6 Cancer Stem Cells

Implications for Development of More Effective Therapies

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SUMMARY

Despite advances in the development of cytotoxic chemotherapies, the fact remains that for most common malignancies, metastatic disease remains incurable. Recent work has suggested that most, if not all, malignancies are driven by a small subpopulation of cells that have stem cell characteristics. These "tumor stem cells" are thought to arise either from normal tissue stem cells or from early progenitor cells through dysregulation of self-renewal pathways. The partial differentiation of cancer stem cells may result in tumor heterogeneity. One of the characteristics of this heterogeneity may be reflected in the resistance of cancer stem cells to cytotoxic chemotherapy. Evidence is presented that current chemotherapeutic regimens selectively target more differentiated cells in tumors, while sparing the tumor stem cell component. This may account for relapse following tumor regression. The mechanisms contributing to the resistance of tumor stem cells to cytotoxic agents may involve increased efficiency of DNA replication and repair mechanisms in stem cells, changes in cell cycle parameters, and the overexpression of antiapoptotic and transporter proteins in these cell populations.

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The tumor stem cell model of carcinogenesis has fundamental implications for the development of new cancer therapeutic agents, as well as for the design of clinical trials utilizing these agents. Strategies aimed at the targeting of cancer stem cell populations may lead to more effective therapies for the treatment of advanced malignancies.

Key Words: Cytotoxic chemotherapy; dysregulation; tumor heterogeneity; tumor stem cells; tumor stem cell model.

1. INTRODUCTION

Despite numerous advances in the development of antineoplastic agents, the fact remains that for most common malignancies, advanced disease remains incurable. Cytotoxic chemotherapies are often able to induce regression of cancer in patients, relieving symptoms, and improving quality of life. However, for most common malignancies, the tumors ultimately recur and become resistant to these agents. Recent work has suggested that most, if not all, malignancies, may contain a small subpopulation of cells that have stem cell characteristics. These "tumor stem cells" may drive tumorigenesis, and may display resistance to agents in our current pharmacologic armamentarium. In this chapter, we review recent evidence suggesting that cancers may arise from normal stem cells or their immediate progenitors, producing tumor heterogeneity and are driven by a "cancer stem cell" population. We explore potential molecular mechanisms accounting for resistance of these cancer stem cells to cytotoxic chemotherapy. Finally, based on an understanding of the biology of basic stem cell processes, we propose new strategies for therapeutic development that specifically target the cancer stem cell population. Targeting of this critical cell population may result in more effective treatments for advanced cancers.

2. TISSUE-SPECIFIC STEM CELLS AND THE ORIGIN OF CANCER

All tissues in the body are derived from the differentiation of organ-specific stem cells. These stem cells are defined by their capacity to undergo self-renewal, as well as to differentiate into the cell types that compose each organ. These tissue-specific stem cells are distinguished from embryonic stem cells in that their differentiation is largely restricted to cell types within a particular organ. Stem cells, by their long-lived nature, are subject to the accumulation of multiple mutations required for carcinogenesis. Over 40 yr ago, it was postulated that these tissue-specific stem cells may be the cell of origin of cancer *(1)*. Normal stem cells and their transformed counterparts share many characteristics, including the capacity for self-renewal, differentiation (although this is dysregulated in tumors), immortality as evidenced by telomerase expression, resistance to apoptosis, and ability to migrate and home to distant organ sites. Several recent reviews have explored the concept of the stem cell origin of tumors *(2–6)*. Recent studies of chronic myelogenous leukemia suggest that progenitor cells may also acquire mutations that allow them to self-renew *(6–8)*. A separate but related issue concerns the generation of tumor heterogeneity and the presence within tumors of tumor stem cells. If tumors arise through the transformation of stem or early progenitor cells and display various levels of differentiation, then tumor heterogeneity may be created, at least in part, by the aberrant differentiation of tumor stem cells and progenitor cells. Indeed, strong evidence has accumulated over the past decade that there exists within most, if not all tumors, a "stem cell population" that drives tumorigenesis. This was first demonstrated in human leukemia by John Dick's group *(9)*. They demonstrated that only a rare population of cells

within leukemias, which expressed cell-surface markers similar to normal stem cells ($CD34^+CD38^-$), were able to transfer the leukemic phenotype to immunosuppressed nonobese, severe combined immunodeficient (NOD-SCID) mice. Furthermore, the tumors that developed in these mice recapitulated the characteristics of the leukemia from which the samples were derived. These studies, and subsequent ones *(10,11)*, have demonstrated that leukemias may contain a cellular hierarchy, with transformed tumor stem cells and other cells in various stages of differentiation. A similar model for stem cells in solid tumors was first demonstrated by our group in collaboration with Michael F. Clarke's laboratory *(12)*. We showed that human breast tumors contain a subpopulation of tumor stem cells that bear the cell-surface phenotype $ESA^+CD44^+CD24^{-/low}Lineage^-$. As few as 100 of these cells could form tumors in NOD-SCID mice, whereas 20,000 cells that did not bear this phenotype failed to form tumors. Furthermore, fitting a stem cell model, the tumors that were generated by the tumorigenic stem cells recapitulated the phenotypic heterogeneity found in the initial tumors.

More recently, several groups have provided evidence for the existence of tumor stem cells in human brain tumors. Dirks' group first demonstrated that human brain tumors contained a subpopulation of cells bearing the neural stem cell marker CD133 (2,13). These tumor stem cells were able to form tumor neurospheres in vitro, as well as to differentiate into tumors resembling those from the initial samples. Furthermore, they demonstrated that these sphere-forming cells are able to produce tumors when injected intracranially into NOD-SCID mice *(2)*. These tumors recapitulated the phenotypic heterogeneity found in the initial tumors. Cancer stem cells have also been isolated from human glioblastomas *(14,15)*. The existence of a tumor stem cell population has recently been described in human multiple myeloma. Richard Jones group *(16)* has found that human myelomas are generated from cells that lack the expression of syndecan (CD138), which is present on mature plasma cells. These "myeloma stem cells" are pre-B cells expressing CD20. All of the above studies point to the existence of a stem cell component within human tumors capable of transferring the malignant phenotype, as well as the more differentiated "nontumorigenic" cells that compose the bulk of the tumor. The percent of tumor stem cells within tumors may vary between different tumor types, as well as within each tumor type. Leukemic and myeloma stem cells may comprise as few as 1 out of 5×10^4 cells, whereas in solid tumors such as breast cancer and brain tumors, cells bearing the stem cell phenotype appear to be more abundant, comprising between 1 and 20% of the tumor cell population. Furthermore, there is evidence in brain tumors that the percent of stem cells within a tumor may be predictive of its clinical aggressiveness *(13)*.

3. BIOLOGICAL IMPLICATIONS OF CANCER STEM CELLS

As indicated above, the stem cell model of carcinogenesis suggests that tumor heterogeneity is generated through partial differentiation of tumor stem cells. In a sense then, tumorigenesis represents a form of abnormal organ development. This contrasts to earlier models that attribute the development of tumor heterogeneity to stochastic processes that result from random mutation and subsequent clonal selection. The development of cellular heterogeneity through differentiation of malignant stem and/or progenitor cells has implications for understanding the process of tumor metastasis, as well as for providing an explanation for the resistance of tumors to therapeutic agents. It has been hypothesized that metastasis results from random mutation and selection and is therefore a late event in tumor evolution. However, recent studies utilizing molecular profiling have cast doubt

on this model. These studies have shown that the propensity of tumors to metastasize can be predicted by the molecular profile of the initial tumor, suggesting that the ability of tumor cells to metastasize is "hard-wired" into the genotype of the tumor. These results are more consistent with a stem cell model, which suggests that the metastatic propensity of a tumor is determined by its cell of origin as well as initial mutation profile, rather than being a late event in tumor evolution.

Another important issue in carcinogenesis is the interaction between transformed stem cells and their surrounding microenvironment. Normal stem cell behavior is tightly regulated by interactions between the stem cells and the surrounding environment. This environment, composed of neighboring cells, extracellular matrix, and soluble factors, has been termed the "stem cell niche." Evidence has accumulated that developing tumors also have important interactions with the surrounding environment. Indeed, the reciprocal interaction between tumor stem cells and their surrounding niche may play a fundamental role in tumor development. Recent studies have indicated that the stroma surrounding tumors has an altered gene expression profile compared to stroma surrounding normal tissue. This profile resembles that found in inflammatory tissue, suggesting similarities between wound healing and tumorigenesis *(17)*. Interestingly, these studies provide a potential explanation for the role of inflammation in carcinogenesis in tumors such as gastric tumors. Interaction between tumor cells and their environment undoubtedly also plays a role in the sensitivity of these tumor cells to therapeutic agents. In this regard, it has been demonstrated that attachment of tumor cells to the extracellular matrix mediated by integrins, regulates their sensitivity to chemotherapy *(18)*.

4. STEM CELLS AND CELL SURVIVAL

The generation of phenotypic heterogeneity through differentiation of tumor stem cells also has profound implications for understanding the sensitivity of these cells to chemotherapeutic agents, and for the development of new agents that target this tumor stem cell population.

By virtue of their fundamental importance in organogenesis, normal stem cells have evolved mechanisms that promote their survival and enhance their resistance to apoptosis. Examples of this can be found in organs where tissues undergo rapid turnover. In the mammary gland during pregnancy, there is marked proliferation and accumulation of mammary epithelial cells. These cells then undergo differentiation and produce milk proteins during lactation. The process of mammary involution that occurs following lactation is accompanied by massive apoptosis of differentiated cells. However, the stem cell component of the mammary gland is resistant to these apoptotic signals. These cells survive the involution process and regenerate the gland during subsequent pregnancies *(19,20)*.

Resistance of stem cells to apoptosis can also be seen in colonic epithelial stem cells. These stem cells give rise to the rapidly proliferating cells, termed transient amplifying cells, which then differentiate and are shed into the intestine after they undergo apoptosis *(21)*. Colonic stem cells are inherently resistant to this apoptotic process.

The inherent resistance of normal stem cells to apoptosis is also observed in patients undergoing cytotoxic chemotherapy treatments. When patients are given nonmyeloablative doses of cytotoxic chemotherapy, they experience transient decreases in their white blood cell counts. This is caused by apoptosis of differentiated neutrophil and myeloid precursors. The stem cells in the bone marrow are not ablated by these doses of

chemotherapy and are able to regenerate a normal hematopoietic system after several weeks. Similarly, many of the gastrointestinal side effects of chemotherapy are caused by the induction of apoptosis in differentiating colonic epithelial cells. These injured cells are regenerated by stem cells that are able to survive these chemotherapeutic insults.

5. TUMOR STEM CELLS AND RESISTANCE TO CYTOTOXIC AGENTS

Just as normal stem cells may be more resistant to the induction of apoptosis by cytotoxic agents and radiation therapy than are more differentiated cells, so too, tumor stem cells may display increased resistance to these agents compared to the more differentiated cells that compose the bulk of the tumor. Supporting this concept, Craig Jordan's group has demonstrated that leukemic stem cells are more resistant to chemotherapy than are the more differentiated myeloblastic cells that constitute the vast majority of cells in leukemia *(22)*. Similarly, Matsui et al. *(16)* have shown that myeloma stem cells are resistant to current therapies being used to treat myeloma, including chemotherapy and proteosome inhibitors. Previous observations regarding the in vitro behavior of "tumor spheroids" may also be related to the enrichment of stem cells in these structures. A number of groups, including Robert Kerbel's *(23)*, have found that when tumor cells are cultured on nonadherent surfaces, they form floating colonies termed tumor spheroids. Cells in these tumor spheroids are considerably more resistant to both chemotherapy and radiation therapy, than are the same cells cultured as monolayers. These effects were not merely because of drug penetration or uptake. Interestingly, a number of laboratories, including our own, have recently shown that both normal and tumor cells growing in spheroids are highly enriched for "stem and early progenitor cells" *(24)*. This raises the intriguing possibility that the relative resistance of tumor spheroids to chemotherapy and radiation therapy is because of enrichment of stem cells in these structures.

6. MECHANISMS OF STEM CELL RESISTANCE TO APOPTOSIS

As described, there is evidence that both normal stem cells and their malignant counterparts are more resistant to apoptosis than are the differentiated cells comprising the bulk of normal organs or tumors. Work in a number of laboratories has begun to elucidate the molecular mechanisms that may account for this resistance, which are described in the following four subheadings.

6.1. Cell Cycle Kinetics

Both normal stem cells and their malignant counterparts are slowly cycling cells that may contain a large fraction of cells that are in G_0 *(25)*. In turn, these cells may give rise to "transit-amplifying cells" that have a substantially higher growth fraction. Chemotherapeutic agents, particularly those with cell cycle specificity, will thus have substantially more effects on transit amplifying, rapidly dividing cells, than relatively quiescent stem cells.

6.2. DNA Replication and Repair Mechanisms

Stem cells are defined by their ability to undergo self-renewal as well as differentiation. Self-renewal divisions are inherently different from divisions that occur in differentiating cells. Stem cell self-renewal may occur by either asynchronous or synchronous division. Asynchronous self-renewal results in a daughter cell with identical phenotype to the parent stem cell, as well as a second daughter cell that then undergoes differentia-

tion. Because only a single stem cell is produced from this division, it can account for stem cell replenishment but not stem cell expansion. In contrast, a symmetric division resulting in two identical stem cells from a single stem cell can result in expansion of stem cell pools. The latter may occur during expansion of tumor stem cells in early tumor development. As first suggested by Cairns *(26)* and more recently confirmed by Potten et al. *(27)*, symmetric cell division of stem cells involves an unusual DNA segregation event in which the parental strand of DNA is retained in the daughter stem cell, whereas the newly replicated strand is passed on to another daughter cell that undergoes differentiation. If this is the case, then DNA damaging agents may have less effect on tumor stem cells undergoing asymmetric cell division, because the DNA replication errors would be passed on to the more differentiated cells, rather than be maintained in the tumor stem cell. In addition, it has also been found that stem cells have increased levels of DNA repair enzymes *(27–29)*. These mechanisms may have evolved to prevent accumulation of detrimental mutations and tumor formation. However, these same repair mechanisms may make tumor stem cells more resistant to DNA damaging therapeutic agents.

6.3. Antiapoptotic Proteins

Normal stem cells express higher levels of antiapoptotic proteins such as members of the Bcl-2 family, than do their more differentiated progeny. These cells also express inhibitors of apoptosis proteins. These proteins contribute to the resistance of stem cells to apoptotic insults. The expression of Bcl2 and or Bcl-X_L antiapoptotic proteins in cancer, has been associated with resistance to different drugs *(30)*.

6.4. Transporter Proteins

One of the properties that has been used to isolate normal stem cells from a variety of organs is their ability to exclude Hoechst dyes. As first described by Goodell et al. *(31)*, it was found that hematopoietic stem cells, are able to exclude Hoechst and rhodamine fluorescent dyes, a process that can be assessed by flow cytometry. These cells, termed the side or "SP population," show lower levels of staining because of the pumping action of ATP-binding cassette (ABC) transporters. The first transporter to be identified for its ability to efflux rhodamine and Hoechst in stem cells was ABCB1 or P-glycoprotein. More recently, the SP population has been redefined by the expression of a particular type of ABC transporter protein known as ABCG2 or breast cancer resistance protein (BCRP) that accounts for most of the Hoechst dye efflux in stem cells *(32)*. SP populations have now been described also in neuronal stem cells and both human and rodent mammary stem cells *(4,33)*. In addition to normal tissue stem cells, the existence of an SP population in tumorigenic stem cells has been demonstrated by recent studies showing that tumor SP cells are capable of generating tumors in mice to a much greater extent than tumor cells that do not exclude Hoechst dye. The specificity of this effect has been demonstrated by blocking these cellular pumps with agents such as verapamil *(34)*. The presence of transporter proteins in both tumorigenic, as well as normal stem cells, may be one of the factors conferring on this stem cell population resistance to chemotherapy-induced apoptosis.

Failures in chemotherapy have been linked to the development of a multidrug resistance. In many cases, the initial shrinkage of a tumor is followed by the development of resistance to drugs to which the tumor was initially exposed, as well as to other drugs to which there was no prior exposure. Multidrug resistance is caused in part by the decrease in the accumulation of drugs inside the cells because of activity of ABC protein transport-

ers *(34–37)*. BCRP, first described in breast cancers that were resistant to chemotherapy, has been found to be overexpressed in normal hematopoietic stem cells. Expression of BCRP may also protect stem cells against hypoxia. In stem cells, hypoxic environments induce the expression of BCRP that in turn prevents the detrimental accumulation of porphyrins (including heme) that can generate reactive oxygen species and damage the mitochondria *(38)*.

In addition to serving a protective mechanism in these cells, it has been suggested that these transporter proteins may play a direct role in stem cell biology by pumping out agents that induce cellular differentiation, thus keeping the stem cells in an undifferentiated state *(35,39)*. For example, ABC transporters have been shown to play a significant role in cell fate determination by exporting differentiation factors in *Dictyostelium (40,41)*.

7. SELF-RENEWAL AND SURVIVAL: ARE THESE PROCESSES LINKED?

As noted previously, stem cells are the only cells capable of undergoing self-renewal. Recent work has shed light on pathways that may regulate this process. A number of pathways that play in important role during development have been implicated in stem cell self-renewal. These pathways include Wnt, Hedgehog, Notch, as well as the transcription factor Bmi-1. Interestingly, each of these pathways when dysregulated has been found to promote carcinogenesis in murine models. Furthermore, there is accumulating evidence for dysregulation of these pathways in a variety of human malignancies. (For review of the role of these pathways in carcinogenesis, *see* refs. *42–45*.) In addition to their role in carcinogenesis, each of these pathways has also been linked to self-renewal of stem cells. For instance, Wnt signaling has been found to be involved in the self-renewal of hematopoietic stem cells. Dysregulation of this pathway has recently been demonstrated to play a role in the generation of chronic myelogenous leukemia. In this case, the activation of the Wnt pathway in myeloid progenitor cells may be responsible for expansion of leukemic clones. Hedgehog signaling has been implicated in a variety of human malignancies, including basal carcinoma of the skin *(46)* small cell lung cancer *(47)* as well as a number of gastrointestinal malignancies *(48)* including gastric cancer *(49)* and pancreatic cancer *(50)*.

Recently, evidence has been provided that this pathway is also dysregulated in human prostate *(51,52)* and breast *(53)* cancer. In addition to their role in self-renewal of stem cells, it now appears that each of these pathways is also linked to cell survival. For example, activation of Wnt signaling increases the generation of insulin-like growth factors, which in turn stimulate Akt, promoting cell survival *(54)*. These pathways may have evolved as important antineoplastic mechanisms, preventing stem cells from forming tumors. Simultaneous activation of self-renewal and survival pathways may be required for stem cell self-renewal and expansion. If this is the case, then specific targeting of the self-renewal pathways may provide an important approach to the induction of cell death in tumor stem cells.

8. CLINICAL IMPLICATIONS

The tumor stem cell model of carcinogenesis has fundamental implications for the development of new cancer therapeutic agents. In the past, antineoplastic agents have largely been developed through testing in animal models, as well as in phase II human

clinical trials. In both of these, the end point has been shrinkage of tumors. Tumor response is usually defined in the clinic as the shrinkage of a tumor by at least 50%. However, if tumor stem cells are inherently resistant to chemotherapeutic agents and if these cells comprise only a minority of the tumor, then the shrinkage of tumors may merely reflect effects of chemotherapy on differentiated cells in a tumor rather than the tumor stem cell population. This may explain why induction of tumor regression often does not translate into clinically significant increases in patient survival. This has been illustrated for many tumor types including solid tumors and well as multiple myeloma, where patient survival does not correlate with changes in the M-protein levels *(55)*. If the tumor stem cell model of carcinogenesis is correct, then we may need to devise new experimental paradigms for evaluation of antineoplastic agents that can target stem cell populations. It will be important to find and validate intermediate end points that accurately predict ultimate patient survival. In this regard, future clinical trial designs may involve such intermediate end points such as time to tumor progression following delivery of an agent that can target tumor stem cells.

The tumor stem cell model also has implications for interpreting molecular profiling studies. These studies have shown that tumor gene expression profiles have important prognostic and predictive value. Molecular profiling of tumors reflects gene expression patterns of a tumor stem cell component, as well as the bulk of the tumor that is derived from these stem cells. The fact that the initial gene expression patterns are predictive of subsequent behavior is consistent with a model in which tumor stem cells and their particular mutation spectrum determine the expression profile of the entire tumor. We have recently described the implications of gene profiling in directing the hormonal therapy of breast cancer *(56)*. Most recently, a 21-gene expression profile of primary breast tumors has been shown to be useful in selecting patients for chemotherapy *(57)*. These genes may reflect the profile of a particular group of breast tumors derived from a common progenitor or stem cell and the mutation subset that share clinical characteristics.

The tumor stem cell model of carcinogenesis also has important implications for understanding metastasis and tumor dormancy. Micrometastasis of tumor stem cells may carry a different prognosis from micrometastasis of more differentiated cells. This may explain why up to 50% of breast cancer and prostate cancer patients with micrometastasis to their bone marrow do not develop overt metastasis over a 10-yr period *(58,59)*. One may postulate that some of these patients have metastasis of more differentiated cells, and only the metastasis of tumor stem cells will carry a poor prognosis. The elucidation of markers that define these stem cell populations will be necessary to confirm this hypothesis.

If the ultimate cure of various cancers depends on the elimination of tumor stem cells, one can question why several malignancies such as testicular carcinoma and choreocarcinoma are curable even in the metastatic setting with chemotherapy, whereas the vast majority of common malignancies are not. One might speculate that the stem cell component of testicular and choreocarcinoma are inherently different from other tissue stem cells because these involve germ cells *(60)*. Indeed, chemotherapy treatment of these tumors also often results in residual masses that are found to be benign teratomas composed of differentiated cells. An understanding of the inherent differences between the stem cells of testicular cancer and choreocarinoma compared to those from other tumors may provide new clues for the development of therapies against these common tumor types.

9. OPPORTUNITIES FOR THERAPEUTIC DEVELOPMENT

The tumor stem cell model suggests that it may be necessary to modify the current paradigm in cancer drug development. If the eradication of cancers requires the targeting and elimination of tumor stem cells, then one must devise therapies that can selectively kill these tumor stem cells while sparing normal stem cells. Because many pathways such as those involved in self-renewal are shared between tumor stem cells and their normal counterparts, this may seem a formidable task. However, recent studies in animal models that have utilized agents that target these pathways indicate the feasibility of this approach. For instance, Notch signaling requires processing by the enzyme γ-secretase. γ-secretase inhibitors have recently been shown to have activity against breast cancers that overexpresses Notch 1 *(61)*. Furthermore, in a murine model, these treatments appear to have little toxicity. Agents targeting Hedgehog signaling have recently been described to have antineoplastic activity. A Hedgehog inhibitor, cyclopamine, that specifically inhibits Hedgehog signaling by binding to the protein smoothend, was utilized to treat animals bearing a variety of tumor xenografts. Administration of cyclopamine to animals bearing prostatic cancer xenografts resulted in a dramatic regression of these tumors *(51)*. Although the specific targeting of tumor stem cells by these agents has not yet been demonstrated, the fact that remissions obtained by this treatment were long lasting is consistent with the potential elimination of tumor stem cells *(62)*. Furthermore, at least over brief periods, the administration of cyclopamine appeared to be nontoxic. A cyclopamine analog with 10 times the activity of the native compound has recently been shown to block medulloblastoma formation in a transgenic murine model *(63)*, and this therapy also appears to be nontoxic. Elements of the Wnt pathway represent other potential tumor stem cell targets. Toward this end, small molecule inhibitors of Wnt signaling have recently been produced that specifically interfere with the binding of β-catenin to ternary complex factor transcription factors *(64)*. It remains to be determined whether these small-molecule Wnt inhibitors have antitumor activity or toxicity.

In addition to targeting self-renewal pathways, it may be possible to target specific molecules present on tumor stem cells utilizing antibodies or antibody conjugated toxins. For example, Jones et al. have found that myeloma stem cells are pre-B cells that express CD20. This suggests that antibodies against CD20, such as the clinically available rituxamib, may have value in the treatment of myeloma by targeting its stem cell population. Furthermore, these studies suggest that the molecular profiling of tumor stem cells may identify new targets for therapeutic development.

10. CONCLUSIONS

In this chapter, we have reviewed evidence for the existence of tumor stem cells in a variety of human malignancies. These tumor stem cells that drive tumorigenesis may be resistant to currently available chemotherapeutic agents. These cells may therefore contribute to resistance of tumors to these agents as well as to relapse following treatment. If this is the case, then the development of more effective cancer therapies will require the targeting of the tumor stem cell population. A paradigm shift in cancer therapeutics may be required to develop agents that selectively target tumor stem cells while sparing their normal stem cell counterparts. Evaluation of these agents may require alterations in

current clinical trial designs. Nevertheless, the recent elucidation of mechanisms that govern key events in both normal and tumor stem cells suggests the feasibility of selectively targeting these pathways to develop more effective cancer therapeutics.

REFERENCES

1. Till JE, McCullouch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961; 14:213–222.
2. Singh SK, Clarke ID, Hide T, et al. Cancer stem cells in nervous system tumors. Oncogene 2004; 23:7267–7273.
3. Al-Hajj M, Clarke M. Self-renewal and solid tumor stem cells. Oncogene 2004; 23:7274–7282.
4. Dontu G, Al-Hajj M, Abdallah WM, et al. Stem cells in normal breast development and breast cancer. Cell Prolif 2003; 36(Suppl 1):59–72.
5. Owens DM, Watt FM. Contribution of stem cells and differentiated cells to epidermal tumours. Nat Rev Cancer 2003; 3:444–451.
6. Reya T, Morrison SJ, Clarke MF, et al. Stem cells, cancer, and cancer stem cells. Nature 2001; 414:105–111.
7. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med 2004; 351:657–667.
8. Passeguâe E, Jamieson CH, Ailles LE, et al. Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? Proc Natl Acad Sci U S A 2003; 100(Suppl 1):11,842–11,849.
9. Bonnet D, Dick JE, Department of Genetics RIHfSCUoTOC. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3:730–737.
10. Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol 2004; 5:738–743.
11. Dorrell C, Takenaka K, Minden MD, et al. Hematopoietic cell fate and the initiation of leukemic properties in primitive primary human cells are influenced by Ras activity and farnesyltransferase inhibition. Mol Cell Biol 2004; 24:6993–7002.
12. Al-Hajj M, Wicha M, Benito-Hernandez A, et al. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100:3983–3988.
13. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003; 63:5821–5828.
14. Yuan X, Curtin J, Xiong Y, et al. Isolation of cancer stem cells from adult glioblastoma multiforme. Oncogene 2004; 23:9392–9400.
15. Galli R, Binda E, Orfanelli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 2004; 64:7011–7021.
16. Matsui W, Huff CA, Wang Q, et al. Characterization of clonogenic multiple myeloma cells. Blood 2004; 103:2332–2336.
17. Mueller MM, Fusenig NE, Group T, et al. Friends or foes—bipolar effects of the tumour stroma in cancer. Nature reviews. Cancer 2004; 4:839–849.
18. Wang F, Hansen RK, Radisky D, et al. Phenotypic reversion or death of cancer cells by altering signaling pathways in three-dimensional contexts. J Natl Cancer Inst 2002; 94:1494–1503.
19. Hennighausen L, Robinson GW. Signaling pathways in mammary gland development. Dev Cell 2001; 1:467–475.
20. Strange R, Metcalfe T, Thackray L, et al. Apoptosis in normal and neoplastic mammary gland development. Microsc Res Tech 2001; 52:171–181.
21. Marshman E, Booth C, Potten CS. The intestinal epithelial stem cell. Bioessays 2002; 24:91–98.
22. Jordan CT, Guzman ML. Mechanisms controlling pathogenesis and survival of leukemic stem cells. Oncogene 2004; 23:7178–7187.
23. Mayer B, Klement G, Kaneko M, et al. Multicellular gastric cancer spheroids recapitulate growth pattern and differentiation phenotype of human gastric carcinomas. Gastroenterology 2001; 121:839–852.
24. Dontu G, Wicha MS. Survival of mammary stem cells in suspension culture: implications for stem cell biology and neoplasia. J Mammary Gland Biol Neoplasia 2005; 10:75–86.
25. Venezia TA, Merchant AA, Ramos CA, et al. Molecular signatures of proliferation and quiescence in hematopoietic stem cells. PLoS Biol 2004; 2:e301.
26. Cairns J. The cancer problem. Sci Am 1975; 233:64–72, 77–68.

27. Potten CS, Owen G, Booth D. Intestinal stem cells protect their genome by selective segregation of template DNA strands. J Cell Sci 2002; 115:2381–2388.
28. Park Y, Gerson SL. DNA repair defects in stem cell function and aging. Annu Rev Med 2004; 56:495–508.
29. Cai J, Weiss ML, Rao MS. In search of "stemness." Exp Hematol 2004; 32:585–598.
30. Wang S, Yang D, Lippman ME. Targeting Bcl-2 and Bcl-XL with nonpeptidic small-molecule antagonists. Semin Oncol 2003; 30:133–142.
31. Goodell MA, Brose K, Paradis G, et al. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 1996; 183:1797–1806.
32. Doyle LA, Yang W, Abruzzo LV, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 1998; 95:15,665–15,670.
33. Alvi A, Clayton H, Joshi C, et al. Functional and molecular characterisation of mammary side population cells. Breast Cancer Res 2002; 5:E1.
34. Ross DD, Doyle LA. Mining our ABCs: pharmacogenomic approach for evaluating transporter function in cancer drug resistance. Cancer Cell 2004; 6:105–107.
35. Bunting KD. ABC transporters as phenotypic markers and functional regulators of stem cells. Stem Cells 2002; 20:11–20.
36. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2002; 2:48–58.
37. Dean M, Allikmets R. Complete characterization of the human ABC gene family. J Bioenerg Biomembr 2001; 33:475–479.
38. Krishnamurthy P, Ross DD, Nakanishi T, et al. The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with heme. J Biol Chem 2004; 279:24,218–24,225.
39. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 2001; 7:1028–1034.
40. Good JR, Cabral M, Sharma S, et al. TagA, a putative serine protease/ABC transporter of Dictyostelium that is required for cell fate determination at the onset of development. Development 2003; 130:2953–2965.
41. Good JR, Kuspa A. Evidence that a cell-type-specific efflux pump regulates cell differentiation in *Dictyostelium*. Dev Biol 2000; 220:53–61.
42. Liu S, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. Breast Cancer Res 2005; 7:86–95.
43. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. Nature 2004; 432:324–331.
44. Kopper L, Hajdu M. Tumor stem cells. Pathol Oncol Res 2004; 10:69–73.
45. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem cell biology to cancer. Nat Rev Cancer 2003; 3:895–902.
46. Wetmore C, Division of Pediatric Hematology Oncology DoP, Adolescent M, et al. Sonic hedgehog in normal and neoplastic proliferation: insight gained from human tumors and animal models. Current Opin Genet Dev 2003; 13:34–42.
47. Watkins DN, Berman DM, Burkholder SG, et al. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 2003; 422:313–317.
48. Oniscu A, James RM, Morris RG, et al. Expression of Sonic hedgehog pathway genes is altered in colonic neoplasia. J Pathol 2004; 203:909–917.
49. Xie K, Abbruzzese JL. Developmental biology informs cancer: the emerging role of the hedgehog signaling pathway in upper gastrointestinal cancers. Cancer Cell 2003; 4:245–247.
50. Thayer SP, di Magliano MP, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003; 425:851–856.
51. Karhadkar SS, Bova GS, Abdallah N, et al. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature 2004; 431:707–712.
52. Fan L, Pepicelli CV, Dibble CC, et al. Hedgehog signaling promotes prostate xenograft tumor growth. Endocrinology 2004; 145:3961–3970.
53. Lewis MT. Hedgehog signaling in mouse mammary gland development and neoplasia. J Mammary Gland Biol Neoplasia 2001; 6:53–66.
54. Sinha D, Wang Z, Ruchalski KL, et al. Lithium activates the Wnt and phosphatidylinositol 3-kinase Akt signaling pathways to promote cell survival in the absence of soluble survival factors. Am J Physiol 2004; 288:F703–F713.
55. Kyle RA, Rajkumar SV, Division of H, et al. Multiple myeloma. New Engl J Med 2004; 351:1860–1873.

56. Dontu G, El-Ashry D, Wicha MS. Breast cancer, stem/progenitor cells and the estrogen receptor. Trends Endocrinol Metab 2004; 15:193–197.
57. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004; 351:2817–2826.
58. Braun S, Vogl FD, Janni W, et al. Evaluation of bone marrow in breast cancer patients: prediction of clinical outcome and response to therapy. Breast 2003; 12:397–404.
59. Braun S, Pantel K. Prognostic significance of micrometastatic bone marrow involvement. Breast Cancer Res Treat 1998; 52:201–216.
60. Masters JR, Koberle B. Curing metastatic cancer: lessons from testicular germ-cell tumours. Nat Rev Cancer 2003; 3:517–525.
61. Weijzen S, Rizzo P, Braid M, et al. Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. Nat Med 2002; 8:979–986.
62. Sanchez P, Hernandez AM, Stecca B, et al. Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. Proc Natl Acad Sci U S A 2004; 101:12,561–12,566.
63. Romer JT, Kimura H, Magdaleno S, et al. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in $Ptc1^{+/-}p53^{-/-}$ mice. Cancer Cell 2004; 6:229–240.
64. Lepourcelet M, Chen YN, France DS, et al. Small-molecule antagonists of the oncogenic Tcf/β-catenin protein complex. Cancer Cell 2004; 5:91–102.