

Cancer Stem Cell: A Potential New Target for Cancer Therapy

Xiaodong Zhu



About the author: Xiaodong (Sheldon) Zhu is currently a Postdoctor of University of California San Francisco. Dr. Zhu received a B.S from Hebei Univeristy, a M.S from Institute of Microbiology, Chinese Academy of Sciences and a Ph.D from Graduate University of the Chinese Academy of Sciences. He worked at National Engineer Research center for microbial drugs (North China Pharmaceutical Corporation) as assistant engineer, Yiling Pharmaceutical Corporation as New drug development manager in China. Before he came to San Francisco in 2004, he was a Research Assistant Professor at Institute of Microbiology, Chinese Academy of Sciences. His research interest is focused on the development and application of therapeutic antibodies or other agents targeting cancer stem cells and emerging virus infection.

A major problem associated with current cancer therapeutics is the eventual recurrence even after the elimination of bulk of the cancer cells. Recent studies on cancer stem cells may provide a potential solution to this problem. Here, we review the current status of cancer stem cell research which has attracted a great deal of attention. We then further discuss the potential therapeutic implication of this rapidly evolving area of cancer biology.

The hypothesis of cancer stem cells

The hypothesis is that there is a small subset of cancer cells within a tumor, termed cancer stem cells (CSCs), which are capable of both self-renewing and differentiating to heterogeneous lineages of cells within a tumor. It also suggests that CSCs have the properties that make them refractory to the current cancer drugs which target rapidly-dividing cells, and as a consequence, CSCs are responsible for the relapse and perhaps metastasis of tumors ¹.

Identification of CSCs

It is well known that tumors are made up by heterogeneous population of tumor cells with divergent morphological and differentiation features. In addition, it has been demonstrated that only a small proportion of the cancer cells are capable of forming colonies in in vitro clonogenic assays or in vivo transplantation assay. There are two alternative explanations to these phenomena, one is that every cell of the tumor has the capacity to proliferate and initiate new tumors but the probability of an individual cell completing the necessary steps in these assays is small (stochastic model). Another explanation is that only a rare, phenotypically-distinct, subpopulation of cells has the ability to initiate and form new tumors (CSC model).

Using an elegant experimental strategy, John Dick and colleagues discovered in 1997 that heterogeneous populations of cells in acute myeloid leukemia (AML) consist of a phenotypically-distinct population that is tumorigenic, and a much larger population that lacks this tumorigenic potential. They transplanted primary AML into NOD/SCID mice and found only rare cells, identified as CD34+CD38-, are capable of initiating and sustaining growth of the leukemia ².

Six years later, Michael Clarke and colleagues from University of Michigan isolated putative CSCs from breast cancer ³. They demonstrated that as few as 100 ESA+CD44+CD24-/lowLineage- cells were able to form tumors in mice, whereas no tumors were formed from tens of thousands of cells with alternate phenotypes. This subpopulation could be serially passaged in mice without loss of tumorigenicity showing their capacity for self-renewal and differentiation. Soon thereafter, a study showed that the CD133 positive sub-

Therapeutic Implication of Cancer Stem Cells

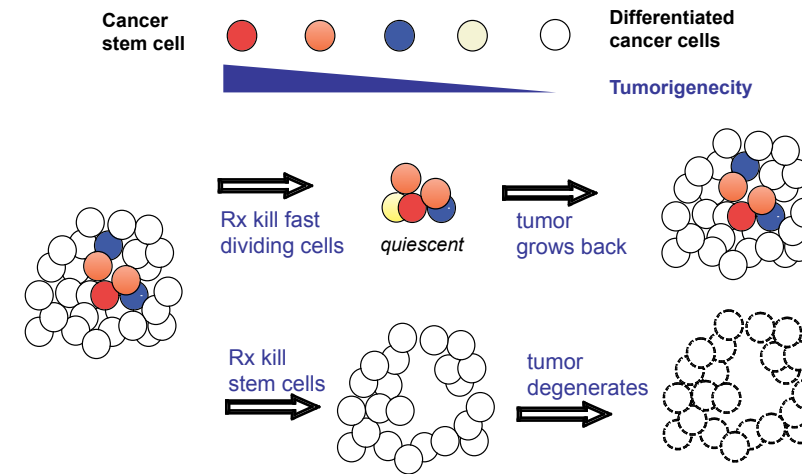


Figure 1. shows the CSCs working model (in blue) and their markers (in *Italic*).

population from human brain tumors has a dramatically enhanced capacity for initiating tumors in NOD-SCID mouse brains ⁴.

Subsequently, putative CSCs have been identified in many other types of tumors based on different surface markers, including colon cancer ⁵, liver cancer ⁶, prostate cancer ⁷ and melanoma ⁸.

Therapeutic implications of CSCs

There have been increasing evidences suggesting that the self-renewing CSCs are responsible for tumor initiation, recurrence, and resistance to conventional chemotherapy or radiation therapy.

Bao S et al demonstrated that brain tumor stem cells derived from human glioblastoma specimens display high resistance to radiation due to increased activation of the DNA damage checkpoint. It might explain the frequent recurrence or progression after radiation therapy. They also linked these tumor stem cells to tumor angiogenesis through increased expression of VEGF, suggesting that antiangiogenic therapies may improve targeting of CSCs and activity of radiation in solid cancer ⁹.

An intriguing property of CSCs is that they express high levels of specific ABC drug transporters. A distinct "side population" of cells with high drug or Hoechst 33342 efflux capacity mediated by ABCG2 and ABCB1 can be

isolated from many tissues including the brain, breast and liver, and these cells might represent lineage-specific stem cells. Studies have demonstrated that the side population of some primary tumors or cancer cell lines possesses CSC-like properties ^{10,11}.

Reported in a cover article in January 2008 issue of Nature, researchers from Harvard University established a direct relationship between tumor stem cells, tumor progression and chemoresistance ⁹. By NOD/SCID mouse xenotransplantation experiments, they demonstrated the subset defined by ABCB5, a novel drug transporter and chemoresistance mediator, was enriched for melanoma malignant initiating cells. There is a significant correlation between melanoma progression and expression level of ABCB5. They also demon-

strated that a monoclonal antibody targeting melanoma stem cells was capable of inducing antibody-dependent cell-mediated cytotoxicity and exerted tumor-inhibitory effects in mice.

Some studies also show that CSCs may play an important role in tumor metastasis and disease progression. Balic et al first showed that the majority of early disseminated cancer cells detected in the bone marrow of breast cancer patients have a putative breast CSC phenotype ¹². In another study, Hermann et al reported small population of CD133+ cells could initiate tumors. By subdividing CD133+ cells into two subsets based on the expression of the CXCR4 molecule, they demonstrated that only CD133+CXCR4+ subset was able to form spontaneous metastases ¹³.

If only a small population of tumor cells drives tumor formation and resist to traditional therapeutic agent, the goal of cancer therapy should be to identify this population and develop novel therapies that target it. First of all, it is critical that agents directed against CSCs discriminate between CSCs and normal stem cells. Rapamycin, a drug approved by FDA, has been shown to deplete leukemia stem cells and restore normal HSC function. It is possible to identify mechanistic differences between CSCs and normal stem cells. The differences could thus be targeted to eradicate CSCs without damaging normal stem cells ¹⁴.

Several other strategies of targeting CSC have been investigated.

1. Identify therapeutic targets that are preferentially expressed in CSCs. Target such markers using antibody-based approach. Several existing markers, although not specific to CSC, such as CD133, CD44, ABCB5, have been used in proof of concept studies.
2. Targeting stem-cell niches that provide a supportive microenvironment for their long-term self-renewal and survival¹⁵.
3. If the malignant cells of cancers are CSCs, then it should be potential to treat cancer by using differentiation promoting agent. Piccirillo et al used bone morphogenetic proteins (BMPs) to prompt differentiation of CD133+ brain tumor stem cells. They found that BMP treatment reduced the size of the tumors grafted into mice and prolonged the animals' survival¹⁶.
4. Targeting developmental signaling pathways, including Notch, Wnt, Hedgehog, Pten-PI3K, TGF β /BMP and LIF. These signaling pathways have been shown to play important roles in controlling normal stem cell self-renewal and implicated in CSC proliferation and development¹⁷.
5. A combination strategy using both the CSC inhibitors and conventional drugs may be more efficacious.

Controversies and questions

It should be emphasized that the CSC hypothesis has not been universally accepted and has many unanswered questions.

1. The origin of cancer: The cellular origin of CSCs remains unclear. Logically, CSCs must have the capacity to self-renew. Normal stem cells are the only cells that possess the ability. They could live long enough to acquire enough genetic abnormalities to become CSCs. However, due to extremely rapid mutation of cancers, it is possible that more differentiated cancer could acquire "stem-ness"- the ability to perpetually renew and generate differentiated progeny.
2. Rarity of CSC: It is possible that in some types of

cancer, CSCs may be less rare than assumed. Williams RT et al investigated the effects of Arf tumor suppressor inactivation on the generation of BCR-ABL-induced leukemia stem cells¹⁸. They found every p185+Arf- cell had leukemic capacity and these leukemia-initiating cells are not rare. The origin, biology of these cells is still not fully elucidated.

3. The current animal model and methodology to study CSC: A study by Australian scientists revealed that showed that mouse primary B lymphomas do not have CSCs and only 10 randomly selected tumor cells can initiate similar tumors following transplantation into congenic mice. They questioned xenotransplantation experimental model and argued that the rarity of human tumor cell function is not due to the existence of rare stem cells, but rather to the rarity with which human cells adapt to the mouse microenvironments¹⁹. This study suggests that it is necessary to develop improved or novel xenograft animal model for identification of CSCs and evaluation of future therapeutic efficacy.
4. Specific CSC markers: currently, there is a lack of specific markers to identify CSC. Several studies use CD133, a marker for normal stem cells, to isolate and identify CSC from various tissues. For example, CD133+ brain tumor stem cells can regenerate the original tumor in vivo after serial transplantation⁵. However, Beier et al reported that CD133- cells from GBM tumors were equally able to form orthotopic tumors as the CD133+ subset²⁰. It should also be noted that many brain tumor cell lines such as U87MG do not express CD133 epitopes but nonetheless form tumors when grafted into immunodeficient mice²¹. One potential issue is that the extensively used commercial anti-CD133 antibodies, AC133 and AC141, have not been well characterized in term of epitopes²² and crossreactivity²³.

Conclusion

Despite of the controversies, the evidence for the CSC hypothesis is increasing. Numerous studies have been published in the recent years supporting the hypothesis. Many scientists now believe that targeting CSC is a promising new strategy for cancer therapy. This emerging area of cancer research has renewed hope for more effective treatment and even cure for this devastating disease.

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Toward Understanding Intracellular Signaling Events in Embryonic Stem Cells

Gen-Sheng Feng



Introduction

Mouse embryonic stem cells (mESCs) are pluripotent cells that are derived from the inner cell mass of blastocyst-stage embryos¹. These undifferentiated cells retain the capacity to generate all cell types in the body, which was vividly demonstrated by their ability to contribute to all tissues of adult mice, including the germ cells, following injection into host blastocysts². Establishment of mouse ES cell lines, in combination with homologous DNA recombination, has allowed creation of mutant mouse strains with targeted gene elimination, which was credited by the 2007 Nobel Prize in Physiology and Medicine to Drs. Mario Capecchi, Martin Evans and Oliver Smithies.

In 1998, Thomson and colleagues successfully generated pluripotent human embryonic stem cell (hESC) lines, which has elicited a great deal of excitement in the fields of stem cell biology and regenerative medicine³. It has been widely recognized that hESCs and/or somatic stem cells might become potential sources of cells to regenerate damaged tissues. However, the molecular mechanisms underlying the control of hESC pluripotency and differentiation are not fully understood. In the past few years, much progress has been made in determining functional requirement of transcription factors Oct4, Sox2 and Nanog in maintenance of human and mouse ES cell identity. It is of interests to note that these factors are found to co-occupy a substantial portion of their target genes^{4,5}.

The essential role of these transcription factors in maintaining ES cell pluripotency was clearly demonstrated by Takahashi and Yamanaka⁶. These two researchers introduced retrovirus-carried transcription factors, *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*, into mouse embryonic or adult fibroblast cells, and created so-called iPS (induced pluripotent stem) cells. The iPS cells exhibit ES cell-like morphology and growth properties, express ES cell markers and retain the potential to differentiate into three germ layer cells in vivo. Upon modification of in vitro selection conditions, several groups have shown that the iPS cells established using this approach retain germ-line competency^{7,8}. Immediately following the success of establishing mouse iPS cells, similar attempts by several groups have proven successful in establishing human iPS cells⁹⁻¹¹. The Yamanaka group again used the same group of four transcription factors (*Oct3/4*, *Sox2*, *Klf4* and *c-Myc*), while Yu and colleagues used another combination: *Oct4*, *Sox2*, *Nanog* and *Lin28*. Notably, these human iPS cells have normal karyotypes and telomerase activity, express unique ES cell markers and retain developmental potential to differentiate into all germ layer cells. Evidently, the iPS cell technology can now allow us to establish customized human ES cells using somatic cells derived from patients. This type of reprogrammed iPS cells will be useful for molecular

analysis of cellular events involved in pathogenesis of diseases and also for drug selection and toxicology tests.

Comparative dissection of signaling events in mouse and human ES cells

The intracellular signaling mechanism for control of human ES cell pluripotency remains to be elucidated. Since mESCs have received intensive attentions in the past twenty years and a great wealth of information is available, characterization of hESC properties has been naturally conducted by reference with mESCs. Comparative analyses between hESCs and mESCs by several groups have defined common and distinct marker gene expression patterns, cellular properties and signaling mechanisms¹²⁻¹⁵. A number of growth factors and cytokines, such as leukemia inhibitory factor (LIF), bone morphogenic protein 4 (BMP4), basic fibroblast growth factor (bFGF) and Wnt are known to play critical roles in regulation of mESC and/or hESC pluripotency in culture¹⁶⁻¹⁸.

In mouse ES cells, LIF is a most critical cytokine for maintenance of pluripotency and self-renewal. Binding of LIF with a complex consisting of the LIF receptor and gp130 induced recruitment of the JAK family kinases that in turn activate signal transducer and activator of transcription 3 (Stat3) and extracellular signal activated kinase 1/2 (Erk1/2). It appears that gp130-mediated activation of Stat3 is sufficient to maintain pluripotent mouse ES cells in serum-containing medium. However, LIF is insufficient to block neural differentiation and sustain self-renewal of mouse ES cells in serum-free medium. Ying et al. found that LIF works in concert with bone morphogenetic proteins (BMPs) to maintain pluripotency, and mouse ES cells can be cultured and propagated in serum-free medium supplemented with BMP and LIF without feeders¹⁹. Furthermore, forced expression of Id genes, downstream factors induced by BMP via the Smad pathway, liberated mouse ES cells from dependence on BMP or serum. Thus, BMP-induced expression of Id proteins support LIF/Stat3 mediated self-renewal of mouse ES cells by suppressing lineage-specific gene expression and cell differentiation.

Shp2, a cytoplasmic tyrosine phosphatase, acts in the molecular switch governing ES cell self-renewal versus differentiation²⁰. A targeted deletion of Shp2 gene in mouse ES cells results in more efficient self-renewal. LIF-stimulated phospho-Stat3 signals are higher in Shp2-deficient mES cells compared to wild-type cells, and expression of wild-type Shp2 in Shp2-deficient

mES cells downregulates LIF-stimulated phospho-Stat3 levels²¹. Consistent with this observation, mES cells engineered to express a G-CSF-gp130 chimeric receptor avoiding a Shp2 binding tyrosyl residue (Y757) of gp130 required lower levels of gp130 stimulation to maintain pluripotency²². Thus, Shp2 facilitates mouse ES cell differentiation by downregulating the Stat3 pathway, while promoting signaling through the Erk pathway. The balance between Stat3 activation, essential for mESC self-renewal, and Erk activation, which favors differentiation, may determine stem cell fate. Whether Shp2 has a similar function in control of human ES cell differentiation and self-renewal remains to be determined.

Interestingly, LIF is unnecessary for maintenance of human ES cell self-renewal²³. Human ES cells undergo rapid differentiation in feeder-free culture medium supplemented with LIF. A similar phenotype of LIF-independency was observed for cynomolgus monkey ES cell lines²⁴. Human ES cells are routinely maintained on fibroblast feeder layers or in fibroblast-conditioned medium (CM). Xu and colleagues showed that human ES cells cultured in unconditioned medium (UM) are subjected to high levels of BMP signaling activity, which is reduced in CM. Indeed, a BMP antagonist Noggin synergizes with basic fibroblast growth factor (bFGF) to suppress BMP signal and stimulate self-renewing proliferation of human ES cells in the absence of feeder cells. These findings suggest a basic difference in the self-renewal mechanism between mouse and human ES cells^{18,25}. Thus, the functional requirement of BMP signaling appears to be opposite between human and mouse ES cells. However, activation of the Erk pathway leads to differentiation in both human and mouse ES cells, as expression of dominant negative mutants or use of pharmaceutical inhibitors of Mek suppressed hESC and mESC differentiation²⁶.

Small molecules that suppress ES cell differentiation and sustain self-renewal

With the recognition that too many variable factors exist in culturing human and mouse ES cells with feeder cells and serum, attempts have been made to find small chemical molecules that are potent to sustain ES cell pluripotency in culture. Meanwhile, work using these molecules has also provided fundamental insights into the critical signaling pathways involved in ES cell differentiation versus self-renewal. Brivanlou's group isolated 6-bromoindirubin-3'-oxime (BIO) as a specific pharmacological inhibitor of glycogen synthase kinase 3 (GSK3) from mollusk Tyrian purple. Activation of