Glioma Models: New GEMMs Add "Class" with Genomic and Expression Correlations

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Malignant gliomas are characterized by accumulated aberrations affecting TP53, PTEN, and RB1 signaling. In this issue, Chow et al. discover that combinatorial loss of these tumor suppressors in mice induces gliomas with unexpected degrees of similarity to human pathologic, genomic, and expression subclasses recently revealed by large-scale genomic studies.

Malignant gliomas represent one of the most studied and well-characterized human cancers at the genetic and expression-profiling level. The most common of these tumors, glioblastoma (GBM), has been the focus of several large scale integrated genomic efforts and was in fact the first cancer genome characterized by the Cancer Genome Atlas project (Cancer Genome Atlas Research Network, 2008). One of the consensus findings of these studies has been the centrality of alterations in the TP53, PI3K/PTEN, and RB1 signaling pathways in human gliomas. While these efforts have yielded an excellent snapshot of GBM at a single point in time, they have generated only indirect hints at the combinatorial hierarchy of genetic events needed for initiation and progression, due to the inaccessibility and limited availability of early stage neoplastic tissue.

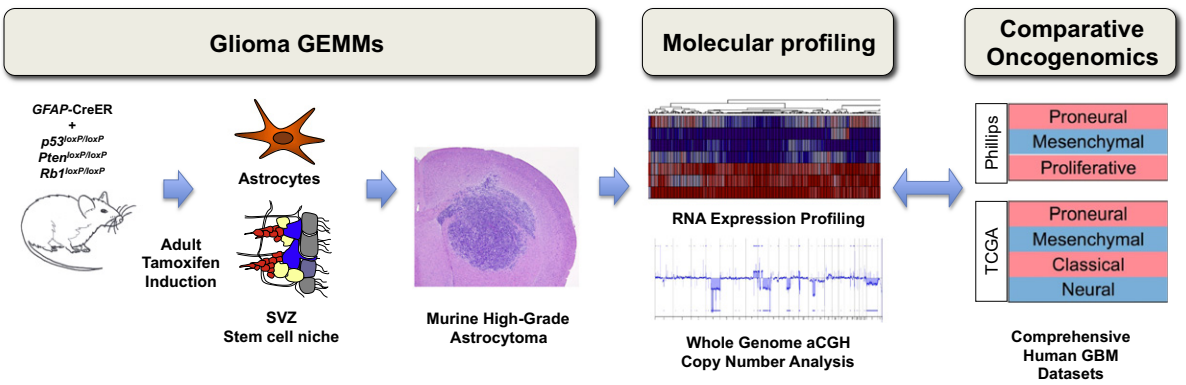
Genetically engineered mouse models (GEMMs) of gliomas are a useful tool in the evaluation of tumor initiation, progression, and origins of gliomas, as the timing and location of mutations may be precisely controlled. Glioma modelers have been fortunate in that many aspects of glioma biology are faithfully reproduced upon introduction of relatively few mutations into the mouse genome. In some instances, mouse model phenotypes have even presaged the future importance of particular genetic alterations, such as inactivating mutations of *TP53* and *NF1*, once thought to represent only infrequent events in adult GBM (Reilly et al., 2000; Zheng et al., 2008). With respect to tumor origins, mouse studies using noninducible *cre*-recombinase systems have established that mouse gliomas readily arise from proliferative Gfap and Nestin positive stem/progenitor cells within the subventricular zone (SVZ), but have generated less clear evidence for origins from mature astrocytes, for which astrocytomas were originally named. Despite these contributions to the field, there remains significant debate about whether simplified mouse models can sufficiently substitute for primary tissue or glioma cell lines in recreating the genomic and expression heterogeneity of the original disease, particularly at the level that would make them useful for preclinical studies and evaluation of targeted therapeutics.

In this issue of *Cancer Cell*, Chow et al. (2011) show that mouse models can still hold their own in this debate. The authors initially sought to carefully dissect the combinatorial interactions of the critical GBM tumor suppressor genes *p53, Pten*, and *Rb1* in the adult brain. Prior work indicated roles for each of these genes in the growth regulation of neural stem/progenitor cells. Combined loss of *p53* and *Pten* has been shown to be sufficient for transforming neural stem cells of the SVZ into malignant gliomas using conditional knockout mice (Jacques et al., 2010; Zheng et al., 2008). However, the techniques employed in these analyses left open questions about the role of these genes in non-stem cells of the adult brain. To address these issues from a different perspective, Chow et al. (2011) used an inducible *GFAP-CreER* mouse driver to achieve combinatorial deletion of all three tumor suppressors ($p53^{loxP/loxP}$, $Pten^{loxP/loxP}$, and $Rb1^{loxP/loxP}$) in up to 50% of the mature astrocytes in the adult brain and only a small percentage of stem/progenitor cells within the SVZ (Figure 1A).

As expected, individual loss of tumor suppressor genes was generally insufficient for glioma formation, although the loss of *p53* alone did cause a low incidence of glioma formation, in concordance with previous reports. However, combined loss of *p53* with either *Pten* and/or *Rb1* in the adult brain led to a high incidence of malignant gliomas with different latencies and histopathologies. One novel result was the finding that layering of *Rb1* loss on top of *p53*/*Pten* loss did not produce very significant changes at either the clinico-pathologic level or in the incidence of tumor formation (87% incidence with *p53:Pten* loss versus 85% in *p53:Pten:Rb1* loss). However, *Rb1* loss produced decreased latency of tumor formation (211 days for *p53:Pten* versus 159 days for *p53:Pten:Rb1*) as well as marked differences in secondary genomic aberrations in the tumors (see below).

The current study by Chow et al. (2011) identifies nearly all of the tumors as anaplastic astrocytomas (WHO grade III) and less frequently glioblastomas with necrosis (WHO grade IV). Of particular value is the finding that the gliomas appeared to develop in the absence of any detectable low-grade precursor—a

A



B

GEMMs	Phenotype	Glioma Incidence	Glioma Latency (Median Survival, days)	Secondary RTK Amplifications	Human Subclass Correlation (Phillips)
Pten	Increased stem proliferation, migration defect, neuronal hypertrophy	0%	-	-	-
Rb1	Increased brain size	0%	-	-	-
p53	Anaplastic astrocytoma (Gr III)	Rare (~17%)	-	-	-
Pten:Rb1	No brain tumor, few pituitary tumors	0%	-	-	-
p53:Rb1	Astrocytoma (Gr III, IV); central PNET, olfactory PNET, (Gr IV)	37%	Long (~350)	-	-
p53:Pten	Anaplastic astrocytoma (Gr III), myxoid glioma , GBM (Gr IV)	87%	Medium (211)	*Pdgfra, Met, Egfr*	Proneural = proliferative = mesenchymal
p53:Pten:Rb1	Anaplastic astrocytoma (Gr III), myxoid glioma, GBM (Gr IV)	85%	Short (159)	*Pdgfra*	Proneural = proliferative > mesenchymal

Figure 1. Combinatorial Tumor Suppressor Loss in Mouse Glioma Models Reveals Novel Correlations to Human Glioma Drivers and Expression Subclasses

(A) Schematic describing conditional and inducible deletion of *p53, Pten,* and *Rb1* in Gfap-positive adult brain astrocytes and stem cells and subsequent comparative genomic correlations with human GBM datasets.

(B) Summary of specific combinations of suppressor deletion reveal hidden secondary genetic and subclass differences in adult murine high-grade gliomas.

desirable characteristic for an accurate model of de novo human primary GBM. The authors make note of interesting phenotypic diversity in the tumors, with some similarities to human tumors such as myxoid phenotype and some features of central primitive neuroectodermal tumors (cPNET). Such diversity was also noted in a prior study using noninducible *p53:Pten* deletion, but this model showed multifocal mixed oligodendroglial and astrocytic tumors in the same brain (Zheng et al., 2008). Future studies may be needed to determine whether the difference in these phenotypes are a result of timing of deletion or of the cell compartments in which tumor suppressor loss occurs. Collectively, it seems that one can conclude that alterations in the p53 pathway are key initiating events in murine and human high-grade glioma formation and also suggest that the role of Pten and Rb1 may be more crucial for tumor progression.

Chow et al. (2011) were particularly interested in determining whether gliomas might originate from mature astrocytes, and in fact uncovered evidence to support this hypothesis. While a subset of gliomas in the *GFAP-CreER:p53:Pten* background arose in contact with the SVZ region, 22% of tumors developed at distant sites including the cerebellum, brainstem, and spinal cord without connection to the SVZ or other known germinal zones in the adult brain. The authors make the cogent argument that these tumors most likely arise from genomic deletion in mature astrocytes; however, even with the advanced techniques used, it remains a challenge to conclusively track the precise origin of the tumors. Certainly these studies do not address the potential contribution of "distributed" progenitors—such as NG2/Olig2+ cells or oligodendroglial progenitors—to tumor formation, despite evidence that such cells may be the most closely related to gliomas. Future studies precisely targeting oligodendroglial and other cellular compartments will likely be helpful in glioma models, given the utility of this approach in dissecting the origins of other brain tumors such as medulloblastoma (Gibson et al., 2010; Schuller et al., 2008).

Clearly, the most unexpected turn in the work came when the authors delved into the hidden genomic and RNA expression patterns in the murine tumors. Using array comparative genomic hybridization (aCGH), the authors discovered focal as well as large-scale genomic aberrations characteristic of human GBM genomic subclasses. For example, *p53:Pten* tumors had acquired secondary amplifications of receptor tyrosine kinases (RTKs), including *Pdgfra, Egfr,* and *Met,* which are hallmark events in human GBM pathogenesis (Figure 1B). Interestingly, *p53:Pten:Rb1* tumors seemed to acquire only *Pdgfra* amplification and had a distinctly different genomic profile compared with *p53:Pten* tumors, suggesting that the loss of *Rb1* removed the selective pressure for development of additional RTK activation. These findings are reminiscent of comparative oncogenomic studies in T-ALL and other cancers, which leads one to conclude that secondary induction of copy number alterations in mouse models may not be a rare event (Maser et al., 2007). Such findings provide immediate opportunities to investigate the basic mechanisms by which tumor suppressor losses might induce secondary genomic alterations in glioma progression and should help

investigators prioritize candidate genes for functional studies.

As a final step, the authors sought to determine to what degree murine gliomas might recapitulate gene expression subclasses of human glioblastoma described in prior studies (Phillips et al., 2006; Verhaak et al., 2010). Unsupervised hierarchical clustering analysis across *p53:Pten* and *p53:Pten:Rb1* tumors generated three clusters of murine gliomas, HC1, HC2, and HC3, with significant similarity to proneural and mesenchymal subclasses of GBM. One interesting deviation from the human data, however, was that correlation of the mouse histology and genetics with the murine expression subclasses was weak, yet such correlations are clearly present in human GBM, particularly for the mesenchymal subclass that is associated with *NF1* loss, necrosis, and inflammation. While further refinement of mouse-to-human expression correlations is likely required, the results support the idea that mouse glioma models could be very helpful in exploring the diversity of human GBM subclasses with implications for better diagnosis and prediction of prognosis in patients. What seems equally clear is that mouse models can still generate novel ways to surprise and inform investigators who seek to understand these most challenging cancers of the brain.

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Escaping Anoikis through ROS: ANGPTL4 Controls Integrin Signaling through Nox1

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Reactive oxygen species (ROS) mediate various cell fate decisions in normal and transformed cells. In this issue of *Cancer Cell*, Zhu et al. demonstrate the ability of ANGPTL4 to engage integrin-dependent survival signals by activation of the NADPH oxidase Nox1, thus mimicking anchorage conditions and bypassing anoikis by controlling ROS.

Cancer cells have long been known to display abnormal redox metabolism, releasing increased levels of reactive oxidants compared with normal cells. The exact significance of such reactive oxygen species (ROS) production as it pertains to malignant transformation, however, has been less clear. ROS cause oxidative stress, which results in mutations to both nuclear and mitochondrial DNA. With loss of fail-safe death and senescence mechanisms, such genomic damage has been proposed to accelerate transformation and cancer progression.

Besides creating a stress response, however, ROS clearly participate in physiologic signaling at a variety of levels, requiring tight spatial and temporal regulation of oxidants by normal cells (Terada, 2006). In this capacity, ROS control proliferation, differentiation, junction formation, and response to cytokines and other soluble factors. Given their propensity to produce increased levels of ROS, are cancer cells able to appropriate these oxidant-dependent signals as a means of dysregulating proliferative and survival pathways? In this issue of *Cancer Cell*, Zhu et al. (2011) uncover a role for angiopoietin-like 4 (ANGPTL4) in activating integrin-related, oxidant-dependent survival pathways, despite the loss of matrix attachment. This highjacking of normal