

IN THE SPOTLIGHT

Apples to Origins: Identifying Brain Tumor Stem Cell Genes by Comparing Transcriptomes of Normal and Cancer Stem Cells

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Summary: The mechanisms whereby medulloblastoma stem cells coordinate tumor propagation are poorly understood. Using microarray analysis, Corno and colleagues draw parallels and distinctions between medulloblastoma stem cells from the *Ptch*^{+/-} mouse and normal neural stem cells, identifying *Ebf3* as a cancer stem cell-specific transcript critical for tumor growth. *Cancer Discov*; 2(6): 492-4. ©2012 AACR.

Commentary on Corno et al., p. 554 (3).

Management of the pediatric brain tumor medulloblastoma presents a significant clinical dilemma. While aggressive therapeutic modalities result in tumor eradication in most standard risk cases, the extent of chemo- and radiotherapy necessary to reliably prevent disease recurrence is devastating to the developing central nervous system. In addition, some aggressive subgroups of medulloblastoma are frequently refractory to treatment (1). The threat of such resistance and recurrence may well be attributed to brain tumor stem cells, which have been implicated in the recovery of tumor mass following radiation therapy (2). Refinement in medulloblastoma treatment must then address the need to eliminate these resilient tumor cells that may not respond to therapies designed to target the tumor bulk. Ultimately, the discovery of vulnerabilities distinct to medulloblastoma stem cells could permit the selective destruction of the tumor while sparing the developing central nervous system.

Presently, the mechanisms of cancer stem cell-mediated tumor maintenance and propagation are poorly understood. The dynamic phenotype and, in most cases, the rarity of cancer stem cells complicate their prospective enrichment for functional or comparative studies. This is generally overcome by either selecting for unique stem cell characteristics *in vitro* or sorting for cells that express particular surface markers that associate with tumor-propagating activity. In this issue of *Cancer Discovery*, Corno and colleagues (3) enriched normal and tumor stem cell populations by culturing under neurosphere assay conditions to compare the transcriptomes of tumor stem cells isolated from medulloblastomas of the *Ptch*^{+/-} mouse strain and normal neural stem cells. With this approach, the extent of similarity between normal and tumor stem cells could be determined, and ultimately a gene expression signature distinctly associated with tumorigenic stem cells was identified. These findings reveal unexpected similarities between medulloblastoma stem

cells and normal neural stem cells and identify various cancer stem cell-specific transcripts that may have potential as tumor-selective therapeutic targets in medulloblastoma.

The authors first generated neural stem cell cultures from various regions of the normal postnatal brain, including the forebrain subventricular zone and from anatomic regions in proximity to the origin of *Ptch*^{+/-} tumors, namely, the cerebellar white matter and the periventricular region at the floor of the fourth ventricle (Fig. 1). Intriguingly, gene expression profiling indicated that cerebellar and fourth ventricle stem cells (hereafter referred to collectively as hindbrain neural stem cells) were indistinguishable from one another yet distinct from forebrain subventricular zone stem cells. The authors then established neurosphere media cultures ("tumor stem cells" hereafter) from tumors of *Ptch*^{+/-} mice with either wild-type, heterozygous-null, or homozygous-null alleles of *p53*. This approach cleverly takes into account a previous observation that self-renewal and multilineage differentiation capacity *in vitro* are shared among all of these cell populations, whereas tumorigenicity is limited to the *p53*-deficient (*Ptch*^{+/-}, *p53*^{+/-} or *Ptch*^{+/-}, *p53*^{-/-}) tumor stem cell lines (4).

Transcriptomes of stem cells isolated from the various *Ptch*^{+/-} tumors were then compared with those of the untransformed neural stem cells. Importantly, the tumor stem cells more closely resembled neural stem cells of the hindbrain than those of the forebrain. Gene signatures distinguishing tumor stem cells from untransformed neural stem cells, as well as signatures distinct to tumorigenic (*Ptch*^{+/-}, *p53*^{-/-}) or nontumorigenic (*Ptch*^{+/-}, *p53*^{+/-}) stem cells of *Ptch*^{+/-} tumors, were then generated. Perhaps unsurprisingly, the authors found that the molecular signature distinguishing both *Ptch*^{+/-} tumor stem cell lines from hindbrain neural stem cells were predominately associated with the Sonic hedgehog (Shh) molecular subgroup of medulloblastoma. Less expected was the finding that the genes upregulated exclusively in the tumorigenic *Ptch*^{+/-}, *p53*^{-/-} tumor stem cells were associated with the Wnt molecular subgroup of medulloblastoma. Although a relationship between *Ptch*^{+/-} mouse tumors and Wnt subgroup medulloblastomas is unlikely (5), a potential role for Wnt signaling in tumor stem cells of Shh subgroup medulloblastomas is intriguing. Indeed, many of the genes distinct to tumorigenic stem cells mediate Wnt signaling, and

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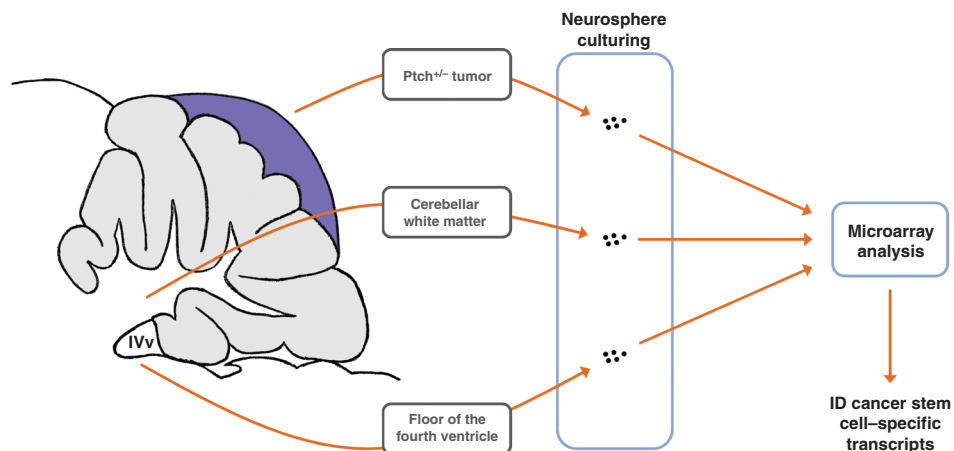


Figure 1. Schematic of normal and tumor stem cell populations of the mouse hindbrain and their downstream characterization by Corno and colleagues (3). Normal neural stem cell populations identified are those present at postnatal day 7. ID, identify; IVv, fourth ventricle.

other studies have found that Wnt pathway genes are overexpressed in Shh subgroup tumors (1).

Beyond the descriptive associations between cancer stem cell-specific transcripts and medulloblastoma subgroups, functional validation of these gene sets as mediators of cancer stem cell function would reveal their potential to serve as therapeutic targets. One promising such gene identified by the authors was the transcription factor Ebf3, which was found to be upregulated in tumor stem cells of *Ptch*^{+/−} tumors and expressed in various histologic subtypes of patient medulloblastomas. In flank allografts of tumor stem cells, Ebf3 overexpression gave rise to larger, more proliferative tumors, whereas knockdown of Ebf3 in combination with its heterodimeric partners inhibited tumor growth. A systematic functional investigation of the genes identified in this study holds promise for identifying additional critical mediators of cancer stem cell function in *Ptch*^{+/−} tumors. Although Ebf3 was found to be expressed across a small cohort of patient medulloblastomas, it is yet to be determined whether the gene sets identified by the authors in an animal model resembling Shh group tumors (5) will be relevant to cancer stem cells of non-Shh subgroups of medulloblastoma. Considering the distinct genetic alterations (1) and cellular origins (5–7) underlying each subgroup, it may be expected that the mechanistic basis for tumor propagation will vary accordingly.

Several complementary approaches will likely be required to understand cancer stem cells of various brain tumor subtypes and anatomic origins. Previous unbiased approaches to identifying mediators of brain tumor stem cell function have compared gene expression signatures between cancer stem cells and their more differentiated progeny (8) or between bulk tumor and the normal stem cells that serve as their cell of origin (6, 9). Identification of a druggable target [the phosphoinositide 3-kinase (PI3K) pathway] via the latter approach (6) lends substantial validation to this strategy of identifying critical mechanisms of cancer stem cell function; however, its applicability to tumors of non-stem cell origin is unknown.

What then is the relationship between *Ptch*^{+/−} tumors and neural stem cells of the postnatal hindbrain? There is substan-

tial evidence that *Ptch*^{+/−} tumors are ultimately derived from committed granule neuron precursors (GNP) of the cerebellum (5, 7, 10) rather than postnatal neural stem cells. In addition, when the Shh pathway is constitutively activated in neural stem cells of the embryonic cerebellum, these cells must first commit to the GNP lineage before transformation occurs (7). However, unlike GNPs, a subset of cells in *Ptch*^{+/−} tumors are capable of self-renewal and multilineage differentiation *in vitro* (4), indicating that over the course of transformation, GNPs can acquire these stem cell characteristics. Supporting these phenotypic observations, Corno and colleagues (3) found that tumor stem cells derived from *Ptch*^{+/−} tumors exhibit gene expression similarities with hindbrain neural stem cells. It would be of interest to determine the extent of similarity of these cells with normal GNPs as well, as it is likely that many differentially expressed genes in the tumor stem cells may have been retained from their GNP cell of origin, as suggested elsewhere for tumor bulk from *Ptch*^{+/−} tumors (10). Regardless, the success of this approach is encouraging with regard to the potential to identify cancer stem cell genes in other tumors that have acquired stem cell characteristics over the course of transformation.

Despite a potential for the discovery of novel cancer stem cell targets using stem cell enrichment by culturing, such experiments must be interpreted with some caution. Long-term culturing, even in neurosphere culture conditions, can alter cellular phenotypes, and thus it is crucial that findings of *in vitro* studies are verified in intact or minimally cultured tumors. In addition, correlates of neural stem cell function such as survival in neurosphere culture conditions, sphere formation, and differentiation plasticity are not necessarily related to tumor-propagating ability, and in some cases, tumor-propagating cells specifically lack these characteristics. Relevantly, and in contrast to the findings of Corno and colleagues (3), it has been shown elsewhere that cancer stem cells that do not survive neurosphere culture conditions can be isolated from *Ptch*^{+/−}, *p53*^{+/+} tumors (8). It is therefore of utmost importance that the operational definition of brain tumor stem cells minimally includes tumor-propagating ability, as it is this defining characteristic that contributes to tumor maintenance or recurrence.

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An appreciation that unipotent cells may co-opt and subvert normal stem cell programs over the course of transformation has broad implications for the study of cancer stem cells. The extent to which cancer stem cells resemble normal stem cells from their cognate anatomic compartments, as investigated by Corno and colleagues (3), is yet to be determined for many cancers. Faithful animal models and protocols for stem cell isolation from the relevant normal tissue will be required to conduct such analogous studies. Critically, cancer stem cell populations must be clearly defined on the basis of their ability to propagate the original tumor. Keeping this in mind, the generalizability of the approach by the authors has potential to improve our understanding of cancer stem cells underlying various malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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