High-grade glioma: Can we teach an old dogma new tricks?

The best efforts of clinicians and biologists battling high-grade glioma (HGG) have been overshadowed by two cruel facts: these tumors are essentially incurable and will kill most patients within months, and emergent knowledge of the genetic alterations in HGG has done nothing to ease this burden of suffering. In this issue of *Cancer Cell*, Phillips et al. report an extensive study of the gene expression profiles of a large cohort of HGG. Their data provide new clues to the origins of this disease and suggest potential targets for novel therapies.

Overlapping patterns of clinical behavior, tumor histology, and chromosomal alteration among high-grade gliomas (HGGs) have been distilled into a central dogma that has governed much of our thinking about this disease over the last 20 years. For example, glioblastoma (GBM), which is the most aggressive form of HGG, is believed to arise through one of two mechanisms. GBMs that develop by progression from lower-grade tumors tend to occur in younger adults and frequently contain mutations in *TP53*. These so-called secondary GBMs have been contrasted with primary GBMs that develop rapidly as de novo high-grade lesions in older patients. Primary GBMs are characterized by a different set of genetic alterations that include the following: amplification of *EGFR* and *HDM2*, inactivating mutations in *PTEN*, and loss of all or a portion of chromosome 10. Unfortunately, this synthesis of the various pathologic characteristics of HGG has failed to provide the level of understanding required to advance the treatment of this disease.

Microarray technologies have provided unbiased approaches to assess the validity of established paradigms of tumorigenesis. In this issue of *Cancer Cell*, Phillips et al. (2006) report the use of gene expression profiling to identify novel subgroups of HGG that are defined by distinct clinical and molecular characteristics. This is not the first attempt to use gene expression profiling to improve understanding of gliomagenesis (Bredel et al., 2005; Freije et al., 2004; Godard et al., 2003; Liang et al., 2005; Mischel et al., 2003; Nigro et al., 2005; Rich et al., 2005; Tso et al., 2006). However, while published studies have provided transcriptional evidence that HGG comprises distinct subgroups, they have generally included too few numbers of tumors to reliably assess the clinical implications of these expression profiles. Phillips and colleagues adopted a supervised approach to guide their analyses. In this manner, they first divided 76 samples of HGG into those obtained from patients who experienced either short or relatively long-term survival. They then used Spearman statistics to select the genes that were most significantly and differentially expressed between these two groups of tumors. Subsequent two-way cluster analysis segregated the 76 tumors into three distinct subclasses based on their preferential expression of genes characteristic of neural tissue (*PN*), proliferating cells

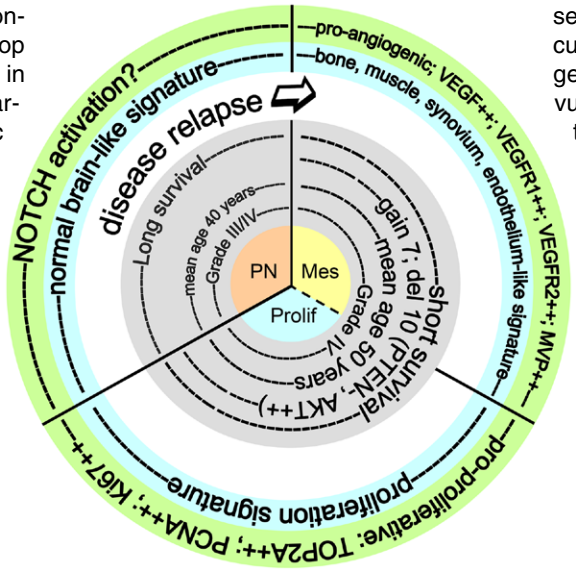

Figure 1. Summary of the major clinical and molecular characteristics of the *PN*, *Prolif*, and *Mes* signature HGG subgroups defined by Phillips et al.

(*Prolif*), or mesenchymal tissues (*Mes*). The *PN* signature was significantly overrepresented among less aggressive forms of HGG, while the *Prolif* and *Mes* signatures overlapped with poor risk disease (Figure 1). Importantly, similar analyses of two independent sets of HGG confirmed that tumors displaying the *PN* signature were associated with a more favorable prognosis. The *PN* signature also correlated closely with established markers of better clinical outcome, including a younger age at diagnosis and grade III histology (Figure 1) (Buckner, 2003). This impact on clinical outcome was independent of tumor grade, since *PN* signature GBMs were associated with longer survival times than either *Mes* or *Prolif* signature GBM.

These data confirm a growing literature indicating that gene expression profiling may predict the clinical outcome of HGG, even among patients with GBM (Freije et al., 2004; Liang et al., 2005; Nigro et al., 2005; Rich et al., 2005). However, more than prognostic markers, we need better treatments of HGG. The anticipated result of subclassifying tumors by expression profiling is that these immense data sets will take our understanding beyond current paradigms to unravel the pathogenesis of the disease and pinpoint vulnerable areas for attack with novel therapies. The generation of genome-wide expression profiles of a large number of well-characterized HGGs (>250) by Phillips and colleagues has provided us with an unprecedented resource with which to do this. As a first step in this process, the authors make some important initial observations from their data set. Of particular note, since the prognosis and gene expression profiles of HGGs are closely correlated, then the authors argue that these gene expression profiles may hold vital clues to the mechanisms that regulate glioma growth. In this regard, Phillips et al. liken the *Mes*, *Prolif*, and *PN* signatures to those of neural stem cells, transit-amplifying cells, and immature neurons, respectively; suggesting that the aggressiveness of glioma growth may be governed by processes that regulate cell fate choices during neurogenesis. These expression data complement recent evidence that gliomas may arise from stem cell-like cancer cells (Sanai et al., 2005). The authors go on to demonstrate that the gene expression signature of glioma cell lines correlates with the EGF/FGF-dependent proliferation of these cells (neurosphere formation) in culture. The authors interpret these findings as an indication that the stem cell-like behavior of HGG might be predicted from tumor gene expression profiles. However,

the presented studies do not test the self-renewal (serial transfer of neurospheres) or potency of glioma cells that are more rigorous analyses of the stem cell fraction. Studies of glioma cells isolated from fresh samples of *PN*, *Prolif*, and *Mes*, signature tumors using more formal tests of stem cell function would better determine whether these expression profiles report the stem cell phenotype of HGG. These studies might also help to determine if the different expression subtypes of HGG are maintained by a common cell type, or represent more distinct forms of the disease that arise from transformed cells at different stages along the neural differentiation pathway. Interestingly, the authors demonstrate that some tumors display a switch in expression signature from *PN* to *Mes* following disease progression (Figure 1). Thus, expression subtypes of HGG may not be entirely distinct, but rather represent different stages or forms of a more common disease process. Comparison of the *PN*, *Prolif*, and *Mes* signatures to those of the available mouse models of glioma that have been derived from cells in different stages of differentiation (Bachoo et al., 2002) may provide further clues to the cellular origins of these HGG subtypes. Finally, the findings of Phillips et al. also have implications for the development of novel therapies of HGG. Perhaps the most significant of these is the demonstration that tumors with *PN* and *Prolif*/*Mes* signatures display evidence of activation of the NOTCH and AKT cell signal pathways, respectively. These cell signaling systems have been identified previously as potential targets for glioma treatment. Therefore, the efficiency of clinical trials that test inhibitors of NOTCH or AKT signaling could be increased significantly by enrolling patients whose tumors display the *PN* or *Prolif*/Med expression signature, respectively.

The extensive expression profiling analysis by Phillips et al. represents an important step forward in our understanding of the biology and treatment of HGG. Their integrated approach has provided important clues that may allow us ultimately to identify the distinct molecular processes that result in the long-recognized clinical and pathologic forms of these devastating diseases.

Richard J. Gilbertson[1,*]

[1]Departments of Developmental Neurobiology and Hematology-Oncology, St. Jude Children's Research Hospital, 332 North Lauderdale Street, Memphis, Tennessee 38105
*E-mail: richard.gilbertson@stjude.org

Selected reading

Bachoo, R.M., Maher, E.A., Ligon, K.L., Sharpless, N.E., Chan, S.S., You, M.J., Tang, Y., DeFrances, J., Stover, E., Weissleder, R., et al. (2002). Cancer Cell *1*, 269–277.

Bredel, M., Bredel, C., Juric, D., Harsh, G.R., Vogel, H., Recht, L.D., and Sikic, B.I. (2005). Cancer Res. *65*, 8679–8689.

Buckner, J.C. (2003). Semin. Oncol. *30*, 10–14.

Freije, W.A., Castro-Vargas, F.E., Fang, Z., Horvath, S., Cloughesy, T., Liau, L.M., Mischel, P.S., and Nelson, S.F. (2004). Cancer Res. *64*, 6503–6510.

Godard, S., Getz, G., Delorenzi, M., Farmer, P., Kobayashi, H., Desbaillets, I., Nozaki, M., Diserens, A.C., Hamou, M.F., Dietrich, P.Y., et al. (2003). Cancer Res. *63*, 6613–6625.

Liang, Y., Diehn, M., Watson, N., Bollen, A.W., Aldape, K.D., Nicholas, M.K., Lamborn, K.R., Berger, M.S., Botstein, D., Brown, P.O., and Israel, M.A. (2005). Proc. Natl. Acad. Sci. USA *102*, 5814–5819. Published online April 12, 2005.

Mischel, P.S., Shai, R., Shi, T., Horvath, S., Lu, K.V., Choe, G., Seligson, D., Kremen, T.J., Palotie, A., Liau, L.M., et al. (2003). Oncogene *22*, 2361–2373.

Nigro, J.M., Misra, A., Zhang, L., Smirnov, I., Colman, H., Griffin, C., Ozburn, N., Chen, M., Pan, E., Koul, D., et al. (2005). Cancer Res. *65*, 1678–1686.

Phillips, H.S., Kharbanda, S., Chen, R., Forrest, W., Soriano, R., Wu, T.D., Misra, A., Nigro, J., Colman, H., Soroceanu, L., et al. (2006). Cancer Cell, this issue.

Rich, J.N., Hans, C., Jones, B., Iversen, E.S., McLendon, R.E., Rasheed, B.K., Dobra, A., Dressman, H.K., Bigner, D.D., Nevins, J.R., and West, M. (2005). Cancer Res. *65*, 4051–4058.

Sanai, N., Alvarez-Buylla, A., and Berger, M.S. (2005). N. Engl. J. Med. *353*, 811–822.

Tso, C.L., Freije, W.A., Day, A., Chen, Z., Merriman, B., Perlina, A., Lee, Y., Dia, E.Q., Yoshimoto, K., Mischel, P.S., et al. (2006). Cancer Res. *66*, 159–167.

DOI 10.1016/j.ccr.2006.02.024

Targeting sphingosine-1-phosphate: A novel avenue for cancer therapeutics

Sphingosine-1-phosphate (S1P) is a pleiotropic lipid mediator that has been shown to regulate cell growth, cell survival, cell invasion, vascular maturation, and angiogenesis, processes that are important for cancer progression. In this issue of *Cancer Cell*, Visentin et al. demonstrate that a monoclonal antibody that binds S1P with extremely high affinity and specificity significantly slows tumor progression and associated angiogenesis in several animal models of human cancer. Their results suggest that S1P not only affects tumor cells themselves, but also is permissive or required for the actions of angiogenic factors, and thus may be a bona fide cancer target.

Sphingosine-1-phosphate (S1P) is the simplest and most intriguing sphingolipid metabolite. Although S1P was initially considered as an intermediate in the ultimate degradation of all sphingolipids, its bewildering nature is rapidly being deciphered, and it is now emerging as a vital lipid mediator of a myriad of cellular processes important for cancer. S1P exerts most of its actions as a specific ligand for a family of five cognate G protein-coupled receptors, designated $S1P_{1-5}$, which regulate cytoskeletal rearrangements and cell movement, angiogenesis and vascular maturation, and immunity and lymphocyte trafficking. This potent lipid may also have intracellular func-