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Cancer "Stem Cells" or Stem Cell "Cancer"?

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The hypothesis of the cancer 'stem cells' is not entirely new. It stemmed from the observation that not all the cells within a tumor can maintain tumor growth, and that large numbers of tumor cells are needed to transplant a tumor, even in an autologous context.

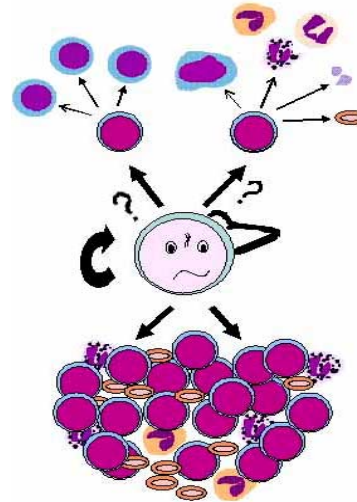
Moreover, most cancers are not clonal, but consist of heterogeneous cell populations, similar to the hierarchical tree of stem cell lineages. Also, a hypothetical long-lived stem cell at the base of tumor outgrowth would allow progression towards malignancy through accumulation of mutations and epigenetic changes.

But only recently, by applying techniques used in the stem cell field to identify self-renewing populations, has it become possible to prospectively isolate cancer stem cells. The prospective isolation and transplantation of defined cells allowed researchers to demonstrate that only a few cells within a tumor can generate heterogeneous tumors, whereas transplantation of the rest of the cells within a tumor did not give rise to tumors upon transplantation.

Interestingly, with a better characterization of tissue stem cells it has emerged that several long-known oncogenic pathways are pivotal to maintenance of normal stem cell self-renewal. Al-Hajj et al., focusing on the hematopoietic system, discuss these pathways, sum up the evidence for the cancer stem cell and discuss its implications for therapy.

Therapeutic implications of cancer stem cells. Al-Hajj M, Becker MW, Wicha M, Weissman I, Clarke MF (2004). *Curr Opin Genet Dev* 14:43.

Nakagawara and Ohira discuss the current knowledge on molecular links between mechanisms regulating neuroblastoma and normal development of synaptic neurons, including several gene expression screening



approaches and a comprehensive overview of differentially regulated genes.

Comprehensive genomics linking between neural development and cancer: neuroblastoma as a model.

Nakagawara A, Ohira M (2004). *Cancer Lett* 204:213.

Last year, several papers demonstrated that cancer stem cells could be isolated and characterized in more detail, thus supporting strongly the cancer stem cell model.

Al-Hajj et al. transplanted human patients' breast cancer cells into mice and identified a subpopulation (CD44 +/CD24 - /low /Lin -) that was the only one capable of generating heterogeneous primary and secondary tumors similar to the original patient specimens, indicative of a cell hierarchy within the solid tumors. Their results suggested that the tumorigenic cells can both self-renew and form non-tumorigenic cancer cells.

Prospective identification of tumorigenic breast cancer cells.

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003). *Proc Natl Acad Sci U S A* 100:3983.

From pediatric brain tumors, Hemmati et al. were able to isolate tumorigenic cells with characteristics similar to neural stem cells. These cells are multipotent, self-renewing and are able to produce proliferating neurospheres which can be differentiated into neurons and glia. These tumor-derived neurospheres share many expressed genes with normal neurospheres. However, unlike neural stem cell-derived neurospheres, these cells were longer lived and gave rise to abnormal dual-phenotype cells.

Cancerous stem cells can arise from pediatric brain tumors. Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, Kornblum HI (2003). *Proc Natl Acad Sci U S A* 100:15178.

Singh et al. identified a cell within different human brain tumors that interestingly expresses the surface marker CD133, which is also expressed on hematopoietic stem cells. This cell self-renews, but also differentiates *in vitro* into tumor cells similar to the original tumor of the patient. Moreover, the authors describe increased self-renewal capacity with increasing aggressiveness of the tumor sample.

Identification of a cancer stem cell in human brain tumors. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB (2003). *Cancer Res* 63:5821.

Pursuing a reverse approach, the following papers show tumor formation by normal adult tissue stem cells upon overexpression of molecules associated with normal self-renewal. Celso et al. overexpress inducible β -catenin targeted to the epidermis of adult mice, and show that continuous and long expression leads to *de novo* hair follicle formation, and to formation of benign tumors. Upon discontinuing β -catenin expression, the tumors regressed, although the skin did not return to normal and retained small epithelial outgrowths and cysts.

Transient activation of beta-catenin signaling in adult mouse epidermis is sufficient to induce new hair follicles but continuous activation is required to maintain hair follicle tumors. Lo Celso C, Prowse DM, Watt FM (2004). *Development* 131:1787.

Serakinci et al. overexpressed telomerase in normal adult tissue stem cells (human mesenchymal stem cells), and show the accumulation of mutations over time in culture, and acquisition of tumorigenicity when transplanted into mice. The authors also analyzed earlier and later passages of the cells by gene array.

Adult human mesenchymal stem cell as a target for neoplastic transformation. Serakinci N, Guldborg P, Burns JS, Abdallah B, Schrodder H, Jensen T, Kassem M (2004). *Oncogene* 23:5095.

Wnt signaling is involved in self-renewal of hemato-

poietic stem cells (HSC) as well as in tumor formation. Liu et al. investigated whether mammary progenitors are also regulated by Wnt signaling and show a correlation between activation of Wnt pathway, increase in progenitor numbers and subsequent tumor formation.

The transforming activity of Wnt effectors correlates with their ability to induce the accumulation of mammary progenitor cells. Liu BY, McDermott SP, Khwaja SS, Alexander CM (2004). *Proc Natl Acad Sci U S A* 101:4158.

And last, what about the old cell lines we all used over the years, and expanded incessantly and extensively? Not surprisingly, they do contain stem-like cells that have been maintained over all these abusive years in culture, sometimes even in low serum conditions. Maybe those are the cells to study in more detail to understand how to maintain self-renewal and obtain expansion of adult stem cells *in vitro*?

Kondo et al. analyzed the regenerative and tumor formation potential six well-known cell lines: C6 (rat glioma), MCF-7 (breast cancer), U-20 and SaOS-2 (osteosarcoma), B104 (rat neuroblastoma) and HeLa (adenocarcinoma, around since over 50 years). They find that, with the exception of U-20 and SaOS-2, these cell lines contain SP cells. Focusing on the C6 line, they identify PDGF and bFGF as *in vitro* maintenance factors in absence of serum and show that sorted SP cells can produce both new SP and non-SP cells in culture, can produce neurons and glia in culture, and form tumors containing both glia and neurons, when injected into nude mice.

Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Kondo T, Setoguchi T, Taga T (2004). *Proc Natl Acad Sci U S A* 101:781.

Now that it has been shown that certain cancers contain stem cell-like subpopulations, and conversely, that manipulation of self-renewal pathways in normal stem cells can lead to cancer, new questions arise. Is cancer a stem cell disease, i.e. a normal stem cell loses its regulatory mechanisms and fuels tumor growth? Or do tumors arise from non-stem cells, like a progenitor cells, that acquire a stem cell-like phenotype during carcinogenesis?

Hope et al. lineage-traced leukemic stem cells from AML patients that were serially transplanted into NOD/SCID mice. They were able to demonstrate

heterogeneity within the SCID leukemia-initiating cells (SL-IC). However, each donor sample gave rise to highly reproducible engraftment kinetics within cohorts of mice and over multiple experiments, as shown by tracing of proviral integration sites within cells isolated from the serially arising tumors. Moreover, the authors demonstrated elegantly that the engrafting cells within the tumors are organized in a hierarchical tree, similar to the hematopoietic stem/progenitor tree, including short-term, long-term and quiescent long-term SL-IC. The latter growing out only in secondary or tertiary recipients. These data suggest that in AML, the initial target cell is within the HSC compartment, with subsequent alterations possibly occurring in downstream progenitors from which the leukemic stem cells emerge.

Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Hope KJ, Jin L, Dick JE (2004). *Nat Immunol* 5:738.

On the other hand, previous work had determined that in CML, hematopoietic stem cells may not be involved, but rather a progenitor cell that re-acquired self-renewal capacities. Although CML is classified as a HSC disorder, the *bcr/abl* fusion gene is expressed in HSCs, direct evidence for HSC malignancy in CML has been lacking.

Jaiswal et al. made transgenic mice with expression of the *bcr/abl* fusion gene targeted exclusively to myeloid progenitors and their myelomonocytic progeny. These mice developed a myeloid disease sharing many characteristics with human CML, despite absence of *bcr/abl* expression in HSCs. Moreover, by crossing these mice with *bcl-2* transgenic mice they obtained a synergistic effect and induced blast crisis in these animals. These results are consistent with the hypothesis that additional modifications are necessary for full transformation, and correlates with the clinical finding that the majority of myeloid blast crisis patients have leukemic cells expressing high levels of *bcl-2*.

Expression of BCR/ABL and BCL-2 in myeloid progenitors leads to myeloid leukemias. Jaiswal S, Traver D, Miyamoto T, Akashi K, Lagasse E, Weissman IL (2003). *Proc Natl Acad Sci U S A* 100:10002.

In a follow-up study, Jamieson et al. demonstrate that despite expression of the *bcr/abl* fusion gene in their HSCs, CML is fueled by progenitor cells which reacquired self-renewal capacities. While CML patients carry the Philadelphia chromosome in their HSCs and have high levels of *bcr-abl* transcript, no increase in HSC numbers can be observed, compared to normal bone marrow. Moreover, the granulocyte-macrophage progenitor (GMP) was identified to acquire self-renewal capacity through activation of the β -catenin pathway. The authors show an increased activation of β -catenin in GMPs of patients in blast crisis, compared to their HSCs or normal marrow HSCs or GMPs. They also show that ectopic expression of β -catenin in normal GMPs confers self-renewal capacity as assessed by replating assay, and that ectopic expression of axin, a specific inhibitor of the β -catenin pathway, reduced the plating capacity of leukemic cells.

Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, Gotlib J, Li K, Manz MG, Keating A, Sawyers CL, Weissman IL (2004). *N Engl J Med* 351:657.

In conclusion, it is still unclear whether cancer is a disease originating purely in stem cells, or whether it is generated by a non-stem cell acquiring *de novo* self-renewal capacities. It is also possible that the stem cell carries the genetic make-up for disaster, and additional mutations are acquired along the differentiation of its progeny, resulting in a cell that acquires stem cell-likeness. Maybe it will depend on the tumor type and the environment in which the tumor originates, or on the genetic background of the individual, as suggested by mouse studies. But in any case, much can be learned by comparing normal stem cells with their tumorigenic counterpart. For future therapeutic approaches it will be important to identify pathways that are different. And for stem cell therapy, study of the 'reverted' progenitors may lead to insight into how reacquisition of self-renewal is regulated, to avoid such occurrences after stem cell/progenitor transplantation. This will be of importance when using adult stem cells from older individuals, as these cells may have accumulated mutations over their life-time.