Khayrullina, T., Yen, J.H., Jing, H., and Ganea, D. (2008). J. Immunol. *181*, 721–735.

Kortylewski, M., Xin, H., Kujawski, M., Lee, H., Liu, Y., Harris, T., Drake, C., Pardoll, D., and Yu, H. (2009). Cancer Cell *15*, this issue, 114–123.

Langowski, J.L., Kastelein, R.A., and Oft, M. (2007). Trends Immunol. *28*, 207–212.

Trinchieri, G., Pflanz, S., and Kastelein, R.A. (2003). Immunity *19*, 641–644.

Vicari, A.P., Chiodoni, C., Vaure, C., Ait-Yahia, S., Dercamp, C., Matsos, F., Reynard, O., Taverne, C., Merle, P., Colombo, M.P., et al. (2002). J. Exp. Med. *196*, 541–549.

Wei, L., Laurence, A., and O'Shea, J.J. (2008). Semin. Cell Dev. Biol. *19*, 394–400.

Yang, J., Liao, X., Agarwal, M.K., Barnes, L., Auron, P.E., and Stark, G.R. (2007). Genes Dev. *21*, 1396–1408.

Yu, H., Kortylewski, M., and Pardoll, D. (2007). Nat. Rev. Immunol. *7*, 41–51.

Zou, W. (2006). Nat. Rev. Immunol. *6*, 295–307.

Identifying the Perpetrator in Medulloblastoma: Dorian Gray versus Benjamin Button

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Tumors contain a subpopulation of tumor-propagating cells (TPCs) that are critical for their growth. In this issue, Read, Wechsler-Reya, and colleagues show that in an animal model of medulloblastoma, TPCs express the surface marker CD15 and have properties distinct from neural stem cells.

The idea that stem cells or their close derivatives might underlie tumor formation has a long history but has come to the fore with the advent of modern stem cell biology. We now know that, for a variety of tumors, there is an essential subpopulation of cells that maintains the growth of neoplastic tissue and can initiate new tumors in vivo when transplanted into a receptive host. These tumor-propagating cells (TPCs) can resemble normal tissue stem cells in their marker expression and their ability to self-renew and produce differentiated progeny. For a given tumor type, defining its constituent TPCs is of paramount importance—first to identify and target them to combat tumor growth, and second to understand their etiology and find ways to prevent tumor formation. Do TPCs arise from normal stem cells that go awry and proliferate uncontrollably, disregarding regulatory mechanisms that keep them in check, or do they arise from later-stage progenitor cells that revert to acquire stem-like features? These two views of cancer formation—reminiscent of the literary characters of Dorian Gray, who didn't age, and Benjamin Button, who was born old and grew young—are an active point of debate. Recent studies indicate that mutations in the stem cell compartment more readily phenocopy colon cancer (Barker et al., 2008). However the same might not be true for cancers in other tissues.

Medulloblastomas are the most common pediatric brain tumors. They occur in the cerebellum, a brain region involved in integrating sensory perception and movement control. The cerebellum forms from two major germinal regions, the ventricular zone and the external granular layer (Figure 1). The most abundant cell in the brain, the cerebellar granule neuron, arises from granule neuron precursors (GNPs) in the external granular layer, largely during the early postnatal period.

The remarkable expansion of GNPs is governed principally by the growth factor Sonic hedgehog (Shh) (Kenney et al., 2003). The signaling cascade is initiated by Shh binding to the cell surface receptor Patched (Ptc). A key player in the cascade is a G protein-coupled receptor-like molecule called Smoothened (Smo). In the absence of Shh, Ptc inhibits the activity of Smo. Shh binding to Ptc relieves its inhibition, and Smo can signal downstream. The targets of Smo include Gli transcription factors, which then translocate to the nucleus and initiate transcription.

Approximately 25% of medulloblastomas result from inappropriate activation of Shh signaling, and a subset of human medulloblastomas harbor mutations of the *Ptc* gene (Zurawel et al., 2000). A useful model of medulloblastoma is the $Ptc^{+/-}$ mutant mouse. *Ptc* haploinsufficiency increases proliferation of neural stem cells (Galvin et al., 2007), and 15%–20% of $Ptc^{+/-}$ mice develop medulloblastomas (Goodrich et al., 1997).

Read et al. (2009) examined medulloblastomas derived from $Ptc^{+/-}$ mice. Using fluorescence-activated cell sorting (FACS) with cell surface markers, they separated subpopulations of live cells and then stereotaxically injected these into SCID/beige mouse cerebella to examine whether they could form tumors. Surprisingly, the investigators found that medulloblastomas were not propagated by cells expressing the neural stem cell (NSC) marker CD133, leading them to search for other candidate markers. A prior study had described CD15, also known as Lewis X (LeX) or stage-specific embryonic antigen-1 (SSEA-1), as a marker of forebrain NSCs and progenitor

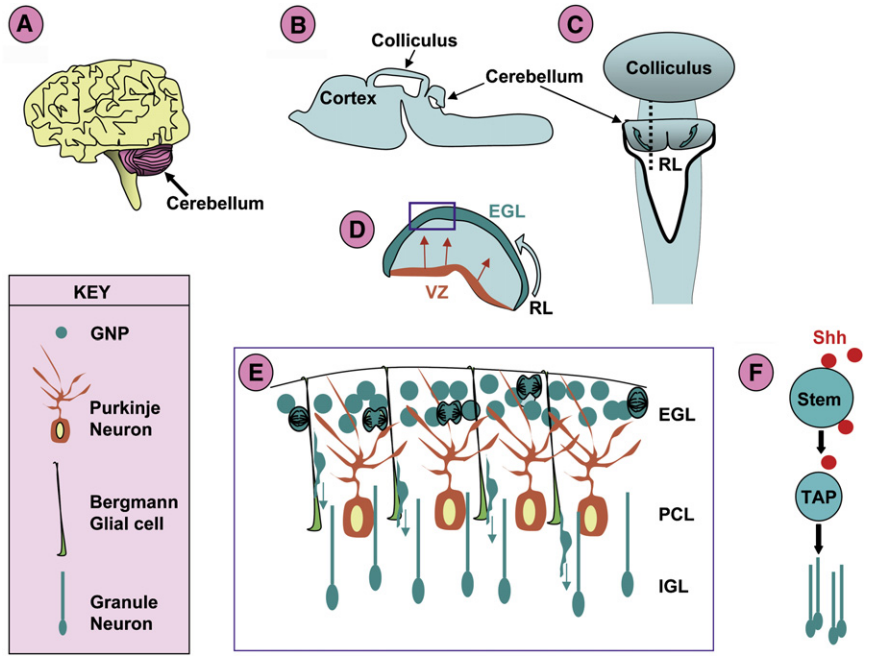

Figure 1. Cerebellum Development

(A) Mature human brain showing the location of the cerebellum.

(B) Midgestation mouse brain indicating the early cerebellum immediately caudal to the colliculus.

(C) Dorsal view showing the developing cerebellum and the direction of progenitor cell migration from the rhombic lip (RL) over the surface of the cerebellum to produce the external granular layer (EGL).

(D) Cross-section (dotted line indicated in [C]) showing the two major cerebellar germinal zones, the ventricular zone (VZ) and the EGL. VZ progenitors are active in the embryonic period, and progeny migrate radially toward the pia to form the early-born cerebellar cells, including the Purkinje neurons and Bergmann glia. Granule neuron precursors (GNPs) arise first in the RL and then proliferate and migrate over the surface of the cerebellum, forming the EGL.

(E) Magnification of boxed area in (D) showing the EGL GNPs. These progenitor cells are stimulated by Sonic hedgehog (Shh) to divide and generate immature granule neurons that migrate down the Bergman glia to the internal granular layer (IGL).

(F) Scheme of cerebellar progenitor cell development in the EGL, identifying the basic cell compartments that could transform into tumor-propagating cells (TPCs).

cells that is highly expressed in the cerebellum anlage (Capela and Temple, 2006). Read et al. found that CD15 was expressed by a subset of normal granule precursor cells and medulloblastoma cells. FACS-sorted $CD15^+$ cells from $Ptc^{+/-}$ medulloblastomas were able to form tumors in vivo containing a characteristic mixture of cells, although they could not form neurospheres in culture. $CD15^+$ cells from $Ptc^{+/-}$ tumors exhibited higher proliferation rates and elevated levels of the Hh target genes *Gli1* and *Cyclin D1* compared to $CD15^-$ cells, consistent with Shh pathway activation. Transcriptome comparison of the $CD15^+$ and $CD15^-$ cells demonstrated that the $CD15^+$ cells expressed higher levels of genes associated with progenitor cell proliferation rather than differentiation and migration, establishing a CD15-associated gene "signature."

The authors then found that CD15 was expressed in a subset of human medulloblastomas. Increased expression of the CD15 gene signature was associated with shorter survival time of medulloblastoma patients. Future studies aimed at understanding the functional significance of CD15 and associated genes should be enlightening in understanding its prognostic value. This carbohydrate moiety can bind Wnt-1 and fibroblast growth factors, so it could enhance progenitor cell activity by supplying bioactive molecules.

Three basic cell compartments could give rise to TPCs after undergoing neoplastic transformation: NSCs, transit-amplifying progenitor (TAP) cells, and differentiated cells. Given the lack of CD133 association, neurosphere formation, and multilineage potential and the expression of CD15, the authors suggest that these medulloblastoma TPCs are related not to normal cerebellar stem cells (Lee et al., 2005) but to later progenitor cells that have acquired tumor-generating properties. There is a growing literature indicating that some brain cancer TPCs lack CD133 (e.g., Wang et al., 2008), lending support to the TAP origin hypothesis—with a couple of caveats. First, lack of CD133 might reflect loss of the marker rather than origination from a negative cell. Indeed, CD133 expression has been linked to hypoxic stress, so expression levels among tumor cells might reflect microenvironment more than lineage origination (Griguer et al., 2008). And second, CD133 expression might not encompass all stem cells—especially in a tissue such as the cerebellum, where development is protracted and the germinal tissue is complex (Figure 1).

The lack of neurosphere generation from these $Ptc^{+/-}$ medulloblastoma TPCs is particularly surprising, as a variety of NSCs and TAP cells can produce neurospheres. It is conceivable that some stem/progenitors require a niche to proliferate and are unable to form neurospheres. Whatever their derivation, it is intriguing that these TPCs appear to have specific growth requirements that depend on cell-cell interactions: this might present an Achilles' heel that can be targeted.

Thus, while $Ptc^{+/-}$ medulloblastoma TPCs lack some NSC features and, we extrapolate, might therefore have a TAP origin, reliance on a handful of markers and functional assays leaves room for different interpretations. A more direct test of origination might be to take the promoter-based approach of Barker et al. (2008) and compare the effect of reduced levels of Patched signaling in targeted $CD133^+$ versus TAP cells in vivo.

We do need to keep in mind that tumor cells might defy the logic of normal lineage relationships: the fact that they can coexpress neuronal and glial differentiation markers (Hemmati et al., 2003)—taboo in normal cells—indicates that they are as abnormal as the fictitious Dorian Gray or Benjamin Button. TPCs in individual tumors may have different cellular origins, and they may be dynamically evolving with unstable marker expression. Although they present a daunting, moving, and complex target, full characterization of TPCs using the powerful tools of cell separation and

comprehensive molecular characterization, as beautifully illustrated in the Read et al. study, along with similar definition of normal progenitor lineages should allow TPC categorization and ultimately a fuller understanding of the disease for design of individualized treatments.

REFERENCES

Barker, N., Ridgway, R.A., van Es, J.H., van de Wetering, M., Begthel, H., van den Born, M., Danenberg, E., Clarke, A.R., Sansom, O.J., and Clevers, H. (2008). Nature. Published online December 17, 2008. 10.1038/nature07602.

Capela, A., and Temple, S. (2006). Dev. Biol. *291*, 300–313.

Galvin, K.E., Ye, H., and Wetmore, C. (2007). Dev. Biol. *308*, 331–342.

Goodrich, L.V., Milenkovic, L., Higgins, K.M., and Scott, M.P. (1997). Science *277*, 1109–1113.

Griguer, C.E., Oliva, C.R., Gobin, E., Marcorelles, P., Benos, D.J., Lancaster, J.R., Jr., and Gillespie, G.Y. (2008). PLoS ONE *3*, e3655.

Hemmati, H.D., Nakano, I., Lazareff, J.A., Masterman-Smith, M., Geschwind, D.H., Bronner-Fraser, M., and Kornblum, H.I. (2003). Proc. Natl. Acad. Sci. USA *100*, 15178–15183.

Kenney, A.M., Cole, M.D., and Rowitch, D.H. (2003). Development *130*, 15–28.

Lee, A., Kessler, J.D., Read, T.A., Kaiser, C., Corbeil, D., Huttner, W.B., Johnson, J.E., and Wechsler-Reya, R.J. (2005). Nat. Neurosci. *8*, 723–729.

Read, T.-A., Fogarty, M.P., Markant, S.L., McLendon, R.E., Wei, Z., Ellison, D.W., Febbo, P.G., and Wechsler-Reya, R.J. (2009). Cancer Cell *15*, this issue, 135–147.

Wang, J., Sakariassen, P.O., Tsinkalovsky, O., Immervoll, H., Boe, S.O., Svendsen, A., Prestegarden, L., Rosland, G., Thorsen, F., Stuhr, L., et al. (2008). Int. J. Cancer *122*, 761–768.

Zurawel, R.H., Allen, C., Chiappa, S., Cato, W., Biegel, J., Cogen, P., de Sauvage, F., and Raffel, C. (2000). Genes Chromosomes Cancer *27*, 44–51.

T Cell Acute Lymphoblastic Leukemia: NOTCHing the Way toward a Better Treatment Outcome

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γ-secretase inhibitors block the activation of NOTCH1 but have limited activity against T cell acute lymphoblastic leukemia (T-ALL) and cause severe gastrointestinal toxicity. In a recent study, Real et al. show that a potent γ-secretase inhibitor potentiates the cytotoxicity of dexamethasone against glucocorticoid-resistant T-ALL cells, while dexamethasone abrogates the gastrointestinal toxicity induced by the γ-secretase inhibitor.

T cell acute lymphoblastic leukemia (T-ALL), a clonal malignant disorder of immature T cells, represents 10%–15% of childhood and 25% of adult ALL cases. Although intensive chemotherapy has done much to improve prognosis for this disease, as many as 30% of childhood cases and approximately 50% of adult cases will relapse. Remarkable progress in understanding the genetic mechanisms underlying T-ALL pathogenesis has opened the way for the development of molecular targeted therapy. It is now clear that genetic abnormalities involving T cell receptor genes, basic helix-loop-helix genes (e.g., *TAL1*, *TAL2*, *LYL1*, *MYC*), cysteine-rich LIM domain-containing genes (*LMO1*, *LMO2*), or homeodomain genes (e.g., *HOX11/TLX1*, *HOX11L2/TLX3*, the *HOXA* gene cluster) can participate in the transformation of normal thymocytes by blocking differentiation. Abnormalities of a different group of genes (e.g., *CDKN2A/2B*, *CCND2*, *LCK*, *RAS*, *PTEN*, *ABL1*, *JAK2*, *FLT3*) appear to increase self-renewal, alter responses to extracellular signals, or impose resistance to apoptosis (Pui et al., 2008; Van Vlierberghe et al., 2008).

NOTCH1 encodes a heterodimeric receptor that regulates normal T cell development beginning as early as the commitment of multipotent hematopoietic progenitors to the T cell lineage (Figure 1). Activating mutations of *NOTCH1* represent one of the most common genetic abnormalities in T-ALL. Indeed, 60% of T-ALL cases possess such mutations (Weng et al., 2004; van Grotel et al., 2008; Asnafi et al., 2008). Among them, activating mutations of the *NOTCH1* heterodimerization domain or juxtamembrane extracellular region induce ligand-independent activation of the receptor. Truncating mutations of the COOH-terminal PEST domain of the intracellular region, on the other hand, extend NOTCH1 signaling by removing the Cdc phosphodegron domains and preventing the proteasome-mediated degradation of the intracellular domains of the receptor (Palomero and Ferrando, 2008). One of the proteins that binds to the Cdc phosphodegron and primes the intracellular subunit for degradation is the F box protein FBXW7. Thus, it is not surprising that mutations in *FBXW7* can also extend