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TARGETING THE PERPETRATOR: CANCER STEM CELL THERAPEUTICS

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Abstract

The hypothesis that tumors may originate from a rare population of cancer stem cells (CSCs) has gained tremendous popularity in recent years and is supported extensively by several pioneering works. Cancer therapies targeting CSCs have unlimited potential for relapse free survival of cancer patients. As a result, knowledge of biological pathways that govern CSCs is very important and this review is focused on the biology of CSCs and recent advances in therapeutic approaches targeting them.

Keywords

Breast cancer; cancer stem cell; cancer therapy; drug resistance; signaling pathway; stem cell

INTRODUCTION

Cancers develop from normal cells that have acquired the ability to grow abnormally, survive, and spread to infiltrate into other tissues, outside of their natural microenvironment. Modern cancer therapies often face a challenge by the development of secondary tumors leading to the relapse of the disease even after successful initial therapy and recovery. This fact indicates that tumors also contain a small sub-population of cells that have characteristics of somatic stem cells. In our body, the stem cells are capable of self-renewal, asymmetric division and multilineage differentiation and have unlimited potential to differentiate into a diverse range of specialized cell types for development and maintenance of the normal tissues. Similarly, cancer cells with stem cell like properties can develop and maintain tumors in similar fashion and are termed CSCs (alternatively named as tumor-initiating cells or stem-like cancer cells). According to the cancer stem cell hypothesis, only a subset of cancer cells within each tumor has the ability to self-renew and undergo abnormal differentiation to develop into a tumor [1, 2]. Like normal stem cells, they are also long-lived cells with unlimited tumorigenic potentials and are responsible for tumor growth, maintenance, and relapse [3].

The hypothesis of CSCs was first developed by observing the similarity between embryonic tissue and cancer with respect to their enormous capacity for proliferation and differentiation [4, 5]. This observation suggested the “Embryonal Rest” hypothesis that the stem cells in adult tissue may acquire the ability to give rise to cancer [4–6]. Later, a few key experimental observations supported this hypothesis. The CSCs model was first established in the leukemia system. In 1963, Bruce *et al* demonstrated a minority of malignant blood

cells could form colonies in the spleen of a mouse [7] and the “CSCs” was proposed by Fiala [8]. Later Pierce *et al* showed experimental evidence of the existence of a cellular hierarchy in squamous cell carcinomas [9]. A pioneering study by Hamburger and Salmon demonstrated CSCs by *in vitro* colony forming assays of tumor cells from different epithelial tumors [10, 11]. In 1990, Fialkow *et al* reported that in chronic myelogenous leukemia (CML) and acute leukemia a single progenitor cell gave rise to replicating clones and created a tumor after sequentially acquiring additional mutations [12]. In 1994, based on the approach of surface marker expression used by Dr Irving Weissman’s laboratory for the identification of hematopoietic stem cells (HSC) [13], John Dick’s group isolated stem cells in acute myeloid leukemia and showed tumorigenic potential utilizing SCID mice as a model [14, 15]. Later several other studies demonstrated the presence of CSCs in various solid tumors [16–22] including breast tumors in which the CSC population is characterized by CD44⁺CD24^{−/low} expression [18].

Tumors may arise from a single cell [23], however, they are composed of heterogeneous populations of cells with differences in morphology, architecture, and developmental potentials [24, 25]. The stochastic model predicts that every cancer cell has the potential to form a new tumor, however, entry into the cell cycle is a stochastic event that occurs with low probability [2, 5]. Based on this model, all cancer cells have similar tumorigenic potential and only a small number of cancer cells would be able to grow a tumor. However, several studies demonstrated that a large number of cells were required to grow a tumor [7, 11], indicating differences in differentiation potentials within the tumor cells [26, 27]. In addition, striking morphological similarities between many primary tumors and their tissues of origin have also been observed [28]. All these observations popularize the CSC theory as the responsible element for tumor development and progression. CSCs [11] are now considered as the tumorigenic counterpart of the normal stem cells and undergo both uncontrolled and differentiated growth patterns detectable in both benign and malignant tumors [28, 29].

CELLULAR ORIGIN OF CSCs

The existence of the CSCs has already been established in different tumors, however, the origin of CSCs is not clear. It is a well-known fact that several mutations are necessary for a cell to become tumorigenic [30, 31]. Thus, the stem cells are likely candidates to accumulate mutations because of their long life span compared to restricted progenitors or differentiated cells. In fact, the leukemic stem cells have a surface marker phenotype similar to its normal counterpart hematopoietic stem cells [15, 32] and colon crypt stem cells have been reported as the cells-of-origin of intestinal cancers [33]. However, it is still unclear whether CSCs are derived from tissue specific stem cells or mature cells that have undergone a de-differentiation process [4]. Besides the acquisition of mutations to achieve the CSC property, the cell-cell fusion theory between any cell including stem/progenitor cells or terminally differentiated cells with and without abnormal properties has been proposed as another possible CSC origin [29]. This theory has been developed based on the observations that hematopoietic stem cells can fuse with several cell types in different tissues including liver, heart, and brain [34–39] both *in vitro* and *in vivo* and further supported by extensive chromosomal disorders detected in early cancers [40, 41].

In breast cancer, the CSC cell population displays a more mesenchymal phenotype [42], however, it is not clear whether breast CSCs are originated from basal or luminal cells. Liu *et al* identified an invasive gene signature (IGS) [43] and 89% of genes that were overexpressed in CSCs were coordinately overexpressed in basal subtype of breast cancers [44], indicating basal-cell breast cancers may be enriched in tumorigenic breast-CSCs or maintain a similar transcriptional profile. Breaking down of epithelial cell homeostasis and

the acquisition of a migratory mesenchymal phenotype is referred to as EMT and is considered a crucial early event in malignancy. A recent report from Gupta *et al* reported that EMT causes increased resistance to chemotherapy and enrichment in breast CSCs [45]. All these studies indicated a possibility that through EMT, epithelial cells (or preferably luminal cells) in breast tissue can achieve CSC properties probably by stepwise accumulation of mutations in oncogenes and tumor-suppressor genes or through yet to discover molecular mechanisms. Villasden *et al* [46] functionally identified a stem cell zone in luminal compartment in breast biopsies as indicated by the presence of cells with a capacity for clonal growth, self-renewal, and bipotency. They reported higher distribution of progenitor cells in the luminal compartment compared to basal compartment as assessed by combined staining for myoepithelial keratin K14 and the luminal keratin K19. This study and the strong link between cell proliferation and cancer risk support the notion that luminal progenitor cells may be the cells of origin for breast cancer and is reviewed extensively by Stingl *et al* [47].

SIGNALING PATHWAYS IN CSCS

The signaling pathways that regulate aberrant stem cell-like properties of CSCs are not well characterized. However, it is likely that there will be some overlap in the key signaling pathways between CSCs and normal adult stem cells. Here we reviewed some of these signaling pathways that are important for CSC biology. Table 1 lists the investigational agents currently in clinical trials that target some key signaling pathways implicated in CSCs that are currently in clinical trials for the treatment of various cancers.

Wnt signaling pathway

The highly conserved Wingless-type (Wnt) signaling pathway is critical in embryogenesis, and also has known roles in regulation of cell fate, proliferation, morphology, migration, apoptosis, differentiation and stem cell self-renewal [48–52]. There are two currently characterized pathways included in Wnt signaling: 1) the canonical or β -catenin-dependent pathway in which Wnt proteins bind to ‘frizzled’ membrane receptors, leading to downstream gene transcription by β -catenin; and 2) the non-canonical or β -catenin-independent which does not involve β -catenin stabilization [53]. The near 20 members of the Wnt family generally are involved in one of the two signaling pathways. Past studies have suggested β -catenin acts as a somatic stem cell survival factor in numerous systems, including the hematopoietic, neuronal, and gastrointestinal systems [28, 54–56]. As recently reviewed [57] there is mounting evidence that the oncogenic effects of Wnt-1 on mammary epithelium are initiated in the progenitor cells [58–60]. Woodward *et al* demonstrated that progenitor cells in the mammary gland are more resistant to clinically relevant doses of radiation compared to nonprogenitors, and that overexpression of the Wnt- β -catenin pathway can enhance this radioresistance [61]. In an analysis of β -catenin cellular localization in human breast cancers, high cytoplasmic expression was associated with recurrence, metastasis, and breast cancer-specific death [62]. Current studies support the exploitation of the Wnt signaling pathway as a potential drug target.

Notch signaling

In mammals the Notch signaling pathway consists of the ligands (Jagged and Delta-like), receptors (Notch 1–4) and several downstream target genes such as *p21*, *Hes-1* and *Deltex*. Notch receptors and their downstream target genes are widely expressed in mammalian tissues including embryonic tissues [63]. The Notch pathway has been implicated in the self-renewal of stem cells in hematopoietic, neural and germ cells [2]. As recently reviewed [57] Notch signaling is essential in determining mammary cell fate. Notch-1 in mice and Notch-3 in humans direct the mammary stem cell towards a luminal as opposed to a myoepithelial

cell lineage [64, 65]. A role for Notch signaling in human breast cancer is supported by both the development of adenocarcinomas in the murine mammary gland following constitutive pathway activation and the loss of Numb expression, a negative regulator of the Notch pathway, in a large proportion of breast carcinomas [66]. A γ -secretase inhibitor and a Notch4-neutralizing antibody reduced DCIS mammosphere formation, indicating that Notch signaling may represent a novel target of stem cell self-renewal directed therapeutics [67].

Bmi-1

Originally identified as an oncogene [68], the Polycomb epigenetic chromatin modifier Bmi-1 regulates expression of genes controlling cell proliferation, survival, and differentiation. Bmi-1 is a component of the Polycomb repressive complex 1, and is involved in the stable maintenance of repression of gene transcription. Two critical tumor suppressor pathways, p16 Ink4a and p19 ARF, with known roles in stem cell maintenance and cancer are repressed by Bmi-1 [69, 70]. As reviewed by Park *et al* [71], Bmi-1 is required for the self-renewal of adult murine hematopoietic stem cells and neuronal stem cells [72, 73]. It is postulated to act by preventing premature senescence of somatic stem cells, possibly by repressing expression of genes involved in senescence or by regulating telomerase expression. Given the range of tissue expression and phenotypic changes in *Bmi-1* deficient mice [74], Bmi-1 likely has a role in maintaining self-renewal of other types of adult stem cells. Mammary gland development is impaired in *Bmi-1* knockout mice [75]. These mice develop a rudimentary mammary tree during embryogenesis that later fails to invade the fat pad at puberty. The wild-type phenotype can be restored, however, either by pregnancy or Ink4a/Arf deletion, indicating the presence of mammary stem cells in *Bmi-1* deficient mice. Although as demonstrated by limiting dilution studies, these mammary stem cells appear to have reduced activity. Recently, Liu *et al* [76] observed increased expression of BMI-1 in human breast CSCs, as identified by the CD44⁺CD24^{-/low}lin⁻ phenotype, leading to speculation of BMI-1's involvement in self-renewal of CSCs. Additionally, they established by mammosphere and transplantation assays that Bmi-1 overexpression promotes mammary stem cell self-renewal and proliferation.

Hedgehog signaling

The Hedgehog (Hh) signaling pathway is another highly conserved developmental pathway that plays a critical role in the maintenance of somatic stem cells. Sonic Hedgehog, Indian Hedgehog, and Desert Hedgehog are the three mammalian Hh ligands and have distinct patterns of tissue expression and likely distinct biological function. Upon ligand binding to the Hh receptor Patched, Smoothened mediates the activation of the transcription factors Gli-1, -2, and -3, allowing alterations in target gene transcription [77]. Hh signaling has been established as a regulator of stem cell renewal in the nervous system [78, 79] and human embryonic skin [80]. As recently reviewed [81], there is increasing evidence that inhibition of Hh signaling in breast tumors may interfere with the maintenance of a putative CSC compartment. Human breast CSCs, as identified by the CD44⁺CD24^{-/low}lin⁻ phenotype, show increased gene expression of PTCH1, GLI1, and GLI2 compared to remaining tumor cells isolated from primary breast cancers. Additionally, Hh signaling increases expression of Bmi-1 in isolated mammary epithelial stem cells and CSCs [76]. Of the discussed signaling pathways, the Hh may show the most promise as a CSC targeting therapy. Inhibition of Hh signaling has been shown to increase the response of cancer cell lines to classical chemotherapies [82]. Early phase clinical trials have already shown that inhibition of Hh signaling at the receptor level can counteract skin cancer and prevent metastasis [83]. Additionally, several small molecule compounds targeting the Hh pathway are under development. Combining cyclopamine and Smoothened-targeting shRNA in a human glioma xenograft model shows that Hh signaling can regulate the self-renewal of the CSC population, and also reduced proliferation and prevents recurrence and metastasis [84].

Additional small molecules are being developed targeting the GLI-mediated transcription of Hh target genes [85].

INVOLVEMENT OF MICROENVIRONMENT

The tissue microenvironment is exceptionally important for the tissue specific stem cells to maintain their stem cell state [86]. The interaction of stem cells with neighboring cells, tissue matrix, and the presence of specific factors and signaling molecules within the stem cell niche/compartments regulate stem cell homeostasis i.e. to self-renew or exit the niche and give rise to a subpopulation of progenitor cells [87, 88]. Several studies have shown developmental limitations of the tissue specific stem cells by specific signals from the microenvironment under specific conditions such as injury or infection [89–91]. Similarly for CSCs, the microenvironment may play a significant role in cancer progression and innate factors within the tumor microenvironment can facilitate tumor growth [92]. Although, ideally, most CSCs will be independent of the niche since they can be propagated *in vitro* with minimal support from exogenous factors [86]. However, tumor regeneration by brain cancer neurospheres has been reported to be more efficient in brain compared to other tissues when injected in the NOD/SCID mice [41, 86]. Thus CSCs may depend on their microenvironment to maintain their CSC fate. In addition, the xenograft model for CSCs is not ideal because of the differences between the mouse and human tissue microenvironment and the lack of an intact immune system when evaluating the tumor-initiating capacity of the human cancer cells [93]. Consistent with this notion, Strasser and colleagues attempted to test the original CSC hypothesis by using nonirradiated congenic animals (an alternative approach to the xenograft system), and found that "tumor growth need not be driven by rare CSCs" [94]. They observed that when lymphomas and leukemias of mouse origin were transplanted into histocompatible mice, a very high frequency (at least 1 in 10) of the tumor cells can seed tumor growth suggesting the low frequency of tumor-sustaining cells (nearly 1 in 10^{5-6}) observed in xenotransplantation studies [14, 15, 95] and indicated the limitation of human tumor cells to adapt and grow in a foreign (mouse) milieu. As a result, it is also reasonable to think that the nontumorigenic population of tumor cells might be tumorigenic in the presence of the appropriate microenvironment [86, 93].

CSC NICHE

Based on the significant role of the normal stem cell niche, the "CSC niche" has already been proposed and increasing evidences support that factors derived from the tumor microenvironment serve to regulate cancer cells [96]. Work in the hematopoietic system suggests a possible role for the niche in regulating CSC maintenance. Using dynamic *in vivo* confocal imaging, Sipkins *et al* suggested that a molecularly distinct vasculature defines a microenvironment for early metastatic tumor spread in murine bone marrow which expresses the adhesion molecule E-selectin and the chemoattractant stromal-cell-derived factor 1 (SDF-1) to influence the homing of a variety of tumor cell lines [97]. Stromal fibroblasts, which represent most of the stromal cells within invasive human breast carcinoma, secrete elevated level of SDF-1 to promote tumor growth through direct binding of SDF-1 to its cognate receptor, CXCR4 and stimulate angiogenesis by recruiting endothelial progenitor cells into carcinomas [98]. Moreover, both adhesion molecules/integrins and soluble factors of the morphogen Wnt pathway can promote cell survival in AML and resistance to chemotherapeutic treatments [99]. Another study supports the existence of the "premetastatic niche", which is marked by ECM components such as fibronectin. In this case, migration of VEGFR1-expressing bone marrow-derived hematopoietic progenitor cells to the sites of future metastasis occurred via the signals from the primary tumor cells as indicated by the prevention of the tumor metastasis using antibodies against VEGFR1 or by depleting VEGFR1⁺ cells from the bone marrow [100].

Moreover, various matrix metalloproteinases are also induced in solid tumors and play important roles in tumor invasion and metastasis through VEGF Receptor (VEGFR) signaling [101]. Recently, Calabrese *et al* showed that endothelial cells interact closely with self-renewing brain tumor cells and secrete factors to promote their long-term growth and self-renewal [102]. In basal cell carcinoma (BCC) of the skin, BMP antagonist Gremlin 1 from tumor-associated stromal cells promotes proliferation of tumor cells *in vivo* [103]. Thus, the ability of a tumor to metastasize may depend on the CSCs tendency to acquire “stemness” by establishing specific niches that are more hospitable [96].

ROLE OF MICRORNA

MicroRNA (miRNA) are small, noncoding RNA molecules that target specific messenger RNA (mRNA) for translational repression by the RNA-induced silencing complex (RISC). One miRNA molecule can target hundreds of distinct mRNA, and therefore miRNA have the ability to drastically affect the proteome, and thus the phenotype, of the cell [104–106]. MiRNA have been demonstrated to be important in many biological processes, including cell proliferation, differentiation, and apoptosis [107–109]. Because these processes are often dysregulated in cancer cells, it is not surprising that miRNA have been shown to play a role in breast cancer progression and metastasis. Furthermore, the recent identification of miRNA that are differentially expressed in breast CSCs compared to non-tumorigenic breast cancer cells suggests that miRNA are orchestrators of CSC function and maintenance [110, 111].

In a study published by Shimono *et al*, miRNA expression in human breast CSCs (CD44⁺CD24[−]lin[−]) was compared to miRNA expression in their differentiated progeny (non-tumorigenic breast cancer cells) [110]. Members of the miR-200 family (a, b, and c) were significantly downregulated in the breast CSCs, suggesting they may play a role in preventing breast cancer cell stemness. Supporting this suggested role, targets of miR-200c include BMI1, which is necessary for stem cell self-renewal, and ZEB1/ZEB2, which are transcriptional repressors of E-cadherin [110, 112]. While the signal that initiates the downregulation of miR-200c to permit differentiation is not known, miRNA-200 expression does appear to be intricately regulated by a balance between AKT1 and AKT2 [113]. An increase in the AKT1:AKT2 ratio results in a increase in miR-200, and a resulting decrease in EMT and stem cell properties [113]. Remarkably, forced expression of miR-200c in breast CSCs by infection of a miR-200c-expressing lentivirus resulted in suppression of tumor formation when the cells were injected into the mammary fat pad of NOD/SCID mice [110].

Yu *et al* identified the tumor suppressor miRNA let-7 as decreased in breast CSCs, and found that infection of a let-7 lentivirus inhibited breast CSC self-renewal and suppressed their ability to form mammospheres *in vitro* and tumors *in vivo* [111]. Targets of let-7 include HMGA2, which promotes EMT, Ras, which promotes self-renewal, and IL6, which activates Stat3 to promote cell motility, growth, and self-renewal [111, 113]. Iliopoulos et al demonstrated that as nontransformed MCF10A cells undergo an epigenetic switch to form mammospheres, epigenetic inheritance occurs through a feedback loop involving Lin28-mediated downregulation of let-7 [113]. Lin28 is an RNA-binding protein that induces uridylation of specific miRNA to block miRNA processing by Dicer [114], and Lin28 expression is sufficient to transform human fibroblasts into pluripotent stem cells [115]. Additionally, miR-200c and another miRNA identified by Yu *et al* as decreased in breast CSCs, miR-107, are also regulated by Lin28 [114], suggesting that Lin28 may coordinately effect the expression of multiple miRNA to promote stem cell properties.

MiRNA-21 is identified as overexpressed in nearly every cancer or tumor examined, and thus has been labeled an “oncomir” [116]. MiRNA-21 has been linked to major cell functions, including apoptosis, cell proliferation, cell growth, cell migration, and protein translation [117–119], and targets include the tumor suppressor genes programmed cell death 4 (PDCD4) and phosphatase and tensin homologue (PTEN) [119, 120]. Bourguignon *et al* recently demonstrated that Nanog activates the processing of miR-21 by Drosha by activation of p68 helicase, which promotes pri-miR-21 cleavage [121]. Interestingly, activation of Nanog was due to the interaction of the extracellular matrix component hyaluronan, which is often abnormally produced during transformation and metastasis, with CD44, a marker of breast CSCs [121, 122]. In addition, inhibition of miR-21 in MCF-7 cells prevented tumor growth in a xenograft mouse model [123].

The identification of specific miRNA that are dysregulated in breast CSCs, and the discovery that overexpression of a specific miRNA, like miR-200c or let-7, can negatively impact tumor formation, suggest that miRNA may provide a promising new approach to cancer therapy. Furthermore, miRNA therapy may affect not only the tumor cells but also the chemo-resistant stem cells [124]. Oncomirs, like miR-21, could be inhibited to allow expression of tumor suppressor genes, while tumor suppressor miRNA could be overexpressed to prevent tumor growth.

Several methods have been developed to knockdown the function of a specific miRNA. Antagomirs are 2'-O-methyl oligonucleotides that are complementary to a specific miRNA, so that when they bind to the miRNA they prevent its interaction with the target. Antagomirs can be conjugated to a cholesterol moiety to allow for delivery into the cell [125]. As another means to knockdown a specific miRNA, “sponges” have been developed [126]. Sponges are long transcripts that have multiple miRNA binding sites, and thus “soak up” the endogenous miRNA molecules.

While inhibition of specific miRNA may work in some instances, in other cases it may be more efficacious, and less detrimental to normal cells, if a tumor suppressor miRNA can be overexpressed, as the normal cells can more easily compensate for excess miRNA rather than the loss of a crucial miRNA. MiRNA can be overexpressed either by adding exogenous pre-miRNA or by infection with a lentivirus that expressed the specific miRNA.

While specific miRNA may be dysregulated in cancer systems, the same miRNA may have an essential role in a normal cell system. Therefore, it is necessary that miRNA effectors are targeted directly to the tumor. One method that has been developed is nanoparticles. Nanoparticles coated with transferrin can bind to transferrin receptor, a receptor found primarily on cancer cells, and thus specifically deliver the miRNA effector to cancer cells [127]. To overexpress a miRNA, Kota *et al* used viral vector delivery to overexpress a miRNA in liver cancer cells, and the vector was packaged into a serotype that is specifically picked up by liver cells [128]. Another option for targeting miRNA effectors to tumors is through mesenchymal stem cells. Dwyer *et al* demonstrated that monocyte-chemotactic protein-1 secretion by breast tumor cells attracts mesenchymal stem cells to the tumor, and thus these cells can be utilized as carriers of miRNA-expressing adenoviruses [129]. Finally, at least one group has had success with intratumoral injections of miRNA antagomirs [130].

CLINICAL RELEVANCE OF CSCS: RESISTANCE TO CURRENT THERAPIES

In several tumors, recurrences are very common after chemo- and radiation therapy, both of which are popularly used as non-surgical method to eliminate tumors. Recent work in leukemia as well in solid tumors showed that the CSCs are particularly resistant to conventional chemo- and radiation therapies compared with the more differentiated cells in the non-CSC compartment that compose tumor bulk [61, 131–133]. Costello *et al* reported

that CD34⁺/CD38⁻ leukemia precursors cells showed reduced sensitivity to daunorubicin, a major drug used in leukemia treatment, compared to leukemic blasts (CD34⁺/CD38⁺ counterpart), and increased expression of multidrug resistance genes [131]. In glioblastoma, CD133⁺ CSC populations are relatively resistant to radiation and chemotherapy via ataxia telangiectasia mutated (ATM) DNA repair pathway. Inhibition of c-src tyrosine kinase (CSK) homologous kinases (CHK1 and 2) with a small molecule inhibitor increased the radiation sensitivity of CSC-enriched cells both *in vitro* and *in vivo* [132]. Recently in breast cancer cell lines, the Wnt/ β -catenin pathway has been reported in the radiation resistance in mammary progenitor cells as well as cells expressing CSC markers [61, 134]. Aberrant Wnt/ β -catenin pathway also promotes genomic instability in colon cancer promoting the ability of CSCs to survive extensive DNA damage [135]. In addition the reactive oxygen species [136], Notch signaling and EGFR signaling has also been linked to radiation resistance in tumor cells [134].

The CSCs are also resistant to several chemotherapeutic agents (temozolamide, carboplatin, VP16 and Taxol) [137] in a variety of different cancers [138] and Akt activity [139] as well as autocrine stimulation of IL-4 receptors have been linked to their chemoresistant phenotype [140]. The CSCs, like normal stem cells, express ATP-binding cassette (ABC) transporters such as multidrug resistance transporter 1 (MDR1) and breast cancer resistance protein (BCRP), which are linked to an amplified drug removal ability of CSCs to pump chemotherapeutic drugs (such as daunorubicin, mitoxantrone, vinblastine, paclitaxel, imatinib mesylate, topotecan and methotrexate) out of the cells [134, 141, 142]. In addition, the CSCs also express molecular metabolic mediators like aldehyde dehydrogenase (ALDH), which have been shown to contribute chemo-resistance both in normal stem cells [143] and in leukemic CSCs [144]. Recently, ALDH has been identified as single biomarker of head and neck squamous cell carcinoma CSCs [145] and is also expressed in the breast CSCs [146] suggested that the chemotherapy resistance is associated with a poor prognosis. Furthermore, CSCs from acute and chronic myelogenous leukemias are relatively quiescent [147] and contribute to chemotherapy resistance since the sensitivity to chemotherapeutic agents relies upon lethal cellular damage during cell cycle progression in highly proliferative cells.

Anti-angiogenic and vascular targeting agents are another modern category of cancer therapies for an increasing number of cancers [134]. Recent studies showed that the CSCs produce much higher levels of VEGF in both normoxic and hypoxic conditions [148] and stimulate angiogenesis by secreting VEGF and other factors leading to tumor growth both before and after therapy [102]. HIF-1, a transcription factor stabilized by hypoxic conditions, increases the production of VEGF in variety of tumors [134] and has been suggested to regulate radiosensitizing effect on tumors through induction of ATP metabolism, proliferation and p53 activation along with stimulated endothelial cell survival for angiogenesis in post-radiation tumors [149]. Consistently, a recent report from Bao *et al* indicated that ionizing radiation (IR) treatment of short-term cultures from human glioma xenografts enriched the CD133⁺ subpopulation and irradiated CSC-derived tumors are vascular and hemorrhagic [134, 148]. In fact, a combination of antiangiogenic therapy and radiation showed more success as an anti-tumor therapy [134]. Thus, successful targeting of molecular mechanisms involved in therapeutic resistance of CSCs will improve the efficacy of current cancer therapies for extended relapse-free survival.

RECENT ADVANCES TARGETING CSC POPULATION

Specific targeting of CSCs with tolerable side effects will be ideal for therapeutic purposes and recently few studies have reported success. Although resistance to common radiation and chemotherapeutic approaches of CSCs are well known, it has been reported that

common chemotherapeutics such as cisplatin, etoposide, and bleomycin are effective against undifferentiated cancer cells in testicular germ-cell cancer patients without severely affecting fertility [150]. It was also reported that the combination of Idarubicin, an anthracycline antileukemic drug, with proteasome inhibitor killed leukemic stem cells without affecting the normal HSCs [151]. A recent report from Samudio *et al* [152] demonstrated that fatty acid metabolism is linked to leukemia cell apoptosis and proliferation. Pharmacologic inhibition of fatty acid oxidation with Etomoxir, which inhibits the entry of fatty acids into the mitochondria by blocking the activity of carnitine palmitoyl transferase 1 or treatment with the fatty acid synthase/lipolysis inhibitor Orlistat inhibited proliferation and sensitized human leukemia cells to apoptosis induction by ABT-737 and Nutlin 3a. They also found that Etomoxir decreased the number of quiescent leukemia progenitor cells in approximately 50% of primary human acute myeloid leukemia samples and provided better therapeutic outcome when combined with either ABT-737 or cytosine arabinoside in a murine model of leukemia.

Very recently Gupta *et al* [45] found a specific compound through chemical screening named salinomycin, which reduces the proportion of breast CSCs by almost 100-fold relative to paclitaxel, a commonly used breast cancer chemotherapeutic drug. Treatment of mice with salinomycin inhibits mammary tumor growth in vivo and induces epithelial differentiation of tumor cells. This study also reported that salinomycin treatment results in the loss of expression of breast CSC genes previously identified in patients [43, 153, 154], confirming the specific toxicity of this compound for breast CSCs. In another recent study by Max Wicha group, has suggested a novel means of targeting and eliminating breast CSCs via blockade of the IL-8 receptor CXCR1 [155]. Blocking of CXCR1 using either anti-CXCR1-specific antibody or pharmacological inhibitor repertaxin selectively depleted the human breast CSC population in vitro. In NOD/SCID xenograft models, repertaxin treatment has been shown to specifically target the CSC population in human breast cancer xenografts, retarding tumor growth and reducing metastasis. When combined with chemotherapeutic agent docetaxel, repertaxin treatment significantly reduced tumor size as well as CSC population indicating a potential therapeutic approach to eliminate breast tumor with minimum potential of relapse.

FUTURE DIRECTIONS

The CSC research still needs to be developed almost in every aspect. The molecular mechanisms that regulate CSC development, self-renewal, survival, and differentiation within its microenvironment will be a major area of interest. Understanding and characterization of these mechanisms will lead the way to develop new therapies for many human cancers.

Current procedures for CSC identification rely on the surface antigen expression profile; however, identification of additional biomarkers of CSCs for more specific in situ localization, isolation and characterization is very essential. It is also important to study the miRNA signatures in the CSCs and other progenitor as well as differentiated cells present in the tumor. This approach will provide more specific information along with genomic signature to discover essential genetic/epigenetic changes present during tumor initiation, and development. In addition, studies on the metabolomic profile of CSCs will be another potential area to understand their cellular energy managements. In fact the recent finding of lower ROS levels in CSCs [136] indicated a more efficient oxidative stress handling system present in CSCs. Thus, dissection and profiling of anti-oxidative pathways in CSCs will be a major area of research to identify key targets for therapy.

Another important area of CSC research will be the characterization of interaction of CSCs with complex microenvironment, which is composed of variety of cells and ECM components. The influences from stroma on CSCs for their development, progression, and overall tumorigenic potential will be another potential area of future research, which will lead to an ideal in vivo model for more accurate drug screening to inhibit the tumorigenic potential of not only the CSCs but also the for the other cells with different developmental potentials present in a single tumor.

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Table 1

A list of investigational agents in clinical trials that target cancer stem cell specific signaling pathways.

Drug name	Cancer type	Signaling pathways	Phase	NCT identification numbers in www.clinicaltrials.gov
MK0752	Advanced Breast Cancer Other Solid Tumors	Notch (γ -secretase inhibitor)	I	NCT00106145
PF-03084014	Leukemia, Lymphoid Leukemia, T-Cell Solid Tumors	Notch (γ -secretase inhibitor)	I	NCT00878189
LBH589	Thyroid Carcinoma	Histone deacetylase inhibitor	II	NCT01013597
Resveratrol	Colon Cancer	Wnt	I/II	NCT00256334
GDC-0449	Metastatic Colorectal Cancer	Hedgehog (Inhibits SMO)	II	NCT00636610
	Solid Cancers	Hedgehog (Inhibits SMO)	I	NCT00968981
	Basal Cell Carcinoma	Hedgehog (Inhibits SMO)	II	NCT00833417
BMS-833923 (XL139)	Basal Cell Carcinoma	Hedgehog (small molecule SMO inhibitor)	I	NCT00670189
PF-04449913	Hematologic Malignancies	Hedgehog (small molecule inhibitor of SMO)	I	NCT00953758
IPI-926	Advanced and/or Metastatic Solid Tumor Malignancies	Hedgehog (Cyclopamine-derived SMO inhibitor)	I	NCT00761696
Rapamycin	HER-2 Receptor Positive Breast Cancer	IL2 receptor (inhibits mTOR)	II	NCT00411788

A list of current investigational agents at various phases of clinical trials that target specific stem cell signaling pathways such as Notch, histone deacetylase, Wnt, Hedgehog, and mTOR.