A Multipronged Approach to the Identification and Study of an Important Oncogene in GBM

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In this issue of *Cancer Cell*, Zheng et al. provide strong evidence that PLAGL2 serves as an oncogene in GBM. They demonstrate that PLAGL2 inhibits differentiation and promotes a persistent, self-renewing state, at least in part because of activation of Wnt signaling.

Glioblastoma multiforme (GBM) is one of the most aggressive and highly lethal cancers. New avenues of treatment are therefore desperately needed. However, a great deal needs to be learned about GBM biology prior to developing rational therapies. In recent years, several different approaches have led to an explosion in the knowledge of how GBM cells proliferate, survive, and invade normal tissue. On one front, there are almost daily new and important discoveries of the ways in which key oncogenic pathways mediate their effects, how they interact, and how they are activated. On another front, advances in array and sequencing technology, along with sophisticated bioinformatics, are being used to identify molecular alterations in GBM as well as to group them into functionally meaningful classifications. Additionally, the identification of stem cell-like tumor initiating cells within GBM has provided both an advance in the conception of how tumors may be initiated as well as new methods to study GBM in vitro. These concepts bring into play the rapidly accumulating knowledge of the fundamental properties of stem cells. Finally, the use of mouse models and cells derived from these models that carry oncogenic mutations has facilitated the study of the process of neoplastic transformation in a manner that brings greater control than through the use of human samples.

In the current issue of *Cancer Cell*, Zheng et al. (2010) make use of all these advances in an extensive and elegant series of studies to set forth the argument that PLAGL2, a putative transcription factor, is a disease-causing or promoting gene in GBM. PLAGL2 (*P*leiomorphic *A*denoma *L*ike *2*) was identified because of its homology to PLAG1, which was originally identified as being associated with pleiomorphic adenomas (Kas et al., 1997). Both PLAG1 and PLAGL2 have been associated with the development of malignancies, especially in acute myelogenous leukemia, in which they cooperate with an aberrant fusion gene created by an inversion of chromosome 16 to promote abnormal growth of hematopoetic progenitors (Landrette et al., 2005). The mechanisms by which PLAGL2 and PLAG1 promote cancer are unknown.

Zheng et al. found amplification of chromosome 20q11.21 in nearly 15% of GBMs studied using the powerful data set of The Cancer Genome Atlas project (http://cancergenome.nih.gov). In order to determine which of the nine genes within this chromosomal region has the oncogenic activity, Zheng et al. performed overexpression of each of these genes in astrocytes derived from Ink4a/Arf$^{-/-}$ Pten$^{-/-}$ mice and found that PLAGL2 promoted anchorage-independent growth. Further studies were then conducted to demonstrate the tumor-promoting actions of PLAGL2, including anchorage independent growth and invasiveness of P53$^{-/-}$ astrocytes as well as the tumorigenesis of transformed rat intestinal epithelial cells. The latter finding, although not directly related to glioma formation, is consistent with a tumor-promoting role for PLAGL2 and also supports observations of PLAGL2 amplification in colorectal cancer.

In order to delineate cellular and potential molecular mechanisms of action, the authors took advantage of detailed knowledge of neural stem cell and cancer stem cell biology and culture methods. Neural stem cells can be cultured from the subventricular zone of mice with relative ease in the presence of epidermal growth factor and basic fibroblast growth factor. Although the cell of origin of GBM is debatable, because stem cell-like cells cultured from GBM have many of the characteristics of neural stem cells (e.g., Hemmati et al., 2003), neural stem cells are now being used as a model to study pathways and processes involved in the development and propagation of brain tumors. Zheng et al. cultured neural stem cells from P53$^{-/-}$ mice and examined the effects of overexpressing PLAGL2. Upon withdrawal of mitogen and adherence to substrate, neural stem cell-containing cultures readily differentiate into neurons and glia. Certain factors can be used to enhance the differentiation of neural stem cells along specific lineages. For example, BMP-2 can be used to promote astrocytic differentiation (Gross et al., 1996). PLAGL2 overexpression in neural stem cell-containing cultures did not result in rapid proliferation, but instead inhibited differentiation, even in the presence of BMP-2. These findings suggest that one mechanism by which PLAGL2 can promote GBM formation or growth is by allowing GBM cells to remain in a self-renewing state, through the prevention of differentiation. Similar results were obtained with cultures obtained from primary GBMs through the same methods as for normal neural stem cells. These cultures, although still subject to in vitro artifacts, represent an advance in the study of GBM compared to either primary patient-derived cells grown in serum or "standard" highly passaged cell lines. They retain much of the cellular heterogeneity of the GBM from which they arise and form invasive tumors after

xenotransplantation into immunodeficient animals (Galli et al., 2004). Consistent with a tumor-promoting role, PLAGL2-transformed GBM cultures more readily formed tumors after xenotransplantation.

In order to elucidate the potential mechanisms of action of PLAGL2, Zheng et al. performed microarray analysis of PLAGL2-expressing neural stem cell-containing cultures. Subsequent bioinformatic analysis demonstrated strong overexpression of members of the Wnt pathway. This pathway, largely through stabilization of β-catenin, is known to promote neural stem cell self-renewal and inhibit differentiation (Shimizu et al., 2008). To more closely link these findings, Zheng et al. utilized neural stem cell and GBM stem cell-like containing cultures to demonstrate that the effects of PLAGL2 could be at least partially reversed by inhibition of the Wnt pathway.

Much remains to be learned about PLAGL2 amplification in GBM. Is such amplification a primary tumor-causing lesion in GBM? Is it found in specific subsets of GBM? To what extent does PLAGL2 cooperate with other genetic and epigenetic alterations to promote tumorigenesis? Does PLAGL2 activate other oncogenic pathways besides the Wnt pathway? Can PLAGL2 be targeted? Despite these and many other remaining questions, the studies outlined in Zheng et al. provide strong support for a developing paradigm in tumor biology. Although many oncogenic mutations are known to promote cell-cycle progression, there is a growing recognition of the importance of the inhibition of differentiation and/or maintenance of self-renewal. For example, BMPs can promote the differentiation of GBM stem cell-like cells under some circumstances, as they do with adult neural stem cells (Piccirillo et al., 2006). However, aberrant persistent expression of specific BMP receptor isoforms inhibits this process, maintaining self-renewal (Lee et al., 2008). Additionally, deletion of Pten (Groszer et al., 2006) or P53 (Meletis et al., 2006), common mutations in GBM and many other cancers, allow neural stem cells to remain in a self-renewing state, in addition to their many other effects. This maintenance of self-renewal may allow for the accumulation of other oncogenic mutations, tipping cells into a cancerous state. Further studies will be needed to determine the entire suite of changes that make at least a subset of GBM cells behave like stem cells that are locked into a persistently self-renewing state. Such a daunting task is made more palatable by the rapid and ever-growing interchange of methods and concepts between stem cell and cancer biology.

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