including most of those available today in the public domain. In contrast to the commonly expressed reservations among cancer researchers, as a group the breast cancer cell lines were found to be surprisingly representative of the primary tumors. Although cell lines had a much higher number of genetic changes per sample than primary tumors, genetic events that were exclusively seen in the breast cancer cell lines were rare. As has been demonstrated with the extensively studied NCI-60 cancer cell line set (http://dtp.nci.nih.gov/index.html), molecular profiles of cancer cell lines could help to identify pharmacogenomic predictors of response to therapeutic compounds. For example, Neve et al. (2006) treated cell lines with Herceptin and identified protein levels and genomic aberrations that were correlated with response and resistance. Indeed, if more cancer drug response data became available, this "UCB-51" set could be much more representative and informative than the NCI-60 data series for targeted exploration of therapeutic hypotheses in breast cancer.

As much as these two studies advance the field, there are also many important aspects that remain to be explored in the future. First of all, every clinical study is dependent on the patient selection and therapies administered. These effects cannot be fully evaluated in retrospective studies. The present studies focused on amplifications, yet deletions and unbalanced translocations inactivating or activating cancer genes may also be important. As compared to the ~1 Mb resolution of the BAC arrays used in these studies, the latest generation oligo-array-based CGH can approach theoretical limits of about 10 kb across the nonrepetitive genomic DNA. Transcriptional profiling technologies also continue to advance. For example, alternatively spliced versions of genes are detectable with exon-level analysis, and detection of noncoding RNAs may pinpoint new information. The present studies focused on genetic profiles, but epigenetic profiling has also been shown to be of significant importance. Metabolic and proteomic fingerprints as well as the mathematical analysis and modeling of all the "omics" data are needed to complete a comprehensive understanding of the molecular deregulation of the breast cancer cells in vitro and in vivo. Finally, taking molecular profiles toward the clinical diagnostic setting is the "final frontier" and will require standardized technologies, quality control, and prospective testing in large series of patient cohorts. This is a major effort for any single molecular profiling platform, and an enormous challenge for the clinical application of integrated multiplatform profiling.

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DOI 10.1016/j.ccr.2006.11.007

Radiation resistance and stem-like cells in brain tumors

The concepts of stem cells being resistant to therapy, stem-like cells existing in brain tumors, and these tumors initially responding to therapy followed by recurrence are well documented. On this foundation, a recent paper in *Nature* has demonstrated that CD133-expressing glioma cells in vivo and in culture are relatively resistant to radiation. The mechanism of resistance involves the cell-cycle-regulating proteins CHK1/CHK2. The data raise many questions about the details of radiobiology of stem-like cells in their native environment within tumors in vivo. These answers may lead to better optimization of radiation treatments and schedules for these patients.

Radiation biologists were the first to formulate the concept of stem cells. The term "stem cell" was coined in the context of clonogenic cells surviving radiation that were able to repopulate the spleen (McCulloch and Till, 1960, 2005). In the gut, cells with a relatively low baseline proliferation rate were found to be relatively resistant to radiation and respond

to it with cell division, giving rise to cells that repopulate the crypt (Hornsey, 1973; Marshman et al., 2002).

Even though gliomas have a dismal prognosis, radiation is the most successful nonsurgical treatment for them. In response to a full course of radiation, gliomas frequently respond but then recur. Medulloblastomas are even more sensitive to radiation than gliomas with cure rates of 70% obtained in children old enough to tolerate the treatment. There has been a long debate over the role of stem or progenitor cells in brain tumors. Stem or progenitors cells are more sensitive to oncogenic stimulation and have been proposed to be the cell of origin for these tumors (Holland et al., 2000). Further, human gliomas and medulloblastomas have been shown to contain cells with stem cell properties such as neurosphere formation and self-renewal (Galli et al., 2004). Recently, expression of the cell surface marker CD133 was shown to stratify glioma cells with stem-like character from those without it (Singh et al., 2004). CD133-expressing cells are much more capable of initiating tumors when transplanted into rodents than cells not expressing CD133. CD133 has since been regarded as a marker for stem cells in gliomas.

A paper recently published in *Nature* (Bao et al., 2006) has determined the effect of radiation on the D456MG and D54MG glioma cell lines and surgical glioma samples. Mice bearing D456MG xenograft tumors were treated with high-dose radiation, resulting in an increased percentage of CD133+ cells and more aggressive tumors on serial transplantation. For ex vivo radiation, cells isolated from D54MG xenografts were treated with 5 Gy, resulting in a similar increase in CD133+ cells. Similar results were obtained with cells isolated from three human glioma surgical samples radiated ex vivo with 2 Gy. The percentage of CD133-expressing cells as analyzed by FACS also correlated with the rate of tumor formation when implanted in mice. CD133+ cells from the D456MG line and from primary tumors show a more robust activation of the DNA damage pathway than CD133– cells in response to 3 Gy, and CD133+ cells repaired DNA damage faster than CD133– cells. Insight into the mechanism was revealed with the use of DBH, an inhibitor of CHK1/CHK2, which sensitized both the CD133+ and CD133– cells from D456MG and surgical specimens to radiation. The data indicate that stem cells within gliomas are resistant to radiation at least in part due to an elevated DNA damage response and more rapid repair of the DNA damage. Higher radiation doses kill some CD133-expressing cells, which are likely to be a heterogeneous population. It is not clear what subset of these cells are relatively radiation resistant and what subset are stem-like, but the data clearly indicate that there is some overlap between these two characteristics.

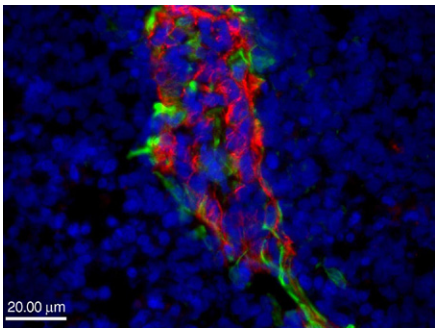

Figure 1. Stem-like cells in brain tumors

Immunofluorescence images of fresh-frozen sections of a human medulloblastoma showing localized regions of cells expressing the stem cell markers nestin (green) and Notch receptor (red) that work well in histochemical staining. Nuclei are stained blue with DAPI. Note that these stem-like cells are located in a specific region, or niche, rather than distributed randomly throughout the tumor. Scale bar, 20 µm.

It is easy to generalize clean and satisfying data such as this, although there are specific details in this study that should be kept in mind. Some of the experiments were done with the D456MG cell line that is wild-type for *p53*, *ink4a-arf*, and *pten* (Rich et al., 2005a), a rare genetic background for adult gliomas (Rich et al., 2005b). Other experiments were done with the D54MG cell line that is mutant for *ink4a-arf* and *pten* but wild-type for *p53*. It is likely that the status of these genes are critical for the radiation effects of both responding and nonresponding cells. In many of the experiments, radiation was done on individual cells ex vivo; it is not known how similar the response of these cells would be if they were in their natural setting in vivo. Stem cells are thought to occupy a specific niche with supportive and interactive stroma; it is not clear what effect the niche has on the relative resistance of stem-like cells to radiation in vivo. Further, the radiation response of cells in the normoxic culture conditions used in this paper may or may not reflect the response of cells in the hypoxic environment of a glioblastoma. In spite of any caveats, it seems clear that cells expressing markers of stem cells exist in gliomas and that these cells are more resistant to radiation than other cells in the tumor.

Important papers such as this one often raise more questions than they answer. For example, where is the niche for glioma stem-like cells in vivo (Figure 1)? Is the DNA damage response and rate of repair for stem-like cells in vivo in their niche also elevated? Does the niche provide additional protections to stem-like cells that are independent of DNA damage response? Presumably, glioma stem-like cells are rarely in cycle in vivo; does this also contribute to their relatively resistant character? Does radiation cause cell cycle arrest in the few stem-like cells that are cycling at the time of treatment? What is the time interval between radiation treatment and the point where stem-like cells reenter the cell cycle? Are these cells sensitive to radiation when they reenter the cycle, and if so, can they be synchronized and reradiated at their most sensitive point? Are there signaling pathways that contribute to the resistance phenotype in these cells? Are there small-molecule inhibitors of signaling pathways already available that could reduce the radiation resistance specifically of the stem-like cell population in vivo? Would the recurrent tumor arising from the resistant stem cells have the same radiation sensitivity as the spectrum of cells in the original tumor? Finally, could a rationally designed dosing schedule for radiation be combined with small-molecule inhibitors to result in a more effective treatment for these patients?

Certainly this paper will stir up discussion in the brain tumor field. It lends support for functional cellular heterogeneity in gliomas and for the existence of stem-like cells as defined by expression of markers, formation of neurospheres, and tumor-initiating capacity. It hopefully will go farther than that. We now need to address some these focused questions of brain tumor radiation biology in vivo. With the answers to these questions we will hopefully then have the tools to rationally change the standard of care for these patients.

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DOI 10.1016/j.ccr.2006.11.008

PU.1 and Junb: Suppressing the formation of acute myeloid leukemia stem cells

Improved understanding of the molecular pathways that suppress the genesis and maintenance of cancer stem cells will facilitate development of rationally targeted therapies. PU.1 is a transcription factor that is required for normal myelomonocytic differentiation in hematopoiesis, and reduced PU.1 activity has been associated with myeloid leukemogenesis in man and in mouse models. A recent study by Steidl et al. demonstrates that Junb and Jun, two AP-1 transcription factors, are critical downstream effectors of the tumor suppressor activity of PU.1, and that reduced expression of *Junb*, in particular, may be a common feature of acute myeloid leukemogenesis.

Tissue-specific stem cells are considered fertile soil for some of the mutations that contribute to the development of human cancers. However, the molecular mechanisms by which these mutations give rise to cancer stem cells, or otherwise lead to neoplastic disease, are less well defined. This issue is of major importance, since the implicated pathways may be targets for molecular therapies, particularly if they are selectively involved in cancer versus normal stem cell maintenance.

Of the many molecular pathologies associated with acute myeloid leukemia (AML), one recurrently implicated gene is *PU.1* (*SPI1*; *Sfpi1*, for Spleen focus-forming virus proviral integration), which codes for a transcription factor that is essential for normal myelomonocytic differentiation and consequently also functions as a tumor suppressor (reviewed in Koschmieder et al., 2005). Repressed *PU.1* transcription has been reported in AMLs harboring *PML-RARα* (Mueller et al., 2006) or *FLT3-ITD* mutations, and the AML-associated oncoprotein AML1-ETO functionally inactivates PU.1 through displacement of its coactivator, JUN (Koschmieder et al., 2005). Heterozygous mutations of *PU.1* have been observed in one series of patients with AML and are postulated to co-operate with reduced PU.1 activity induced by other mechanisms to promote leukemogenesis (Koschmieder et al., 2005). Consistent with these observations, reduced or abrogated *PU.1* expression in mouse models results in AML (Rosenbauer et al., 2004; Metcalf et al., 2006). Thus, PU.1 activity appears to be a target of several oncogenic signaling pathways in AML (Figure 1). However, the downstream genes that are critical mediators of its leukemia-suppressive role have hitherto been undefined.

In an elegant series of experiments, Steidl et al. (2006) have recently solved a significant piece of this puzzle by identifying Jun and Junb, members of the activator protein-1 (AP-1) family of transcriptional regulators, as critical effectors of the PU.1 tumor suppressor pathway. Their studies employed a PU.1 knockdown (PU.1 KD) mouse model that develops highly penetrant AML as a consequence of reduced *PU.1* expression caused by deletion of a critical upstream regulatory element in the *PU.1* gene. Global transcriptional analysis of an immature subfraction of bone marrow cells obtained from preleukemic PU.1 KD mice identified *Jun* and *Junb*, in addition to a number of previously known PU.1 targets, to be downregulated compared with cells obtained from wild-type mice. The compared cell populations,

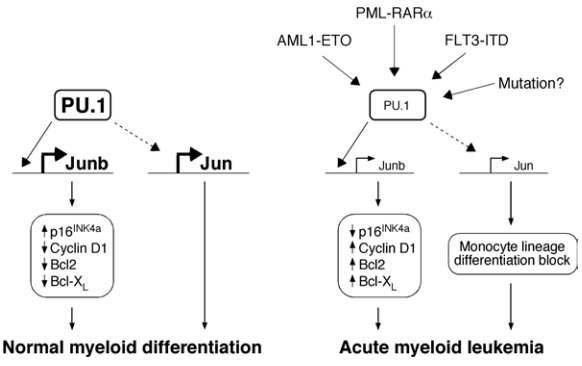

Figure 1. PU.1 in normal and leukemic hematopoiesis

In normal hematopoiesis, PU.1 promotes myelomonocytic differentiation through positively regulating expression of the AP-1 transcription factors Junb and Jun. In acute myeloid leukemia, a variety of mechanisms contribute to a reduction in PU.1 activity, leading to reduced *Junb* and *Jun* expression, with consequent dysregulation of differentiation, programmed cell death, and cellular proliferation.