MINI-REVIEW

Cancer Stem Cells and Response to Therapy

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Abstract

The cancer stem cell (CSC) model states that cancers are organized in cellular hierarchies, which explains the functional heterogeneity often seen in tumors. Like normal tissue stem cells, CSCs are capable of self-renewal, either by symmetric or asymmetric cell division, and have the exclusive ability to reproduce malignant tumors indefinitely. Current systemic cancer therapies frequently fail to eliminate advanced tumors, which may be due to their inability to effectively target CSC populations. It has been shown that embryonic pathways such as Wnt, Hedgehog, and Notch control self-renewal and cell fate decisions of stem cells and progenitor cells. These are evolutionary conserved pathways, involved in CSC maintenance. Targeting these pathways may be effective in eradicating CSCs and preventing chemotherapy or radiotherapy resistance.

Keywords: Cancer stem cells - chemotherapy response - radiotherapy response - targeted therapy

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Introduction

For the past few decades, cancer stem cells (CSCs) have been the subject of intensive research. The CSC model states that cancers are organized in cellular hierarchies which explains the functional heterogeneity often seen in tumors (Harrison et al., 2010; Takebe and Ivy, 2010). Like normal tissue stem cells, CSCs are capable of self-renewal, either by symmetric or asymmetric cell division, and have the exclusive ability to reproduce malignant tumors indefinitely. Stem cells give rise to transit-amplifying cells or progenitor cells by asymmetric cell division which in turn generate more differentiated cells in a given tissue. CSCs constitute a minority population in tumors and have low proliferative rate compared to progenitor cells (Reiman et al., 2010).

In contrast to CSC model, the clonal evolution model which includes a stochastic component explains tumor progression as a result of continued selection of the fittest and the most resistant clones. Thus every cell in a tumor has the capability to acquire sufficient mutations to become invasive and metastatic (Hart and El-Deiry, 2008).

It is generally accepted that cancers arise as a result of a series of genetic and epigenetic events in single cells within a tissue which abrogate the normal physiological control on cell proliferation or initiate a state of genomic instability. It has been proposed that abnormal clones that give rise to malignancy, at least at the initial stages, preserve many of the features of hierarchical structure of the original tissue. According to CSC model, Transit-amplifying cells produce non-dividing end-cells which are more differentiated, even though readily detectable signs of differentiation are usually lost (Valent et al., 2012).

CSCs are generally recognized by the presence or absence of different cell surface markers. For example, breast CSCs are defined by $CD^{44+}/CD^{24-/low}$ cell population (Al-Hajj et al., 2003). These CSC markers can be identified by staining cells with antibodies against them, or by flowcytometry. Table 1 summarizes the list of commonly used CSC markers for various tissues.

A group of recently identified tumor antigens named cancer-testis (CT) antigens has gained attention as stem cell markers (Ghafouri-Fard and Modarressi, 2009; Ghafouri-Fard, 2012). These antigens have been used as cancer biomarkers as well as target molecules for

Table 1. Markers used to Identify CSCs in Various Tissues

Tumor	CSC marker	References
Breast	$CD44^+/CD24^-/lin^-/ALDH1^+$	Al-Hajj et al., 2003; Ginestier et al., 2007
Leukemia	$CD34^+/CD38^-$	Bonnet and Dick, 1997
Colon	$CD133^+/CD44^+/ALDH1^+$	Ricci-Vitiani et al., 2007
Head and Neck	$CD44^+$	Prince et al., 2007
Brain	$CD133^+$	Singh et al., 2004
Lung	$CD133^+$	Eramo et al., 2008
Prostate	$CD133^+/CD44^+/\alpha_2\beta_1^{high}$	Collins et al., 2005
Pancreas	$CD133^+/CD44^+/CD24^+/ESA^+$	Hermann et al., 2007; Li et al., 2007
Liver	$CD90^+$	Yang al., 2008

*CD44: hyaluronate receptor (p-glycoprotein 1); CD24: heat stable antigen; lin: linage markers; ALDH1: aldehyde dehydrogenase 1A1; CD34: hematopoietic progenitor cell antigen (GP105-120); CD38: cyclic ADP ribose hydrolase; CD133: prominin 1; α2β1: integrin α2β1; ESA: epidermal surface antigen; CD90: Thy-1

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immunotherapy of different cancers (Ghafouri-Fard and Ghafouri-Fard, 2012). It seems that the existence of CT gene-expressing cells in tumor cell population may be the result of clonal proliferation of an aberrant CSC (Ghafouri-Fard and Modarressi, 2012).

CSCs may originate from tissue stem cells which have gained cancerous properties through genetic and epigenetic changes. Alternatively, they may arise from transformed progenitor cells that have acquired self-renewal capabilities (Hart and El-Deiry, 2008; Takebe and Ivy, 2010). It was shown that lineage-restricted granule cell progenitors, and not neural stem cells, can give rise to Hedgehog-induced medulloblastoma which points to the latter concept (Schuller et al., 2008).

CSCs reside in specialized microenvironments called niches, which have an important role in stem cell maintenance. It has been shown that response of CSCs to antitumor drugs is different *in vivo* and *in vitro*, which may be due to the effect of the niche. The constituents of niche include fibroblasts, endothelial cells, perivascular cells, tissue macrophages, extracellular matrix, and soluble factors excreted from cells or released from stroma. There is cross talk between CSCs and the niche, in a way that CSCs instruct the niche and they are governed by the niche to proliferate, differentiate, invade and metastasize (Takebe and Ivy, 2010; Nguyen, 2012). CSCs may generate niches as nascent domains or they may use existing tissue stem cell niches (LaBarge, 2010). Molecules like cytokines and their receptors, adhesion molecules and various chemotactic factors may play a role in CSC-niche interactions. An example is CXCR4 which is expressed on many cancer cells, and its ligand is SDF1 (stromal cell-derived factor 1, also known as CXCL12). SDF1 is released from niche and is chemoattractant for $CXCR4^+$ cells, so has a role in entry of cancer cells into the bone marrow (Domanska et al., 2012).

Epithelial-mesenchymal transition (EMT) is a process by which epithelial cells acquire mesencymal phenotype, like becoming spindle-shaped and motile, express mesenchymal markers such as vimentin and N-cadherin, lose epithelial characteristics like cytokeratins and E-cadherin, and detach from their original tissue. It can be seen during embryonic development and tumor metastasis. Many of the constituents of tumor microenvironment can initiate EMT, such as matrix metaloproteinases, growth factors, and transforming growth factor β (TGFβ) (Reiman et al., 2010). Release of TGFβ from stroma can induce properties like invasion and metastasis in tumor through downstream signaling of transcription factors like Snail and Twist. Expression of defined set of transcription factors (eg. Snail and Twist) can induce stem cell characteristics in human mammary carcinoma cells (Mani et al., 2008).

Tumor microenvironment can influence the state of stemness and differentiation of cancer cells. Tumor hypoxia can induce stem like characteristics through hypoxia inducible factor-1 (HIF-1) in many tumors. This is achieved by activation of transcription factors involved in reprogramming of induced pleuripotent stem cells (ie. Oct4, Sox2, Nanog and KLF4) and miRNA-302. It seems that hypoxic locations in the tumor can function as CSC niches. Inflammation can also induce stemness through IL-6 secreted by tumor-infiltrating macrophages and activation of Stat3 signaling (Li and Laterra, 2012). It was shown that IL-6 can induce stem-like phenotype in breast cancer progenitor cells via a positive feedback loop involving NF-κB, Lin28, Let-7 microRNA (Iliopoulos et al., 2011).

Cancer Stem Cells and Chemotherapy Response

Tumor recurrence after initial response to chemotherapy is a major clinical issue. Recurrent tumors usually show heterogeneity in both the population of CSCs and non-CSCs, and also in histologic and cytogenetic appearances. This may be due to the survival of CSCs within the original tumor, which despite chemotherapy and removal of the bulk of the tumor, have repopulated the recurrent tumor (Figure 1). Although cancer stem cells constitute about 1% of tumor cells, they can generate tumors similar to the original one when xenotransplanted into non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. It has been reported that this property is not observed with the remaining non-CSC bulk tumor cells (Eyler and Rich, 2008).

Most cytotoxic therapies induce DNA damage or disrupt mitosis leading to cell death in dividing cancer cells. CSCs are protected against anti-neoplastic drugs through multiple defense mechanisms. These mechanisms can be divided into two groups: CSC-intrinsic and CSC-extrinsic. CSC-intrinsic mechanism can be due to more efficient DNA repair mechanisms, expression of drug pumps, and altered cell cycle. CSC-extrinsic mechanisms refer to the effects of tumor microenvironment on CSCs (Maugeri-Sacca et al., 2011). Furthermore, it seems that CSC population is enriched following chemotherapy, as in a study it was shown that breast cancer stem cell markers (CD^{44+}/CD^{24-}) were expressed more abundantly after neoadjuvant chemotherapy of primary breast cancer patients (Li et al., 2008). In another study, glioma CSCs showed resistance to multiple chemotherapeutic drugs

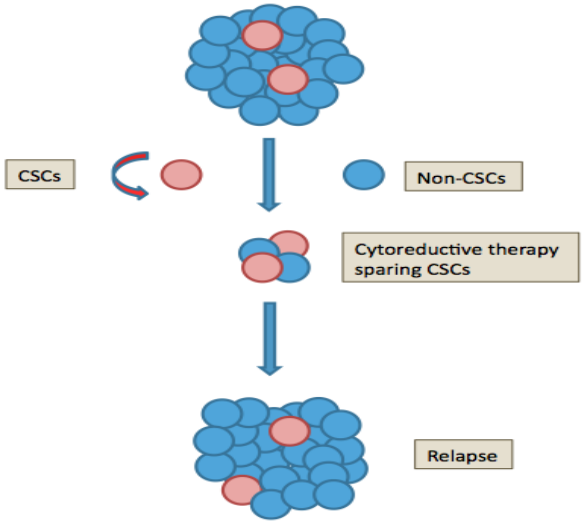

Figure 1. When Cancer Stem Cells (CSCs) Survive Chemotherapy or Radiotherapy, Relapse Follows

including temozolomide, etoposide, carboplatin and paclitaxel, whereas non-CSCs were responsive (Liu et al., 2006).

Embryonic and adult stem cells have more robust DNA repair systems compared to progenitor and differentiated cells (Bracker et al., 2006; Maynard et al., 2008). However, aged stem cells have reduced capability to repair DNA lesions and as a result accumulate genetic and epigenetic mutations. This may lead to increased incidence of various cancers with aging (Rossi et al., 2007). Activation of DNA-damage checkpoint and DNA-damage repair pathways have been proposed as a mechanism of chemotherapy resistance in various cancers (Gallmeier et al., 2011). When lung CSCs are exposed to genotoxic agents, they activate CHK1 and CHK2, but more differentiated lung cancer cells are responsive to these drugs. Use of CHK1 inhibitors along with chemotherapy, can induce cell death in CSC compartment (Maugeri-Sacca et al., 2011).

Epithelial tumor cells secrete Interleukin-4 which contributes in an autocrine manner to apoptosis resistance (Todaro et al., 2008). Colon CSCs showing resistance to fluorouracil and oxaliplatin, were made responsive to these chemotherapy drugs with the use of antibodies against IL-4 (Todaro et al., 2007). It would appear that autocrine IL-4 may function as a survival factor in cancer stem cells and thereforeclearly could play a role in chemotherapy resistance.

Expression of ATP-binding cassette (ABC) transporters is elevated in both normal stem cells and cancer stem cells. These include multidrug resistance transporter 1 (MDR1) and breast cancer resistance protein (BCRP) (Moitra et al., 2011). Acute myeloid leukemia (AML) CSCs can extrude daunorubicin and mitoxantrone more efficiently than non-CSCs (Wulf et al., 2001). Likewise, neuroblastoma stem cells can extrude mitoxantrone with high efficiency (Hirschmann-Jax et al., 2004). Vinblastine and paclitaxel can be expelled by MDR1 and imatinib mesylate and methotrexate can be removed by BCRP (Eyler and Rich, 2008).

Metabolic alterations also may contribute to drug resistance. Aldehyde dehydrogenase 1 (ALDH1) is overexpressed in leukemic stem cells (Pearce et al., 2005) and it was shown that ALDH1 gene transfer can lead to cyclophosphamide resistance in normal stem cells (Magni et al., 1996); so ALDH1 may also play a role in chemotherapy resistance.

Normal stem cells are usually in a state of quiescence and do not exhaust their proliferative ability, unless the tissue encounters injury. During this period of quiescence, CSC population can repair damaged DNA. Likewise, CSCs are mostly quiescent and therefore can escape chemotherapy-induced cytotoxicity which acts on dividing cells (Maugeri-Sacca et al., 2011). It was shown that ovarian CSCs proliferate more slowly and display more resistance to cisplatin relative to non-CSC population (Gao et al., 2010).

EMT has a role in development of metastasis and chemotherapy resistance. EMT can be induced by the activation of a transcriptional network involved in stem cell self-renewal, including Notch, Wnt, and Hedgehog (Maugeri-Sacca et al., 2011). Cells undergoing EMT usually reside at the tumor-stroma interface and acquire stem cell markers and clonogenic properties (Mani et al., 2008). Hypoxia within the tumor can induce angiogenesis through HIF-1 pathway, but abnormal organization of the newly formed vessels leads to low concentration of the chemotherapeutic drugs in the tumor and can be a possible mechanism of therapeutic resistance (Maugeri-Sacca et al., 2011).

Cancer Stem Cells and Radiotherapy Response

Radiotherapy yields a curative potential in many solid tumors. Radiotherapy alone or in combination with chemotherapy can cure locally advanced, unresectable head and neck carcinoma and non-small cell lung cancer in about 10-50 percent of cases. In early stages of tumor progression, radiotherapy alone or in combination with chemotherapy can control local recurrence of tumor similar to surgery (Krause et al., 2011). Radiotherapy is the most efficient non-surgical modality for glioblastoma treatment; however, all of them recur and lead to patient death. In a study, CSC population was enriched after radiotherapy and it was shown that CSC population survived more compared to non-CSC compartment. Radiotherapy induced the same amount of DNA damage to both CSCs and non-CSCs, but CSCs were able to repair damage more robustly. Furthermore, non-CSC population went through apoptosis more after radiation. Genotoxic stress activates ATM, CHK1 and CHK2 checkpoint proteins which in turn activate DNA repair pathway. CSCs display a basal level of checkpoint activation, which means that they are ready to respond to genotoxic insults. Use of CHK1 and CHK2 inhibitors resulted in radiosensitivity of CSC population (Bao et al., 2006).

It seems that Wnt/β-catenin has a role in radiotherapy resistance. Radiation led to enrichment of stem cells in a murine mammary epithelial cell line, which had high levels of activated β-catenin and survivin (an antiapoptotic protein).These cells displayed elevated self-renewal in mammosphere formation assay (Chen et al., 2007; Woodward et al., 2007). Radiation treatment of breast CSCs led to lower levels of reactive oxygen species (ROS) relative to non-CSCs. This reduced ROS levels may be due to increased radical scavenger properties in breast CSCs. Acute irradiation caused Jagged-1 expression on the surface of CSCs and Notch-1 activation (Phillips et al., 2006).

It was shown that in primary breast carcinoma, loss of phosphatase and tensin homologue (PTEN) causes Akt phosphorylation which in turn phosphorylates Chk1 and localizes it in the cytoplasm. This results in defective response to radiation and finally leads to genomic instability (Puc et al., 2005). In another experiment, Akt inhibitors decreased the number of brain CSCs more efficiently compared to non-CSCs, while another group showed that Ras/PI3K/Akt pathway is involved in radioresistance, and PI3K inhibitors caused prolongation of DNA damage in glioblastoma cells (Grana et al., 2002; Kao et al., 2007; Eyler et al., 2008).

CSCs and Angiogenesis

Glioblastoma CSCs secrete vascular endothelial growth factor (VEGF) much more compared to non-CSCs population and its secretion is increased further under hypoxic conditions. This results in enhanced endothelial cell migration and tube formation *in vitro*. It was shown that xenografts from CSCs injected into mice, respond well to anti-VEGF monoclonal anitibody, bevacizumab, with decreased tumor growth and vascularity, whereas xenografts from non-CSCs did not respond to bevacizumab (Bao et al., 2006).

It seems that CSCs themselves depend on factors secreted by their vascular niches as normal stem cells do. Normal stem cells rely on factors like leukemia inhibitory factor, brain-derived neurotrophic factor, so CSC niche maintenance may also depend on these factors. Therefore, there may be a positive feedback between CSCs and angiogenesis (Riquelme et al., 2008).

Tumor hypoxia stabilizes HIF-1, which induces transcription of VEGF and leads to increased vascularization. It was shown that radiation induces HIF-1 activation, which causes endothelial cell survival and is involved in radioresistance (Moeller et al., 2005). CSC population is increased in hypoxic conditions, which means that HIF-1 may be stabilized in these cells. This can have a role in tumor radioresistance (Blazek et al., 2007).

Targeted Therapy Directed toward CSCs

Taken together, there seems to be a stem-like population in different neoplasms which current cancer therapies are not adequately effective against them. In order to completely cure cancer, it is necessary to eradicate these CSCs. It has been shown that embryonic pathways such as Wnt, Hedgehog, and Notch control self-renewal and cell fate decisions of stem cells and progenitor cells. These are evolutionary conserved pathways that play important roles during development, and are involved in CSC maintenance (Figure 2) (O'Brien et al., 2010).

The Notch pathway has a major role in stem cell growth and differentiation. Contact between Notch receptor on the surface of one cell and the Notch ligand on an adjacent cell initiates Notch signaling pathway which in the cell expressing the notch receptor (and possibly in both cells) sends cell fate regulatory signals. These signals result in activation of a transcriptional cascade which affects hundreds of genes. Notch receptors (Notch 1-4) are noncovalent heterodimers including an extracellular segment (N^{EC}) and a transmembrane segment (N^{TM}). Notch ligands include Jagged-1,-2 and Delta-1,-3,-4 and are transmembrane. Ligand binding results in dissociation of N^{EC} from N^{TM}, and exposes a disintegrin and metalloprotease (ADAM) cleavage site on N^{TM}. N^{EC} is trans-endocytosed by the cell expressing the ligand, and N^{TM} is cleaved by ADAM and **g**amma-secretase to produce notch intracellular domain (NICD) which is translocated into the nucleus. NICD is the active fragment and interacts with multiple ubiquitous transcription factors and chromatin-modifying enzymes to form notch transcriptional complex (NTC) and activate transcription (Kopan and Ilagan, 2009).

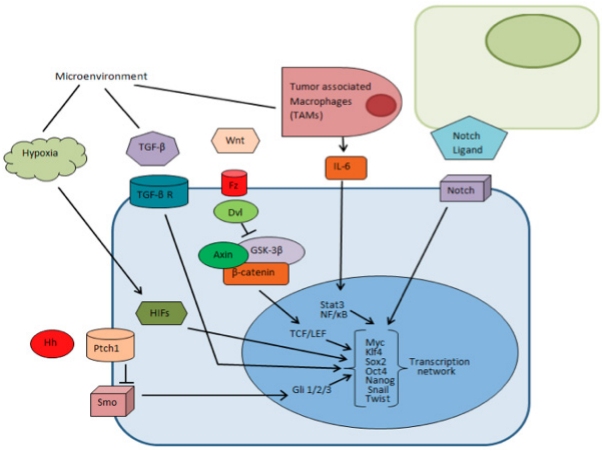

Figure 2. Signal Transduction Pathways, Microenvironment Signals, and Molecular Circuits Involved in Cancer Stem Cell (CSC) Self-renewal and Maintenance

Notch activation induces tumor growth, and prevents apoptosis. Notch signaling is especially important in CSCs of breast and glioma tumors (Kakarala and Wicha, 2008; Fan et al., 2010; Harrison et al., 2010; Wang et al., 2010). Notch seems to play an important role in radioresistance in gliomas. Inhibition of Notch pathway by gamma secretase inhibitors (GSIs) resulted in improved sensitivity of glioma CSC population to radiotherapy (Wang et al., 2010). It was also shown that Notch pathway inhibition in glioblastoma CSCs resulted in decreased proliferation and increased apoptosis as well as decreased AKT and STAT3 phosphorylation (Fan et al., 2010). GSIs can also prevent the formation of mammospheres from primary breast tumors and breast cancer cell lines (Kakarala and Wicha, 2007).

It seems that there is a relationship between HER-2 and Notch signaling pathways as HER2 promoter contains Notch binding sites (Chen et al., 1997). Furthermore, Notch signaling is activated in HER-2 overexpressing cells. SiRNA or GSI inhibition of Notch signaling causes decreased expression of HER-2 and reduced mammosphere formation of breast cancer tumor initiating cells (Magnifico et al., 2009).

Notch pathway is also involved in other malignancies, including hepatocellular carcinoma where Notch, STAT3 and TGF-β paly roles in CSC maintenance (Yao and Mishra, 2009). Another group has demonstrated a relationship between EMT and Notch pathway activation in gemcitabine-resistant pancreatic cancer cells, and as cells undergoing EMT acquire stem cell properties, there may be a link between stemmness and EMT in pancreatic cancer (Wang et al., 2009). These results suggest targeting Notch pathway with inhibitors like GSIs or monoclonal antibodies to Notch receptor or Delta-4 ligand may be effective in eradicating CSCs and preventing chemotherapy or radiotherapy resistance (Pannuti et al., 2010).

Another regulator of self-renewal is sonic hedgehog (Hh) pathway. Hedgehog signaling is active during embryonic period and is specifically important in development of neural tube and skeleton, but is silenced in

most adult tissues (Merchant and Matsui, 2010). Binding of Sonic (SHh), Desert (DHh), and Indian Hedgehog (IHh) to Patched (PTCH1) receptor initiates this pathway. Unbound Patched constitutively represses the activity of smoothened (SMO) which is a transmembrane protein. When Hh ligand binds to Patched, this repression is released and modulates the activity of GLI transcription factors. GLI1 is a transcriptional activator and GLI3 is a repressor, but GLI2 can act as a repressor or activator (Ingham, 2008).

Germinal mutations in PTCH1 occurs in Gorlin syndrome or basal cell nevus syndrome and these patients are in particular predisposed to develop basal cell carcinoma (BCC), rhabdomyosarcoma and medulloblastoma (Johnson et al., 1996). Somatic mutations in PTCH1 and SMO have been identified in sporadic BCC and medulloblastoma (Pietsch et al., 1997; Xie et al., 1998). Other components of the Hedgehog pathway are mutated in other cancers, including GLI1 amplification in glioblastoma, GLI1 and GLI3 mutations in pancreatic adenocarcinoma, and SUFU (a component of a corepressor complex that acts on DNA-bound GLI1) mutations in medulloblastoma (Taylor et al., 2002; Jones et al., 2008; Merchant and Matsui, 2010). Level of Hedgehog ligands are increased in several human cancers, including small-cell lung, colon, prostate cancer, and melanomas (Watkins et al., 2003; Karhadkar et al., 2004; Stecca et al., 2007; Varnat et al., 2009). This ligand-dependent activation functions through either autocrine or paracrine signaling and tumor stroma may be involved in this signaling (Merchant and Matsui, 2010).

Glioblastoma CSCs show activated Hedgehog signaling, and inhibition of this pathway with siRNA or cyclopamine (SMO antagonist) resulted in loss of tumorigenicity (Clement et al., 2007). Increased expression of PTCH1, GLI1, and GLI2 was demonstrated in breast CSCs and administration of cyclopamine or siRNA against GLI1 and GLI2 resulted in reduced BMI-1 (an important regulator of normal stem cells) and decreased tumorigenicity (Liu et al., 2006). In colon carcinoma, elevated expression of GLI1, GLI2 and PTCH1 have been identified in CSC component and it was shown hedgehog pathway activation is involved in colon carcinoma recurrence and metastasis. There is an association between higher expression of hedgehog pathway components and their target gene, Snail1, and epithelial-mesenchymal transition (EMT) in CSCs. Administration of cyclopamine or siRNA against GLI1, GLI2 and SMO decreased tumor growth and induced apoptosis (Varnat et al., 2009).

It seems that Hedgehog pathway is important in EMT and metastasis. Cells undergoing EMT have active Hedgehog pathway; they become motile and invade their surrounding tissue and metastasize. When they settle in their new location, they may further need Hedgehog pathway for self-renewal and growth (Merchant and Matsui, 2010).

Wnt signaling is involved in proliferation, differentiation, survival and apoptosis. The amount of β-catenin determines the activity of this pathway. A multiprotein complex including adenomatosis polyposis coli (APC), axin and glycogen synthase kinase-3β (GSK-3β) normally degrades β-catenin through ubiquitin-proteasome degradation pathway, therefore the amount of β-catenin is kept low. When Wnt binds to its receptor complex including Frizzled (Fz) and low-density lipoprotein receptor-related protein (LRP), a cytoplasmic protein named dishevelled (Dvl) is phosphorylated and in turn inhibits GSK-3β. Therefore β-catenin accumulates in the cytoplasm and translocates into the nucleus, where it forms a complex with members of the transcription factors T-cell transcription factor (TCF)/lymphoid enhancer binding factor (LEF) family. β-catenin recruits co-activators like p-300 or c-AMP response element binding protein (CREB) –binding protein (CBP), leading to transcription of downstream genes (Hecht et al., 2000; Nelson and Nusse, 2004).

The importance of abnormal Wnt signaling is evident in some neoplasms especially colorectal cancer; but its association with some other malignancies is also present, even though classical mutations in this pathway (APC truncation, β-catenin mutations) do not exist. There are many reports of aberrant Wnt signaling in breast cancer. Dishevelled (Dvl) is amplified and overexpressed in 50 percent of ductal breast carcinomas (Nagahata et al., 2003). Frizzled related protein 1 (FRP1) which is a secreted Wnt inhibitor at locus 8p11-21, is frequently deleted in breast cancer. Likewise FRP1 downregulation occurs in 80 percent of breast neoplasms (Ugolini et al., 1999). Axin is also downregulated in some breast neoplasms (Roh et al., 2004). Loss of expression of APC, due to mutation or methylation, occurs in 36 to 50 percent of breast cancers (Virmani et al., 2001).

Wnt/β-catenin pathway is important in maintenance of pleuripotency in embryonic stem (ES) cells, however, it also plays a role in neural differentiation of embryonic stem cells and in cell-fate decision in neural crest stem cells (Hari et al., 2002; Zechner et al., 2003; Sato et al., 2004). There is an association between increased nuclear amount of β-catenin, which is a hallmark of Wnt pathway activation, and progression from chronic phase of chronic myelogenous leukemia to blast crisis and imatinib resistance (Jamieson et al., 2004). Furthermore, it has been shown that Wnt pathway is important in ABCB1/MDR1 transcription, as ABCB1 promoter has a putative TCF/LEF binding element (–1,813 to –275 bp) (Yamada et al., 2000). Therefore, Wnt pathway may play a role in chemotherapy resistance.

There are two categories of Wnt pathway inhibitors: 1) small molecule inhibitors such as nonsteroidal anti-inflammatory drugs (NSAIDs) and molecular targeted agents like CBP/β-catenin antagonist ICG-001, 2) biologic inhibitors like antibodies and siRNA (Takahashi-Yanaga and Kahn, 2010). Aspirin and other NSAIDs have cancer preventive effects, especially in colorectal cancer; for example, sulindac and celecoxib inhibit the growth of adenomas in patients with adenomatous polyposis coli (APC) (Thun et al., 2002). It was shown that sulindac decreased nuclear localization of β-catenin and downregulated the expression of β-catenin/TCF target genes like MET and cyclin D1(Boon et al., 2004). Another group showed indomethacin and aspirin induced reduced

Table 2. Inhibitors of Self-renewal Pathways

Agent	Target	References
Notch Pathway		(Pannuti et al., 2010)
GSIs	γ-secretase	
GSMs	γ-secretase	
Notch mAbs	Notch receptors	
DLL4 mAbs	Delta-4 ligand	
Notch soluble receptor decoys		
siRNA, miRNA-based therapeutics		
Hedgehog Pathway		(Merchant and Matsui, 2010)
GDC-0449 (Vismodegib)	PTCH and/or SMO	
IPI-926 (Saridegib)	SMO	
PF-04449913	SMO	
XL139/BMS833923	SMO	
LDE225	SMO	
Wnt Pathway		(Takahashi-Yanaga and Kahn, 2010)
Aspirin	β-catenin	
Sulindac	β-catenin	
Celecoxib	TCF	
Retinoids	β-catenin	
1α25,-dihydroxy-Vitamin D3	β-catenin	
PNU 74654	β-catenin/TCF	
2,4-diamino-quinazoline	β-catenin/TCF	
NSC668036	Dvl	
FJ9	Dvl	
IWR	Axin	

*GSIs: Gamma Secretase Inhibitors; GSMs: Gamma Secretase Modulators

expression of β-catenin/TCF responsive genes, with no significant effect on nuclear localization of β-catenin (Dihlmann et al., 2001). In another study, it was shown a monoclonal antibody against frizzled receptor, OMP-18R5, inhibited tumor growth in xenograft models of several human tumors including pancreatic, breast, and lung cancer and decreased tumor-initiating cell frequency (Gurney et al., 2012). Table 2 summarizes a list of inhibitors of self-renewal pathways, some of which are already used in the clinic.

Conclusion

As noted, current systemic cancer therapies frequently fail to eliminate advanced tumors, which may be due to their inability to effectively target CSC population (Boman and Wicha, 2008). It has been demonstrated that CSCs exist in various tumor types and the signaling pathways involved in CSC self-renewal and differentiation seems to be common in different tumor types (Boman and Huang, 2008; Dirks, 2008; Huff and Matsui, 2008; Kakarala and Wicha, 2008; Prince and Ailles, 2008; Sell and Leffert, 2008; Takaishi et al., 2008). Therefore, there is the possibility that therapies targeting these common pathways be effective against a wide spectrum of tumor types. The great promise is that this may eventually end in more effective and curative cancer treatment and preventive modalities. This would be challenging as CSCs probably use the same pathways as normal stem cells for self-renewal and maintenance. However, some initial studies have shown that there may be some mechanistic differences between cancer stem cell and normal stem cell maintenance which can be exploited in devising targeted therapies (Yilmaz et al., 2006).

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