. *Stem Cells Dev*. 2014;23:2700–11.
11. Kim YY, Min H, Kim H, Choi YM, Liu HC, Ku SY. Differential microRNA expression profile of human embryonic stem cell-derived cardiac lineage cells. *Tissue Eng Regen Med*. 2017;14:163–9.
12. Mosna F, Sensebé L, Krampera M. Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide. *Stem Cells Dev*. 2010;19:1449–70.
13. Sachs PC, Francis MP, Zhao M, Brumelle J, Rao RR, Elmore LW, et al. Defining essential stem cell characteristics in adipose-derived stromal cells extracted from distinct anatomical sites. *Cell Tissue Res*. 2012;349:505–15.
14. Du Y, Roh DS, Funderburgh ML, Mann MM, Marra KG, Rubin JP, et al. Adipose-derived stem cells differentiate to keratocytes in vitro. *Mol Vis*. 2010;16:2680–9.
15. Lange-Consiglio A, Rossi D, Tassan S, Perego R, Cremonesi F, Parolini O. Conditioned medium from horse amniotic membrane-derived multipotent progenitor cells: immunomodulatory activity in vitro and first clinical application in tendon and ligament injuries in vivo. *Stem Cells Dev*. 2013;22:3015–24.
16. Plock JA, Schnider JT, Solari MG, Zheng XX, Gorantla VS. Perspectives on the use of mesenchymal stem cells in vascularized composite allotransplantation. *Front Immunol*. 2013;4:175.
17. Insausti CL, Blanquer M, García-Hernández AM, Castellanos G, Moreda JM. Amniotic membrane-derived stem cells: immunomodulatory properties and potential clinical application. *Stem Cells Cloning*. 2014;7:53–63.
18. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–76.
19. 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;131:861–72.
20. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol*. 2008;26:101–6.
21. Lam MT, Longaker MT. Comparison of several attachment methods for human iPS, embryonic and adipose-derived stem cells for tissue engineering. *J Tissue Eng Regen Med*. 2012;6:s80–6.
22. Navone S, Cristini S, Canzi L, Parati EA, Invernici G. Stem cell patents: an innovative approach to anti-cancer drug discovery. *Recent Pat Anticancer Drug Discov*. 2010;5:14–21.
23. Crisostomo PR, Markel TA, Wang Y, Meldrum DR. Surgically relevant aspects of stem cell paracrine effects. *Surgery*. 2008;143:577–81.
24. Kilroy GE, Foster SJ, Wu X, Ruiz J, Sherwood S, Heifetz A, et al. Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors. *J Cell Physiol*. 2007;212:702–9.
25. Caplan AI. Why are MSCs therapeutic? New data: new insight. *J Pathol*. 2009;217:318–24.
26. Sarojini H, Estrada R, Lu H, Dekova S, Lee MJ, Gray RD, et al. PEDF from mouse mesenchymal stem cell secretome attracts fibroblasts. *J Cell Biochem*. 2008;104:1793–802.
27. Schinköthe T, Bloch W, Schmidt A. In vitro secreting profile of human mesenchymal stem cells. *Stem Cells Dev*. 2008;17:199–206.
28. Liu CH, Hwang SM. Cytokine interactions in mesenchymal stem cells from cord blood. *Cytokine*. 2005;32:270–9.
29. Phan TT, Lim JJ. Isolation, cultivation and uses of stem/progenitor cells. EP2597149 (2013).
30. Buehring HJ, Trembl S, Lammers R. Isolation and/or identification of stem cells having adipocytic, chondrocytic and pancreatic differentiation potential. WO2010000415 (2010).
31. Malcuit C, Lemieux L, Holmes W, Huertas P, Vilner L. Methods of producing human RPE cells and pharmaceutical preparations of human rpe cells. WO2011063005 (2011).
32. Lengner CJ. iPS cell technology in regenerative medicine. *Ann N Y Acad Sci*. 2010;1192:38–44.
33. Wilmut I, Sullivan G, Chambers I. The evolving biology of cell reprogramming. *Philos Trans R Soc Lond B Biol Sci*. 2011;366:2183–97.
34. Brown RC, Lockwood AH, Sonawane BR. Neurodegenerative diseases: an overview of environmental risk factors. *Environ Health Perspect*. 2005;113:1250–6.
35. Lunn JS, Sakowski SA, Hur J, Feldman EL. Stem cell technology for neurodegenerative diseases. *Ann Neurol*. 2011;70:353–61.
36. Mironov N. Stem cell therapy for the treatment of central nervous system disorders. US20090214484 (2009).
37. Varney T, Mills C, Danilkovitch A. Use of mesenchymal stem cells for treating genetic diseases and disorders. US20070253931 (2007).
38. El-Badawy A, El-Badri N. Clinical efficacy of stem cell therapy for diabetes mellitus: a meta-analysis. *PLoS One*. 2016;11:e0151938.
39. Riordan N, Ichim T. Treatment of insulin resistance and diabetes. US20080260703 (2008).
40. Atala A, Milanesi A, Soker S. Regeneration of pancreatic islets by amniotic fluid stem cell therapy. US20070031384 (2007).
41. Lee EJ, Kim HS. Composition for preventing and treating liver fibrosis or liver cirrhosis, containing, as active ingredient, mesenchymal stem cells derived from human embryonic stem cells. EP3020405 (2017).
42. Wagner SC, Kim AJ, Ma H, Theofilopoulos D. Immunological treatment of liver failure. US20160074437 (2016).
43. Fraser J, Hedrick M, Zhu M, Strem B, Daniels E, Wulur I. Methods of using adipose tissue-derived cells in the treatment of cardiovascular conditions. US20050008626 (2005).
44. Franco WP. Combination growth factor therapy and cell therapy for treatment of acute and chronic heart disease. US20050214260 (2005).
45. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68:7–30.
46. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin*. 2007;57:43–66.
47. Seyfried TN, Huysentruyt LC. On the origin of cancer metastasis. *Crit Rev Oncog*. 2013;18:43–73.

48. Wang W, Goswami S, Lapidus K, Wells AL, Wyckoff JB, Sahai E, et al. Identification and testing of a gene expression signature of invasive carcinoma cells within primary mammary tumors. *Cancer Res.* 2004;64:8585–94.
49. Wang W, Goswami S, Sahai E, Wyckoff JB, Segall JE, Condeelis JS. Tumor cells caught in the act of invading: their strategy for enhanced cell motility. *Trends Cell Biol.* 2005;15:138–45.
50. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer.* 1981;47:207–14.
51. Hannun YA. Apoptosis and the dilemma of cancer chemotherapy. *Blood.* 1997;89:1845–53.
52. Chahar MK, Sharma N, Dobhal MP, Joshi YC. Flavonoids: a versatile source of anticancer drugs. *Pharmacogn Rev.* 2011;5:1–12.
53. Giuffrida D, Rogers IM. Targeting cancer stem cell lines as a new treatment of human cancer. *Recent Pat Anticancer Drug Discov.* 2010;5:205–18.
54. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature.* 2009;461:1071–8.
55. Dillman RO. Cancer immunotherapy. *Cancer Biother Radiopharm.* 2011;26:1–64.
56. Tsiatas M, Mountzios G, Curigliano G. Future perspectives in cancer immunotherapy. *Ann Transl Med.* 2016;4:273.
57. Ojha R, Bhattacharyya S, Singh SK. Autophagy in cancer stem cells: a potential link between chemoresistance, recurrence, and metastasis. *Biores Open Access.* 2015;4:97–108.
58. Esmatabadi MJ, Bakhshinejad B, Motlagh FM, Babashah S, Sadeghizadeh M. Therapeutic resistance and cancer recurrence mechanisms: unfolding the story of tumour coming back. *J Biosci.* 2016;41:497–506.
59. Etzioni R, Urban N, Ramsey S, McIntosh M, Schwartz S, Reid B, et al. The case for early detection. *Nat Rev Cancer.* 2003;3:243–52.
60. Huff CA, Matsui W, Smith BD, Jones RJ. The paradox of response and survival in cancer therapeutics. *Blood.* 2006;107:431–4.
61. Lotfi R, Eisenbacher J, Solgi G, Fuchs K, Yildiz T, Nienhaus C, et al. Human mesenchymal stem cells respond to native but not oxidized damage associated molecular pattern molecules from necrotic (tumor) material. *Eur J Immunol.* 2011;41:2021–8.
62. Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. *Cancer Res.* 2002;62:3603–8.
63. Gao Z, Zhang L, Hu J, Sun Y. Mesenchymal stem cells: a potential targeted-delivery vehicle for anti-cancer drug, loaded nanoparticles. *Nanomedicine.* 2013;9:174–84.
64. Dwyer RM, Potter-Beirne SM, Harrington KA, Lowery AJ, Hennessy E, Murphy JM, et al. Monocyte chemotactic protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. *Clin Cancer Res.* 2007;13:5020–7.
65. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells.* 2007;25:2739–49.
66. Loebinger MR, Eddaoudi A, Davies D, Janes SM. Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer. *Cancer Res.* 2009;69:4134–42.
67. Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, et al. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res.* 2005;65:3307–18.
68. Menon LG, Picinich S, Koneru R, Gao H, Lin SY, Koneru M, et al. Differential gene expression associated with migration of mesenchymal stem cells to conditioned medium from tumor cells or bone marrow cells. *Stem Cells.* 2007;25:520–8.
69. Xin H, Kanehira M, Mizuguchi H, Hayakawa T, Kikuchi T, Nukiwa T, et al. Targeted delivery of CX3CL1 to multiple lung tumors by mesenchymal stem cells. *Stem Cells.* 2007;25:1618–26.
70. Studeny M, Marini FC, Dembinski JL, Zompetta C, Cabreira-Hansen M, Bekele BN, et al. Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. *J Natl Cancer Inst.* 2004;96:1593–603.
71. Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II, et al. Human mesenchymal stem cells exert potent antitumorogenic effects in a model of Kaposi's sarcoma. *J Exp Med.* 2006;203:1235–47.
72. Komarova S, Kawakami Y, Stoff-Khalili MA, Curiel DT, Pereboeva L. Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. *Mol Cancer Ther.* 2006;5:755–66.
73. Alcayaga F, Khoury M. Menstrual blood-derived stem cells for the treatment of human pancreatic carcinoma. *US20170143764* (2017).
74. Mohammadpour H, Pourfathollah AA, Nikougoftar Zarif M, Khalili S. Key role of Dkk3 protein in inhibition of cancer cell proliferation: an in silico identification. *J Theor Biol.* 2016;393:98–104.
75. Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta.* 2003;1653:1–24.
76. Zhang X, Yaccoby S, Abramson S, Hariri RJ. Treatment of bone-related cancers using placental stem cells. *US9121007* (2015).
77. Günther C, Peter S. Mesenchymal stem cells for the treatment of metastatic liver disease. *WO2017036925* (2017).
78. Housley RM, Morris CF, Boyle W, Ring B, Biltz R, Tarpley JE, et al. Keratinocyte growth factor induces proliferation of hepatocytes and epithelial cells throughout the rat gastrointestinal tract. *J Clin Invest.* 1994;94:1764–77.
79. Ra JC, Kang SK, Woo SK, Youn HY, Lee HW, Seo KW. Anti-tumor composition comprising human-derived adult stem cells. *US20120263685* (2012).
80. Hermann F, Günther C, Geumann U. Mesenchymal stem cells to enhance anti-tumor activity of immunotherapy. *WO2017167959* (2017).
81. Herberts CA, Kwa MS, Hermsen HP. Risk factors in the development of stem cell therapy. *J Transl Med.* 2011;9:29.
82. Zhu W, Xu W, Jiang R, Qian H, Chen M, Hu J, et al. Mesenchymal stem cells derived from bone marrow favor tumor cell growth in vivo. *Exp Mol Pathol.* 2006;80:267–74.
83. Xu WT, Bian ZY, Fan QM, Li G, Tang TT. Human mesenchymal stem cells (hMSCs) target osteosarcoma and promote its growth and pulmonary metastasis. *Cancer Lett.* 2009;281:32–41.
84. Tsai KS, Yang SH, Lei YP, Tsai CC, Chen HW, Hsu CY, et al. Mesenchymal stem cells promote formation of colorectal tumors in mice. *Gastroenterology.* 2011;141:1046–56.
85. Roorda BD, ter Elst A, Kamps WA, de Bont ES. Bone marrow-derived cells and tumor growth: contribution of bone marrow-derived cells to tumor micro-environments with special focus on mesenchymal stem cells. *Crit Rev Oncol Hematol.* 2009;69:187–98.
86. Plumas J, Chaperot L, Richard MJ, Molens JP, Bensa JC, Favrot MC. Mesenchymal stem cells induce apoptosis of activated T cells. *Leukemia.* 2005;19:1597–604.
87. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumourstroma promote breast cancer metastasis. *Nature.* 2007;449:557–63.
88. Frey-Vasconcellos J, Whittlesey KJ, Baum E, Feigal EG. Translation of stem cell research: points to consider in designing

- preclinical animal studies. *Stem Cells Transl Med.* 2012;1:353–8.
89. Sensebé L, Fleury-Cappellesso S. Biodistribution of mesenchymal stem/stromal cells in a preclinical setting. *Stem Cells Int.* 2013;2013:678063.
  90. Baraniak PR, McDevitt TC. Stem cell paracrine actions and tissue regeneration. *Regen Med.* 2010;5:121–43.
  91. Spring H, Schüller T, Arnold B, Hämmerling GJ, Ganss R. Chemokines direct endothelial progenitors into tumor neovessels. *Proc Natl Acad Sci U S A.* 2005;102:18111–6.
  92. Klopp AH, Spaeth EL, Dembinski JL, Woodward WA, Munshi A, Meyn RE, et al. Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. *Cancer Res.* 2007;67:11687–95.
  93. Sagar J, Chaib B, Sales K, Winslet M, Seifalian A. Role of stem cells in cancer therapy and cancer stem cells: a review. *Cancer Cell Int.* 2007;7:9.
  94. Dwyer RM, Khan S, Barry FP, O'Brien T, Kerin MJ. Advances in mesenchymal stem cell-mediated gene therapy for cancer. *Stem Cell Res Ther.* 2010;1:25.
  95. Hodgkinson CP, Gomez JA, Mirotsoy M, Dzau VJ. Genetic engineering of mesenchymal stem cells and its application in human disease therapy. *Hum Gene Ther.* 2010;21:1513–26.
  96. Cihova M, Altanerova V, Altaner C. Stem cell based cancer gene therapy. *Mol Pharm.* 2011;8:1480–7.
  97. Fischer U, Steffens S, Frank S, Rainov NG, Schulze-Osthoff K, Kramm CM. Mechanisms of thymidine kinase/ganciclovir and cytosine deaminase/5-fluorocytosine suicide gene therapy-induced cell death in glioma cells. *Oncogene.* 2005;24:1231–43.
  98. Snyder EY, Aboody KS, Brown AB, Breakefield XO. Systemic delivery of neural stem cells to treat cancer. *CA2406664* (2011).
  99. Kim SU. Neural stem cell composition capable of treating cancer and method of treatment. *US20120107282* (2012).
  100. Kim D, Niculescu-Duvaz I, Hallden G, Springer CJ. The emerging fields of suicide gene therapy and virotherapy. *Trends Mol Med.* 2002;8:S68–73.
  101. Denny WA. Prodrugs for gene-directed enzyme-prodrug therapy (suicide gene therapy). *J Biomed Biotechnol.* 2003;2003:48–70.
  102. Kievit E, Bershad E, Ng E, Sethna P, Dev I, Lawrence TS, et al. Superiority of yeast over bacterial cytosine deaminase for enzyme/prodrug gene therapy in colon cancer xenografts. *Cancer Res.* 1999;59:1417–21.
  103. Heidelberger C, Danenberg PV, Moran RG. Fluorinated pyrimidines and their nucleosides. In: Meister A, editor. *Advances in enzymology and related areas of molecular biology.* Wiley; 1983. p. 57–119.
  104. Vermees A, Guchelaar HJ, Dankert J. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J Antimicrob Chemother.* 2000;46:171–9.
  105. Jain KK. Role of nanobiotechnology in developing personalized medicine for cancer. *Technol Cancer Res Treat.* 2005;4:645–50.
  106. Nie S, Xing Y, Kim GJ, Simons JW. Nanotechnology applications in cancer. *Annu Rev Biomed Eng.* 2007;9:257–88.
  107. Sengupta S, Sasisekharan R. Exploiting nanotechnology to target cancer. *Br J Cancer.* 2007;96:1315–9.
  108. Wang MD, Shin DM, Simons JW, Nie S. Nanotechnology for targeted cancer therapy. *Expert Rev Anticancer Ther.* 2007;7:833–7.
  109. Cady C, Mcasey M. Stem cell targeting of cancer, methods and compositions therefor. *WO2009052394* (2009).
  110. Craperi D, Vicat JM, Nissou MF, Mathieu J, Baudier J, Benabid AL, et al. Increased bax expression is associated with cell death induced by ganciclovir in a herpes thymidine kinase gene-expressing glioma cell line. *Hum Gene Ther.* 1999;10:679–88.
  111. Li G, Lee YW, Lau PY. Cell-mediated gene therapy for cancer using mesenchymal stem cells expressing a suicide gene. *US20130171115* (2013).
  112. Deng Q, Zhang Z, Feng X, Li T, Liu N, Lai J, et al. TRAIL-secreting mesenchymal stem cells promote apoptosis in heat-shock-treated liver cancer cells and inhibit tumor growth in nude mice. *Gene Ther.* 2014;21:317–27.
  113. Attar R, Sajjad F, Qureshi MZ, Tahir F, Hussain E, Fayyaz S, et al. TRAIL based therapy: overview of mesenchymal stem cell based delivery and miRNA controlled expression of TRAIL. *Asian Pac J Cancer Prev.* 2014;15:6495–7.
  114. Sung YC, Kim SW, Kim SJ, Park SH. Vector simultaneously expressing dodecameric trail and HSV-TK suicide genes, and anticancer stem cell therapeutic agent using same. *EP2811023* (2015).
  115. Kim SW, Kim SJ, Park SH, Yang HG, Kang MC, Choi YW, et al. Complete regression of metastatic renal cell carcinoma by multiple injections of engineered mesenchymal stem cells expressing dodecameric TRAIL and HSV-TK. *Clin Cancer Res.* 2013;19:415–27.
  116. Maghazachi AA, Al-Aoukaty A, Schall TJ. CC chemokines induce the generation of killer cells from CD56+ cells. *Eur J Immunol.* 1996;26:315–9.
  117. Chang A, Nolta J. Mesenchymal stem cells for targeted cancer therapy. *US20170000886* (2017).
  118. Niu G, Chen X. Vascular endothelial growth factor as an anti-angiogenic target for cancer therapy. *Curr Drug Targets.* 2010;11:1000–17.
  119. Yong TK. Stem cells for anti-angiogenic therapy in age-related macular degeneration, diabetic retinopathy, corneal vascularisation and cancer. *US20170020958* (2017).
  120. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Lett.* 2016;371:48–61.
  121. Srivastava A, Babu A, Filant J, Moxley KM, Ruskin R, Dhannasekaran D, et al. Exploitation of exosomes as nanocarriers for gene-, chemo-, and immune-therapy of cancer. *J Biomed Nanotechnol.* 2016;12:1159–73.
  122. Shtam TA, Kovalev RA, Varfolomeeva EY, Makarov EM, Kil YV, Filatov MV. Exosomes are natural carriers of exogenous siRNA to human cells in vitro. *Cell Commun Signal.* 2013;11:88.
  123. Nagathihalli NS, Nagaraju G. RAD51 as a potential biomarker and therapeutic target for pancreatic cancer. *Biochim Biophys Acta.* 2011;1816:209–18.
  124. Ohno S, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Mol Ther.* 2013;21:185–91.
  125. Hatfield S, Ruohola-Baker H. microRNA and stem cell function. *Cell Tissue Res.* 2008;331:57–66.
  126. Zhang W, Dahlberg JE, Tam W. MicroRNAs in tumorigenesis: a primer. *Am J Pathol.* 2007;171:728–38.
  127. Papagiannakopoulos T, Kosik KS. MicroRNAs: regulators of oncogenesis and stemness. *BMC Med.* 2008;6:15.
  128. Epenetos A, Prokopi M. Delivery of microRNA using mesenchymal stem cell microparticles. *WO2016166600* (2016).
  129. 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.
  130. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer.* 2003;3:895–902.
  131. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. *Oncogene.* 2004;23:7274–82.
  132. Passegué E, Jamieson CH, Ailles LE, Weissman IL. Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a

- reacquisition of stem cell characteristics? *Proc Natl Acad Sci U S A*. 2003;100:11842–9.
133. Jordan CT. Cancer stem cell biology: from leukemia to solid tumors. *Curr Opin Cell Biol*. 2004;16:708–12.
  134. Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea—a paradigm shift. *Cancer Res*. 2006;66:1883–90.
  135. Tang C, Ang BT, Pervaiz S. Cancer stem cell: target for anti-cancer therapy. *FASEB J*. 2007;21:3777–85.
  136. Pierce GB, Speers WC. Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. *Cancer Res*. 1988;48:1996–2004.
  137. Wong RM, Scotland RR, Lau RL, Wang C, Korman AJ, Kast WM, et al. Programmed death-1 blockade enhances expansion and functional capacity of human melanoma antigen-specific CTLs. *Int Immunol*. 2007;19:1223–34.
  138. Wu K, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8⁺ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res*. 2009;69:8067–75.
  139. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, White DE, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood*. 2009;114:1537–44.
  140. Fourcade J, Sun Z, Benallaoua M, Guillaume P, Luescher IF, Sander C, et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8⁺ T cell dysfunction in melanoma patients. *J Exp Med*. 2010;207:2175–86.
  141. Matsuzaki J, Gnjjatic S, Mhawech-Fauceglia P, Beck A, Miller A, Tsuji T, et al. Tumor-infiltrating NY-ESO-1-specific CD8⁺ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. *Proc Natl Acad Sci U S A*. 2010;107:7875–80.
  142. Frank MH, Frank NY. Therapeutic and diagnostic methods relating to cancer stem cells. EP3130923 (2017).
  143. Bhutani D, Vaishampayan UN. Monoclonal antibodies in oncology therapeutics: present and future indications. *Expert Opin Biol Ther*. 2013;13:269–82.
  144. Tuccillo FM, de Laurentiis A, Palmieri C, Fiume G, Bonelli P, Borrelli A, et al. Aberrant glycosylation as biomarker for cancer: focus on CD43. *Biomed Res Int*. 2014;2014:742831.
  145. Yan Y, Zuo X, Wei D. Concise review: emerging role of CD44 in cancer stem cells: a promising biomarker and therapeutic target. *Stem Cells Transl Med*. 2015;4:1033–43.
  146. Castellà EM, Ariza A, Pellicer I, Fernández-Vasalo A, Ojanguen I. Differential expression of CD44v6 in metastases of intestinal and diffuse types of gastric carcinoma. *J Clin Pathol*. 1998;51:134–7.
  147. Liu L, Zhang L, Yang L, Li H, Li R, Yu J, et al. Anti-CD47 antibody as a targeted therapeutic agent for human lung cancer and cancer stem cells. *Front Immunol*. 2017;8:404.
  148. Bergstein I. Methods of cancer diagnosis and therapy targeted against a cancer stem line. US8715945 (2014).
  149. Roberts DR, Kaur S, Liu C. Methods to eliminate cancer stem cells by targeting cd47. EP3204420 (2017).
  150. Hong KP, Yoon S, Koukoulas I, Cristiano B, Wilson DS, Kopsidas G. Anti-CD43 antibody and use thereof for cancer treatment. WO2017065493 (2017).
  151. Battula VL, Shi Y, Evans KW, Wang RY, Spaeth EL, Jacamo RO, et al. Ganglioside GD2 identifies breast cancer stem cells and promotes tumorigenesis. *J Clin Invest*. 2012;122:2066–78.
  152. Cheung NK, Saarinen UM, Neely JE, Landmeier B, Donovan D, Coccia PF. Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. *Cancer Res*. 1985;45:2642–9.
  153. Chang HR, Cordon-Cardo C, Houghton AN, Cheung NK, Brennan MF. Expression of disialogangliosides GD2 and GD3 on human soft tissue sarcomas. *Cancer*. 1992;70:633–8.
  154. Grant SC, Kostakoglu L, Kris MG, Yeh SD, Larson SM, Finn RD, et al. Targeting of small-cell lung cancer using the anti-GD2 ganglioside monoclonal antibody 3F8: a pilot trial. *Eur J Nucl Med*. 1996;23:145–9.
  155. Battula V, Andreeff M, Mani SA, Sarkar TR. Ganglioside GD2 as a marker and target on cancer stem cells. US20150044233 (2015).
  156. Yeung G, Mulero JJ, Berntsen RP, Loeb DB, Drmanac R, Ford JE. Cloning of a novel epidermal growth factor repeat containing gene EGFL6: expressed in tumor and fetal tissues. *Genomics*. 1999;62:304–7.
  157. Buchner G, Orfanelli U, Quaderi N, Bassi MT, Andolfi G, Ballabio A, et al. Identification of a new EGF-repeat-containing gene from human Xp22: a candidate for developmental disorders. *Genomics*. 2000;65:16–23.
  158. Wang X, Gong Y, Wang D, Xie Q, Zheng M, Zhou Y, et al. Analysis of gene expression profiling in meningioma: deregulated signaling pathways associated with meningioma and EGFL6 overexpression in benign meningioma tissue and serum. *PLoS One*. 2012;7:e52707.
  159. Buckanovich RJ, Sasaroli D, O'Brien-Jenkins A, Bothyl J, Hammond R, Katsaros D, et al. Tumor vascular proteins as biomarkers in ovarian cancer. *J Clin Oncol*. 2007;25:852–61.
  160. Buckanovich R. Compositions and methods relating to inhibiting cancer cell growth and/or proliferation. US9850300 (2017).
  161. Hofer MD, Fecko A, Shen R, Setlur SR, Pienta KG, Tomlins SA, et al. Expression of the platelet-derived growth factor receptor in prostate cancer and treatment implications with tyrosine kinase inhibitors. *Neoplasia*. 2004;6:503–12.
  162. Mani SA, Hollier BG, Evans KW, Werden SJ, Sarkar TR, Tinnirello A. Identification of cancer stem cell markers and use of inhibitors thereof to treat cancer. US20140275201 (2014).
  163. Schepers AG, Snippert HJ, Stange DE, van den Born M, van Es JH, van de Wetering M, et al. Lineage tracing reveals Lgr5⁺ stem cell activity in mouse intestinal adenomas. *Science*. 2012;337:730–5.
  164. Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, et al. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature*. 2009;457:608–11.
  165. Yamazaki T, Okabe H, Kobayashi S, Watanabe T, Matsubara K, Natori O, et al. Cancer stem cell-specific molecule. US20160159904 (2016).
  166. Giannakis M, Stappenbeck TS, Mills JC, Leip DG, Lovett M, Clifton SW, et al. Molecular properties of adult mouse gastric and intestinal epithelial progenitors in their niches. *J Biol Chem*. 2006;281:11292–300.
  167. May R, Riehl TE, Hunt C, Sureban SM, Anant S, Houchen CW. Identification of a novel putative gastrointestinal stem cell and adenoma stem cell marker, doublecortin and CaM kinase-like-1, following radiation injury and in adenomatous polyposis coli/multiple intestinal neoplasia mice. *Stem Cells*. 2008;26:630–7.
  168. May R, Sureban SM, Hoang N, Riehl TE, Lightfoot SA, Ramanujam R, et al. Doublecortin and CaM kinase-like-1 and leucine-rich-repeat-containing G-protein-coupled receptor mark quiescent and cycling intestinal stem cells, respectively. *Stem Cells*. 2009;27:2571–9.
  169. Anant S, Houchen C, Ramalingam S, Ramanujam R, Subramaniam D. Compositions useful for cancer detection and treatment, a cancer stem cell model, and methods of production and use thereof. US8936941 (2015).
  170. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol*. 1998;141:1539–50.

171. Sahin U, Tureci O, Walter K, Wagner M, Kreuzberg M, Hacker S, et al. Diagnosis and therapy of cancer involving cancer stem cells. *US20160159901* (2016).
172. Nakahata K, Uehara S, Nishikawa S, Kawatsu M, Zenitani M, Oue T, et al. Aldehyde dehydrogenase 1 (ALDH1) is a potential marker for cancer stem cells in embryonal rhabdomyosarcoma. *PLoS One*. 2015;10:e0125454.
173. 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;1:555–67.
174. Rahadiani N, Ikeda J, Mamat S, Matsuzaki S, Ueda Y, Umehara R, et al. Expression of aldehyde dehydrogenase 1 (ALDH1) in endometrioid adenocarcinoma and its clinical implications. *Cancer Sci*. 2011;102:903–8.
175. Nishikawa S, Konno M, Hamabe A, Hasegawa S, Kano Y, Ohta K, et al. Aldehyde dehydrogenase high gastric cancer stem cells are resistant to chemotherapy. *Int J Oncol*. 2013;42:1437–42.
176. Wicha MS, Dontu G, Ginestier C, Charafe-Jauffret E, Liu S. Aldehyde dehydrogenase 1 (ALDH1) as a cancer stem cell marker. *US8435746* (2013).
177. Asuthkar S, Gondi CS, Nalla AK, Velpula KK, Gorantla B, Rao JS. Urokinase-type plasminogen activator receptor (uPAR)-mediated regulation of WNT/ β -catenin signaling is enhanced in irradiated medulloblastoma cells. *J Biol Chem*. 2012;287:20576–89.
178. Romer J, Nielsen BS, Ploug M. The urokinase receptor as a potential target in cancer therapy. *Curr Pharm Des*. 2004;10:2359–76.
179. Sidenius N, Blasi F. The urokinase plasminogen activator system in cancer: recent advances and implication for prognosis and therapy. *Cancer Metastasis Rev*. 2003;22:205–22.
180. Mazar AP. Antibodies to urokinase- type plasminogen activator receptor(uPAR)bind cancer stem cells: use in diagnosis and therapy. *WO2007134274* (2008).
181. Lopez J, Meier P. To fight or die—inhibitor of apoptosis proteins at the crossroad of innate immunity and death. *Curr Opin Cell Biol*. 2010;22:872–81.
182. Tamm I, Kornblau SM, Segall H, Krajewski S, Welsh K, Kitada S, et al. Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. *Clin Cancer Res*. 2000;6:1796–803.
183. Buchsbaum DJ, LoBuglio AF, Zhou T, Foreman K. Targeting cancer stem cells with DR5 agonists. *US8703712* (2014).
184. Benderra Z, Faussat AM, Sayada L, Perrot JY, Chaoui D, Marie JP, et al. Breast cancer resistance protein and P-glycoprotein in 149 adult acute myeloid leukemias. *Clin Cancer Res*. 2004;10:7896–902.
185. Kamiyama N, Takagi S, Yamamoto C, Kudo T, Nakagawa T, Takahashi M, et al. Expression of ABC transporters in human hepatocyte carcinoma cells with cross-resistance to epirubicin and mitoxantrone. *Anticancer Res*. 2006;26:885–8.
186. Tsunoda S, Okumura T, Ito T, Kondo K, Ortiz C, Tanaka E, et al. ABCG2 expression is an independent unfavorable prognostic factor in esophageal squamous cell carcinoma. *Oncology*. 2006;71:251–8.
187. Yoh K, Ishii G, Yokose T, Minegishi Y, Tsuta K, Goto K, et al. Breast cancer resistance protein impacts clinical outcome in platinum-based chemotherapy for advanced non-small cell lung cancer. *Clin Cancer Res*. 2004;10:1691–7.
188. Chu P, Peach R. Cancer stem cells. *US20150017677* (2015).
189. Ono M, Tsuda H, Kobayashi T, Takeshita F, Takahashi RU, Tamura K, et al. The expression and clinical significance of ribophorin II (RPN2) in human breast cancer. *Pathol Int*. 2015;65:301–8.
190. Manandhar S, Kim CG, Lee SH, Kang SH, Basnet N, Lee YM. Exostosin 1 regulates cancer cell stemness in doxorubicin-resistant breast cancer cells. *Oncotarget*. 2017;8:70521–37.
191. Ochiya T. Method and composition for the treatment, prevention, and diagnosis of cancer containing or derived from cancer stem cells. *US20170240902* (2017).
192. Lee YM. Method for treating breast cancer by targeting breast cancer stem cell. *US9617545* (2017).
193. Karsten U, Goletz S. What makes cancer stem cell markers different? *Springerplus*. 2013;2:301.
194. Kim WT, Ryu CJ. Cancer stem cell surface markers on normal stem cells. *BMB Rep*. 2017;50:285–98.
195. Ciurea ME, Georgescu AM, Purcaru SO, Artene SA, Emami GH, Boldeanu MV, et al. Cancer stem cells: biological functions and therapeutically targeting. *Int J Mol Sci*. 2014;15:8169–85.
196. Skrtić M, Srisanthadevan S, Jhas B, Gebbia M, Wang X, Wang Z, et al. Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. *Cancer Cell*. 2011;20:674–88.
197. Schimmer AD, Skrtic M. Use of tigecycline for treatment of cancer. *EP2544692* (2013).
198. Yeh CT, Wu AT, Chang PM, Chen KY, Yang CN, Yang SC, et al. Trifluoperazine, an antipsychotic agent, inhibits cancer stem cell growth and overcomes drug resistance of lung cancer. *Am J Respir Crit Care Med*. 2012;186:1180–8.
199. Huang CY. Pharmaceutical composition for elimination of cancer stem cells. *US20140294994* (2014).
200. Mongan NP, Gudas LJ. Diverse actions of retinoid receptors in cancer prevention and treatment. *Differentiation*. 2007;75:853–70.
201. Gudas LJ, Sporn MB, Roberts AB. Cell biology and biochemistry of the retinoids. In: Sporn MB, Roberts AB, Goodman DS, editors. *The retinoids: biology, chemistry, and medicine*. 2nd ed. New York: Raven; 1994. p. 443–520.
202. Gudas LJ. Retinoids and vertebrate development. *J Biol Chem*. 1994;269:15399–402.
203. Chambon P. A decade of molecular biology of retinoic acid receptors. *FASEB J*. 1996;10:940–54.
204. Kurisaki A, Wang YY, Takada H, Ekimoto H. Cancer stem cell proliferation inhibitor. *EP3146978* (2017).
205. Arya SS, Salve AR, Chauhan S. Peanuts as functional food: a review. *J Food Sci Technol*. 2016;53:31–41.
206. The French National Institute of Health and Medical Research (Inserm). Use of heterosidic flavonoid derivatives for therapy of stem cell cancers. *EP2119434* (2009).
207. Zhang Y, Tang L. Discovery and development of sulforaphane as a cancer chemopreventive phytochemical. *Acta Pharmacol Sin*. 2007;28:1343–54.
208. Chen Q, Ganapathy S, Singh KP, Shankar S, Srivastava RK. Resveratrol induces growth arrest and apoptosis through activation of FOXO transcription factors in prostate cancer cells. *PLoS One*. 2010;5:e15288.
209. Pandey PR, Okuda H, Watabe M, Pai SK, Liu W, Kobayashi A, et al. Resveratrol suppresses growth of cancer stem-like cells by inhibiting fatty acid synthase. *Breast Cancer Res Treat*. 2011;130:387–98.
210. Srivastava R. Compositions and methods for treating and preventing cancer by targeting and inhibiting cancer stem cells. *US20160374944* (2016).
211. Hsu YL, Kuo YC, Kuo PL, Ng LT, Kuo YH, Lin CC. Apoptotic effects of extract from *Antrodia camphorata* fruiting bodies in human hepatocellular carcinoma cell lines. *Cancer Lett*. 2005;221:77–89.
212. Hsu YL, Kuo PL, Cho CY, Ni WC, Tzeng TF, Ng LT, et al. *Antrodia cinnamomea* fruiting bodies extract suppresses the invasive potential of human liver cancer cell line PLC/PRF/5

- through inhibition of nuclear factor kappaB pathway. *Food Chem Toxicol.* 2007;45:1249–57.
213. Huang CC, Chen CC, Chen LG. Method for treating a cancer caused by cancer stem cells. US20130089627 (2013).
  214. Yoon JS, Hwang DW, Kim ES, Kim JS, Kim S, Chung HJ, et al. Anti-tumoral effect of arsenic compound, sodium metaarsenite (KML001), in non-Hodgkin's lymphoma: an in vitro and in vivo study. *Invest New Drugs.* 2016;34:1–14.
  215. Burger A. Cancer stem cell-targeted and drug resistant cancer therapy. EP2475362 (2012).
  216. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev.* 1998;19:225–68.
  217. Chen WY, Lee EH. Method for treating breast cancer with prolactin receptor agonists. US9370554 (2016).
  218. Wang J, Sun Q, Morita Y, Jiang H, Gross A, Lechel A, et al. A differentiation checkpoint limits hematopoietic stem cell self-renewal in response to DNA damage. *Cell.* 2012;148:1001–14.
  219. Chung YJ, Park BB, Kang YJ, Kim TM, Eaves CJ, Oh IH. Unique effects of Stat3 on the early phase of hematopoietic stem cell regeneration. *Blood.* 2006;108:1208–15.
  220. Lin L, Liu A, Peng Z, Lin HJ, Li PK, Li C, et al. STAT3 is necessary for proliferation and survival in colon cancer-initiating cells. *Cancer Res.* 2011;71:7226–37.
  221. Ho PL, Lay EJ, Jian W, Parra D, Chan KS. Stat3 activation in urothelial stem cells leads to direct progression to invasive bladder cancer. *Cancer Res.* 2012;72:3135–42.
  222. Guryanova OA, Wu Q, Cheng L, Lathia JD, Huang Z, Yang J, et al. Nonreceptor tyrosine kinase BMX maintains self-renewal and tumorigenic potential of glioblastoma stem cells by activating STAT3. *Cancer Cell.* 2011;19:498–511.
  223. Su YJ, Lai HM, Chang YW, Chen GY, Lee JL. Direct reprogramming of stem cell properties in colon cancer cells by CD44. *EMBO J.* 2011;30:3186–99.
  224. Li CJ, Rogoff H, Li Y, Liu J, Li W. Group of STAT3 pathway inhibitors and cancer stem cell pathway inhibitors. US9745278 (2017).
  225. Arora A, Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol Exp Ther.* 2005;315:971–9.
  226. Prost S, Kirszenbaum M, Le Dantec M, Rousselot P, LeBoulch P. Combination of an anti-cancer agent such as a tyrosinekinase inhibitor and a STAT5 antagonist, preferably a thiazolidinedione, for eliminating hematologic cancer stem cells in vivo and for preventing hematologic cancer relapse. US9623015 (2017).
  227. Shah SN, Cope L, Poh W, Belton A, Roy S, Talbot CC Jr, et al. HMGA1: a master regulator of tumor progression in triple-negative breast cancer cells. *PLoS One.* 2013;8:e63419.
  228. Smith Resar LM, Huso D, Cope L. Methods of inhibiting cancer stem cells with HMGA1 inhibitors. US9545417 (2017).
  229. Cao X, Fang L, Gibbs S, Huang Y, Dai Z, Wen P, et al. Glucose uptake inhibitor sensitizes cancer cells to daunorubicin and overcomes drug resistance in hypoxia. *Cancer Chemother Pharmacol.* 2007;59:495–505.
  230. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol.* 2000;1:11–21.
  231. Kondratskyi A, Yassine M, Kondratska K, Skryma R, Slomianny C, Prevarskaya N. Calcium-permeable ion channels in control of autophagy and cancer. *Front Physiol.* 2013;4:272.
  232. Cheong JH, Park ES, Park KC. Composition for treating cancer stem cells. US20170312333 (2017).
  233. Croce CM, Calin GA. miRNAs, cancer, and stem cell division. *Cell.* 2005;122:6–7.
  234. Zhou X, Yue Y, Wang R, Gong B, Duan Z. MicroRNA-145 inhibits tumorigenesis and invasion of cervical cancer stem cells. *Int J Oncol.* 2017;50:853–62.
  235. Chiou SH, Hsu HS, Cherng JY. Method for inhibiting cancer stem cell like properties and chemoradioresistant properties of cancer or tumor cells with microrna145. US8846633 (2014).
  236. Fukuhara H, Ino Y, Todo T. Oncolytic virus therapy: a new era of cancer treatment at dawn. *Cancer Sci.* 2016;107:1373–9.
  237. Martuza R, Rabkin S, Wakimoto H, Kanai R. Use of oncolytic herpes viruses for killing cancer stem cells. US8703120 (2014).
  238. Buchholz C, Bach P, Abel T. Enhanced tumor therapy by tumor stem cell targeted oncolytic viruses. US20140065694 (2014).
  239. O'Brien T, Barry FP. Stem cell therapy and regenerative medicine. *Mayo Clin Proc.* 2009;84:859–61.