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# Cancer and Stem Cell Signaling: A Guide to Preventive and Therapeutic Strategies for Cancer Stem Cells

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## Introduction

This issue of Stem Cell Reviews is directed toward the identification of signal transduction pathways shared by embryonic stem cells, tissue determined stem cells and cancer stem cells. The practical goal is to identify trans-activating pathways that are over-expressed in cancer stem cells as compared to normal stem cells and to explore the possibility that blocking or modifying these pathways will allow targeted cancer stem cell therapy.

## Cancers are Caricatures of Normal Tissue

Normal tissues and cancer tissues consist of the same populations of cells: resting stem cells, proliferating transit-amplifying cells, and terminally differentiated cells [1]. During normal tissue renewal, new mature tissue cells arise from the differentiation of proliferating transit-amplifying cells. Normal tissue stem cells are usually held in reserve, but can respond to tissue loss by producing more transit amplifying cells. Cancer results when transit-amplifying cells fail to differentiate fully, continue to proliferate, and do not die. The outcome is an accumulation of undifferentiated or poorly differentiated cancer transit-amplifying cells.

At present, the most effective non-surgical treatments for cancer are directed against the cancer transit-amplifying cells: chemotherapy and radiation therapy kill them, differentiation therapy forces them to undergo terminal differentiation and apoptosis, and anti-angiogenic therapy limits their blood supply [2–4]. However, even if the cancer transit-amplifying cells are completely inhibited or eliminated, the cancer may eventually re-form from cancer stem cells, which are resistant to therapy directed against the proliferating transit-amplifying population (Fig. 1). For effective elimination of a cancer, the cancer stem cells must also be eliminated, or permanently blocked. Therefore, cancer therapy must be directed not only against the proliferating cells of the cancer, but also against the resting cancer stem cells. This can be done by interrupting the cell signaling pathways that allow cancer stem cells to survive and become proliferating cancer transit-amplifying cells. The current issue of Stem Cell Reviews brings together papers on stem cell signaling that provide the first step in identifying those signals that are critical not only for normal stem cell maintenance and differentiation, but also for cancer stem cells. The hypothesis is that if the cancer stem cell signaling pathways can be blocked, without seriously compromising normal tissue renewal, the cancer can be eliminated or permanently inhibited [4].

An excellent place to begin is the paper by Dreesen and Brinvanlou. These authors systematically analyze the role of seven major signaling pathways implicated in the maintenance of both cancer and normal embryonic stem

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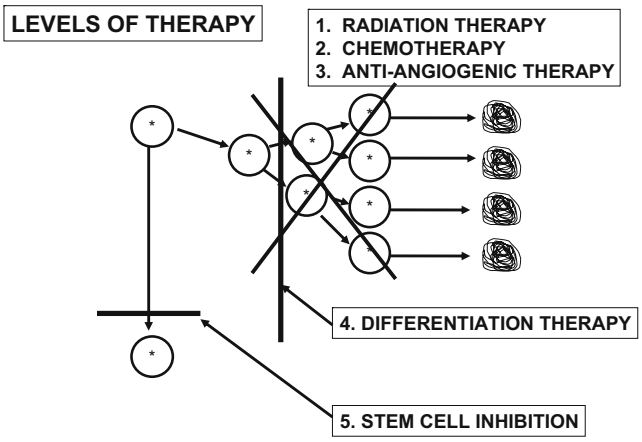


**Fig. 1** Levels of Therapy for Cancer. Radiation therapy (1) and chemotherapy (2) kill the proliferating transit-amplifying cells of the cancer. Anti-angiogenic therapy (3) blocks the blood supply to the proliferating cells, thereby inhibiting growth. Differentiation therapy (4) inactivates signals that cause maturation arrest and thereby allow the cancer cells to terminally differentiate. Even if these approaches are effective cancers will regrow from the resting cancer stem cells if the therapies are discontinued. The goal of stem cell inhibition therapy (5) is to prevent cancer stem cells from reformining the cancer. Modified from [4]

cells: JAK/STAT, Notch, MAPK/ERK, PI3K/AKT, NF-κB, Wnt and TGF-β (see Fig. 1 in Dreesen and Brivanlou, this issue). STAT3 is active in many lung, breast and head and neck cancers. Mutations in the Notch signaling pathway are found in over half of human T-cell tumors. MAPK/ERK pathway mutations (RAS) are found in about half of colon cancers and over 90% of pancreatic cancers. Deregulated PI3K/AKT signaling is seen in glioblastomas, lung carcinomas and melanomas, as well as in breast, ovarian, and thyroid cancers. The Nf-κB pathway is activated in Hodgkin's lymphoma and B-cell lymphomas. Aberrant Wnt signaling is found in intestinal carcinomas, gastric polyps, and myeloid leukemias. The TGF-β pathway is active in lung cancers. Associated with each of these signaling pathways are naturally occurring inhibitors that control activation of the pathway; such inhibitors are candidates for investigator induced inhibition.

Peter Neth and co-authors focus on Wnt signal transduction in stem cells and cancer cells involved in the control of migration and in the invasive behavior of human mesenchymal stem cells; they access the relationship of this property to the invasive phenotype of cancer cells. They find a correlation between loss of epithelial characteristics of a tumor (epithelial–mesenchymal transition) and activation of the Wnt/β-catenin pathway that leads to invasion and metastasis. This transition can be at least partially reversed by blocking production of molecules involved in the signaling pathway with small inhibitor RNA (iRNA) directed to the messenger RNA of β-catnenin or the Wnt co-receptor, LRP5. This inhibition reduces proliferation and invasiveness of treated cells, thereby providing proof of principle for inhibition of stem cell signaling pathways for treatment of cancer.

Further extensive characterization of the Wnt signaling system is accomplished by Katoh. His studies clearly show the complexities of one important signaling pathway, and multiple interactions between this signaling pathway and other signaling pathways. He points out the critical importance of a balance between Wnt-FGF-Notch and BMP-Hedgehog signaling for maintenance of homeostasis between stem and progenitor cells. His presentation shows that attempts to inhibit one or a few signaling molecules can lead to unexpected results, due to the degree of "cross-talk" that exists between the signaling pathways. He predicts the future use of a personalized transcriptome for each individual that could be used for genetic screening, prognosis prediction, and therapeutic optimization of that individual's cancer. Thus, each human cancer may have a set of signals that once known, can be exploited in an individualized therapy, to block the signals than activate the stem cells of the cancer.

The end point of the Wnt pathway is discussed by Yi and Merrill, who describe the downstream DNA-binding transcription factors in the Wnt signaling pathway (Tcf proteins). These factors either activate or inhibit the transcription of target genes. An understanding of how the Tcf proteins regulate gene expression will offer us the means to determine how stem cells regulate their self-renewal, differentiation and proliferative potential. For an intervention in the Wnt pathway to be effective, we may need to consider the pathway at multiple levels: not only the phosphorlyation steps and preservation of β-catenin, but also the interaction of β-catenin with its co-transcription factor Tcf, to form a sequence-specific bipartite transcription activator. However, Tcf proteins do more than form a transcription complex with β-catenin. Other functions or these proteins include activation of cancer from normal tissue stem cells. For example, decreased Tcf levels may be related to activation of breast cancer stem cells. Nanog is believed to be a major factor for self-renewal and survival of embryonic stem cells and is also a potential oncogenic signal. Nanog is activated by Oct4-Sox2, but is suppressed by Tcf3. Thus, manipulation of Tcf-related proteins may be another way to control cancer stem cell signaling.

Amander Clark points out that somatic germ cells retain core molecules essential for stem cell pluripotency during the initial phases of germ cell development, at the primiordial germ cell (PGC) stage. These include the transcription factors Nanog, Oct4, and Sox2. Invasive

seminomas (germ cell tumors) are associated with high levels of fetal germ cell markers, including Oct4, Nanog and growth differentiation factor 3 (GDF3). At the same time there is no detectable expression of differentiation meiotic or post meiotic germ cell factors, supporting the hypothesis that seminoma arises from an abnormal fetal PGC. Nevertheless, seminomas do not have stem cell-like differentiation capabilities (pluirpotency), in contrast to embryonal carcinoma cells. The most striking feature of human testicular cancer is the acquisition of duplication at chromosome 12p, where pluripotent transcription factors such as Nanog are physically located. It is possible that this abonormality maintains the transformed stem cell phenotype universal among testicular cancer stem cells.

The paper by Byrnes and commentary by Sherley discusses the proposal that Nanog is critical for reprogramming nuclei during altered nuclear transfer-oocyte assisted reprogramming (ANT-OAR). Induction of overexpression of Nanog in an enucleated oocyte and a somatic cell designated for nuclear transfer has been proposed as a means of enhancing somatic cell nuclear transfer, and maintaining pluripotency. Byrnes argues that this proposal is too simple, and that other factors must be involved. This paper again emphasizes the interplay of signaling pathways in normal development of the embryo. The answer to the question of whether or not ANT-OAR, or another signal, or a combination of signals, will actually work awaits critical experimental development. For those with religiously based objections, whether this strategy is an alternative acceptable to derive new human embryonic stem cells lines from normal blastocysts in vitro, remains to be seen. It has been the policy of Stem Cell Reviews to present these controversial issues. Only when the experiments are done will we know the answer to the technical reservations. Whatever the result, the ethical arguments are likely to continue.

## Stem Cell Signals and Cancer Therapy

Given that at least seven interacting signaling pathways that are active in cancers and presumptive cancer stem cells have been identified, how can this information be used to design therapies for cancers? As outlined above, cancer treatments now in general use are directed toward killing the transit amplifying cells (radiation therapy and chemotherapy), preventing proliferation of transit amplifying cells (anti-angiogenesis) or forcing cancer cells to differentiate (differentiation therapy). The effectiveness of differentiation therapy convincingly demonstrates the role of maturation arrest in cancer and the role of cancer stem cells in regrowth of cancers [2].

## Differentiation Therapy

Therapy to reverse maturation arrest of cancers and allow terminal differentiation of cancer transit amplifying cells, i.e., differentiation therapy, has been shown to be effective against some cancers, in particular, myeloid leukemias [3]. Perhaps the best example is the use of imatinib (Gleevec) for treatment of chronic myeloid leukemia (CML) [5]. Continuous treatment of chronic-phase CML with imatinib as initial therapy has induced long-lasting clinical responses. Such treatment is targeted, because imatinib blocks the specific cell signaling tyrosine kinase (*BCR/ABL* fusion product) that is constitutively activated in CML. It is differentiation therapy, because blockade of the BCR/ABL mediated signal prevents continued activation of the cells, and allows the leukemic cells to differentiate and die [3]. Differentiation therapy is also possible for other forms of leukemia [3]. However, differentiation therapy is not permanent; the leukemic stem cells are not destroyed by this treatment, and will re-form the leukemia if therapy is discontinued [5]. Thus, a logical next step in the advance of cancer treatment is stem cell inhibition [4].

It appears possible for an embryonic microenvironment to "reprogram" malignant melanoma cells, as it does embryonic cells or teratocarcinoma cells. Abbott et al. describe phenotypic similarities between embryonic stem cells and multipotent, tumorigenic melanoma cells, not only in signaling, but also in response to an embryonic microenvironment. Furthermore, in zebrafish embryo model, multipotent melanoma cells placed in the animal pole of the blastula induced the formation of an ectopic cranial outgrowth of zebrafish progenitor cells, and were no longer tumorigenic. The molecular signature of aggressive melanoma cells shares some commonality with that of human embryonic cells. For example, Nodal, an embryonic morphogen and a member of the TGF-β superfamily involved in maintenance of stem cell pluripotency, is expressed by embryonic stem cells, and aberrantly overexpressed by multipotent melanoma cells. Lefty, secreted by embryonic cells, is an inhibitor of Nodal, but this inhibitor is not expressed by melanoma cells, thus allowing them to sustain an unregulated plastic phenotype. The authors propose that Lefty, or other inhibitors of the Nodal pathway (see Table 1), could be used for therapy of melanoma. Nodal expression is correlated with melanoma pathogenesis, and may serve as a new biomarker for disease progression.

## Stem Cell Inhibition Therapy

Inhibition of cancer stem cells may be mediated by molecules that block cancer stem cell signaling pathways

**Table 1** Some inhibitors of major stem cell signaling pathways

| Signaling pathways | Inhibitors |
|---|---|
| JAK-STAT | APS |
| Notch | γ-secretase inhibitor (DAPT) |
| MAPK/ERK | RAF kinase inhibitors/U0126 |
| PI3-K/Akt | Rapamycin (LY294002) |
| NF-κB | I-κB, PTDC |
| Wnt/β-catenin | NSAID, GSK-3, sFRPs, DKK, Axin |
| TGFβ (BMP) | SMAD6,7; Lefty1,2; Gremlin, SM16, etc. |
| Sonic hedgehog (sHH) | Cyclopamine |
| Oct-4/Sox2/Nanog | Tcf3 |

or by the use of iRNA molecules that block synthesis of the signaling molecules.

## Molecular Inhibitors

Table 1 lists some major signaling pathways for normal and cancer stem cells, and some inhibitors currently under investigation. In Table 2, the full names of these inhibitors are given. While these agents have been shown to inhibit stem cell signaling pathways, much more work needs to be done to determine whether any of these are effective in vivo, how they interact with one another, what the effects on both normal and cancer stem cells will actually be, and even whether they can be administered to a living animal without loss of activity, or without production of untoward effects.

## iRNAs and Cancer Stem Cell Signals

iRNAs have been shown to block stem cell signals effectively during normal development [6]. For application of iRNA to cancer treatment, it is first necessary to identify which signals are active in cancer stem cells. In his paper in this issue, Glinsky identifies particular patterns of gene expression of "stemness"/differentiation pathways in 12 types of cancer [7]. Stemness expression patterns are associated with a short interval to disease recurrence, the presence of distant metastasis, early death and therapy-resistant cancers. Differentiation pathways are associated with are associated with more benign disease. For example, it is possible to predict relapse-free survival of human breast and prostate cancer patients through assessment of the levels of expression of a minimal set of four stem cell genes: BMI1, Oct4, EED and Lmo4. With the use of a microarray-based cancer therapy outcome predictor alogorithm, it may be possible to obtain an expression pattern for each cancer, and to then test the effect of selected iRNAs on cultured cancer cells from individual patients. Once these data are available, it may be possible to design a means of delivery of a selected set of iRNAs directed to the cancer cells that will have little or no effect on normal tissue renewal. To be effective, these iRNAs will most likely need to be used in combination with other forms of cancer therapy, including the molecular inhibitors of cancer stem cells listed above. Studies on how to block cancer stem cell signals effectively, while not disrupting normal stem cell signals, promise to identify approaches that can be used in

**Table 2** Some stem cell signaling pathway inhibitors

| Stem cell signaling pathway inhibitors | |
|---|---|
| APS | Adaptor molecule (pleckstrin homology and SH-2 domains) |
| NSAID | Non-steroidal anti-inflammatory drugs |
| GSK-3 | Glycogen synthesis kinase-3 |
| sFRPs | secreted Frizzled-related proteins |
| DKK | Dickkopf family (WIF-1, Cerebus) |
| DAPT | γ-secretase inhibitor, *N*-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester |
| SMAD6,7 | Related to Drosophila Mad (Mothers against decapentaplegic), Inhibitor of SMAD transcription factors for TGF-β pathway |
| Lefty1,2 | Inhibitor of Activin activation of TGF-β pathway |
| Gremlin | Inhibitor of BMP activation of TGF-β pathway |
| LY294002 | selective PI3 kinase (PI3K) inhibitor |
| I-κB | Inhibitor of κB |
| PTDC | sodium pyrrolidinethiocarbamate |
| U0126 | MAP kinase inhibitor-1,4-Diamino-2,3-dicyano-1,4-bis(o-aminophenylmercapto)butadiene ethanolate |
| SM16 | Small molecular inhibitor of TGF-β type I receptor kinase (ALK5) |
| Tcf3 | Repressor of Wnt target genes |

In lieu of an extensive bibliography covering these agents, the reader is advised to look them up in a standard internet search program, such as Google.

**Table 3** Some vectors for delivery of iRNA

| Vectors |
|---|
| CYCLODEXTRIN POLYMERS |
| CHO MODIFIED |
| LIPOSOMAL NANOPEPTIDES |
| BIOLOGIC NANOPEPTIDES |
| LENTIVIRUSES |
| ADENOVIRUSES (ONCOLYTIC) |

combination with other therapeutic modalities, to finally provide cures for cancer. However, many pitfalls must be overcome, before this gains clinical application.

## Delivery of Gene Therapy

A major problem is how to deliver iRNAs or non-RNA molecular inhibitors to cancer stem cells while maintaining integrity of the inhibitors, and not negatively affecting normal tissue stem cells.

## Route

Some specificity can be determined by the route of administration: systemic administration (intravenous injection) for hematologous cancers (leukemias, lymphomas), intrahepatic for hepatocellular carcinoma, intraperitoneal for ovarian cancer, or nasal inhalation for lung. It may be possible to inject the inhibitory molecules directly into some cancers, such as breast and prostate cancers, or directly into some metastatic cancer sites, but it is unlikely that the agent will reach all the cells of the tumor. Preclinical models are now being tested to determine the most efficacious way to combine the route of injection with vectors to deliver iRNA.

## Vectors

Protected delivery of iRNA may be accomplished using liposomal or biological nanoparticle carriers, as well as virus-based expression vectors [8] (see Table 3). Delivery of genes by lentiviral vectors has the advantage that the vectors infect both non-cycling and post-mitotic cells and the transgenes expressed from lentivirus are not silenced during differentiation of embryonic stem cells [9, 10]. Cancer stem cells can be targeted by receptors that are known to be over expressed on cancer stem cells as compared to normal stem cells. For example, biological nanoparticles containing iRNA may be coated with antibodies to Her2/neu, EGFR, epithelial specific antigen (ESA) or the CD44 phenotype on breast cancer stem cells [11, 12]. Other markers that may be addressed in this way are c-kit [13, 14], Notch 1 [15, 16], CD-133 [17], chemokine receptor CXCR4 [18], CD34, SCA-1, Thy-1 [19], and the SLAM family members CD48, CD150 and CD 244 [20]. Nano-immunoliposome complexes of siRNA to HER-2 encapsulated by a cationic liposome and decorated with Anti-TfR single-chain antibody fragment targets the complex to primary and metastatic lesions in a xenograft mouse model of human pancreatic and breast cancers [21].

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