Targeting Signal Pathways active in Cancer Stem Cells to Overcome Drug Resistance

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1 Introduction

Over the last several decades, although there have been advances in the treatment of diseases such as children leukemia, Hodgkin's disease, and testicular cancer, however, the survival of patients with the most common malignancies such as lung, breast, liver, and colon cancers has not changed significantly[1-4]. Most of patients will relapse and ultimately died of the disease. The scarce efficacy of current treatments indicates the resistance of cancer cells to cytotoxic agents, radiation, and even immunotherapy and survival from the treatment without major injure.

It is believed that tumors is initiated and maintained by a rare population of cancer cells with stem cell properties known as cancer stem cell (CSC). The concept that a rare population of the tissue stem cells maybe the cellular origin of cancer was proposed almost 150 years ago. Approximately 50 years ago the concept that only a small subpopulation of so-called leukemic stem cells (LSCs) may be connected to the maintenance and evolution of myeloid leukemia emerged. Conclusive evidences for the existence of LSCs come from the function assay using SCID-leukemia and NOD/SCID-leukemia xenotransplantation models in which mice were transplanted with leukemic cells from the bone marrow and peripheral blood of AML patients. These studies demonstrated that the leukemic grafts were highly representative of the original patients disease and the SCID/leukemia initiating cell presented at a frequency of 0.2-100/10⁶ mononuclear cells[5]. More recently, this principle has also been extended to other tumors, such as breast, brain, prostate, pancreas, colon, lung, liver, and head and neck tumors[1-11].

CSCs have been reported to be the only tumorigenic population and play a central role in relapse because of the failure of current chemotherapy to eradicate them. The existence of CSCs highlights the critical need for the new therapeutic strategies to directly target the CSC population for ultimately curing cancer.

Based on the solid evidences that cancers are stem cell diseases, the view of drug resistance change. It is believed that CSCs are naturally resistant to conventional chemotherapy and serve as the main mediators of drug resistance[2,3,12-14]. Moreover, it is accepted that drug resistance is governed by the mutations that confer protection mechanism through modulation of cell survival factors. To that end, a number of signal pathways involved in CSCs viability and survival, namely the Hedgehog, Ras, FLT3, PI3K/AKT, NF-κB, mTOR are aberrantly regulated in CSCs. Because of their wide-ranging biological effects, deregulation one or more of these pathways may give rise to a failure of current chemotherapy. Others and we have long been interested in exploring the mechanisms of drug resistance of CSCs influenced by these cell survival pathways and molecular interaction networks. Thus we can determine the critical elements and the general rules driving the network to guide the use of specific inhibitors of a given pathway. This review will focus on the drug resistance of CSCs and the signal pathway and their potential cross-talk (Fig 1).

Fig 1 Signal transduction pathways important in cancer stem cells
2 Cancer stem cells and drug resistance

According to the hierarchy model, cancers consist of a heterogeneous population, within which only a rare population of CSCs sustains the disease. CSCs share some properties of normal stem cells, such as self-renewal potential, proliferation and essential property of self-protection. The whole drug resistance concept has been revised incorporating the CSCs paradigm. CSCs play the key role in the drug resistance of cancers. CSCs present in the original tumour mass and survive chemotherapy, whereas the committed but variably differentiated cells are killed. Several mechanisms make CSCs more resistant to conventional chemotherapeutic agents. For example, CSCs exhibited higher expression of drug resistance proteins, such as lung resistance-related protein (LRP) and multiple resistance-associated protein (MRP). Recent work from our group suggests that LSCs are resistance to mitoxantrone and daunorubicin via up-regulation of ABCG2 and MRP. Another group of investigators have demonstrated that LSCs isolated from human leukemia are predominantly in the G0 phase of the cell cycle that made it resistance to cell cycle specific chemotherapeutic agents such as Ara-c. Furthermore, CSCs have capacity for DNA repair. As a result, at least some of CSCs can survive chemotherapy including DNA damage agents such as alkylating agents. Moreover, CSCs are resistant to chemotherapy through impaired apoptosis pathway. Our unpublished data show that LSCs up-regulated Bcl2 protein and Bcl2 siRNA enhanced the sensitivity of LSCs to mitoxantrone cytotoxicity. The properties of CSCs suggest that the current chemotherapy drugs will not be curative. Current studies focus on a number of signaling pathways that regulate chemoresistance of CSCs through survival pathway. We will outline some of these pathways and their potential in drug resistance.

2.1 Hedgehog pathway "Hedgehog" (HH) molecules are secretory signaling proteins that were first discovered in Drosophila. Three HH homologs have been identified in humans including Sonic hedgehog (SHH), Indian hedgehog (IHH) and Desert hedgehog (DHH). Secreted hedgehog molecules bind to and inhibit the cell surface receptor Patched 1 protein on target cells. Smoothened is a transmembrane protein primarily located in the membrane endosomes. It is proposed that the endogenous agonist of SMO is a small intracellular molecule transported out of the cell by PTCH1, a mechanism preventing binding to SMO. Upon binding an HH ligand, PTCH1 is internalized and inactivated so that the endogenous agonist of SMO accumulates in cytoplasm and activates SMO. Activated SMO causing release of the Gli family of transcription factors (Gli 1, Gli 2 and Gli 3), which can then translocate into the nucleus and activate gene transcription that control the cell cycle, signal transduction, and apoptosis. HH pathway, which is one of the main pathways that control stem cell fate, self-renewal and maintenance, plays a central role in drug resistance of cancer cell.

HH pathway make CSCs more resistance to chemotherapy through several mechanisms. First, HH controls the cell cycle fate during cell proliferation. Second, HH signaling may act as upstream of other signal pathway that regulate self-renewal of stem cell. Furthermore, HH pathway contributes to the survival of tumor progenitor cells by opposing the activation of both intrinsic and extrinsic apoptosis cascades. Gli-1 is considered the positive transcriptional transactivator in the shh pathway. Gli-1 was also able to induce endogenous Bcl2 expression. Moreover, HH signal also up-regulates the expression of Bcl2 through activated PI3K and AKT. We have been demonstrated that Bcl2 was high expression via up-regulation Gli in LSCs. These findings suggest that in addition to regulating proliferation of tumor progenitor cells, HH signaling may support the survival of tumor progenitor cells.

Moreover, HH pathway regulates the expression of two ABC proteins, multidrug resistance protein-1 and breast cancer resistance protein and leads to the efflux of various chemotherapeutic drugs.

2.2 Ras signaling pathway Ras, the protein product of the ras proto-oncogenes, is localized to the inner surface of the cell membrane, in which it becomes functional in transducing the mitogenic signals of tyrosine kinase receptors that regulate diverse signaling pathways involved in cell growth, differentiation and apoptosis. The family of ras includes N-ras, K-ras, and H-ras. Ras mutations are most commonly associated with th cancer including leukemia. Once activated, ras is able to trigger several signaling including Raf-Mek-Map kinase pathway, FMS-like tyrosine kinase 3 (FLT3) pathway, and phosphoinositide 3-kinase (PI3K)/cytoplasmic protein kinase B (AKT) pathway. The potential relevance of the Raf-MEK-MAP kinase pathway to abnormal hematopoiesis is highlighted by the ability of a constitutively activated mutant Raf to eliminate growth factor dependence of hematopoietic cells. Ras also activates the PI3K pathway, which can result in suppression of apoptosis by directly activating AKT. The PI3K/AKT pathway is important for relaying survival signals in hematopoietic cells by Ras. Mutations of ras in LSCs result in refractory and relapse of leukemia.

2.3 FMS-like tyrosine kinase 3 signaling The FLT3 gene, also known as fetal liver tyrosine kinase 2 (PLK2), encodes a membrane-bound receptor tyrosine kinase (RTK). FLT3 have been shown to play a role in leukemogenesis. In most examined patients cohorts, FLT3 is consistently associated with unfavorable prognosis and relapse of AML patients. In recent studies, it was also shown that FLT3 was expressed in LSCs. FLT3 activates special anti-apoptotic signal by up-regulating Bcl2 family. In additionally, FLT3 mediates drug resistance through activating PI3K/AKT survival pathway. Interestingly, simultaneous mutations of ras and FLT3 are rare, sug-
Phosphatase and tensin homolog (PTEN) is negative regulated in CSCs and high levels of PI3K/AKT have been linked to poor prognosis and chemoresistance. The intermediates of the PI3K/AKT survival pathway are activated in CSCs and high levels of PI3K/AKT has been linked to poor prognosis and chemoresistance. Tumor suppressor gene Phosphatase and tensin homolog (PTEN) is negative regulator of AKT pathway. The increasing evidences have supported that PI3K plays critical roles in the chemotherapy-resistance in CSCs. Furthermore, the downstream effector of PI3K, AKT (a subfamily of the serine/threonine protein kinases), have been associated with the cell growth and survival of CSCs. Three AKT isoforms (AKT1, AKT2, and AKT3) have been identified, all of which share an N-terminal PH domain, with central kinase domain, and a serine/threonine-rich C-terminal region. The intermediates of the PI3K/AKT survival pathway are activated in CSCs and high levels of PI3K/AKT has been linked to poor prognosis and chemoresistance. Tumor suppressor gene Phosphatase and tensin homolog (PTEN) is negative regulator of AKT pathway. Mutations or losses of PTEN have been found in a large number of cancers including brain, breast, prostate and leukemia. Loss of PTEN function results in AKT activating and cancer resistance to conventional therapy and a relapse following initial regression. Shoman et al have reported a strong correlation between down-regulation of PTEN expression and failure to respond to tamoxifen treatment in estrogen receptor-positive tumors. In the hematopoietic system, recently studies show that conditional deletion of PTEN result in leukemia. Thus PI3K/Akt pathway plays the critical role in the CSC resistance to a number of anti-tumor agents. 2.5 NF-κB signaling pathway Nuclear factor of xB (NF-κB) is a family of closely related dimeric transcription factors that bind to the xB sites. NF-κB is an inducible and ubiquitously expressed transcription factor that regulates cell survival, inflammation, and differentiation. It is becoming increasingly clear that NF-κB signaling plays critical roles in cancer development and progression. Cancer cells especially poorly differentiated cancer cells shows activated NF-κB in the nucleus, suggesting that activated NF-κB regulates its downstream genes to promote cancer cell growth. The exciting results have shown that NF-κB is constitutively activated in LSCs whereas it is strikingly not activated in their normal counterpart, suggesting this transcription factor is preferentially in LSCs. This provides a possible that specific target the LSCs while spare the normal HSCs. More importantly, it has been well known that many chemotherapeutic agents such as nucleoside analogs and anthracyclines induce the activity of NF-κB, which causes drug resistance in cancer cells. Therefore, targeting NF-κB would be promising strategy to overcome the drug resistance of CSCs. 3 Strategies to overcome drug resistance through regulating survival signal pathways of CSCs The concept that cancer is a stem cells disease has the potential to change the view of drug resistance. As the understanding of the signaling pathway involved in the survival and chemoresistance of CSCs, it is likely to identify new mechanism-based effective therapy directed at CSCs to cure cancer. 3.1 Targeting of hedgehog pathway As indicated above, the HH pathway is activated in many human CSCs and plays the central role in drug resistance. Cyclopamine is a natural steroid alkaloid that inhibits the HH pathway by directly binding and suppressing the Smo receptor. Recent studies showed that cyclopamine inhibits various human malignancies including breast, prostate, liver, pancreas, small cell lung cancer, and glioma. Importantly, continuous cyclopamine eliminated PC3 cancer-initiating cells. Similarly, cyclopamine treatment also counteracts the expansion of multiple myeloma (MM) stem cell and decrease the number of MM stem cell. Cyclopamine inhibited the self-renewal ability of glioma CSCs and enhanced the anti-proliferative and apoptotic effect of the current drug temozolomide on the gliomasphere cells. Furthermore, blocking the HH signal pathway by Gli siRNA or humanized anti-SHH antibodies has been shown to induce apoptosis in a wide variety of tumors through activation of intrinsic and extrinsic apoptosis cascades and resensitized the chemoresistant CSCs. Taken together, these studies suggest that selective target HH pathway may lead to more effective cancer therapies. 3.2 Targeting of the ras pathway The emerging evidences shown that increase in ras activity may be an early step in the development of cancer. The preclinical concept of farnesyltransferase blockade as a targeted therapy against oncogenic Ras has clearly evolved with the recognition that many proteins involved signaling pathways in tumor cells undergo farnesylation. Several farnesyltransferase inhibitors as monotherapy in cancer in vitro or in clinical trial demonstrate encouraging responses and good tolerability. BMS-214662, a cytotoxic farnesyltransferase inhibitor, previously reported to selectively kill nonproliferating subpopulation in tumor cells. Recent studies have also been shown that BMS-214662, alone or in combination with imatinib or dasatinib, effectively induced apoptosis of resistant CML stem cells and potently induced apoptosis of both proliferating and quiescent CML stem/progenitor cells with less than 1% recovery of Philadelphia-positive long-term culture-initiating cells. Normal stem/progenitor cells were relatively spared by BMS-214662. Our unpublished data also showed that manumycin enhanced mitoxantrone-induced apoptosis in LSCs. 3.3 Regulation of the PI3K/AKT pathway The increasing
evidence has shown that activated FLT3, PI3K/AKT pathway is critical for drug resistance of CSCs, therefore, downregulation of FLT3, PI3K, and AKT could sensitize CSCs to chemotherapy and overcome drug resistance. The PI3K/AKT pathway may be inhibited with PI3K (LY294002, PX-866), PDK1 (OSU-03012, celecoxib), AKT (A-443654, perifosine, tricribine) or downstream mTOR inhibitors such as rapamycin and modified rapamycins (CCI-779 and RAD001). Inhibition of the PI3K/AKT pathway by the specific pathway inhibitors LY294002 leads to a dose-dependent decrease in survival of LSCs[42]. LY294002 also significantly reduced the survival of SP fraction within MCF7 cells and decrease cancer stem-like cells[43]. Wortmannin are able to inhibit CML and AML cell proliferation and to synergize with targeted tyrosine kinase inhibitors. Additionally, dual PI3K/PDK-1 inhibitor BAG956 have been demonstrated effective against leukemia[44]. Recently, publication by Yilmaz and colleagues demonstrated that mammalian target of rapamycin (mTOR) inhibition with rapamycin not only depleted leukemia-initiating cells but also restored normal HSC function[32]. In conclusion, inhibition of this pathway leads to an increase in apoptosis in CSCs, and that it potentiates the response to cytotoxic chemotherapy.

3.4 Targeting of NF-κB signaling pathway Previous studies have demonstrated that NF-κB, a known regulator of growth and survival, is constitutively active in LSCs but not in normal hematopoietic stem cells (HSCs). These suggest that LSC-specific targeted therapy should be feasible using a variety of strategies. Guzman et al.[36] have previously shown that a combination of the proteasome inhibitor MG-132 and the anthracycline idarubicin was sufficient to preferentially ablate human LSCs in vitro while sparing normal HSCs. These studies demonstrate that LSC-specific targeting can be achieved. Recently, Guzman et al.[40] also demonstrated that the single plant-derived compound parthenolide (PTL) effectively eradicates AML LSCs by inducing robust apoptosis via induce oxidative stress and inhibit NF-κB while sparing normal HSCs. These properties make these compound an attractive agent for clinical evaluation. However, the poor solubility of PTL makes pharmacologic use of the compound difficult. Thus, more recently, orally bioavailable Dimethylamino-parthenolide (DMAPT) induces rapid death of primary human LSCs from both myeloid and lymphoid leukemias, and is also highly cytotoxic to bulk leukemic cell populations[46]. Servida et al. also reported that PS-341 induced apoptosis in leukemia progenitor cells[47]. In an effort to expand strategies for selectively targeting LSCs, the recent study has been shown that the compound TDZD-8 (4-benzyl,2-methyl,1,2,4-thiadiazolidine, 3,5 dione), which was originally developed as a non-ATP competitive inhibitor of GSK-3β, was strongly and selectively cytotoxic to multiple types of primary leukemia cells, as well as phenotypically and functionally defined LSCs. The cytotoxicity is associated with a rapid loss of membrane integrity, induction of oxidative stress, and inhibition of several signal transduction pathways including NF-κB and FLT3[48].

4 Conclusions

Altogether, these recent investigations have revealed that cancers originate from cancer stem cells. The cancer stem cells can provide critical functions in cancer initiation and progression into metastatic and recurrent disease states. CSCs are often resistant to standard chemotherapy, which make cancer refractory and relapse. The concept of cancer as a stem cell disease has the potential to change significantly the view of the problem of drug resistance. Research efforts to discover the specific signal pathway serving to resistance of CSCs should lead to more effective and safe cancers therapeutic treatments for ultimately curing cancer. Future studies will focus on the identifying and targeting of critical signal pathway to overcome the drug resistance of CSCs for improvement of the current cancer treatments.

Reference


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