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Bmi1 and Cell of Origin Determinants of Brain Tumor Phenotype

Peter Dirks[1,*]
[1]Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada
*Correspondence: peter.dirks@sickkids.ca
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Glioblastomas frequently express oncogenic EGFR and loss of the *Ink4a/Arf* locus. *Bmi1*, a positive regulator of stem cell self renewal, may be critical to drive brain tumor growth. In this issue of *Cancer Cell*, Bruggeman and colleagues suggest that brain tumors with these molecular alterations can be initiated in both neural precursor and differentiated cell compartments in the absence of *Bmi1*; however, tumorigenicity is reduced, and tumors contain fewer precursor cells. Surprisingly, tumors appear less malignant when initiated in precursor cells. *Bmi1*-deficient tumors also had fewer neuronal lineage cells, suggesting a role for *Bmi1* in determination of cell lineage and tumor phenotype.

Glioblastomas remain among the most aggressive human cancers. The application of the conceptual and methodological framework of neural stem cell biology to brain cancer (Bachoo et al., 2002; Holland et al., 1998) and the identification of human brain tumor initiating cells (Singh et al., 2004) has opened up fresh approaches to interrogate the cell of origin for brain tumors. Particularly in mouse model systems, investigators can address the effect of expression of oncogenes or loss of tumor suppressors in normal precursor or differentiated cell compartments.

Current understanding of the mechanisms of tumor progression and initiation remain limited, particularly the cell context of recognized molecular signaling pathways implicated in the disease, such as aberrant EGFR signaling. Does oncogene expression drive a stem cell or a progenitor compartment in the tumor? Determinants of tumor phenotype and relationship to prognosis are also poorly understood. How do distinct molecular alterations specify the ultimate histopathologic tumor picture? How does the expression of neural precursor or differentiated lineages in the tumor correlate with tumor behavior? Are tumors that express more markers of differentiation less aggressive? As well, the relationship of tumor behavior to the putative cell of origin is not understood. Do tumors arise in a stem cell or a more differentiated cell compartment, and does the behavior and phenotype of the tumor depend on the cell compartment of origin? Are tumors that arise in stem cell compartments more malignant than those arising in progenitors, or vice versa? These questions come in to focus in the study by Bruggeman and colleagues (Bruggeman et al., 2007).

Bmi1 has been implicated in control of stem cells in multiple tissues, particularly as a positive regulator of self renewal, and *Bmi1*-deficient mice have deficiencies in their stem cell compartments, including the brain (Molofsky et al., 2003; Park et al., 2003). *Bmi1* promotion of proliferation and self renewal is thought to relate to suppression of the *Ink4a/Arf* locus (Bruggeman et al., 2005), although other loci have recently been shown to be targeted as well (Fasano et al., 2007). *Ink4a/Arf* loss itself, consistent with its tumor suppressor role, causes increased neural stem cell activity in vivo (Molofsky et al., 2006). Although *Ink4a/Arf* is lost genetically in a large fraction of human glioblastoma samples, mice deficient for *Ink4a/Arf* rarely develop spontaneous brain tumors.

The current study by Bruggeman et al. (2007) attempts to further probe the functional role of *Bmi1* together

with *Ink4a/Arf* locus in an ex vivo oncogenic transduction model of glioma. Are tumors that can be initiated in the context of *Ink4a/Arf* deficiency dependent on *Bmi1*?

The authors transduced either mouse adult-derived adherent neural precursor cells or early postnatal (day 7)-derived cortical mouse astrocytes with a brain tumor-associated oncogene to test the effects of tumorigenicity in the presence of a *Bmi1*-deficient background. The main finding of this study is that in the presence of *Ink4a/Arf* and *Bmi1* deficiency, transformation by oncogenic EGFR results in different growth kinetics and tumor cell lineages depending on whether neural precursor cells or astrocyte cells are transformed. Importantly, both cell compartments are permissive for transformation, and therefore, *Bmi1* expression is not required for tumor initiation. However, loss of *Bmi1* attenuates overall tumorigenicity (in both cell compartments) in an *Ink4a/Arf*-deficient background and also affects lineage determination in resulting tumors; Bmi1 seems to be required for specification of neuronal lineages in the resulting tumors. In addition, Bmi1-deficient tumors have fewer cells expressing the neural precursor marker nestin, suggesting that one reason they may be less aggressive is that they have fewer stem cells. It remains uncertain whether in vivo tumors that lack *Bmi1* are critically impaired in their self renewal ability, as defined by serial transplantation studies (Lessard and Sauvageau, 2003).

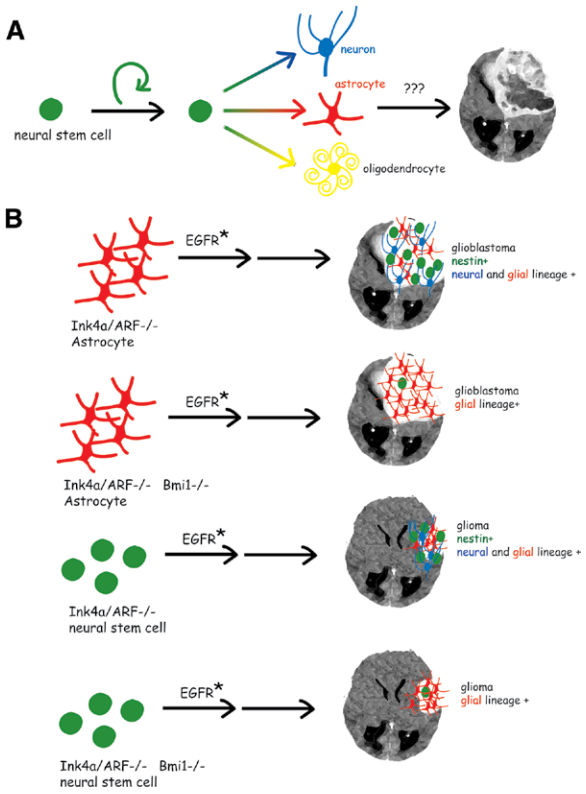

Figure 1. Tumor Phenotype Depends on Bmi1 Status and Cell of Origin

(A) Neural stem cells self renew and generate differentiated cell types. It remains unclear which cell in the normal stem cell hierarchy is the cell of origin for brain tumors. Tumors of distinct phenotypes could have different cells of origin.

(B) The effect of transformation of cultures of neural precursor cells enriched for stem cells versus differentiated astrocytes was tested. Astrocytes deficient in *Ink4a/Arf* that are transformed with oncogenic EGFR give rise to malignant glioblastoma-like tumors. In the absence of *Bmi1*, mice survive longer, and their tumors have few nestin+ cells and few neuronal lineage+ cells. In contrast, neural precursors, transformed with the identical mechanisms, give rise to tumors that showed less-malignant features. Although mice died from their tumors, those with an absence of *Bmi1* lived longer, had fewer nestin+ cells, and few neuronal lineage+ cells.

The results suggest most strongly that the brain tumors that emerge in these models depends on the cell context, and perhaps surprisingly, tumors originating from cultures highly enriched for neural stem cells appear less malignant than those arising from astrocytes, although mice died at about the same time (Figure 1). As normal neural stem cells are thought to be predominantly quiescent in vivo, could transformation events in these cells cause a more slowly proliferating (although not shown here) and less aggressive tumor?

These findings are very interesting, but there are questions concerning the purity of cell types that are transformed, which reflects our generally poor understanding of the normal neural stem cell hierarchy and possible problems with cultures. The normal neural stem cell hierarchy is largely undefined, and a paucity of cell surface markers makes progress challenging. The markers used to identify cell populations in the brain are also not specific; GFAP marks stem cells as well as differentiated astrocytes. Consequently, the populations tested for transformation ability in this study are not directly purified from the brain but are derived from cultures, which may alter the function of the stem cells and more differentiated cells. The precursor and differentiated populations are not matched for age; could the astrocyte cultures derived from P7 brain contain more primitive cells than the adult derived precursors? Also, the effect that *Bmi1* deficiency has on the normal neural stem cell compartment suggests that the starting cell populations in wild-type versus the deficient *Bmi1* cells may be different. Despite these concerns, the authors do use state-of-the-art techniques to obtain enriched populations of stem cells or differentiated cells.

This study also suggests that *Bmi1* may have a role outside control of neural stem cells in the brain. Bmi1 is widely expressed in the postnatal brain, and it is expressed in the bulk population of the human brain tumor samples. This data would suggest that Bmi1 has a functional role in both normal stem cells and differentiated cells of the brain or different functions in these distinct cell populations. *Bmi1* may be important for specifying differentiated neuronal lineages in the brain as shown by data in vitro and in the tumors in vivo.

In summary, these studies, which are challenging to perform, raise further interesting questions about

cell context of molecular alterations that play a role in brain tumors and do provide important further insight into the role of *Bmi1* in brain tumorigenesis. The finding that distinct tumor phenotypes that arise from transformation of different cell populations in the brain suggests that knowing the cell of origin may be important for understanding the prognosis of the tumor. It will likely also be important to determine the signaling mechanisms that subsequently become operational in the different cell contexts to devise new therapies.

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PI3 Kinase Activation and Response to Trastuzumab Therapy: What's neu with Herceptin Resistance?

Ben Ho Park[1] and Nancy E. Davidson[1,*]
[1]Breast Cancer Program, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans Street, Baltimore, MD 21231, USA
*Correspondence: davidna@jhmi.edu
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Trastuzumab is an established therapy for women with breast cancers that overexpress HER2. Despite its proven benefit in treating breast cancer, not all women derive benefit from this monoclonal antibody, and resistant disease can develop. In this issue of *Cancer Cell*, Berns et al. present evidence that activation of the PI3 kinase pathway, either via loss of the tumor suppressor PTEN or through oncogenic stimulation of PIK3CA, can mediate trastuzumab resistance. This study extends important work of others and forms the rationale for future investigations combining inhibitors of the PI3 kinase pathway in conjunction with trastuzumab therapy.

One of the most successful examples of targeted therapies for epithelial cancers has been the demonstration that breast cancers with amplification of the ERBB2/HER2 oncogene are responsive to trastuzumab (Herceptin), a humanized monoclonal antibody directed against the transmembrane HER2 protein. As a result, patients whose breast cancer cells demonstrate overexpression of HER2 protein by immunohistochemistry and/or gene amplification by fluorescence in situ hybridization (FISH) are candidates for this therapy in both the adjuvant and metastatic settings (reviewed in Hudis, 2007). However, as with many cancer therapies, not all women whose tumors overexpress HER2 will respond to trastuzumab. Indeed, only about one-third of women with newly diagnosed advanced breast cancer that overexpresses HER2 demonstrate tumor regression with trastuzumab monotherapy (Vogel et al., 2002). Also, trastuzumab treatment is not without cost in both economic and human terms. Although it is generally well tolerated, rare but significant cardiac toxicity can develop in patients receiving this therapy, especially when it is given in close proximity to anthracycline chemotherapy. One year of trastuzumab therapy, the current standard regimen in early breast cancer, costs approximately $50,000 (USD). Therefore, the identification of predictive biomarkers that can more accurately select responders or nonresponders is vital to improve the therapeutic index of this agent. Also,