Stem cells in malignancy

Stem cells in the adult organism are responsible for tissue renewal and repair of aged or damaged tissue. The hypothesis of stem cells as a “spring” of cancer cells has come from Fialkow et al. and Hamburger and Salmon (1, 2). The concept of cancer as a stem cell disease has the potential to dramatically change our view of cancer management (3). This study is aimed to review the possibilities of targeting the growth of cancer stem cells, including analysis of the most promising compounds leading to more efficient anti-cancer therapy in the view of multiple drug resistance. Cancer stem cells are probably refractory to therapies that have been developed to eradicate the tumor which majority is a non-stem cell compartment. The cancer stem cell hypothesis will influence on the diagnosis and cancer treatment. The question is; should we turn from eliminating the bulk of rapidly dividing but terminally differentiated tumor cells and be refocused on the minority stem cell population that fuels tumor growth?

Signal transduction within the stem cell

Biochemical pathways that are active in the majority of tumor cells might be of minor relevance for the biology of CSCs (Cancer Stem Cells), whereas biochemical pathways active only on a small minority of cancer cells might play key roles in CSCs biology and thus in the overall long-term behavior of a tumor (4).

The BMI1 oncogene, a member of the Polycomb group ring finger (PCGF) gene family, was shown to be expressed at high levels in HSCs (Hematopoietic Stem Cells) and progressively down-regulated during hematopoietic differentiation (5, 6). A second molecule that is likely to play a key role in the molecular machinery of both HSC and LSC (Leukemia Stem Cells) self-renewal is the protein phosphatase and tensin homologue (PTEN), a known tumor suppressor (6). Genes required for self-renewal of normal HSCs 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). 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 (7-9). Additional studies implicate the Wnt beta-catenin pathway in the
maintenance of stem-cell self-renewal in other tissues as well (10, 11). Hedgehog (HH) and WNT signaling molecules, contains a large number of genes that can act as tumor suppressor genes or oncogenes in mammalian cells (12). For example, Patched (PTCH) codes for the receptor that binds HH molecules and is mutated in patients with nevoid basal cell carcinoma syndrome (13, 14). PTCH is also mutated in nearly all sporadic basal cell carcinomas and in some medulloblastomas (15). The mammalian HH genes, i.e. sonic hedgehog (SHH), Indian hedgehog (IHH), and desert hedgehog (DHH) are over-expressed in a wide variety of cancers, including small-cell lung, pancreas, gastric, breast, and prostate (16-19). HH family over-expression and PTCH mutation both have the effect of constitutive action of smoothened (SMO) 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.

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 proliferation. This model leads to hypothesis that directly targeting the growth of stem cells could be promising in cancer management (20).

**Stem cells, multi-drug resistance and cancer disease relapse**

It is generally accepted that normal stem cells show properties that provide for a long lifespan such as relative quiescence, resistance to drugs and toxins through the expression of several ATP binding cassette (ABC) transporters, an active DNA repair capacity, and a resistance to apoptosis. One particularly intriguing property of stem cells is that they express high levels of specific ABC drug transporters. ABC drug transporters are responsible for drug resistance following chemotherapy (Table 1). For example, hematopoietic stem cells express high levels of ABCG2, but the gene is turned off in most committed progenitor and mature blood cells. The two ABC transporter-encoding genes that have been studied most extensively in stem cells are ABCB1, which encodes P-glycoprotein, and ABCG2 (21-25). Along with ABCC1, they represent the three principal multidrug-resistance genes that have been identified in tumor cells. These genes, members of the ABC-transporter superfamily, are promiscuous transporters of both hydrophobic and hydrophilic compounds (26, 27). Important physiological role for these ABC transporters in protecting cells from toxins.

It follows that cancer stem cells might also possess these resistance mechanisms. The paradigm that drug resistance originates in the stem-cell phenotype might stimulate new strategies for the development of anticancer therapies. For example, non-stem-cell tumor cells often express ABCG2 and ABCB1. These proteins are highly expressed in drug-resistant cells, and histopathological studies have reported increased expression of the ABCB1 transporter in more differentiated tumors (28, 29). In addition, in a range of cell lines, differentiating agents induce expression of ABCB1, inhibit cell growth, and increase the expression of markers of maturation (30, 31). Leukemic stem cells (LSCs) are in quiescence state (32), resistance to drugs and toxins through the expression of ATP-associated transporters (20), and resistance to apoptotic stimuli (33). Cytotoxic chemotherapy eliminates most cells in a tumor, but cancer stem cells survive due to their relative high resistance to drugs and because of their silent replication. Despite the small number of such cells, their property of being immortal is expected to be sufficient to allow tumor recurrence. The relapse can occur many years after initial treatment by means of chemotherapy or radiotherapy. It is hypothesized that if tumors are derived from an early stem cell or its progenitor cells, the metastases are formed readily and are phenotypically more heterogeneous. Metastases derived from a later stem cell are more homogenous and have more restricted metastatic potential (34). Cellular heterogeneity of metastases, their growth in distinct areas of body under different environment might be a consequence of cancer stem cell differentiation and/or dedifferentiation. The CSC model can also shed new light on the biology of metastases and explain why, despite extensive intra-tumor heterogeneity (35, 36), comparison of paired samples of primary tumors and autologous lymph node and/or distant-site metastases usually reveals striking similarities over a wide range of parameters, including tissue morphology (35, 36), repertoire of somatic genetic mutations (37-39), expression of tumor-suppressor and immunomodulatory proteins (40), expression of epigenetically controlled genes (41), and overall tran-
scriptional profile as defined by gene expression arrays (42, 43).

**Overcoming drug resistance**

It was thought that drug resistance could be avoided and tumor cells eliminated by inhibiting the main transporters of chemotherapy drugs. Therefore, much effort has been devoted to the development of inhibitors of ABC transporters. First-generation compounds included drugs identified as ABCB1 inhibitors, such as verapamil and cyclosporine (multi-protein, universal inhibitor), that were used to treat other diseases. These inhibitors were combined with a range of chemotherapy regimens for many cancers (27). As the results were not convincing, subsequent clinical trials were attempted with second-generation inhibitors such as PSC 833 and VX-710. The results of these trials were largely negative, because of pharmacokinetic interaction between the chemotherapeutic agent and the ABCB1 inhibitor in some cases. These studies might also have failed because of the presence of additional transporters, such as ABC1 and ABC2, that were not inhibited. Although the results of these trials were negative, correlative studies did show that transport by ABCB1 could be inhibited. Efflux activity was assessed with a radionuclide-imaging agent (99mTc-Sestamibi), confirming that some human tumors have ABCB1 activity that can be suppressed with the ABCB1 inhibitors VX 710, PSC 833 and tariquidar (XR9576) (44-47). The increased 99mTc-Sestamibi retention in the entire tumor following treatment with tariquidar indicates that the transporter expressing phenotype of the cancer stem cell persists in the committed, abnormally developing progenitors that comprise the proliferative pool of cancer cells. Third generation inhibitors include zosuquidar.

As cancer stem cells express drug transporters that make them resistant to many chemotherapy agents, anticancer strategies should include efforts to target these cells with their special properties. Clinical studies have attempted to overcome drug resistance through combination therapies in which a cytotoxic drug was given along with an ABC-transporter inhibitor. In a new paradigm, transport inhibitors might be thought of as tumor stem cell sensitizing agents, that allow the most crucial and most drug-resistant cells in a tumor to be destroyed. If the stem cells are the main mediators of drug resistance, ABC inhibitors would not necessarily reduce tumor burden immediately. Thus, the efficacy could be observed using alternative end points, such as the frequency of relapse or the time to relapse.

However, it is possible that the cytotoxic drugs or ABC inhibitors tested were inefficient in killing cancer stem cells. An inhibitor of drug transport might be most beneficial when combined with an anticancer agent that specifically targets the stem cells, such as imatinib, which targets the leukemia stem cells that carry the BCR-ABL fusion protein. Another potential reason that clinical trials involving drug-transport inhibitors have not proven successful is that the wrong transporter was inhibited. Most of the studies evaluating cells with the SP (side population) phenotype have shown that stem cells overexpress ABCG2, rather than ABCB1, which has been the transporter targeted in most studies.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Drugs transported by the protein</th>
<th>Other drugs and substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1</td>
<td>PGP/MDR</td>
<td>Doxorubicin, etoposide, vinblastine, paclitaxel</td>
<td>Digoxine</td>
</tr>
<tr>
<td>ABCC1</td>
<td>MRP1</td>
<td>Doxorubicin, daunorubicin, vincristine, etoposide, camptothecine, methotrexate</td>
<td>Rhodamine</td>
</tr>
<tr>
<td>ABCC2</td>
<td>MRP2</td>
<td>Vinblastine, cisplatin, doxorubicin, methotrexate</td>
<td>Sulfirpyrazone</td>
</tr>
<tr>
<td>ABCC3</td>
<td>MRP3</td>
<td>Methotrexate, etoposide</td>
<td></td>
</tr>
<tr>
<td>ABCC4</td>
<td>MRP4</td>
<td>6-mercaptopurine, 6-thioguanine, methotrexate and its metabolites</td>
<td>cAMP, cGMP</td>
</tr>
<tr>
<td>ABCC5</td>
<td>MRP5</td>
<td>6-mercaptopurine, 6-thioguanine, methotrexate and its metabolites</td>
<td>cAMP, cGMP</td>
</tr>
<tr>
<td>ABCC6</td>
<td>MRP6</td>
<td>Etoposide</td>
<td></td>
</tr>
<tr>
<td>ABCG2</td>
<td>MXR/BCRP</td>
<td>Mitoxantrone, topotecan, doxorubicin, daunorubicin, irinotecan, methotrexate, imatinib</td>
<td>Hoechst 33342, Rhodamine</td>
</tr>
</tbody>
</table>
Circumvention of drug resistance

The treatment of acute myeloid leukemia (AML) often failed, because anthracyclines, alkylating agents, nucleoside analogs, and topoisomerase inhibitors may not target leukemic stem cells (LSCs) very effectively (52). For example, Ara-C, which is a cycle-active agent commonly used in treating leukemia, shows virtually no activity with isolated LSCs (53). From a therapeutic perspective, the nature of the LSC may vary depending upon the stage during which it arose. Accordingly, drug resistance and various characteristics that are relevant to therapy may also differ, based on the origin of the neoplastic cell (54). The properties of leukemic stem cells indicate that current chemotherapeutic drugs will not be effective. The use of current cytotoxic agents is not effective in leukemia because the agents target both the leukemic and normal stem cell populations (54).

Evaluation of treatment efficacy

Traditionally, anti-tumor treatments are screened based on their capacity to induce a clinical response (i.e., a dramatic regression, either complete or partial, of the tumor lesion). This approach, however, tends to select for treatments that are active on the bulk of tumor cell populations but not necessarily on CSCs. From a purely theoretical point of view, anti-tumor treatments that selectively target the CSC subset might actually be unable to induce rapid shrinkage of tumor masses but might eliminate their capacity for long-term growth and therefore cause their arrest or slowly reduce their size. It is therefore likely that, alongside new treatment strategies, new approaches for the preclinical evaluation of their efficacy will need to be devised (4). Consequently, new strategies are required that specifically and preferentially target the malignant stem cell population, while sparing normal stem cells (54). More and more data support the concept that anti-neoplastic therapies are curative only when all cancer stem cells in a given tumor are eliminated. This new concept may soon change dramatically our view regarding the treatment of solid tumors. Significant effort is directed on evaluation of target expression profiles in cancer stem cells in various tumors and leukemias and on the potential of tumor stem cells to escape therapy through differential expression of targets, plasticity and quiescence (55).

Therapeutic CSC targeting

According to the CSC model, therapeutic approaches that do not eradicate the CSC compartment are likely to achieve little success; they might kill the majority of tumor cells and induce temporary regression of gross tumor lesions but fail to prevent disease relapse and metastatic dissemination (4, 56). In support of this hypothesis is the finding that, in the hematopoietic system, both normal stem cells and CSCs (ie. hematopoietic and leukemic stem cells from AML patients) mainly appear to be in a quiescent, non-dividing, G0 state, and therefore inherently resistant to the toxic effect of traditional chemotherapeutic regimens (32, 57, 58).

Although stem cells can self-renew, they are generally quiescent, spending most of their time in G0 phase. Due to self-renew properties, stem cells can repair their DNA. Unfortunately, they have also the potential to accumulate mutations acquired after exposure to carcinogens (20).

A further concern is that normal stem cells and progenitor cells may prove to be more sensitive than cancer stem cells to the effects of chemotherapy. Normal colon stem cells, for example, can inhibit DNA repair mechanisms and thereby undergo apoptosis in response to DNA damage; this mechanism avoids the accumulation of harmful mutations (59). If, however, colon cancer cells evade this protective mechanism, then chemotherapy could preferentially spare them. Recent studies have demonstrated that normal hematopoietic stem cells undergo premature senescence (ie., cellular “aging”) when exposed to ionizing radiation or busulfan (60, 61).

Implications for cancer therapy: opportunities and challenges

The cancer stem cell hypothesis posits that cancer stem cells are a minority population of self-renewing cancer cells that fuel tumor growth and...
Strategies to overcome resistance to chemotherapy

As implied by the multi-step theory of carcinogenesis, leukemic stem cells (LSCs) are likely to have the fewest number of molecular aberrations in the population of the malignant cells and, as such, biologically most similar to normal, hematopoietic stem cells (HSCs). The primary challenge in developing treatment strategies targeted toward LSCs is to identify pro-apoptotic stimuli that spare the normal HSCs while exerting the desired effect on LSCs. 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 LSCs. Several agents effective in inhibiting the ATP-binding cassette transporters have been studied and found to have limited clinical efficacy (63, 64). The biggest obstacle to this approach is the similarly high expression of these transporters in normal HSCs, making them equally susceptible to the inhibitors (20). As such, strategies directed at pathways that specifically regulate LSC survival would probably be more effective (65). The search for identification of survival pathways that are preferentially over-expressed in LSCs is ongoing and several recent studies have described means of differential activation of apoptosis mechanisms in LSCs (57, 66, 67). The transcription factor NF-xB was found to be constitutively activated in LSCs but not in normal HSCs (66). Therefore, NF-xB inhibitors were added to anti-leukemic agents such as idarubicin in experimental models (57, 66). Recently, single-agent parthenolide, a potent inhibitor of NF-xB, was found to induce apoptosis in AML and CML LSCs and progenitors while sparing normal HSCs (67). Notably, parthenolide was much more selective in eliminating LSCs and sparing normal hematopoietic cells than was the standard chemotherapy agent cytarabine (67). Constitutive activation of the phosphatidylinositol-3 kinase is also necessary for the survival of LSCs and its pharmacologic inhibition by LY294002 leads to a dose-dependent decrease in survival (68). The fate of LSCs depends on the relative expression of transcription factors and their regulation, usually by aberrant signaling pathways (69, 70). Although it has been proposed that recruitment of LSCs from the G0 to the S phase of the cell cycle might contribute to their eradication by cell cycle-specific cytotoxic agents, it is possible that prolonging the ‘‘quiescent phase’’ could be beneficial. One could envision a scenario in which LSCs are maintained in a state of hibernation, thereby prolonging relapse-free survival. The best evidence for this possibility is the existence of patients with clinically distinct indolent or proliferative forms of AML as well as the wide variation in the duration of relapse-free interval in individual patients.

New agents to target the leukemic stem cell for therapeutic purposes

A major cellular target of cancer therapy is directed against neoplastic stem cells. Current investigations are attempting to combine molecular targeted therapies with the concept of the cancer stem cells. Molecular targeted therapy can be divided into 6 categories that correspond to the following types of targets: 1) surface molecules; 2) ABC proteins; 3) specific oncoproteins; 4) normal stem cell pathways; 5) survival factors, present in cancer stem cells only (NF-xB pathway, PI3K, downstream signaling molecules and related signal-transduction pathways); 6) oxidative stress (3, 20, 54, 55) (Table 2).

Surface molecules. The ideal target would be expressed on all neoplastic stem cells but not on normal stem cells. The best examples are IL-3 receptor a chain and CD33, since it is regarded that these antigens are not expressed on normal stem cells. However, only selected subpopulation of neoplastic stem cells express these antigens. Another example...
is CD44 antigen, which is expressed both on normal and cancer stem cells. In such cases, targeted therapy has to be combined with stem cell transplantation. Anti-CD33 consists of calicheamicin and a humanized anti-CD33 antibody (CMA-676). Anti-CD44 is a monoclonal antibody, acting through upregulation of p27. Other possible agents include antibodies conjugated with diphtheria toxin against GM-CSF (CD116) granulocyte-macrophage-colony stimulating factor, IL-3(CD123) and urokinase plasminogen-activator receptor (CD87).

**ABC proteins.** The most important targets include: PGP1 and BCRP, with the best known inhibitors: zosuquidar and fumitremorgin, respectively.

**Specific oncoproteins.** Probably, disease-related oncogenic proteins play an important role in the initiation and progression of leukemia and solid tumors. Such oncoproteins are expressed early in tumor development, and are expressed in neoplastic stem cells. The best known include BCR-ABL (imatinib is its inhibitor), PML-RARα (inhibitor: all-trans retinoid acid), HER-2/neu (inhibitor: trastuzumab), mutated RAS, mutated Kit. However, this therapy is not associated with the elimination of the entire neoplastic clone, due to drug resistance, mutated forms, other mutations, and also BCR-ABL-negative clones.

**Normal stem cell pathways.** Genes that have demonstrated their involvement in the regulation of self-renewal in normal stem cells include the BMI-1, NOTCH, WNT, and Sonic Hedgehog pathways. These genes are potential targets for therapy. Cyclopamine is a compound originally discovered in the Corn Lily (*Veratrum californicum*), a plant teratogenic to sheep (71). Cyclopamine specifically inhibits the SMO protein and can suppress the growth of cells and tumors that contain activated HH (72). Human prostate tumors grown as xenografts in mice were completely eliminated following twenty-one days of treatment with cyclopamine, and UV-induced basal cell carcinomas were suppressed in mice given low levels of cyclopamine in their drinking water (73, 74). Both cyclopamine and SMO inhibitors are in development as anticancer agents. Several companies have developed γ-secretase inhibitors and these drugs may have applications to cancer therapy. Rapamycin is a bacterially derived molecule that inhibits the target of rapamycin (TOR) family of kinases (75). Rapamycin has immunosuppressive properties and is also used to treat certain leukemias (76). Yilmaz et al. have shown that rapamycin can selectively inhibit leukemia initiating cells in leukemias generated in mice harboring a conditional deletion of PTEN (phosphatase and tensin homolog deleted on chromosome 10), a phosphatidylinositol phosphate (PIP) phosphatase often mutated in human tumors (77). Rapamycin depletes the leukemia-initiating cells while restoring the normal hematopoietic stem cells, suggesting therapies can be devised specific for cancer stem cells.

### Table 2. Targets in leukemic stem cells.

<table>
<thead>
<tr>
<th>General target</th>
<th>Specific target</th>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface molecules</td>
<td>IL-3 receptor α chain, CD3, CD44</td>
<td>zosuquidar, fumitremorgin</td>
</tr>
<tr>
<td>ABC proteins</td>
<td>PGP1, BCRP</td>
<td></td>
</tr>
<tr>
<td>Specific oncoproteins</td>
<td>BCR-ABL, PML-RARα, HER-2/neu, Mutated RAS, Mutated Kit</td>
<td>imatinib, all-trans retinoid acid, trastuzumab</td>
</tr>
<tr>
<td>Stem cell pathways</td>
<td>Bmi-1, Notch, Wnt, Sonic Hedgehog pathways, SMo protein</td>
<td>cyclopamine, SMO inhibitors</td>
</tr>
<tr>
<td>Survival factors</td>
<td>NF-κB</td>
<td>proteasome inhibitors, parthenolide (or its analogs), TDZD-8, inhibitors of mTOR</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>DNA damage response</td>
<td>idarubicin or other chemotherapeutics, parthenolide (or its analogs), TDZD-8, heat shock</td>
</tr>
</tbody>
</table>
Additional strategies to directly target the growth of tumor stem cells can now be developed and may prove superior in effectiveness. **Survival factors.** These factors are present in cancer stem cells only, such as NF-kB pathway. Proteasome inhibitors down-regulate NF-kB expression. Inhibition of survival factors (NF-kB/PI3K) include proteasome inhibitors, parthenolide and its analogs, TDZD-8 and ET-18-OCH3 (54). Another pathway is phosphoinositide-3 kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway. Whether mTOR is activated in stem cells and whether inhibitors of mTOR (rapamycin) can counteract the growth of LCS is under investigation. Other downstream signaling molecules and related signal-transduction pathways are possible to known. **Oxidative stress.** DNA damage response might be influenced by idarubicin or other chemotherapeutics, parthenolide and its analogs, TDZD-8 and heat shock (54).

**CONCLUSIONS**

The concept of cancer as a stem cell disease has the potential to dramatically change our view of the problem. The discovery of cancer stem cells in solid tumors has already changed our view of carcinogenesis and chemotherapy. 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 a new understanding. Research efforts to understand 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 aided by this new insight. Normal stem cells are being intensively studied to develop approaches for replacing damaged cells and tissues in the body. The insight from such work is likely to aid the understanding of cancer stem cells.

**Acknowledgment**

The study was supported by grant MNiSW N407 078 32/2964 and Nicolaus Copernicus University Grant 551-Ch/CM.

**REFERENCES**


Received: 23. 01. 2008