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Review

Oncogene interactions are required for glioma development and progression as revealed by a tissue specific transgenic mouse model

Lynette M. Moore, Kristen M. Holmes, Gregory N. Fuller, and Wei Zhang

Abstract

The aggressive and invasive nature of brain tumors has hampered progress in the design and implementation of efficacious therapies. The recent success of targeted therapies in other tumor types makes this an attractive area for research yet complicating matters is the ability of brain tumors to circumvent the targeted pathways to develop drug resistance. Effective therapies will likely need to target more than one signaling pathway or target multiple nodes within a given pathway. Key to identifying these targets is the elucidation of the driver and passenger molecules within these pathways. Animal models provide a useful tool with many advantages in the study of these pathways. These models provide a means to dissect the critical components of tumorigenesis, as well as serve as agents for preclinical testing. This review focuses on the use of the RCAS/tv-a mouse model of brain tumors and describes their unique ability to provide insight into the role of oncogene cooperation in tumor development and progression.

Key words Glioma, animal models, RCAS/tv-a

Gliomas arise in ~18 000 new patients each year in the United States according to the Central Brain Tumor Registry of the United States; and present unique issues with regard to treatment. Survival rates are particularly dismal, with an average survival of one year from time of diagnosis for the most aggressive form of glioma, glioblastoma multiforme (GBM), and 3–5 years for anaplastic oligodendroglioma (AO)[1,2]. The current standard treatment consists of surgical resection, radiotherapy, and chemotherapy[3]; however the rapid progression and highly invasive nature of gliomas makes treatment difficult. Cancer stem cells (CSCs) may also be factors which complicate treatment. Two reports demonstrate that $CD133^+$ cells (a population of cells thought to be enriched for CSCs) isolated from human gliomas were more resistant to ionizing radiation than their $CD133^-$ counterparts[4]. The $CD133^+$ cells appeared to more effectively repair DNA damage, and the irradiated $CD133^+$ cells retained their ability to form tumors in mice. Another study determined that this cell population exhibited enhanced checkpoint activation[5]. Both of these reports implicate radioresistant CSCs in glioma therapeutic resistance and recurrence. Current research towards improving glioma therapy now focuses on both the role of CSCs in tumor development and treatment response and the development of targeted therapies and identification of key pathway targets for the treatment of gliomas. The focus of this review is the use of mouse models to elucidate key nodes of oncogene cooperation in the signaling pathways driving tumor development and progression.

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Genetic Changes Involved in the Development of Glioma

Glioma classification is currently based primarily on histological features. Recently there has been considerable focus on characterizing the genetic and molecular alterations that occur during tumor development in an effort to more accurately classify and treat these diseases[6,7]. In glioma as well as other cancers, many of the deregulated molecules are components of receptor tyrosine kinase (RTK) signaling pathways. Human gliomas frequently contain alterations

in these pathways by the over-expression or amplification of the receptors themselves, such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR)[7,8], or growth factors such as vascular endothelial growth factor (VEGF)[9].

The elucidation of common genetic changes that occur in tumors has led to recent advances in more targeted therapies. RTK and VEGF inhibitors have proven successful in many tumor types. Unfortunately, these therapies have been less successful in the treatment of glioma. RTK inhibitors targeting EGFR have not been successful in glioma clinical trials, despite achieving significant benefit in other neoplastic diseases such as colon cancer[10-12]. VEGF inhibitors have been approved for the treatment of several tumor types, but are not yet approved for the treatment of glioma[13]. The relatively minimal improvement over the past several decades in the survival of patients with glioma highlights the importance of modulating existing therapeutics and identifying novel targets to develop more efficacious treatments.

Given the complexity of genetic changes that occur throughout the development of GBM, it has become clear that no single pathway is responsible for this disease. Monotherapies such as those described above have provided modest or no survival benefit, likely due to the ability of gliomas to circumvent the targeted pathway. A recent study of GBM cell lines identified three or more activated RTKs in 19 of the 20 cell lines examined, illustrating the use of multiple pathways for the rapid growth and invasive properties of glioma[14]. Multitargeted kinase inhibitors or combination therapies will be more likely to elicit a long-term, favorable response. It is becoming increasingly clear that in order to develop specific and successful therapies, we must understand the cooperative effects of the proliferation, migration, and invasion signaling pathways that are utilized by both normal and malignant glial cells.

The wealth of data available for study with the availability of high throughput genomic studies has provided a great deal of information with regards to the loss, mutation, overexpression, and amplification of common genetic loci, oncogenes, and tumor suppressors; yet overexpression and amplification do not always translate into drugable targets, as appears to be the case for EGFR in glioma. Dissecting these pathways so as to delineate "driver" and "passenger" oncogenes or tumor suppressors will provide invaluable information to aid in the development and testing of novel single and combination therapies.

Models for the Study of Glioma Development and Progression

While the therapeutics described above are currently in human clinical trials, it follows that there exists a need for efficient systems in which to model this disease. Mouse models are critical for furthering our understanding of the genetic and molecular changes, the altered signaling pathways, and the pathologic features of glioma. Animal studies aid in the discovery of new targets for therapeutics as well as serve as valuable pre-clinical models in which to test novel therapeutics. To model glioma in the mouse, several methods have been utilized (reviewed in reference [15]). Most frequently, xenograft models, whereby cell lines are allowed to develop into tumors within immune compromised mice, are utilized to test new therapeutics. This has a number of advantages including relative ease and speed; however, these models often fail to accurately recapitulate the true tumor due to the use of passaged cell lines and subcutaneous injection sites. Xenograft models also fail to recapitulate interactions between the tumor cells and organ-derived stromal cells. It has become increasingly apparent that these interactions play a major role in tumor cell biology. Genetically engineered mouse models (GEMMs) can recreate the genetic events that are frequently perturbed in brain tumors and more closely resemble human tumors, however these can be costly and time consuming to create.

The approach we and others have taken is a GEMM which utilizes the avian retrovirus, RCAS, to deliver and overexpress genes of interest (Figure 1). In this model, the receptor for the RCAS virus, tv-a, is driven by the Nestin promoter (Ntv-a) or the glial fibrillary acidic protein (GFAP) promoter (Gtv-a). The Nestin promoter is active in neural/glial stem cells, whereas the GFAP promoter is active in neural stem cells/astrocytes; therefore RCAS infection (and oncogene expression) is directed specifically to the glial cells of the mouse. More recently, an additional mouse model has been generated, which drives tv-a expression from the 2',3'-cyclic nucleotide 3'-phosphodiesterase (*Cnp*) promoter (Ctv-a). In this mouse, tv-a is expressed in oligodendrocyte progenitor cells[16]. By placing tv-a expression under the control of various promoters, specific cell types can be targeted and information regarding glioma cell of origin can be gained. The virions expressing the desired genes of interest are directly delivered to the mouse brain to induce gene expression in Nestin, GFAP, or Cnp-expressing cells. This system provides a number of key advantages. Somatic cell gene transfer provides a system to study the effects of low frequency events that take place in the adult, rather than tissue-wide as in typical mouse models. Importantly, this model also allows for the simultaneous delivery and study of multiple oncogenes without the need to generate multiple animal models, which is both costly and time consuming. This provides the unique ability to study oncogene cooperation. Additionally, tv-a transgenic mice can be used alone, or crossed to one or

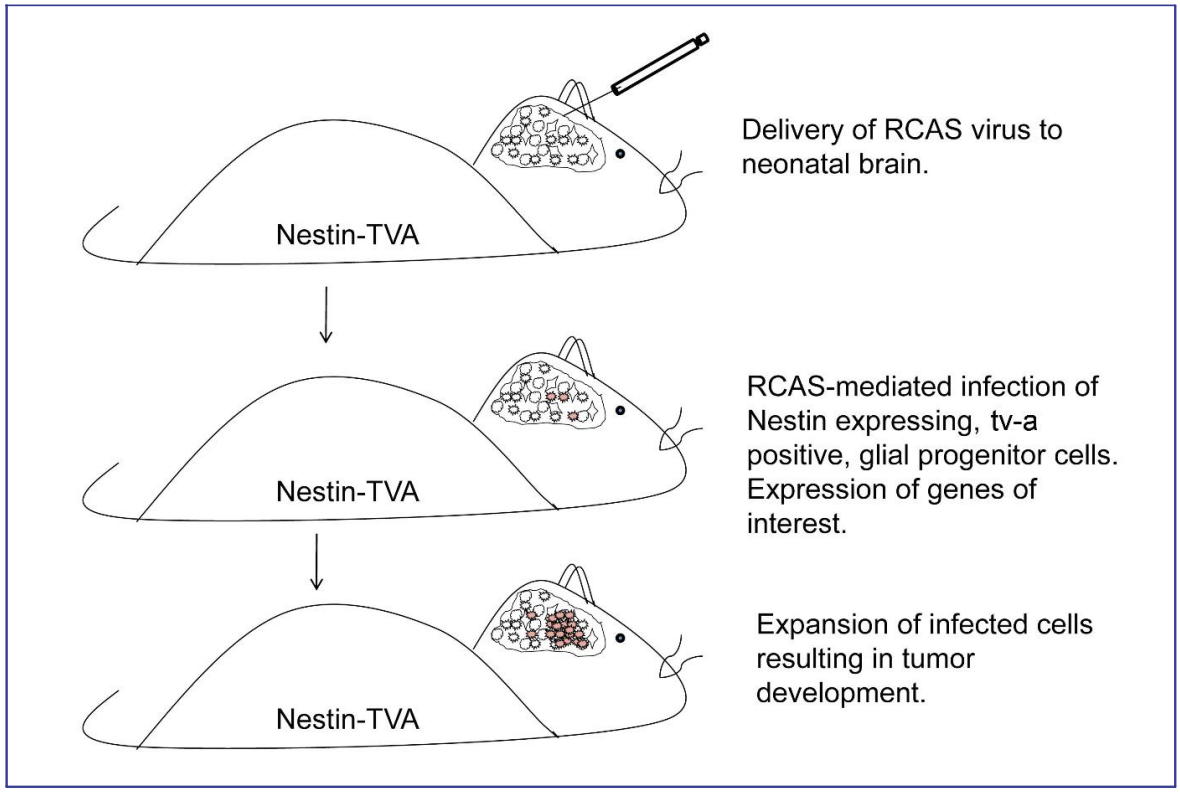

Figure 1. The RCAS/tv-a mouse model of glioma. Cell-type specific expression of the RCAS viral tv-a receptor is achieved by placing receptor expression under the control of the Nestin promoter, driving expression of the receptor in glial progenitors. The RCAS virons expressing genes of interest are delivered to the glial progenitors via direct injection of the virus-producing cells. The RCAS virus infects the target cells, resulting in expression of the delivered genes. Expression of a driver oncogene or combination of oncogenes leads to transformation of the infected cells and promotes tumor development and progression over time.

more knockout animals. This provides a powerful tool to evaluate oncogenes in the context of tumor suppressor gene loss to more closely model the multiple, cooperative genetic changes that occur in glioma.

The RCAS/tv-a model has been used successfully to generate a range of brain tumor subtypes. By targeting specific cell populations and modifying the genes delivered, models of medulloblastoma, brainstem glioma, astrocytoma, oligodendroglioma, and GBM have been described (Table 1). The tumors that arise accurately recapitulate human disease in terms of gene expression and pathologic features of human glioma, including increased cellularity, mitotic figures, and secondary structures of Scherer such as perineuronal satellitosis[17]. High grade tumors also display regions of microvascular proliferation and necrosis surrounded by pseudopalisading cells (Figure 2).

Oncogene Cooperation in the Development of Glioma in Mouse Models

Tumorigenesis is typically described as a multi-step process involvin g genetic aberrations which deregulate oncogenes and tumor suppressor genes [18]. In fact, a recent study highlighted the importance of oncogene cooperation by defining a class of genes critical for the transformation of colon cancer cells in response to synergistic oncogenic alterations [19]. This study identified genes that respond synergistically to Ras and mutant p53 in colon cells. This class of genes was critical for the resulting malignant phenotype, and in some cases, modulation of even one of these genes was sufficient to inhibit tumor formation[19]. The RCAS/tv-a model provides a valuable tool in which to study cooperating events such as these and others.

The first study of oncogene cooperation in this system came from the examination of mutant Kras (G12D) and activated Akt[20]. Singly neither Kras nor Akt was sufficient to induce gliomas in Ntv-a mice. However, when both the Ras and Akt signaling pathways were activated, tumors arose in the Ntv-a mice which demonstrated features of GBM such as increased cell density, high numbers of mitotic figures, microvascular proliferation, and pseudopalisading necrosis. Our group also examined the cooperation between Kras and Akt1 in

Table 1. Oncogene cooperation in the RCAS/Ntv-a mouse model

Oncogenes	Mouse strain	Tumors	References
KRas + Akt	Ntv-a	GBM	20
KRas + Akt + c-Myc	Gtv-a	GBM	22
EGFR*	Ntv-a, *INK4a-ARF*$^{-/-}$	A	34
PDGFB	Ntv-a	O, BSG	21, 25, 50
PDGFB + IGFBP2	Ntv-a	AO	21
IGFBP2 + KRas + Akt	Ntv-a	A	21
IGFBP2 + Akt	Ntv-a	NT	21
Akt + Inducible KRas	Ntv-a	GBM	40
PDGFB	Ntv-a, *INK4a-ARF*$^{-/-}$	AO, BSG	33, 50
PDGFB	Ctv-a	O	16
Kras + Akt	Ctv-a	NT	16
Shh + Myc, IGFII, Akt, or HGF	Ntv-a	MB	45–47, 49
Raf + Akt	Ntv-a	AO/GBM	23, 24

RCAS/tv-a mouse models of glioma demonstrate oncogene cooperation. EGFR, epidermal growth factor receptor; PDGFB, platelet-derived growth factor B; IGFBP2, insulin-like growth factor binding protein 2; IGFII, insulin growth factor II; HGF, hepatocyte growth factor; GBM, glioblastoma; A, astrocytoma; O, oligodendroglioma; BSG, brainstem glioma; AO, anaplastic oligodendroglioma; MB, medulloblastoma; NT, no tumor.

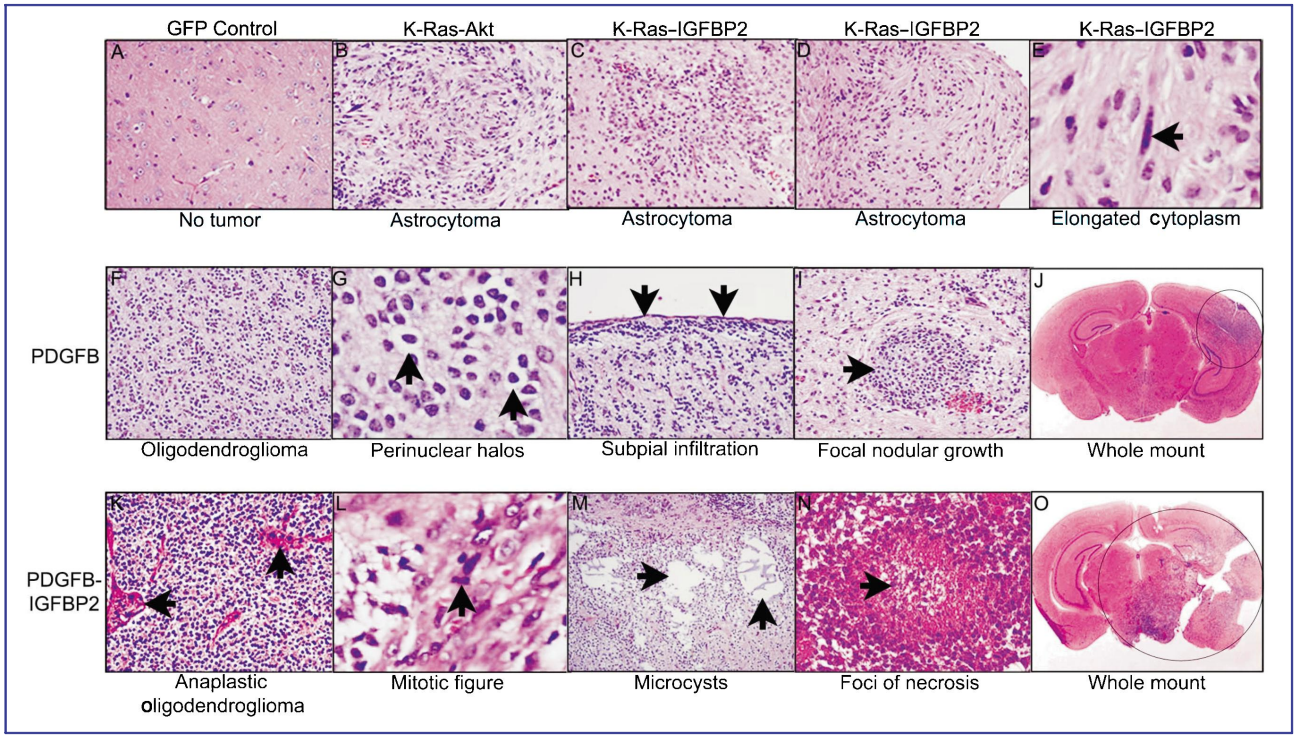

Figure 2. The RCAS/Ntv-a mouse model recapitulates human glioma. H&E staining of several representative tumors from *N-tva* mice shows RCAS-GFP vector control-injected gray matter (A), astrocytoma resulting from the combined injection of RCAS vectors for K-Ras and Akt (B), and astrocytomas resulting from the combined injection of RCAS vectors for K-Ras and insulin like growth factor binding protein 2 (IGFBP2) (C and D). Original magnification is ×200 in all figures unless otherwise noted. E, enlargement of K-Ras–IGFBP2 injection showing elongated fibrillary cytoplasm and oval elongated nuclei (arrow) seen in the astrocytomas. (Enlargement at ×400.) Platelet-derived growth factor B (PDGFB)–driven oligodendrogliomas exhibit uniform, round nuclei surrounded by perinuclear halos (F–J; arrows in F and G), subpial infiltration (H, arrows), and focal nodular growth pattern (I, arrow). (Enlargement of perinuclear halos is shown in G at ×400.) J, whole mount of a PDGFB-driven tumor shows a relatively small tumor limited to the cerebral cortex of one hemisphere (×1.5 original magnification, with tumor area indicated by circle). Anaplastic oligodendrogliomas resulting from the combined injection of RCAS vectors for PDGFB and IGFBP2 (K–O) show areas of microvascular proliferation (K, arrows), mitotic figures (L, arrow), microcysts (M, arrows), and foci of necrosis (N, arrow). (Enlargement of mitotic figure is shown in L at ×400.) O, whole mount of a PDGFB-IGFBP2–driven tumor shows a much larger tumor with involvement of the cortex, deep gray nuclei, and bilateral brainstem (×1.5 original magnification, with tumor area indicated by circle).

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Ntv-a mice and confirmed the development of high-grade astrocytoma, however we did not observe GBM in this study[21].

While introduction of Kras and Akt led to glioma formation in Ntv-a mice, no tumors developed from the same combination in Gtv-a mice [20]. Holland's group examined this further by delivering c-Myc in combination with Kras and Akt to Gtv-a and Ntv-a mice [22]. The addition of Myc to Kras and Akt did not increase the grade or incidence of tumors arising in Ntv-a mice. However, this combination in Gtv-a mice resulted in the development of gemistyocytic astrocytomas in 22% of injected mice. Many of these tumors contained features of GBM such as microvascular proliferation and pseudopalisading necrosis[22]. These studies demonstrate that the response to particular oncogenic stress differs with the cell of origin and that specific combinations of oncogenes may be required to drive glioma formation.

The RAF family of kinases, which function downstream of Ras in the Ras-MAPK signaling pathway, have also been implicated in glioma, and studied in the context of the RCAS/Ntv-a mouse model. Delivery of a constitutively active Raf-1 mutant to glial progenitors together with activated Akt led to the development of tumors which were very similar to those that arose from the Kras and Akt combination[23]. It is also interesting to note that while Kras alone was not able to initiate tumor formation, some animals injected with Raf-1 alone did develop small hyperplastic lesions, although no evidence of these lesions developing into tumors was identified[23]. Delivery of BRAF containing an activating mutation ($BRAF^{V600E}$) was found to cooperate in a similar manner with activated Akt[24]. These tumors were similar to those arising from the cooperation of Kras and Akt, although the BRAF tumors were more pleomorphic [24]. These studies indicate that RAF family members can substitute for Ras activation in promoting tumor development; however, the activation of additional Kras effectors may explain some of the morphological differences between the tumors which arise from the different combinations.

While oncogenic Ras promotes the development of astrocytoma, platelet-derived growth factor B (PDGFB) appears to drive tumors towards oligodendroglioma in this system. PDGFB expression in myelinating oligodendrocyte progenitors in Ctv-a mice led to tumor formation in 33% of animals, most classified as low-grade oligodendroglioma[16]. However, in this model, Kras and Akt did not lead to tumor formation. Additionally, delivery of PDGFB to glial progenitors in Ntv-a mice led to the formation of oligodendrogliomas in 60% of injected animals, whereas the delivery of PDGFB to Gtv-a mice led to the formation of oligodendroglioma or mixed oligoastrocytomas in 40% of mice[25]. A related study from our laboratory confirmed the formation of oligodendroglioma upon delivery of PDGFB to Ntv-a mice[21]. Furthering this analysis, we examined the cooperation of PDGFB with insulin-like growth factor binding protein 2 (IGFBP2). IGFBP2 cooperated with PDGFB in Ntv-a animals to promote progression to AO (Grade III), identifying IGFBP2 as a driver of glioma progression[21].

IGFBP2 is frequently overexpressed in high-grade gliomas and its expression is associated with poor patient survival, highlighting its importance as a key oncogene in gliomagenesis[26-28]. The high expression of IGFBP2 can be detected in the serum of patients affected with several types of high-grade tumors and may be useful as a biomarker for high-grade tumors and, more specifically, for gliomas lacking PTEN or the *INK4a-ARF* locus [29-33]. Further studies have found that IGFBP2 in combination with Kras resulted in the development of astrocytoma in Ntv-a mice, indicating that IGFBP2 cooperates with Kras to initiate tumor formation. However, contrary to the results with PDGFB and IGFBP2, IGFBP2 did not cooperate with Kras and Akt to enhance glioma progression. Recent studies from our lab have determined that integrin linked kinase (ILK) cooperates with PDGFB in a manner similar to IGFBP2. A dominant negative ILK was capable of blocking IGFBP2-mediated progression of PDGFB induced gliomas, indicating that ILK may be a key downstream mediator of IGFBP2 signaling (Kristen Holmes and Wei Zhang, manuscript in preparation).

This model can also be used to study the cooperation of oncogenes with tumor suppressor loss. Early studies with this system have generated gliomas in mice by delivering a constitutively active form of EGFR (EGFR*) to Ntv-a and Gtv-a animals[34]. EGFR* introduced into Ntv-a or Gtv-a mice did not develop gliomas, however tv-a transgenic animals containing disrupted *INK4a-ARF* locus did develop lesions displaying features of glioma upon EGFR* delivery. These tumors arose at a higher frequency in Ntv-a mice than in Gtv-a mice, suggesting that specific populations of cells respond differently to a particular oncogene.

The *INK4a-ARF* locus is well studied in the RCAS/tv-a model. Loss of this locus is a frequent event in glioma, and leads to the loss of the $p16^{INK4a}$ cyclin-dependent kinase inhibitor and $p14^{ARF}$ ($p19^{ARF}$ in the mouse) tumor suppressors which regulate the RB and p53 pathways[6,7,35]. RAF family members cooperate with the *Ink4a-Arf* loss. Activated Raf-1 in the context of Arf loss led to the development of gliomas in Ntv-a mice, similar to Kras in the same context[23]. BRAF combined with *Ink4a-Arf* loss in Ntv-a mice also led to the development of gliomas, however these tumors were less infiltrative and lacked the necrosis and vascular proliferation which are frequently observed in GBM[24]. The delivery of PDGFB to Ntv-a or Gtv-a;*Ink4a-Arf*$^{-/-}$ mice leads to increased tumor incidence and progression as compared to wild-type mice[25]. A recent study from our lab identified IGFBP2

as a key player in the increased tumor incidence and progression in PDGFB-induced gliomas from Ntv-a; *Ink4a-Arf*$^{-/-}$ mice[33]. An inverse relationship between the levels of p16^{INK4a} and IGFBP2 was identified in human glioma, as well as cell lines from many different cancer cell types. Endogenous IGFBP2 was upregulated in the PDGFB-induced tumors of Ntv-a; *Ink4a-Arf*$^{-/-}$ mice, and inhibition of IGFBP2 by antisense technology provided a survival benefit to the mice.

PDGFB also cooperates with the loss of the tumor suppressor, p27^{Kip1}, another cyclin-dependent kinase inhibitor[36,37]. p27 deficiency accelerated the development of PDGFB-induced gliomas[37]. Ntv-a–positive, p27-deficient glial progenitor cell lines were established and cell culture studies indicate that p27 loss has an inhibitory effect on DNA damage repair which may promote chromosomal instability[37]. Despite the accelerated tumor development, the tumors that arose from PDGFB cooperation with p27 loss were less invasive than the PDGFB-p27 wild-type tumors, and cell culture studies revealed migration defects in these cells[36,37].

This model has also been utilized in combination with PTEN loss, another frequently disrupted molecule in glioma. Kras cooperates with PTEN loss in the Ntv-a model to produce gliomas similar to those arising from the delivery of Kras with activated Akt[38]. This study confirmed that the activation of Akt and PTEN loss function similarly with respect to glioma formation. A recent study also reported the ability of this system to direct expression of miRNAs. The authors reported that the RCAS-mediated expression of the PTEN targeting miR-26a in glial progenitor cells enhanced tumor formation and grade, similar to the loss of PTEN itself[39].

These studies of cooperating oncogenes and tumor suppressors can shed light on the molecular pathways utilized for tumor initiation and progression. One interesting observation arising from the studies of PDGFB with IGFBP2 was the upregulation of endogenous Akt in the resulting AOs, suggesting that the cooperation of these two oncogenes leads to activation of downstream Akt growth promoting pathways. When IGFBP2 was co-delivered with Akt to Ntv-a mice, no tumors developed, suggesting that IGFBP2 and Akt may lie in the same pathway[21]. Further, inhibiting tumor progression by blocking ILK signaling in PDGFB and IGFBP2 induced tumors has uncovered new mechanisms of cell signaling utilized by these tumors (unpublished data). Studies of PDGFB-induced gliomas in Ntv-a;*Ink4a/Arf*$^{-/-}$ mice revealed up-regulation of endogenous IGFBP2, identifying a novel link between two key players in glioma development[33].

The studies described here provide important information with regard to oncogene cooperation and the links between oncogenes and their downstream signaling partners. PDGFB appears to be the only oncogene capable of initiating glioma development alone. All others tested thus far require the cooperation of either an additional oncogene or the loss of one or more tumor suppressor genes. Further, the cell type and specific oncogene combinations play critical roles in determining tumor outcome. Delineating these pathways is critical for future therapeutics as targeting these downstream molecules, alone or in combination, may aid in circumventing the issues that arise with tumor resistance to single agents targeted at upstream signaling components.

Oncogene Dependence

One key modification of the RCAS/tv-a model is the addition of an inducible component. Holmen's group utilized this system to investigate the dependence of Akt and Kras driven gliomas on Ras signaling[40]. By modifying the RCAS viral vectors to place Kras under the control of tetracycline, Kras overexpression can be tightly controlled in the context of Akt activation. This key study demonstrated that continued Kras expression was required for the maintenance of glioma, and that inhibition of Kras led to tumor regression and increased survival.

The term "oncogene addiction" has been used to describe the dependency of tumor cells on a single oncogenic activity. There have been several mouse models of tumorigenesis that demonstrate this phenomenon[41]. The dependency of the tumors on a single oncogenic activity suggests that targeted therapies to inactivate the oncogene might prove useful. The success of Gleevec, a kinase inhibitor that affects c-kit, PDGFR, and the BCR-ABL fusion protein, in treating gastrointestinal stromal tumors (GIST) and chronic myeloid leukemia, indicates that this is indeed the case[42]. However, the key factors involved in glioma are still in the process of being uncovered. Additionally, the issue of tumor dormancy in response to oncogene inactivation arises and has been most thoroughly addressed in studies with Myc inactivation. In several mouse model systems of different tumor types, inactivation of Myc results in tumor regression, and in some cases, even a brief inactivation can result in long-term to permanent tumor regression (reviewed in reference [43]). However, this does not occur in all tumor types, suggesting that other factors, intrinsic to the tumor type, play a role in the response of the tumor to oncogene inactivation. For instance, in liver tumors, Myc reactivation resulted in tumor regrowth[44]. Therefore,

understanding how gliomas respond to both sustained and brief inactivation of oncogenes provides critical information pertinent to the design and implementation of future therapies.

RCAS/tv-a System to Model Other Brain Tumors

In addition to gliomas, the RCAS/tv-a system has been utilized to model medulloblastoma. Many of these studies have focused on Shh/Patched signaling, a key pathway in the development of medulloblastoma. Delivery of Shh to Nestin expressing cells was sufficient to promote tumor formation[45]. The tumorigenicity was greatly enhanced by co-delivery of c-Myc, N-myc, IGF-II, or Akt[45-47]. Shh also cooperates with apoptotic inhibition in a model of RCAS-mediated Shh and Bcl-2 delivery[48]. Hepatocyte growth factor (HGF) and its receptor, c-met, both frequently overexpressed in medulloblastomas, may serve as additional key components of medulloblastoma development. HGF also cooperates with Shh to induce tumor formation, and the inhibition of HGF with a monoclonal antibody was able to increase survival in this mouse model demonstrating the potential for HGF-targeted therapies[49]. The Ntv-a mice have also been used to model brainstem gliomas (BSGs) by a brainstem specific injection of PDGFB to the Ntv-a mice[50]. The resultant tumors were diffusely infiltrating and expressed markers similar to those observed in pediatric BSGs.

Conclusions: the Future of Mouse Models and Preclinical Research

Mouse models provide an excellent resource for study in both basic science and preclinical research. The RCAS/tv-a model has many advantages over traditional transgenic and xenograft mouse models. This system allows for the introduction of somatic oncogene delivery to a small subset of cells to more accurately recapitulate tumor initiation and further development. The ability to deliver multiple genes simultaneously provides a convenient model in which to study oncogene cooperation. This model will allow researchers to mimic many of the genetic changes that occur in glioma in the mouse, providing a model to study how these changes interact and affect tumor initiation, tumor progression, downstream signaling pathways, and cellular changes such as proliferation, migration and invasion.

However, there are some limitations of this model. While the model accurately recapitulates human disease in terms of pathology and specific genetic events, the r esulting tumors likely do not represent the large number of genetic and molecular changes that occur within any given human glioma. In patients, these additional changes may mediate drug effectiveness. Further, in the RCAS/tv-a model, the same limitations apply with respect to drug delivery as one must develop drugs which can circumvent the blood brain barrier. Despite these limitations, the model provides a unique system in which to identify new potential targets and test their effectiveness, *in vivo*.

Future studies with these animal models will provide useful information with regard to important questions such as how particular oncogene combinations produce specific glioma subtypes and identifying the cells-of-origin for a given oncogene or transgenic line. These studies can be furthered by including tumor suppressor gene loss and miRNA expression. The results from these studies provide valuable insight into the molecular pathways that are activated or altered upon oncogene overexpression (Figure 3). Specific combinations of events may determine glioma phenotype, and translate into patient survival. A greater understanding of these actions is critical for patient stratification and the careful design of therapeutics. Additionally, these models prove to be an invaluable tool for preclinical studies of new and existing therapeutics, both singly and in combination to aid in the development of the most effective treatments. The RCAS/tv-a model has already been utilized in some pre-clinical testing. The BSG model was examined for tumor responsiveness to the Akt inhibitor, perifosine, both singly and in combination with ionizing radiation[50]. Hu and colleagues[38] utilized the RCAS/Ntv-a model to examine the effects of the mTOR inhibitor, CCI-779, on GBMs that arose from the delivery of Ras and Akt. This study demonstrated the ability of CCI-779 to inhibit mTOR signaling and induce apoptosis in a subset of tumor cells *in vivo*[38]. Additionally, MRI methodologies have been demonstrated to distinguish high-grade and low-grade gliomas, predict overall survival, and were subsequently used to assign mice to treatment groups[51]. These studies demonstrate the unique utility of this mouse model to accurately recapitulate human glioma and to provide a valuable resource for the development and testing of current and novel therapeutics.

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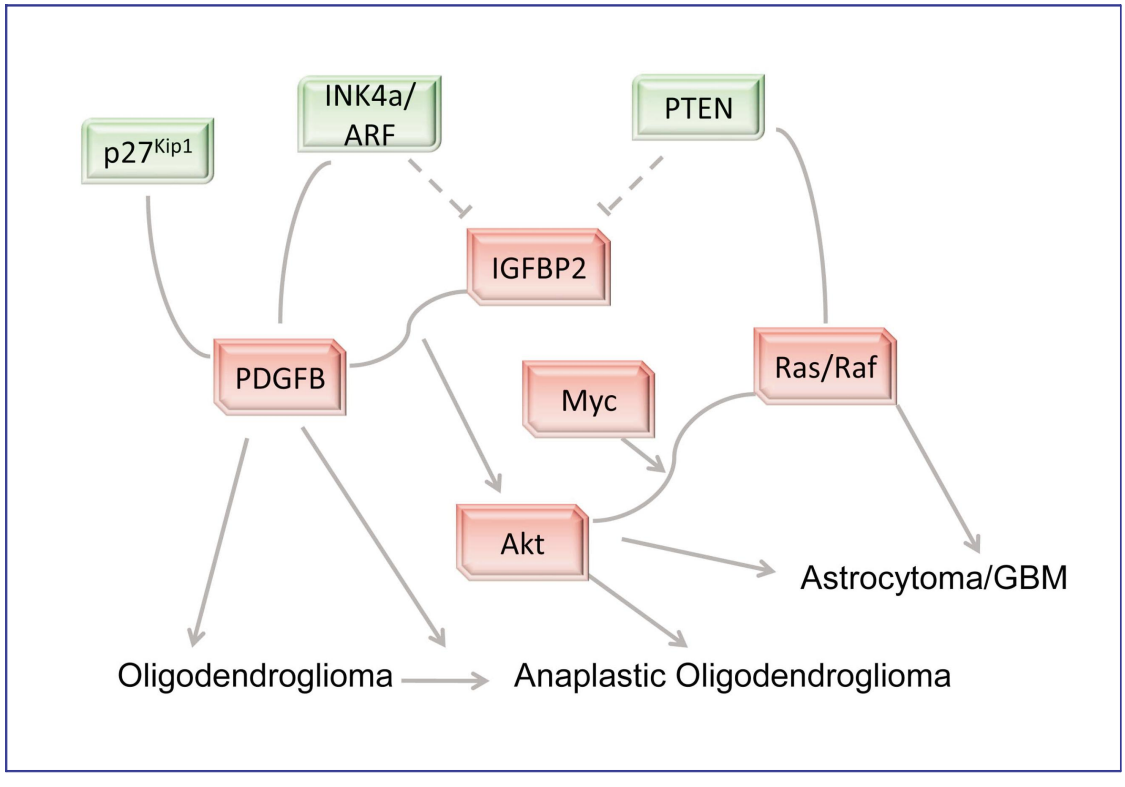

Figure 3. Oncogene and tumor suppressor cooperation in glioma. The RCAS/Tv-a mouse model has elucidated novel links between oncogenes (red) and tumor suppressors (green) and shed light on their roles in the development and progression of glioma. PTEN, phosphatase and tensin homologue; GBM, glioblastoma.

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