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Successful Cancer Treatment: Eradication of Cancer Stem Cells

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INTRODUCTION

The increasing incidence of cancer in many countries is a consequence of our success as a species. Otherwise, cancer would be a rare event. This is no accident, and its justification can be found at the root of the evolution of multicellular organisms. Indeed, the emergence of multicellular organisms required coordination and cooperation between cells that became increasingly specialized resulting in an overall benefit for the organism. Multicellularity also brings with it the risk of cancer, viewed as the deregulated proliferation of a new and particular cell-type population that can threaten the integrity and survival of the organism (Hanahan and Weinberg, 2000).

Studies during the last 40–50 years have shown that cancer is an acquired genetic disorder due to mutations that activate proto-oncogenes, silence tumor suppressor genes or induce genomic instability (Vogelstein and Kinzler, 2004). Genetic mutations can be due to exogenous genotoxic agents such as ionizing radiation or chemotherapeutic agents that interact with DNA. However, the genome replication machinery is also prone to errors that inevitably result in mutations being incorporated in cells (Kunkel and Bebenek, 2000). Hence, individuals with mutations in DNA-repair enzymes have an intrinsically higher risk of cancer.

TISSUE ORGANIZATION AND STEM CELLS

The path selected throughout evolution to mitigate the risk of persistent mutations in tissues led to a hierarchic architecture where the majority of cells have a limited life-span and are continuously being replaced (Nowak *et al.*, 2003). Tissue integrity is maintained by relatively small populations of long lived cells that replicate at a relatively slow pace. This architectural organization is present in all epithelia (skin, respiratory, and gastrointestinal tract) and in the hematopoietic system (Figure 10.1). These constitute the most common sites for tumor development in humans. Indeed, all tissues exposed to the external environment with its genotoxic agents exhibit high cell turnover. Every tissue is composed of a variety of cell types which together give the tissue its specific structure and function. At the

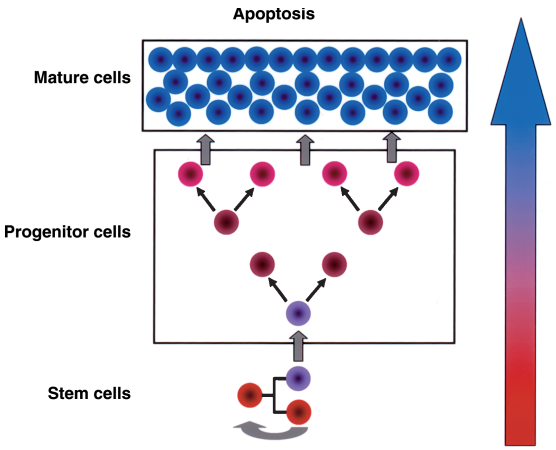

FIGURE 10.1. Normal tissue architecture. The majority of cells in epithelia are short lived and die by apoptosis or are shed. Tissue integrity is maintained by the slow replication of tissue specific stem cells that give rise to progenitor cells that proliferate for a short period of time to produce differentiated cells. Many tumors have a similar hierarchical organization with cancer stem cells responsible for the maintenance of the tumor

root of this tree there is a small population of cells: these are the stem cells (SC). Tissue specific SC are defined by two properties: a capacity for long-term self-renewal to maintain their own population, and their ability to differentiate into various types of specific cells that are found in any given tissue. The best studied SC are those present in the hematopoietic system, although our knowledge regarding skin and gastrointestinal stem cells is increasing rapidly. Stem cells not only provide a mechanism for maintenance of the tissue but are also required for repair after injury (e.g., trauma including surgery, bleeding, chemotherapy, and transplantation).

Tissue specific SC are only a very small fraction of all tissue-specific cell types, being organized within micro environments known as SC niches (Moore and Lemischka, 2006; Scadden, 2006). The niche is a complex structure composed of cytokines and growth factors, extracellular stroma, and supporting cells that, depending on location, may include osteoblasts, fibroblasts and other mesenchymal cells, cells of the monocyte lineage, and immune system cells (Moore and Lemischka, 2006). Stem cell behavior is heavily influenced by the particular niche in which they reside. Current thinking suggests that the interaction between intracellular genetic and epigenetic factors within the SC coupled with extracellular stimulation from the surrounding niche determine the fate of a specific SC, including its rate of replication, (a)symmetry of division and survival. The number of SC present in any given niche is not known and may be variable depending on the site (location) and state (function) of the niche. For example, a colonic crypt has an estimated number between 1 to 10 SC that are responsible for the maintenance of each crypt.

In the absence of an external genotoxic agent, the main cause of genetic mutations is the intrinsic error rate of the DNA replication machinery (Kunkel and Bebenek, 2000). In such a case, a small pool of SC that replicate slowly serve to minimize the risk of acquiring mutations that would persist in the progeny cell population.

EVIDENCE FOR CANCER STEM CELLS

The neoplastic cells that define the origin of the tumor (e.g., epithelial, mesenchymal etc) require the support of a stroma that provides the vascular supply and scaffolding on which the tumor cells grow. Moreover, it is becoming increasingly

clear that even within the neoplastic population, there is an underlying functional hierarchy (architecture) with tumor cells exhibiting various stages of differentiation and survival. Based on this observation, the existence of cancer stem cells (CSC) was initially postulated almost 50 years ago to explain the heterogeneous population of tumor cells. Recent studies using clonogenic assays and flow cytometric cell sorting of established tumor cell lines or human tumors explanted at surgery show that not all cancer cells are created equal. Similar to normal tissues, in any given tumor only a small fraction of cells have long term self-renewal capability and can engraft to form tumor xenografts when implanted into receptive immunodeficient hosts. Tumor cells that have these two capabilities are known as CSC (or cancer initiating cells) and are the subject of intense research (Clarke *et al*., 2006). Initially, CSC were identified in hematopoietic neoplasms such as acute myeloid leukemia (AML) (Lapidot *et al*., 1994). However, to date, CSC have been reported in tumors from the breast (Al-Hajj *et al*., 2003), gastrointestinal tract (O'Brien *et al*., 2007; Ricci-Vitiani *et al*., 2007), lung (Kim *et al*., 2005), prostate (Gu *et al*., 2007), pancreas (Li *et al*., 2007), brain (Singh *et al*., 2004), liver (Suetsugu *et al*., 2006), malignant melanoma (Monzani *et al*., 2007) and multiple myeloma (Matsui *et al*., 2004). Injection of a single breast CSC (LA7) in the foot pad of NOD/SCID mice efficiently induces the growth of mammary tumors in these animal models. The tumors have a complex architecture compatible with differentiation of the progeny cells, and demonstrate both the proliferative potential of these cells as well as the clonal nature of cancer (Zucchi *et al*., 2007). Given the increasing recognition of tumor specific SC, it is not surprising that the current hypothesis is that the majority and perhaps all tumors have CSC at their root to maintain the growing tumor population (Reya *et al*., 2001).

ORIGIN OF CANCER STEM CELLS

A fundamental question in carcinogenesis is the origin of the CSC (Reya *et al*., 2001). In principle, these cells can arise either by the malignant transformation of normal SC, or more differentiated cells can acquire SC – like properties. The favored hypothesis has been that CSC arise due to mutations within the most primitive SC (Wang and Dick, 2005). Because normal SC already have self-renewal capability, a *sine qua non* for the development of the tumor (Hanahan and Weinberg, 2000), CSC originating from mutations of normal SC may free-ride on the self-renewal capacity while expressing the malignant phenotype (Wang and Dick, 2005), and are thus thought to require fewer mutations to reach the malignant phenotype. If the origin of the tumor is within more differentiated progenitor cells, then acquisition of SC-like properties (including unlimited self-renewal) must be an early event in the process of malignant transformation, otherwise the mutant cells would be washed out before significant expansion of the clone could occur. However, there is evidence for the reacquisition of 'SC-like' properties in more committed progenitor cells, depending on the type of tumor and the mutations that drive its evolution and expansion (Huntly *et al*., 2004; Krivtsov *et al*., 2006). It has been

shown that *MOZ-TIF2* expression can confer sustained self-renewal in hematopoietic progenitor cells, but *BCR-ABL* cannot induce long-term self-renewal in the same cell population. However, it is known that chronic myeloid leukaemia (CML) arises by mutation of normal SC. Expression of the *bcr-abl* oncoprotein may be enough to explain the behaviour of the myeloid cells necessary to induce the chronic phase of the disease (Michor *et al.*, 2006). Subsequently, the development of CML blast crisis is associated with the emergence of a new SC that has its origin in CFU-GM cells (Jamieson *et al.*, 2004). In the case of colon cancer, the cell population at risk of neoplastic transformation is thought to be restricted to the (normal) SC that maintain the colonic crypts (Tomlinson *et al.*, 2002). Here mutations in the *APC* gene may be an early event in the path to malignant transformation.

STOCHASTIC DYNAMICS OF CANCER STEM CELLS

It has been the general impression that many of the cancer inducing mutations give an advantage to the tumor (stem) cells by enhancing either proliferation or increasing resistance to apoptosis (Hanahan and Weinberg, 2000; Vogelstein and Kinzler, 2004). In terms of evolutionary dynamics, such mutations give the cell a higher reproductive fitness ($r > 1$) compared to the (normalized to 1) fitness of normal SC. Cells with a higher fitness give rise to a larger number of progeny since they are selected for reproduction more often. If the population of SC were infinite, this would inevitably lead to the extinction of normal SC, that is, invasion of the entire population by CSC. However, given that the size of the SC pool is often small, stochastic effects become relevant. As a consequence, while a mutation that leads to higher reproductive fitness increases the probability that the mutant clone will expand in size and take over the whole population, such an increase does not guarantee invasion. Stochastic considerations show that extinction, latency, or invasion (complete dominance) by the mutant clone is possible during the finite lifetime of the individual (Figure 10.2). It is pointed out that for an infinite lifetime there are only two possible outcomes (absorbing states): either clonal extinction or complete invasion by the mutant cells. However, often these limiting scenarios are not met in practice, because all living systems have a finite lifetime that scales allometrically with mass (Lopes *et al.*, 2007). Even when the relative advantage of CSC is $r = 2$, considered by many to be a very large advantage (Tomlinson *et al.*, 2002), there is a 50% probability that such a clone will die out rather than expand

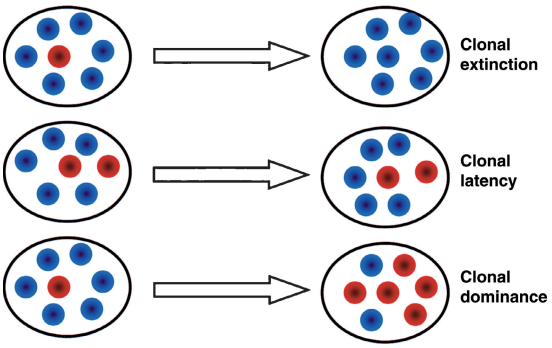

FIGURE 10.2. Stochastic stem cell dynamics. Whenever the pool of stem cells is small, stochastic effects become important. Among the different possible scenarios, three possibilities are detailed, given their experimental realization: extinction of the mutant clone, clonal latency or clonal expansion to cause disease

and take over the compartment where it arises (Dingli *et al*., 2007c). Similarly, it can be shown that a mutation in the SC need not confer a fitness advantage to that cell for the clone to expand. Neutral drift may be enough to expand the population to a size that may be clinically significant (Dingli *et al*., 2007c). This theoretical conclusion may explain several observations in CML where it is thought that the *bcr-abl* oncogene does not give an advantage to the CML SC themselves but only to progenitors further downstream. In such a case, expansion of the CML SC occurs only by neutral drift and perhaps is one reason why the pool of cells driving this disease is so small (Dingli *et al*., 2007b). Moreover, gene expression studies in putative AML SC show that the cells up-regulate two important tumor suppressor genes (interferon regulatory factor 1, IRF-1 and death associated protein kinase, DAPK) that act to suppress proliferation of the malignant SC (Guzman *et al*., 2001b). In other words, these mutations do not necessarily confer to the AML SC an enhanced reproductive fitness. However, they may provide an advantage to downstream progenitors that drive the disease. If this hypothesis is true, it would further support that the number of SC driving a AML is small because their number will increase due to neutral drift as in CML (Dingli *et al*., 2007c). Finally, under stochastic evolutionary dynamics a mutant clone can appear 'stable' for a long time even if the mutation provides a fitness advantage (Dingli *et al*., 2007c). These features are supported by clinical observations from patients with essential thrombocythemia where $JAK2^{V617F}$ clones that remain stable for extended periods of time have been observed (Gale *et al*., 2006).

Stochastic SC dynamics (normal as well as malignant) also show that the time required by a mutant clone to reach a given fraction of the SC pool is highly variable and depends on the fitness provided by the mutation. For any tumor, it is reasonable to assume that the mutant clone must reach a certain fraction for the disease to appear. Simulations of the dynamics of such a population provide data for time probability distributions as a function of fitness. The shapes of such time probability distributions are one humped, but they can be very broad, the width depending on the relative fitness advantage of mutated cells. With increasing fitness advantage these curves become narrower, but the underlying stochastic behavior of these cells (Dingli *et al*., 2007c) will always lead to a range of times during which the process can occur.

Given the widespread impression that cancer inducing mutations give an advantage to the cell, perhaps extinction of tumor SC or tumors would be considered an impossible clinical scenario or simply a theoretical concept. Such conventional wisdom, however, disregards the impact of stochastic effects on the dynamics of SC populations. As discussed before, the probability that a given clone will be eliminated depends on the size it acquires (Dingli *et al*., 2007c). Hence, subclinical clones are more likely to be eliminated while the probability that a clinically evident tumor will undergo spontaneous extinction is small. Again, clinical observations (although rare) show that well established clonal disorders that can threaten the life of the individual can go extinct, even with minimal or no therapy. Perhaps the best example of this is transient myeloproliferative disorder (TMD) seen in patients with Downs' syndrome soon after birth

(Massey *et al*., 2006). Patients with this disease may have very high circulating blast counts (high disease burden that may be life threatening), and yet in almost 85% of these patients, the disorder disappears without recurrence. To date, no explanation has been proposed for this well documented clinical phenomenon, but we propose that stochastic clonal elimination provides the required mechanism. Another example where stochastic tumor extinction may be possible is in the myelodysplastic syndromes where spontaneous resolution has been reported, although this seems to happen quite rarely (Tricot *et al*., 1986).

Stem cells can divide to give rise to two daughter cells that have the same (symmetric division) or different (asymmetric division) fate. A mutation that increases the probability of SC self-renewal, confers a higher reproductive fitness to these cells similar to a scenario where they are selected to reproduce more often. Such mutations (e.g., *adenomatous polyposis coli*, APC) enable the mutant cell to efficiently expand within its environment. An increase in the size of the population of cells at risk increases the probability of acquisition of additional mutations facilitating the full development of the malignant transformation (Dingli *et al*., 2007a).

MARKERS OF CANCER STEM CELLS

As already alluded to, the number of cancer initiating (clonogenic) cells in a given tumor is usually very small, often $< 2\%$ based on flow cytometric cell sorting. The initial screen for these cells is based on their ability to exclude fluorescent DNA binding dyes such as rhodamine or Hoechst 33342. As a result, SC exhibit very low fluorescence with these dyes and appear as negatively stained cells to the side of the majority of cells in a density dot plot; hence, the name 'side population'. Even then, these highly purified cell populations are probably themselves heterogeneous, and it has been shown that acute leukaemia can be induced by the injection of < 100 highly purified cells (Clarke *et al*., 2006). We have recently estimated that for the classic hematopoietic SC disorder CML, perhaps 1–8 active cancer SC may be enough to drive the disease during the chronic phase (Dingli *et al*., 2007b). The small number of cells creates technical difficulties in the identification of cancer SC specific markers that perhaps will be suitable targets for therapy (see below). This notwithstanding, several cell surface markers seem to be over-expressed by these cancer SC. CD133 (prominin-1) recently has been identified on several of the putative cancer SC (Neuzil *et al*., 2007). Prominin-1 is a cell-surface glycoprotein that spans the plasma membrane five times and is expressed in all metazoans. Initially described on hematopoietic SC, it was found to be expressed in normal and malignant SC, although the latter tend to overexpress the protein on their surface (Neuzil *et al*., 2007). Another proposed marker is CD44 that is over-expressed by prostate and pancreatic cancer cells as well as leukemic SC (Jin *et al*., 2006). CD44 is a receptor for hyaluronic acid or chondroitin sulfate while variants of this protein can bind to fibronectin, laminin, and collagen (Ponta *et al*., 2003). Prostate cancer SC have the phenotype $CD44^{+}/\alpha_2\beta_1^{hi}/CD133^{+}$ (Collins *et al*., 2005), while brain tumor SC are often $CD133^{+}$/musashi-1^{+}/$nestin^{+}$ (Vescovi *et al*.,

2006). Normal breast SC have the phenotype $Lin^-/CD29^{hi}/CD24^+$ (Shackleton *et al*., 2006), while breast cancer SC have the phenotype $CD44^+/CD24^{-/low}/Lin^-$ (Al-Hajj *et al*., 2003). Pancreatic cancer SC have the phenotype $CD44^+/Cd24^+/ESA^+$ and seem to express CD133, at least at the level of mRNA (Olempska *et al*., 2007).

In the case of tumors derived from the hematopoietic system, characterization of the immunophenotype of putative cancer SC markers has identified several potential antigens. Acute myeloid leukaemia (AML) SC often express CD123, the α chain of the interleukin-3 receptor (Jordan *et al*., 2000). Other putative markers for AML SC include CD33 (Taussig *et al*., 2005) and CD44 (Jin *et al*., 2006), although these are also expressed, albeit at low levels, on normal hematopoietic stem and progenitor cells. There is a growing consensus that AML SC have the phenotype $CD34^+/CD38^-/CD71^-/CD90^-/CD117^-/CD123^+/HLA\text{-}DR^-$ irrespective of the subtype (Jordan, 2002). While some of these markers are shared with normal SC, CD90, CD117, and CD123 are thought to be fairly specific for the malignant cells. More recently, the C-type lectin like molecule-1 (CLL-1) has been identified on AML but not normal hematopoietic stem and progenitor cells (Van Rhenen *et al*., 2007). Injection of $CD34^+/CD38^-/CLL\text{-}1^+$ cells isolated from patients with AML into NOD/SCID mice led to the development of leukaemia, although serial transplantation was not performed. The majority of malignant plasma cells normally do not express CD20, the target for rituximab, but clonogenic cells isolated from highly purified multiple myeloma cell samples express this therapeutically relevant target (Matsui *et al*., 2004).

TREATING CANCER STEM CELLS

Current chemotherapy is often successful in eliminating the bulk of the malignant cell population; however, often the tumor returns. Several, non-mutually exclusive explanations have been proposed to explain disease recurrence including selection for chemotherapy resistant cells (due to mutation) and the presence of cancer SC that are not eliminated by therapy. However, it is also a fact that many patients have been cured of cancer with current chemotherapy alone. Operationally, successful cancer therapy can be defined as long-term survival without evidence of disease recurrence. To our knowledge, no one has shown that curative therapy requires or is associated with complete elimination of all tumor cells from within the body because currently there are no techniques that can reliably prove this point. However, given the present understanding of the importance of CSC for the development and maintenance of the tumor, it makes sense to consider this population of cells as important targets of therapy that could translate into higher cure rates.

Using mathematical models where tumor growth is maintained by a CSC compartment, it was shown that therapy directed at the more differentiated cancer cells will not be able to cure the cancer (Dingli and Michor, 2006). In this theoretical scenario, therapeutic agents that completely stop CSC replication could in principle operationally cure the tumor (but this can take a very long time). It was shown that only agents that actively kill the CSC and the bulk of the tumor cells will effectively cure the disease in a reasonable time frame.

Genomic instability is an important characteristic of cancer that may occur early in the disease process (Bielas *et al*., 2006). A higher mutation rate increases the risk of acquired drug resistance; therefore rapid elimination of the CSC pool seems a desirable goal (Dingli and Michor, 2006).

Problems with Targeting Cancer Stem Cells

There are several hurdles that hinder therapeutic approaches designed to eliminate neoplastic SC. It is likely that the number of cancer SC responsible for tumor growth and maintenance is quite small (Dingli *et al*., 2007b). For example, we estimate that between 1 and 8 cancer SC may be enough to fuel the chronic phase of CML. The small number of these cells and mass action considerations suggest that it is very difficult to effectively reach therapeutically this critically important cell compartment. Often these cells replicate slowly and at any time, the vast majority of them are in G_0 and, therefore, insensitive to all cell-cycle specific agents (Dean *et al*., 2005). It is also becoming increasingly clear that adhesion to the SC niche itself affords protection from the effects of chemotherapy. Moreover, cancer SC, like their normal counterparts, express multi-specific drug efflux pumps on their plasma membrane that effectively protect them from chemotherapeutic and other genotoxic chemicals. Among these membrane proteins, those that belong to the ATP binding cassette (ABC) such as *ABCB1*, also known as *MDR1* or P-glycoprotein, and *ABCG2* seem to be particularly important. These proteins have broad substrate specificities and couple ATP hydrolysis with drug efflux to the outside of the cell against a chemical gradient (Dean *et al*., 2005). Another mechanism of drug resistance is the intrinsic ability of SC to repair DNA damage. Finally, it has been shown that at least in CML, neoplastic SC that harbour the Philadelphia chromosome, do not even express *bcr-abl*, and therefore are not dependent on this mutation for their survival (Holyoake *et al*., 1999). However, such cells cannot be sensitive to imatinib mesylate.

Safe elimination of CSC requires that there is minimal toxicity to non-malignant stem and progenitor cells that maintain normal tissues. Thus, suitable targets must be identified that provide as wide a therapeutic index as possible between the normal and malignant cells. Recent studies suggest that this might be possible. Most of the evidence is based on studies in hematopoietic neoplasms. Primitive AML cells express an active form of the nuclear factor kappa B (NF-κB) independent of their cell cycle status (Guzman *et al*., 2001a). In these cells, NF-κB plays an important role in preventing apoptosis. However, NF-κB is not expressed by normal SC and therefore, provides an exciting target for therapy. There is also encouraging data regarding the role of NF-κB in preventing apoptosis in other neoplasms such as lymphoma and in malignant melanoma, non-small cell lung cancer and pancreatic carcinoma (Jordan, 2002).

Overcoming Drug Resistance

With the recognition of the ABC transporters and their role in drug resistance, several agents have been tested to block the function of these proteins. The calcium channel blocker verapamil was studied in various tumors and did show some activity in patients with breast (Belpomme *et al*., 2000) and non-small cell lung cancer (Millward *et al*.,

1993), although it did not provide any benefit in multiple myeloma (Dalton *et al*., 1995). The drug had low potency and was often poorly tolerated due to hypotension. Cyclosporine A did not improve the efficacy of VAD chemotherapy in patients with advanced multiple myeloma (Sonneveld *et al*., 2001). Moreover, the drug is plagued by a multitude of potentially serious drug-drug interactions as well as nephrotoxicity that limit its use. More recently, several 'second generation' ABC transporter inhibitors have been developed (Dean *et al*., 2005). Some inhibitors such as zosuquidar have been tested in clinical trials and results are awaited.

Evidence for Effective Anti-Cancer Stem Cell Therapy

If we consider that all tumors are maintained by neoplastic SC, then one cannot but conclude that there are occasions when chemotherapy seems to eliminate these cells since many patients with advanced cancer have been cured. There are many examples from acute leukaemia to testicular cancer and Hodgkin and non-Hodgkin lymphoma. All of these diseases are typically advanced when treated with chemotherapy and many patients have been cured. In CML, there are patients that have been treated with interferon alone that not only lost the Philadelphia chromosome but seem to be in remission for many years, even after stopping interferon therapy and perhaps some have been cured. Recently, Van Rhenen *et al*. (2007) showed that patients with AML have a side population of cells that express CLL-1. If chemotherapy leads to eradication of these cells, the patients experience long remissions. However, if these cells are not eradicated by chemotherapy, patients experience very rapid relapse.

Various strategies to eliminate these cells have been implemented or are currently being tested in clinical trials. Experiments in relevant animal models have explored the use of specific targets to eliminate the tumor SC population. Several groups have evaluated the potential use of surface antigens as therapeutic targets for monoclonal antibodies or fusion proteins. Jin *et al*. (2006) showed that a monoclonal antibody directed at CD44, that is often expressed by AML SC, can prevent the development of disease in serially transplanted mice. A monoclonal anti-CD33 antibody, gemtuzumab, is available and has been used in patients with $CD33^+$ AML, but it can have significant toxicity and is associated with marrow suppression probably because even normal hematopoietic SC and recovering progenitor cells express this antigen. Another approach currently under investigation is to target CD123 by a fusion protein between IL-3 and diphtheria toxin (Cohen *et al*., 2005). The initial studies with this agent suggest that therapeutically meaningful serum concentrations can be achieved, although it induced myelosuppression as well as vasculitis in female cynomolgus monkeys.

As discussed before, it appears that malignant SC depend on NF-κB to prevent apoptosis while normal SC do not express this nuclear factor. Intracellular signalling by NF-κB is blocked by the inhibitor of κB (IκB). Thus, prevention of IκB degradation by proteasome inhibition was evaluated in combination with idarubicin. This strategy led to apoptosis of AML SC in culture (Guzman *et al*., 2001a) with a considerable decrease in NOD/SCID repopulating cells (Guzman *et al*., 2002). Importantly, the same treatment did not harm normal SC suggesting that this approach may be effective and safe.

The Future

Despite the lack of efficacy of initial ABC transporter inhibitors, the combined use of second and third generation agents holds promise, because some of these novel agents can block multiple transporters simultaneously and will soon enter clinical trials (for a recent review, see Dean *et al*., 2005). Agents such as Ko143 and GF120918 seem to inhibit both ABCB1 and ABCG2 (Cisternino *et al*., 2004). They may enhance CSC eradication when combined with drugs such as the anthracyclines. Parthenolide is a natural sesquiterpene lactone and the major active component in feverfew, a product of *Tanacetum parthenium*. The drug has broad antitumor activity because it inhibits DNA synthesis, sensitizes tumor cells to other anti-tumor agents and is a potent inhibitor of NF-κB. In addition, it enhances production of reactive oxygen species and interferes with signaling from key tumor associated pathways including signal transducers and activators of transcription 3 (STAT3), and c-Jun N-terminal kinase activation (Jordan, 2007). The drug has potent activity against both blast crisis CML and AML progenitor and SC populations and significantly inhibit NOD/SCID repopulating cell growth. At the same time, the agent had no significant detrimental effect on normal SC and progenitor cells suggesting that it may be fairly specific to tumor cells. A water soluble derivative of this compound is under development. More recently, TDZD-8 was identified as another potent inhibitor of NF-κB with selective activity against AML SC (Jordan, 2007).

Monoclonal antibodies directed against CSC specific markers are also under development and results are expected soon. In one study, the putative myeloma SC is being targeted with rituximab, an anti-CD20 antibody. The advent of antibodies against CLL-1 will surely drive studies for disease eradication in AML. Finally, the last few years have ushered in the era of genomics. The technology is now available for genome wide searches for CSC specific defects that could be the targets of therapy. This approach holds great promise for the development of safe and effective cancer curing therapies. To this end, mathematical models of tumor development incorporating essential realistic features of the neoplasms may prove insightful towards a full understanding of the origin and evolution of these disorders.

It is concluded that curative therapy for any disease relies on a proper understanding of the underlying mechanisms that drive that disorder. During the last few years, our understanding of tumor growth and evolution has been transformed by the discovery of CSC. They are rapidly emerging as the preferential target of antitumor therapy. Cancer stem cell specific markers, either on their surface or within the inner workings of the cell are being identified that will provide many potential therapeutic targets to increase cure rates for this group of diseases.

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