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*Review*

# Leukemic stem cells: from metabolic pathways and signaling to a new concept of drug resistance targeting

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**Cancer stem cells are a small subset of cancer cells constituting a reservoir of self-sustaining cells with the exclusive ability to self-renew and maintain the tumor. These cells are identified by specific stem cell markers: antigens, molecules and signaling pathways. Transcription factors and molecules associated with oncogenesis, such as NF-κB, Bmi-1, Notch, WNT beta-catenin, Sonic hedgehog and their biochemical pathways, active only in a small minority of cancer cells might play key roles in determining the biology and the overall long-term behavior of a tumor. The molecules and pathways specific for cancer stem cells, which contribute to their drug resistance, are potential targets for new therapeutic strategies.**

**Keywords**: cancer stem cells, leukemic stem cells, drug resistance, metabolic pathways, stem cell markers

## THE CANCER STEM CELL HYPOTHESIS

The observation of similarities between the self-renewal mechanisms of stem cells and of cancer cells has led to the new concept of the cancer stem cell. There are more and more data showing that the differentiation features of a tumor, morphological and architectural, are the key parameters used in routine clinical practice to define a tumor's primary origin. Tumors are not only monoclonal expansions of cells but might be sustained by a diseased "cancer stem cell" (CSC) population, which is endowed with the ability to self-renew and undergo aberrant differentiation (Reya *et al.,* 2001; Clarke & Fuller, 2006).

The cancer stem cells constitute a small subset of cancer cells being a reservoir of self-sustaining cells with the exclusive ability to self-renew and maintain the tumor. These cancer stem cells have the capacity to both divide and expand the cancer stem cell pool and to differentiate into the heterogeneous cancer cell types that in most cases appear to constitute the bulk of the cancer cells within the tumor.

Cancer stem cells, including leukemic stem cells (LSC), are supposed to arise from two potential pathways: (A) a stem cell losing growth regulation could directly become a cancer stem cell, or (B) a mature (i.e., differentiated or committed) cell could acquire the properties of self renewal and become a cancer stem cell (Cozzio *et al.,* 2003; Korbling & Estrov, 2003; Jamieson *et al.,* 2004; Dean, 2006). A fraction of cells in a tumor are known to survive radiation treatment and cytotoxic drug exposure (Dean, 2006). Stem cells express drug transporters, DNA repair systems, and are refractory to programmed cell death. All these properties would allow cancer stem cells to resist our efforts to eliminate them (Zhou *et al.,* 2001; Kim *et al.,* 2002; Scharenberg *et al.,* 2002).

## IDENTIFICATION OF STEM CELLS

Most cells accumulate the fluorescent dyes Hoechst 33342 and rhodamine 123, but stem cells do not, as these compounds are effluxed by multi-

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**Abbreviations**: AML, acute myelogenous leukemia; CML, chronic myeloid leukemia; CSC, cancer stem cell(s); HSC, hematopoietic stem cell; LSC, leukemic stem cell; SP, side population.

drug resistance proteins ABCG2 (BCRP) and ABCB1 (PGP), respectively. Thus, stem cells can be sorted out from among a population of non-stem ones because only they do not accumulate these fluorescent dyes. These cells might contain only a low level of Hoechst 33342 fluorescence and are referred to as "dull cells" or "side population" (SP) cells. The term side population was coined because during flow cytometry analysis, SP cells are visualized as a negatively stained "side population" to one side of the majority of cells on a density dot plot. A large fraction of hematopoietic stem cells (HSC) are found in the SP fraction. These SP cells can reconstitute the bone marrow (Goodell *et al.,* 1996). SP cells can be isolated from many tissues, including the brain, breast, lung, heart, pancreas, testes, skin and liver, and these cells might represent lineage-specific stem cells (Goodell *et al.,* 1996; Zhou *et al.,* 2001; Asakura & Rudnicki, 2002; Lechner *et al.,* 2002; Alvi *et al.,* 2003, Summer *et al.,* 2003, Terunuma *et al.,* 2003; Martin *et al.,* 2004; Lassalle *et al.,* 2004). However, currently there is no experimental evidence to support the claim that cancer stem cells from human solid epithelial tumors are selectively comprised within SP populations, and there is no robust information on their expression of multidrug resistance proteins. Hoechst 33342 staining of bone marrow from ABCG2-null mice fails to detect SP cells. However, the lack of SP cells in this case occurs not because these cells are absent, but because the lack of ABCG2 expression, which causes the accumulation of Hoechst dye (Dean *et al.,* 2005).

Some of the stem cell markers are distributed widely throughout different tissues. Examples of broadly expressed stem cell markers are CD133, CXCR4, BMI-1, Musashi-1 antigen, and Oct-4 (Majka *et al.,* 2005; Dean, 2006; Dalerba *et al.,* 2007). Although stem cells have been discovered in many tissues, little is known regarding their phenotype. An exception is the hematopoietic stem cell (HSC), which represents a well defined cell in terms of function and phenotype. HSC is characterized as a Lin-negative/CD45-positive/CD34-positive/CD38-negative cell.

## STEM CELL MARKERS

All the different hematopoietic lineages can be fully and permanently reconstituted by transplantation of a very small population of cells, representing as little as 0.05% of total bone marrow. This cell population is characterized by a specific surface marker phenotype that, remarkably, is negative for expression of all lineage-specific differentiation antigens (Lin-negative) (Spangrude *et al.,* 1988). Studies of acute myelogenous leukemia have shown that only 0.1–1% of all cells have leukemia-initiating activity (Lapidot *et al.,* 1994).

Stem cell are not defined based solely on surface markers. None of the markers used to isolate stem cells in various normal and cancerous tissues is expressed exclusively by stem cells. For example, CD34 is present both on HSC and on acute myelogenous leukemia (AML) stem cells. CD133 was used to successfully enrich for brain tumor stem cells, but it is also present on normal brain stem cells and on many non-stem cells in various tumors and tissues. The same is true for other commonly used markers, such as CD44, Sca1, and Thy1. The vast majority of cells that express these markers are not stem cells. In addition, markers used to identify stem cells from one tissue are frequently not useful for identifying stem cells in other tissues: Sca-1 is useful for the identification of murine blood stem cells, but it is not consistently expressed by murine mammary duct stem cells. A given marker may or may not be useful for identifying stem cells from other tissues or tumor types (Clarke *et al.,* 2006).

A common immunophenotype of leukemic stem cells with self-renewal potential has been identified. The surface phenotype of LSC in human AML is characterized by the CD34+CD38- phenotype in many AML subtypes, i.e. M1, M4, M5 (Bonnet & Dick, 1997). In most cases their phenotype can be further specified as CD34+/CD38-/CD123+, while the CD123 antigen is also the $\alpha$ chain of IL3R (Jordan *et al.,* 2000).

Another study demonstrated that most AML stem cells are quiescent (Guan *et al.,* 2003). This finding means that these cells will survive standard chemotherapy directed to dividing cells. Isolation of quiescent leukemic stem cells was also reported in chronic myeloid leukemia (CML) (Holyoake *et al.,* 1999). Treatment of CML patients with imatinib mesylate, which is otherwise highly effective in induction of remission, only suppresses the disease, since the drug is not able to destroy the leukemic stem cells, which are the root cause of the CML (Graham *et al.,* 2002; Bhatia *et al.,* 2003).

## THE LEUKEMIC STEM CELLS

Primitive human LSC populations can be selected by cell surface CD34+/CD38-/CD123+ antigens. These cells are almost entirely quiescent, mimicking normal stem cells. As a result, agents that are active in dividing cells will not be effective in the quiescent population. As an alternative, it might be appropriate to consider as the target, other unique mechanisms that maintain the viability and survival of these cells. Two such pathways have been suggested: the PI3 kinase pathway (Xu *et al.,* 2003)

and NF-κB (Guzman *et al.,* 2002), which are evident in LSC. Inhibiting these two pathways might have therapeutic relevance. Purified populations of LCS have been characterized by activation of the NF-κB pathway. Normal hematopoietic stem cells do not show activation of NF-κB. This is only a leukemia-specific phenomenon. While no particular mutations or specific genetic events are associated with activation of the NF-κB or PI3-kinase pathways, converging events, such as multiple different mutations, may feed into these pathways. However, such pathway modification may not be the only mechanism to produce leukemia.

## MOLECULES AND SIGNALING PATHWAYS OF STEM CELL

Biochemical pathways that are active in the majority of tumor cells might be of little functional relevance for the biology of CSC, whereas biochemical pathways active only in a small minority of cancer cells might play key roles in CSC biology and thus in the overall long-term behavior of a tumor (Dalerba *et al.,* 2007). Differential expression of several transcription factors controls the fate of HSC and plays a critical role in the determination of self-renewal, differentiation, and lineage commitment. These pathways are under the control of various intracellular stimuli as well as cytokines and stromal factors from adjacent cells in the bone marrow microenvironment.

It was found that the Bmi-1, Notch, WNT and Sonic hedgehog pathways, tumor suppressor genes and oncogenes are involved in regulation of self-renewal of both normal and cancer stem cells (Spink *et al.,* 2000; Taipale & Beachy, 2001; Andl *et al.,* 2002; Jamora *et al.,* 2003; Willert *et al.,* 2003). Epigenetic signals play an important role, such as modification of chromatin structure, histone deacetylation, etc. Several studies suggest that epigenetic reprogramming is responsible for the loss of the neoplastic cell capacity to form tumors (Surani, 2001; Li *et al.,* 2003a). The stem cell origin will determine the tumor type, with contributions by the genetic alternations of the individual and microenvironmental influences.

Transcription factors and cell cycle regulators associated with oncogenesis, such as Bmi-1 and Sonic hedgehog (SHH), may play roles in the regulation of proliferation of both HSC and LSC (van der Lugt *et al.,* 1996; Taipale & Beachy, 2001). The *BMI-1* oncogene is a member of the Polycomb group ring finger (*PCGF*) gene family. Its transcriptional activity was shown to be high in HSC and progressively down-regulated during hematopoietic differentiation (Lessard *et al.,* 1998; Dimri *et al.,* 2002). It is highly expressed in purified HSC and its expression declines with differentiation (Park *et al.,* 2003). Bmi-1 seems to regulate stem cell renewal by modulating other genes that are important in cellular functions such as proliferation, survival, and lineage commitment (Park *et al.,* 2003). BMI-1 has an essential role in regulating the proliferative potential of leukemic stem cells (Lessard & Sauvageau, 2003). Although direct evidence for the role of SHH in the regulation of stem cell renewal is lacking, *in vitro* studies have shown increased self-renewal of HSC in response to SHH, albeit in combination with other growth factors (Bhardwaj *et al.,* 2001). Another molecule that is likely to play a key role in the molecular machinery of both HSC and LSC self-renewal is the protein phosphatase and tensin homologue (PTEN), a known tumor suppressor (Di Cristofano & Pandolfi, 2000). Genes required for self-renewal of normal HSC can play opposite roles in the development of leukemia. In some cases they are necessary for long-term expansion of the transformed clone (*BMI-1*), but in others they act as tumor suppressors and prevent leukemic transformation (*PTEN*).

Other transcription factors such as the Homeobox (HOX) family members, including HOXB4, as well as the WNT signaling pathway have well-described roles in regulating the self-renewal and differentiation of HSC (Reya *et al.,* 2003; Zhu *et al.,* 2003). HOXB4 promotes the expansion of HSC without losing their ability to differentiate into normal lymphoid and myeloid cells (Sauvageau *et al.,* 1995). It is abundantly expressed in HSC but declines as terminal differentiation proceeds (Sauvageau *et al.,* 1995). Notably, deregulated expression of HOX family members such as HOXA9 is commonly observed in AML (Golub *et al.,* 1999; Lawrence *et al.,* 1999). The WNT signaling pathway has been shown to be critical to the development of several organs and recent studies have illustrated its important role in the regulation of hematopoietic stem and progenitor cell function (Reya *et al.,* 2003; Staal & Clevers, 2005).

The NOTCH protein is another protein important to the growth and differentiation of stem cells. NOTCH is processed by the enzyme γ-secretase, the same enzyme that processes the APP protein important in Alzheimer disease. The NOTCH/Jagged pathway is important in regulating the integration of extracellular regulatory signals controlling HSC fate. Ligand binding leads to proteolytic cleavage and transport of the intracellular domain of NOTCH into the nucleus, where it is a transcription factor. In humans, contrary to what is observed in *Drosophila*, the NOTCH family of proteins comprises at least four members, and NOTCH-1 is the protein involved in HSC self-renewal and mutated in T-lineage acute lymphoblastic leukemia (Ellisen *et al.,* 1991). Members of the NOTCH family have critical roles in keeping HSC in an undifferentiated state

and may act as gatekeepers for factors governing self-renewal and lineage commitment (Ellisen *et al.*, 1991; Pui *et al.*, 1999).

Overexpression of β-catenin, a downstream activator of the WNT signaling pathway, expands the transplantable HSC pool in long-term cultures (Reya *et al.*, 2001). Furthermore, activation of WNT signaling also increases the expression of other transcription factors and cell cycle regulators important in HSC renewal, such as HOXB4 and NOTCH-1 (Reya *et al.*, 2003; Duncan *et al.*, 2005).

One additional pathway important for the growth and differentiation of stem cells is the Hedgehog-Patched (HH-PTCH) pathway. Studies of the HH-PTCH pathway in tumors provide support for the importance of tumor stem cells in cancer, indicating that proliferation of normal stem cells is regulated by signals from surrounding normal cells. Transformation of these stem cells can lead to a premalignant stem cell with abnormal HH expression or deficient PTCH activity. Such cells can grow in an unrestrained manner, leading to local overgrowth. Additional genetic events give rise to a tumor stem cell that can generate more tumor stem cells as well as mature tumor cells. This model leads to specific hypotheses that can be tested as well as new avenues for therapeutics (Dean *et al.*, 2005).

Studies on *Drosophila* and other developmental systems have identified regulatory pathways operative in embryonic cells (Nusslein-Volhard & Wieschaus, 1980). One such pathway, involving the Hedgehog (HH) and WNT signaling molecules, contains a large number of genes that can act as tumor suppressor genes or oncogenes in mammalian cells (Dean, 1997). Thus, Patched (*PTCH*) codes for the receptor that binds HH molecules and is mutated in patients with nevoid basal cell carcinoma syndrome (Hahn *et al.*, 1996; Johnson *et al.*, 1996). *PTCH* is also mutated in nearly all sporadic basal cell carcinomas and in some medulloblastomas (Bale & Yu, 2001). The *HH* genes (sonic hedgehog (*SHH*), Indian hedgehog (*IHH*), and desert hedgehog (*DHH*)) are overexpressed in a wide variety of cancers, including small-cell lung, pancreas, gastric, breast, and prostate (Berman *et al.*, 2003; Thayer *et al.*, 2003; Watkins *et al.*, 2003; Karhadkar *et al.*, 2004). HH family overexpression and *PTCH* mutation both have the effect of constitutive action of SMO (smoothened), a G protein–coupled receptor that is a key signaling component of the pathway. Constitutive HH family expression could lead to stem cell activation and appears to be a common feature of many cancers.

The *in vivo* expression of human telomerase reverse transcriptase (hTERT) has repeatedly proven to be extremely heterogeneous among cancer cells (Yan *et al.*, 2004; Dalerba *et al.*, 2005). hTERT expression in epithelial cells can be upregulated by the Bmi-1 protein during stem cell renewal (Dimri *et al.*, 2002).

Recent research has delineated molecular pathways that regulate the self-renewal capacity of HSC. Self-renewal is the hallmark property of stem cells in both normal and neoplastic tissues. Several genes which encoded transcription factors have been identified, as well as cell cycle regulators that modulate the self renewal and differentiation of HSC (Zhu & Emerson, 2002; Stein *et al.*, 2004). Genes such as *SCL*, *GATA-2*, *LMO-2*, and *AML-1* (also known as *CBFA2* or *RUNX1*) govern the transcriptional regulation of early hematopoiesis, and the deregulation of these genes through chromosomal aberrations leads to several hematopoietic malignancies. The gene encoding the transcription factor SCL is the most frequent target of chromosomal rearrangements in children with T-cell acute lymphoblastic leukemia (Lecuyer & Hoang, 2004). SCL is normally expressed in HSC and immature progenitors and is down-regulated as differentiation proceeds. As a result of chromosomal translocations, SCL is inappropriately expressed and, through collaboration with other oncoproteins, initiates malignant transformation (Lecuyer & Hoang, 2004). Similarly, transcriptional activation of the *AML-1* gene is required for definitive hematopoiesis. As a result of translocation t(8;21), the fusion protein AML-ETO, which is the result of one of the most frequent chromosomal abnormalities in AML, is generated (Licht, 2001). Constitutive expression of AML-ETO has been shown to increase the rate of self-renewal in stem cells (Mulloy *et al.*, 2002). Of interest, such increased self-renewal is of no apparent pathogenic consequences, presumably because secondary mutations are necessary for the formation of the leukemic phenotype (de Guzman *et al.*, 2002).

## MECHANISMS OF MULTIDRUG RESISTANCE

Regarding possible mechanisms of drug resistance, an almost endless number can be envisaged along the signal transduction pathways triggered by these drugs. The most important groups of drug resistance mechanisms is shown in Fig. 1. Although chemotherapy kills most cells in a tumor, it probably leaves tumor stem cells behind, which might be an important mechanism of resistance. For example, the ATP-binding cassette (ABC) drug transporters have been shown to protect stem cells from chemotherapeutic agents (Styczynski *et al.*, 2002; Borowski *et al.*, 2005; Dean *et al.*, 2005). In a wide variety of stem cells, the ABC transporters are expressed causing drug resistance (Zhou *et al.*, 2001; Styczynski, 2007; Styczynski *et al.*, 2007). Normal HSC possess several characteristics that protect them from potential insults. LSC have similar properties, including

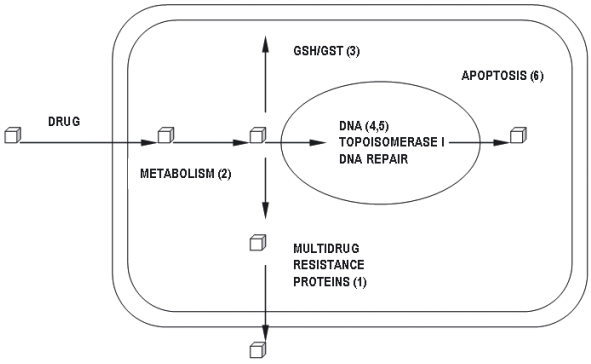


**Figure 1. Main drug resistance mechanisms.**
1. Multidrug resistance proteins. 2. Drug metabolism. 3. Glutathione and its transferases. 4. Molecular target. 5. DNA repair. 6. Apoptosis and its regulation.

quiescence (Guan *et al.,* 2003), resistance to drugs and toxins through the expression of ATP-associated transporters (Dean *et al.,* 2005), and resistance to apoptotic stimuli (Konopleva *et al.,* 2002).

## ROLE OF ABC PROTEINS IN DRUG RESISTANCE

One of the innate resistance mechanisms of stem cells is the expression of one or more out of 48 identified ATP-binding cassette (ABC) transporters (Table 1). These pumps play a role in protecting stem cells from xenobiotic toxins (Gottesman *et al.,* 2002; Dean *et al.,* 2005; Steinbach *et al.,* 2006; Steinbach & Legrand, 2007). Stem cells have many properties that distinguish them from mature, differentiated cells. In addition to their ability to self-renew and differentiate, they are quiescent, dividing infrequently. They also require specific environments comprising other cells, stroma and growth factors for their survival (Blanpain *et al.,* 2004). One particularly intriguing property of stem cells is that they express high levels of specific ABC drug transporters. For example, hematopoietic stem cells express high levels of ABCG2, while the *ABCG2* gene is turned off in most committed progenitor and mature blood cells (Scharenberg *et al.,* 2002). The two ABC transporter-encoding genes that have been studied most extensively in stem cells are *ABCB1*, which encodes P-glycoprotein (Gottesman *et al.,* 2002), and *ABCG2* (Doyle *et al.,* 1998; Miyake *et al.,* 1999; Kim *et al.,* 2002; Scharenberg *et al.,* 2002). *ABCG2* and *ABCB1/MDR1* genes are active in the vast majority of stem cells and in most tumor stem cells (Zhou *et al.,* 2001; Kim *et al.,* 2002; Scharenberg *et al.,* 2002). These transporters can efflux fluorescent dyes such as rhodamine and Hoechst 33342, and this property allows the stem cells to be separated from non-stem cells on a cell sorter (Goodell *et al.,* 1996). The combined use of chemotherapy drugs and ABC transporter inhibitors could be emplyed to specifically target cancer stem cells (Dean, 2005; Dean & Annilo, 2005; Dean *et al.,* 2005). There are already highly specific inhibitors of ABCB1 (PGP) in clinical use and ABCG2 (BCRP) inhibitors in development (Nowak *et al.,* 2005; Pleban *et al.,* 2005; Henrich *et al.,* 2006). These therapies would be predicted to have toxic effects on the patient's normal stem cells. Both ABCG2 and ABCB1 play a role in the blood-brain barrier, which suggests that this approach would have to be carefully titrated to avoid excessive toxicity.

Along with *ABCC1*, they represent the three principal multidrug-resistance genes that have been identified in tumor cells. Members of the ABC-transporter protein superfamily are promiscuous transporters of both hydrophobic and hydrophilic compounds (Dean *et al.,* 2001; Gottesman *et al.,* 2002). These transporters also have important roles in normal transport of drugs across the placenta and the intestine (more accurately, the retention of drugs in the intestinal lumen), and are important components of the blood–brain and blood–testis barriers. An important physiological role of ABC transporters is protecting cells from toxins. The drug-transporting property within stem cells conferred by these ABC transporters is an important marker for isolation and analysis of hematopoietic stem cells.

**Table 1. Most important multidrug resistance protein belonging to ABC family**

| Gene | Protein | Drugs transported by the protein |
|---|---|---|
| *ABCA2* | ABCA2 | Estramustine |
| *ABCB1* | PGP/MDR | Doxorubicin, etoposide, vinblastine, paclitaxel |
| *ABCC1* | MRP1 | Doxorubicin, daunorubicin, vincristine, etoposide, camptothecine, methotrexate |
| *ABCC2* | MRP2 | Vinblastine, cisplatine, doxorubicin, methotrexate |
| *ABCC3* | MRP3 | Methotrexate, etoposide |
| *ABCC4* | MRP4 | 6-Mercaptopurine, 6-thioguanine, methotrexate and its metabolites |
| *ABCC5* | MRP5 | 6-Mercaptopurine, 6-thioguanine, methotrexate and its metabolites |
| *ABCC6* | MRP6 | Etoposide |
| *ABCC11* | MRP8 | 5-Fluorouracil |
| *ABCG2* | MXR/BCRP | Mitoxantrone, topotecan, doxorubicin, daunorubicin, irinotecan, methotrexate, imatinib |

## MODELS OF DRUG RESISTANCE IN CANCER CELLS

Cancer cells can acquire resistance to chemotherapy by a range of mechanisms, including gene mutation or over-expression of the drug target, inactivation of the drug, or elimination of the drug from the cell. Usually, tumors that recur after an initial response to chemotherapy are resistant to multiple drugs. Several models of drug resistance which can be connected with stem cells have been proposed (Fig. 2).

The first model postulates that a small percentage of cells in a population harbouring intrinsic mutations confer drug resistance (Goldie & Coldman, 1979). This hypothesis would theorize that the cell acquiring the mutation is the stem cell. Although the expression of ABC transporters could render stem cells resistant to drugs, other determinants of resistance might be the DNA-repair capacity of the cell or the reluctance to enter apoptosis. Generally regarded as quiescent and non-dividing, stem cells would be expected to be inherently refractory to drugs that target the cell cycle, especially in rapidly dividing cells. New potent agents are able to overcome this mechanism of resistance. The ability of imatinib to induce apoptosis was unchanged in BCR–ABL-positive cells, previously blocked at G1/S phase. This indicates that imatinib is effective in non-dividing cells as well (Paterson *et al.,* 2003).

The second, conventional model of drug resistance indicates that one or several cells in the tumor population acquire mutations and aberrations that confer drug resistance (Dean *et al.,* 2005). These cells have a selective advantage that allows them to overtake the population of tumor cells following cancer chemotherapy.

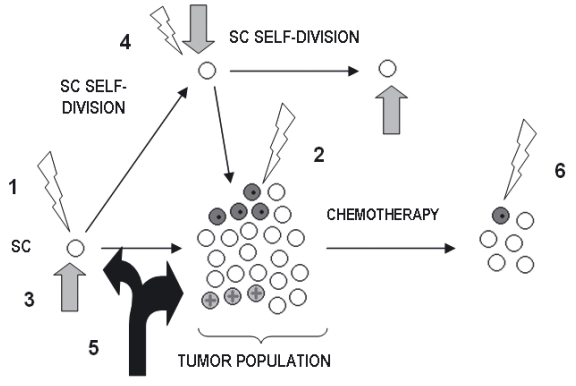


**Figure 2. Models of drug resistance in cancer cells.**
1. Stem cells (SC) harbor intrinsic mutations conferring drug resistance. 2. One or several cells in tumor population acquire mutation that confer drug resistance. 3. Resistance as a natural property of stem cell. 4. Stem cells accumulate mutations over time. 5. Intrinsic drug resistance of SC and differentiated tumor cells. 6. Pluripotent cancer cells surviving chemotherapy develop drug resistance.

The third, alternative model based on the tumor stem cell concept, shows that the cancer stem cells are naturally resistant to chemotherapy through their quiescence, their capacity for DNA repair, and ABC-transporter expression. As a result, at least some of the tumor stem cells can survive chemotherapy and support regrowth of the tumor. Thus, model of resistance suggests that drug-resistant variants of the tumor stem cell or its close descendants arise, producing a population of multidrug-resistant tumor cells that can be found in many patients who have recurrence of their cancer following chemotherapy.

The fourth mechanism, based on the concept that stem cells accumulate mutations over time as a consequence of a long-term exposure to irradiation or chemical carcinogens, and cancer stem cells accumulate mutations that confer drug resistance to their abnormally developing offspring (Reya *et al.,* 2001). Genetic alterations, as those that upregulate ABCB1 expression in human leukemia and lymphoma cells, could have originated in the stem cell (Mickley *et al.,* 1997; Knutsen *et al.,* 1998).

The fifth model indicates that both the stem cells and the variably differentiated cells are inherently drug-resistant, thus therapies have little or no effect, resulting in tumor growth ("intrinsic resistance"). An example of the latter is an intrinsically resistant cancer such as renal cancer, in which ABCB1 is expressed in all cells and contributes to chemotherapy tolerance. In this case, the resistance phenotype of the cancer stem cell persists in the committed, abnormally developing progenitors that comprise the proliferative pool of cancer cells.

In the sixth model, tumors have a built-in population of drug-resistant pluripotent cells that can survive chemotherapy and regrow as cancer relapse (the cancer stem cell model of drug resistance). Normal stem cells can be found in stem cell-driven recovery of normal tissues following chemotherapy. This model can explain the rapid relapse observed clinically. It is also related to the repopulation of the bone marrow by normal hematopoietic stem cells or the recovery of the mucosa of the gastrointestinal tract, both of which usually occur within one 3-week cycle (Cotsarelis & Millar, 2001; Sato *et al.,* 2001).

## TARGETING DRUG RESISTANCE OF CANCER STEM CELLS

The cancer stem cell hypothesis suggests that therapeutic approaches that do not eradicate the CSC compartment are not likely to achieve success. Only a majority of tumor cells might be killed and temporary regression can be achieved but this therapy will fail to prevent relapse or metastatic disease (Reya *et al.,* 2001; Dalerba *et al.,* 2007). In patients with acute

myeloid leukemia, both normal and cancer stem cells mainly appear to be in a quiescent, nondividing, G0 state, thus being inherently resistant to the toxic effect of traditional chemotherapeutic regimens (Morrison & Weissman, 1994; Guzman *et al.*, 2002; Guan *et al.*, 2003). Some agents, such as busulfan, are able to target cells in G0 state, causing a myeloablative effect. Ionizing radiation or busulfan can cause premature senescence of normal hematopoietic stem cells (Narita & Lowe, 2005; Wang *et al.*, 2006). The only rescue in these situations is hematopoietic stem cell transplantation.

The properties of leukemic stem cells indicate that current chemotherapy drugs will not be curative. The use of current cytotoxic agents is not effective in leukemia because the agents target both the leukemic and normal stem cell populations (Jordan, 2007). Such agents as cytarabine, anthracyclines, alkylating agents, nucleoside analogs, and topoisomerase inhibitors currently used in the treatment of acute leukemia show no activity with isolated LSC (Kantarjian *et al.*, 1996; Li *et al.*, 2003b). Since the nature of the LSC may vary depending upon the stage during which it arose, also the drug resistance and various characteristics that are relevant to therapy may differ, based on the origin of the malignant cell (Jordan, 2007).

Possibly, normal stem cells and progenitor cells are more sensitive than cancer stem cells to chemotherapy. DNA repair mechanisms within normal colon stem cells can be inhibited and thereby these cells undergo apoptosis in response to DNA damage. Due to this mechanism cells are protected from the accumulation of harmful mutations (Cairns, 2002).

## STRATEGIES TO OVERCOME LSC RESISTANCE TO THERAPY

The primary challenge in developing treatment strategies targeted toward LSC is to identify pro-apoptotic stimuli that spare the normal HSC, while exerting a cytotoxic effect on LSC. A primary concern in the development of tumor stem cell-specific drugs is to overcome the inherent drug efflux pumps that are highly expressed in LSC. Several agents effective in inhibiting the ATP-binding cassette transporters have been studied and found to have limited clinical efficacy, such as cyclosporine or zosuquidar (List *et al.*, 2001; Baer *et al.*, 2002; Styczynski & Wysocki, 2006). The biggest obstacle to this approach is the similarly high expression of these transporters in normal HSC, making them equally susceptible to these inhibitors (Dean *et al.*, 2005).

Strategies directed at pathways that specifically regulate LSC survival would probably be more efficient (Jordan & Guzman, 2004). The identification of survival pathways that are preferentially over-expressed in LSC suggests that differential activation of apoptosis mechanisms in LSC should be possible (Guzman *et al.*, 2001; 2002; 2005). The transcription factor NF-κB was found to be constitutively activated in LSC but not in normal HSC (Guzman *et al.*, 2001). The antileukemic agent idarubicin is an NF-κB inhibitor used in experimental models (Guzman *et al.*, 2001; 2002). Another potent inhibitor of NF-κB, parthenolide, can induce apoptosis in LSC while sparing normal HSC (Guzman *et al.*, 2005). Constitutive activation of phosphatidylinositide-3 kinase is also necessary for LSC and its pharmacologic inhibition by LY294002 leads to a dose-dependent decrease in survival (Xu *et al.*, 2003). Expression of transcription factors and their regulation by aberrant signaling pathways can influence the survival of LSC (Tani *et al.*, 1996; Blair & Pamphilon, 2003).

## CONCLUSIONS

The concept of cancer as a stem cell disease has the potential to change significantly the view of the problem of drug resistance. There is now abundant evidence that stem cell properties are highly relevant to the biology of several human cancers. By separating the disease into a stem cell activation phase and a tumor progression phase, historical cancer studies can be reinterpreted with new understanding. Research efforts to discover the rules that govern the growth of tumor stem cells as well as to identify tumor stem cell antigens could lead to new targeted approaches. Cancer diagnostics, prevention, and therapeutics are likely to be greatly influenced by this new insight.

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