

# PRE-CLINICAL TRANSGENIC MOUSE MODELS OF NERVOUS SYSTEM TUMORS

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## Abstract

The most common primary CNS tumors are gliomas, where other than a few subtypes such as oligodendrogliomas, the survival has remained unchanged despite advances in surgical, chemo- and radiation therapy, especially for the most malignant and common glioma; glioblastoma multiforme (GBM). Recent novel therapies like immuno- and gene therapy have shown some promise in existing pre-clinical models, but have failed to demonstrate therapeutic benefit in patients. The reason(s) for such failures include our incomplete understanding of the molecular pathogenesis of these tumors and also due to testing of novel biological therapies in less than ideal pre-clinical models, which for the most part have included xenografts established in mice from glioma cell lines or patient explants. Transgenic mouse models offers an opportunity to develop and utilize an easily replenished, reproducible, manipulated spontaneous and more appropriate pre-clinical model of human cancers. Here we highlight on how mouse models are generated using several techniques and how mouse models have come to the forefront to address several issues such as identifying novel tumour modifier genes of central and peripheral nervous system tumours. Lastly we discuss how mouse models may provide an invaluable tool in pre clinical drug screening and testing.

## Keywords

Mouse Models • Cre-Lox • ES cell transgenesis • Pro-nuclear injection • Gliomas • Peripheral Nervous Tumours  
Glioblastoma Multiforme • Cancer genomics • Central Nervous tumours • Neuro-oncology

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## 1. Introduction

### 1.1 Brief Introduction

The most common primary CNS tumors are gliomas, where other than a few subtypes such as oligodendrogliomas, the survival has remained unchanged despite advances in surgical, chemo- and radiation therapy, especially for the most malignant and common glioma; glioblastoma multiforme (GBM). Recent novel therapies like immuno- and gene therapy have shown some promise in existing pre-clinical models, but have failed to demonstrate therapeutic benefit in patients. The reason(s) for such failures include our incomplete understanding of the molecular pathogenesis of these tumors and also due to testing of novel biological therapies in less than ideal pre-clinical models, which for the most part have included xenografts established in mice from glioma cell lines or patient explants. These xenografts fail to resemble human gliomas

especially with respect to the inherent invasive nature of gliomas, their vascularity and molecular heterogeneity, all of which are the main biological hurdles that have led to their resistance to the multitude of attempted therapies.

Schwannomas and neurofibromas are the most common peripheral nerve tumors and unlike gliomas are usually localized non-invasive benign tumors, which are potentially surgically respectable when indicated. However, due to their location such as in cranial nerves, their multiplicity or chance of malignant conversion when they are in the context of cancer pre-disposition syndromes such as Neurofibromatosis1 (NF-1: neurofibromas, other CNS tumors) or Neurofibromatosis2 (NF-2: multiple schwannomas, other CNS tumors), significant morbidity and mortality can arise with surgical therapy. Hence, increased molecular understanding and appropriate pre-clinical models to test biological

therapies in these peripheral nerve tumors are also desirable.

### 1.2 Small Summary

Transgenic mouse models offers an opportunity to develop and utilize an easily replenished, reproducible, manipulated spontaneous and more appropriate pre-clinical model of human cancers, which we can use to add to our molecular knowledge and test promising therapies. The recent completion of the human and mouse genomes have revealed a high homology (>95%) between the two genomes. Taking advantage of this resource, several techniques to manipulate the mouse genome have been developed to suppress the expression of candidate tumor suppressor genes, or over express putative gain of function genes. In this outline, these techniques and how they have been utilized to develop mouse models for gliomas and peripheral nerve tumors in the context of NF1 and NF2, will be discussed in further detail.

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## 2. Techniques for mouse transgenesis

### 2.1 Homologous recombination

Homologous recombination was a breakthrough that rapidly progressed the use of mouse models as it allowed for an unprecedented manipulation of the mouse genome. The mouse genome could be effectively manipulated by targeted insertion of a replacement allele, leading to deletion of a relevant gene of interest. It takes advantage of flanking homologous regions around the targeted allele, which can undergo normal homologous recombination during meiosis. Creation of a targeting vector containing these homologous regions flanking the replacement and non-functional allele, with appropriate selection conditions (i.e.: neomycin and puromycin), can allow for the selection of cells harboring the appropriate homologous recombination event, see Figure 1. The reader is referred to review articles for further details of this extremely useful technology [1,2]. This technique has been further refined, using Cre-recombinase (see below) and other transgenic technologies, to allow more cell/tissue specific and regional targeted deletions of an allele.

### 2.2 Pro-nuclear injection

Micro-injection has been used extensively to introduce foreign DNA into the male pronuclei of fertilized mammalian eggs, which are then implanted into a foster mother for the remainder of embryonic development, Figure 2. One of the major drawbacks of this method is the high frequency of multiple-copy integration of a transgene into the host genome, which can result in variation or sometimes even inhibition of the transgene expression. In comparison, ES (embryonic stem) cell mediated transgenesis provides a higher frequency of low copy number or even single copy transgene integration. A few models of gliomas have been developed using pro-nuclear injection technology with relevant oncogenes [3-5].

### 2.3 Embryonic Stem (ES) cell mediated transgenesis

ES cells are derived from early embryos (blastocyst stage), and can be maintained in culture under the proper growth conditions as undifferentiated, pluripotent cells. ES cells can be manipulated to alter their genetic composition by introducing foreign gene of interest under ubiquitous or cell/tissue-specific promoters using various transfection

techniques, and selected with antibiotics and selectable markers such as LacZ. Next the ES cells are injected into the host blastocyst, followed by aggregation as a morula-stage embryo into a pseudo-pregnant mouse. The resulting pups are chimeras, consisting of transfected ES cell lineage cells, and those cells derived from non-transfected wild type ES cells in the host blastocyst, Figure 3. Due to this chimeric representation in all tissue

### Gene Targeting By Homologous Recombination

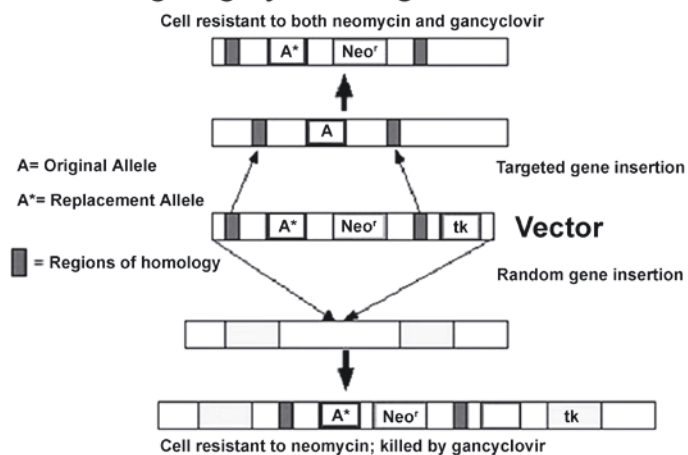


Figure 1. Homologous recombination used to target and knockout specific genes. This technique has been employed to knockout globally a variety of tumor suppressor genes. Recent use of Cre-loxP technology can be combined with homologous recombination, to knockout genes in a cell specific manner, a useful adjunct when global knockout is embryonic lethal.

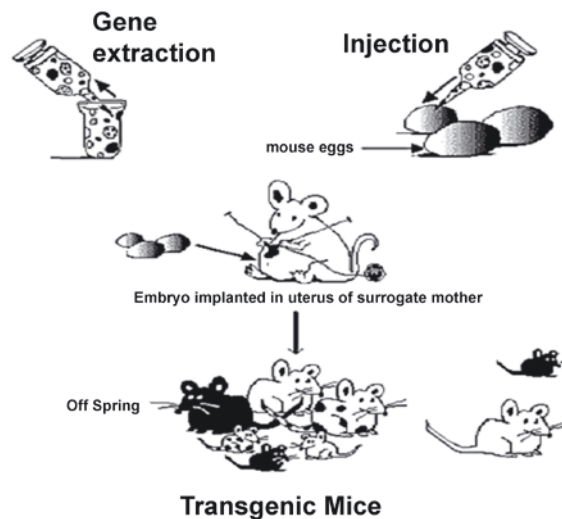
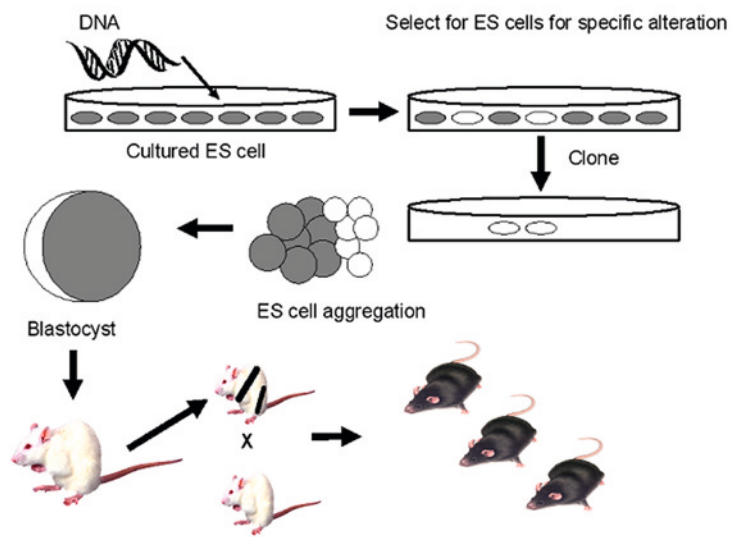


Figure 2. Pro-nuclear injection technique to develop transgenic mice. Nuclei of fertilized eggs are injected with genes of interest, with random and multiple integration sites, and subsequently re-implanted in surrogate mothers. Transgenic offspring harboring the transgene in every cell type are obtained with the potential of germline transmission.

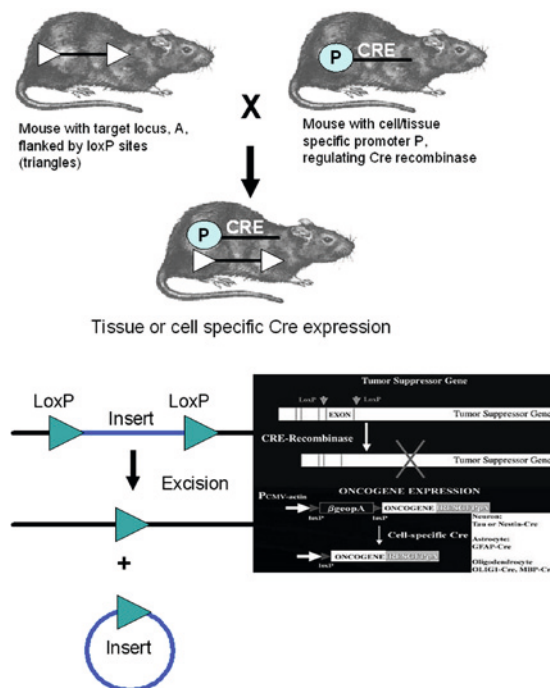
types, many transgenes, which if ubiquitously expressed would be lethal, does not cause lethality unlike pro-nuclear injections. Depending on the effects of the transgene on viability and fertility, the chimeric pups can go on to germline transmission, allowing development of established mouse lines carrying the altered gene(s). This technique has been utilized in the creation of astrocytomas and oligodendrogliomas in transgenic mouse models by our group and several others [6,7].

#### 2.4 Cell/Tissue specific transgenesis

This is one of the most popular and powerful techniques for producing mouse models where the altered gene is expressed or deleted in the cells/tissues of interest and not in all cell types. Advantages of this system include, by-passing potential embryonically lethality phenotype or lack of germline transmission since only a specific cell type will be affected. Secondly, targeting cell types/tissue at different time points can also be investigated for studying the role of specific gene(s) during development or disease progression. These systems depend on the use of a bacteriophage derived recombinase enzyme, such as Cre or Flp recombinase, to specifically excise out a portion of a gene that is flanked by specific nucleotide sequences recognized by these recombinases. The loxP recombination site is 34 bp long and consists of two 13 bp palindromes separated by an asymmetric 8 bp core sequence. A consensus LoxP sequence is the following: ATAAGTTCGTATA (13 bp) ATGTATGC (8bp core) TATACGAAGTTAT (13 bp palindrome to first 13 bp) [8]. FLP recombinase is used to a lesser extent but is similar in principle and recognizes a unique 34bp sequence from Cre-recombinase. The targeted region of a gene, which is crucial for its transcription or function, is flanked by recombinase recognition nucleotide sequence sites (i.e.: loxP-recognized by Cre recombinase or FRT-recognized by Flp recombinase), that are placed in a way that gene function is not compromised when the respective recombinase is absent, Figure 4. In the presence of the recombinase, which is both necessary and sufficient for catalyzing recombination between the recognition sites,



**Figure 3.** Embryonic stem cell transgenesis DNA Vector carrying gene of interest is transfected into cultured mouse ES cells. In our case we used a retrovirus carrying the V12Ha-Ras gene under the control of the astrocyte-specific human GFAP promoter and a neomycin selection marker expression cassette. Selection of ES cells with the transgene of interest is first accomplished by selecting for neomycin resistant clones. Thereafter NeoR ES clones are tested for their ability to express the transgene by *in vitro* differentiation to astrocytes. This process confirms activation of the GFAP promoter expression and specificity. Positive ES cells undergo aggregations and are transferred to a pseudo-pregnant female mouse to create chimeric embryos (striped mouse in picture). The chimeric mice are bred to normal mice and those mice having incorporated the transgene in their germ-line will generate transgenic offspring.



**Figure 4.** Cre-loxP system. Mouse with gene of interest flanked by loxP recognition sites (top). The loxP sites can flank a critical coding exon, which if excised would lead to a non-functioning protein such as a tumor suppressor gene (bottom). It can also flank a stop codon (such as b-geopA- bottom), which after excision would allow expression of an oncogene. Cre-recombinase, to excise out the loxP flanked regions, can be delivered by cell or tissue specific promoters such as GFAP etc. These can be in the form of other transgenic mice, adenoviral or other gene delivery techniques.

the intervening DNA segment is deleted. The Cre-loxP recombinase system is at present the most widely system used in mammals. Using specific cell/tissue-type promoters, and expressing recombinase in the desired cells/tissues, targeted manipulation of the gene of interest in only those cells/tissues can be undertaken, thereby avoiding embryonic or other developmental defects due to more global alterations of an important gene. For example, one can excise out the flanked portion of a gene to delete it or remove a stop sequence to express a gene in astrocytes, by expressing Cre-recombinase under the astroglial specific GFAP promoter. In addition, recent technology allows for inducible expression of the recombinase under regulation of agents such as Tamoxifen or Tetracyclin in the adult mouse, if expressing or deleting a particular gene even restricted to a specific cell/tissue such as astrocytes leads to embryonic lethal (for example by severely interfering with brain development).

### 2.5 Somatic gene transfer using RCAS-tva system

This system uses an avian leukemia retrovirus vector (RCAS) carrying the transgene, which binds to its specific cell surface tva receptor, resulting in incorporation and random integration of the retrovirus in the cells genome, with subsequent expression of the transgene. The transgene is expressed from a spliced message under control of the constitutive retroviral LTR promoter, with the retroviral genes spliced out to prevent viral replication in mammalian cells. The specificity arises from cell type specific promoters used to create transgenic mice, where expression of the tva is only in the cells where that promoter is active. Hence, only these cells in the adult transgenic tva mouse can be transfected with the intracranial injected RCAS vector carrying the transgene. For example the GFAP-tva or Nestin-tva mice have been used to express relevant transgenes in differentiated GFAP+ve and progenitor Nestin+ve glial precursor cells in several models of gliomas [9-14], Figure 5.

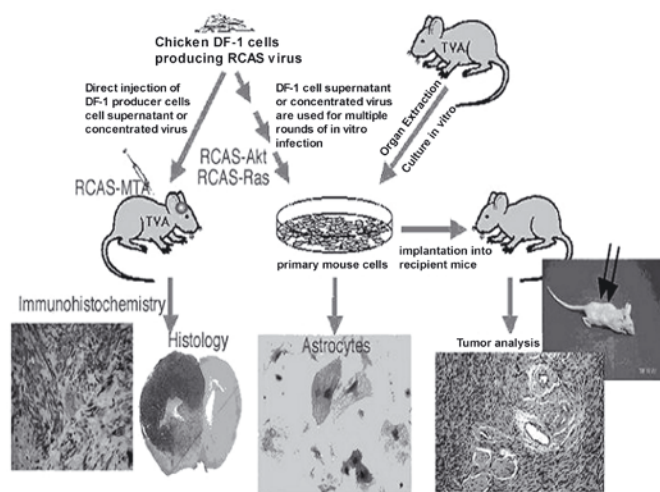
## 3. Animal Models of CNS Tumours

### 3.1 Strategies utilizing transgenic and knockout mice

Molecular progression of gliomas, like most solid tumors, involves accumulation of genetic aberrations, which results in the in-activation of tumor suppressor genes (PTEN, p53, p16, p19, Rb) or activation of oncogenic pathways (p21-Ras, PI3-Kinase, EGFR, CDK4, MDM2) [15],

Table 1). Majority of the current strategies for creating transgenic mouse models is based on manipulating the mouse genome, using some of the techniques discussed above, to over express and/or loose expression of these relevant molecular alterations that have been deciphered from human tumor data (Table 2).

For example, there are transgenic mouse glioma models that target the Rb or p53 cell cycle regulatory pathways. Astrocyte-specific



**Figure 5.** RCAS-tva system. The tva receptor is specific for recognizing and incorporating the RCAS retrovirus. Cell or tissue specific transgenic tva mice (such as GFAP-tva) are made and can be mated with other genetically modified mice (such as Ink4A/Arf null mice). The RCAS retrovirus, with the transgene of interest, can be inoculated into the adult tva mice, either alone or in combination, to yield focal gliomas, as undertaken by Holland et al.

**Table 1.** Genetic aberration in human astrocytomas

Gain of function:	Loss of function:
Aberrant receptors:	Cell cycle pathway gene:
- EGFR/ EGFRvIII	- p53, p19
- PDGFR	- Rb, p16
Aberrant signaling pathway:	In-activators of signaling pathway:
- p21-Ras	- PTEN inhibits PI3-Kinase
- PI3-Kinase	- NF1 inhibits p21-Ras
- Src	
Aberrant cyclin activators	
- CDK4	
Aberrant in-activators of p53	
- MDM2	

inactivation of pRb and also pRb-related proteins, p107 and p130 [16], by expression of a single copy of the viral T121 oncogene driven by the GFAP promoter, led to gliomas which are pathologically similar to Grade III anaplastic astrocytomas. One hundred percent of GFAP:T121 transgenic mice developed these high-grade astrocytomas at ~6 mths of age, a latency which was shortened with the addition of a single null PTEN allele. This supported the critical role of the pRb regulated pathways in astrocytoma formation and loss of PTEN in astrocytoma progression.

The relevance of the p53 regulated cell cycle pathways, which also involves p19 and MDM2, has also been demonstrated in mouse models. p53 null mice by themselves do not develop gliomas, though they develop and die from a variety of other non-CNS tumors. However, a combined deletion of *Nf-1* (neurofibromatosis 1 gene that encodes for neurofibromin, an inactivator of the important mitogenic p21-Ras signaling pathway) and *p53* [17,18] results in generating malignant gliomas. Loss of neurofibromin by itself results in astrogliosis, but not glioma formation, however, when p53 expression is first lost and then followed by loss of neurofibromin, malignant astrocytomas develop [17]. The importance of aberrations in the p53 pathway early in gliomagenesis is

also demonstrated by the small incidence of gliomas in mice null for p19, a protein involved in regulating the stability of p53 through its negative regulation of MDM2 [19].

Over expression of relevant oncogenic aberrations such as receptors or downstream signaling pathways have also been employed in developing mouse glioma models. These have been either alone or in combination with mice harboring specific knockouts of relevant cell cycle regulatory proteins. For example, the S100 glial precursor promoter regulated *v-erbB* (an activated member of the EGFR family) transgenic mice develop oligodendrogliomas, which is potentiated in terms of shorter latency and increased malignancy when undertaken in mice deficient for both p16 and p19 (*Ink4a/ARF* null mice) [5]. Since wild type EGFR and mutant EGFRvIII are the most common gain of function alterations in malignant human astrocytomas, our group has recently created mice expressing these proteins under regulation of the GFAP promoter [7]. Mice with GFAP regulated expression of EGFR and EGFRvIII, both did not result in gliomas, suggesting that like in human gliomas, these are not initiation factors. However, when these mice were mated with a mouse that is prone to develop gliomas, such as the GFAP regulated activated p21-Ras mouse described below, the EGFRvIII but not

the EGFR mice potentiated glioma formation. In addition, expression of EGFRvIII also altered the glioma sub-type, with the double transgenic mice (activated p21-Ras + EGFRvIII) developing oligodendrogliomas and mixed oligo-astrocytomas tumors, compared to the mainly astrocytic lineage tumors in the single transgenic activated p21-Ras mouse described in further detail below.

There are several examples of aberrant expression of relevant downstream signaling pathways, in mouse glioma modeling. These include astrocytomas of varying grades resulting from GFAP regulated expression of *v-src* [3]. Our laboratory has utilized our initial observation of aberrant activation of the p21-Ras signaling pathway in astrocytomas to develop glioma models using ES transgenesis [6,7]. GFAP regulated oncogenic/activated p21-Ras led to multi-focal astrocytomas of varying grades, in a p21-Ras activity dose dependent manner. Mice with extreme high levels of p21-Ras activity in the brain died as chimeras with GBM like tumors. Moderate levels of p21-Ras activation led to germline transmission, with mice that are born normally but start to develop and die from their multi-focal astrocytomas of varying grades commencing at ~12-14wks. Of interest, expression of activated p21-Ras in adult astrocytes leads to senescence, so

Table 2. Transgenic or knockout mouse glioma models

Tumor type	Transgene	Knockout	Cell of origin	References
Astrocytomas	v-Src		GFAP	Weissenberger et al., 1997
Astrocytomas	V <sup>12</sup> Ha-ras		GFAP	Ding, Guha et al., 2001
Oligodendroglioma Mixed Oligo-astrocytoma	V <sup>12</sup> Ha-ras & EGFRvIII		GFAP	Ding, Guha et al., 2003
High grade astrocytomas	T121	pRb (-/-) PTEN(+/-)	GFAP	Xiao, vanDyke et al., 2002
Oligodendroglioma	v-erbB	Ink4a-ARF (-/-)	S-100	Weiss, Israel et al., 2003
GBM (strain specific)		Nf1;Trp53 (cis KO)	All cells	Reilly, Jacks et al., 2000
Astrocytomas		p53 (+/-), NF1 (-/-)	GFAP	Zhu, Paragda 2002
Oligodendrogliomas		P19 (-/-)	All cells	Kamijo, Sherr et al., 1999



the developmental expression of p21-Ras in early glial progenitor cells is of importance. Furthermore, expression of activated p21-Ras in the early glial progenitors leads to genetic instability, but we believe does not suffice alone for transformation, since the astrocytomas occur after a period of development post birth. When the GBM like tumors are examined in these mice, additional genetic alterations such as those found in human GBMs (over-expression of EGFR, CDK4, MDM2; decreased expression of p16, p19, p53, PTEN) [15] are present, which we hypothesize are facilitated by the genetic instability created by activated p21-Ras. As described above, experiments involving mating these p21-Ras mice to other genetically defined mice such as EGFRvIII have already commenced and are continuing, with the potential of these mouse models not only serving as useful pre-clinical reagents, but also to increase our understanding of the in-vivo interactions of these molecular alterations in the pathogenesis of gliomas.

### 3.2 Strategies utilizing viral and transgenic mice

The models described above, for the most part involve modulating the mouse genome commencing at an early embryonic stage. This has the potential advantage of generating a renewable resource of genetically defined mice, which develop gliomas at a certain frequency and hence can be used for a variety of experiments. However, cancer is mainly an adult somatic disease arising from a clone of cells that due to accumulation of genetic alterations have become transformed. Viral transduced expression of relevant gain of function alterations, in combination with transgenic mouse technology, allows one to model such somatic alterations at a later stage in life, though it does not lead to germline colonies.

Although the link between a viral etiology and human gliomas is weak, retroviruses that have been engineered to express relevant gain of function genes have been used to create glioma models in mice and other mammals [9-14]. This includes members of the Rous sarcoma virus family and simian sarcoma virus,

whose transforming properties are due to over expression of the viral oncogene *v-sis*, the cellular counterpart of which is *c-sis* or PDGF-B (Platelet Derived Growth Factor)[20]. Over expression of PDGF and activation of PDGF-Receptors are well recognized in gliomagenesis [9,12,20]. Retroviruses carrying *v-sis* (PDGF-B), when injected into normal mice have yielded astrocytic tumors, with varying glioma types when injected in *Ink4a/ARF* null mice [9]. The frequency of gliomas using this retroviral strategy was between 40-80%, with the most frequent histology being high-grade gliomas that have characteristics similar to GBMs.

One of the best examples of coupling retroviruses to express somatically defined gain of function genes in varying cell lineages and genetic backgrounds to model gliomas, is the RCAS-*tva* system, detailed previously [9-14, Table 3, Figure 3]. This system develops focal gliomas, the sub-type and grade of which varies with the injected retroviral transduced gene (i.e.: PDGF-B, EGFRvIII, activated p21-Ras, activated Akt), the lineage of the cell expressing the *tva* receptor (GFAP, Nestin) and underlying genetic cell cycle alterations in the mice (null for *Ink4a/ARF*, p53 etc.). For example, retroviral transduced expression of *v-sis* or PDGF-B in GFAP-*tva* mice developed oligodendroglioma or mixed oligo-astrocytoma in 40% of the mice, with 60% developing similar gliomas in Nestin-*tva* mice [10]. When these experiments were undertaken in *Ink4a/ARF* null mice, the gliomas formed with a shorter latency and were of higher grade. Another example with the RCAS-*tva* system are experiments with retroviral transduction of activated p21-Ras and Akt, representing the two most implicated aberrant signaling pathways in gliomas, as previously discussed. Neither of these activated signaling molecules by itself formed gliomas, but when expressed together in more primitive Nestin+ve precursor glial cells, astrocytomas developed. Furthermore, activated p21-Ras by itself could give rise to astrocytomas if transduced in Nestin+ve cells in *Ink4a/ARF* null mice. This is consistent with the results we obtained and described above with ES transgenesis of activated p21-Ras [6]. Activated p21-Ras is transforming only if

expressed in early glial lineage cells, with the transformed astrocytes deficient in the gene products encoded by the *Ink4a/ARF* locus (p16 and p19) and expressing Nestin. Similar results with EGFRvIII, have also been obtained by our ES transgenesis model [7] and those with the RCAS-*tva* system [13]. In both, expression of EGFRvIII by itself is not transforming, as it is a progression not an initiation factor. In the RCAS-*tva* system, retroviral transduction of both EGFRvIII and CDK4 in mice null for p53, was required for formation of mixed gliomas.

Recently, The Cancer Genome Atlas (TCGA) initiative involved integrative analysis of DNA copy number variation, gene expression and DNA methylation aberrations in 206 glioblastomas [21]. This study provided new insights into the roles of ERBB2, NF1 and

TP53 as p53 mutations were discovered to be more widespread in primary GBM's than previously thought. Also, novel NF-1 point mutations were discovered helping support the case for NF-1 being a critical GBM tumor suppressor gene. The p53 mutation observations were furthered investigated in mouse models in which co-loss of p53 and Pten in murine neural stem cells (NSCs) resulted in the formation of a penetrant acute-onset high grade glioma with a pathological and molecular profile resembling primary GBMs. In addition to the TCGA study, another group sequenced over 20,661 protein coding genes in GBM operative samples and human xenograft models [22]. P53 once again was observed to have a high mutation rate in primary GBMs. Interestingly, Isocitrate Dehydrogenase1 (IDH1) an enzyme involved in the oxidative carboxylation of isocitrate to  $\alpha$ -ketoglutarate, resulting in the production of nicotinamide adenine dinucleotide phosphate (NADPH) was found to be mutated in 12% of GBMs(22-24). Of the 5 IDH genes, only IDH1 was found to be mutated and that point mutations were consistently observed at residue R132, the substrate binding site. Interestingly, no insertion or deletion mutations (InDels) were identified at the IDH1 locus suggesting a critical role for the point mutation. Lastly, IDH1 mutations were seen in a younger population of GBM patients and that patients with IDH1 mutations had

better overall survival compared to a patients with wild type intact [25]. Mouse models would truly provide an invaluable insight into the role of this gene in gliomagenesis and these studies show how high throughput tumour profiling coupled with mouse models and be an excellent combination of tools.

## 4. Specific Mouse Models of Peripheral Nerve Tumors

### 4.1 Neurofibromatosis 1

After cloning of the *Nf-1* gene on chromosome 17q, knockout technology was utilized by two groups to delete the *Nf-1* gene in all cell types, resulting in identical phenotypes [26,27]. In both, the *Nf-1* (-/-) null mice were embryonically lethal, with major cardiovascular developmental disorders, in support of the critical function of neurofibromin in development. The heterozygous *Nf-1* (+/-) mice were born normally, however, died of non-neuroectodermal tumors slightly earlier than their matched control littermates. Chimeric mice generated from *Nf-1* (-/-) and *Nf-1* (+/+) ES cells were embryonically viable, as per discussion previously of one of the potential benefits of this technology [6,7]. Expectant neurofibromas did form in these chimeric mice, from derivatives of the *Nf-1* (-/-) ES cells, proving that loss of neurofibromin expression in schwann cells was indeed causal towards developing neurofibromas in NF-1 patients. The embryonic lethality was also overcome with cell specific deletion of *Nf-1*, using the previously described Cre-loxP system. For example, the normal NF-1/*lox* mouse can be used to specifically delete neurofibromin in

astrocytes, by breeding the NF-1/*lox* mouse to GFAP:Cre mouse. From this kind of manipulation much has been learned about development of astrocytomas in the context of NF-1. Astrocyte specific deletion of neurofibromin only led to increased number of astrocytes, but not ones that were transformed. However, if neurofibromin was deleted in the presence of existing p53 null astrocytes, then astrocytomas of varying grades formed [17]. Similar strategies have been used to examine the biological consequences due to loss of neurofibromin in other cell types. For example, by mating the NF-1/*lox* mouse with endothelial cell specific Cre-recombinase expressing transgenic mice (Tie2:Cre), insights into the crucial effects of loss of neurofibromin on vascular development have been deciphered [28]. Lastly, mouse models have also given insights into the molecular basis of progression of benign neurofibromas to malignant peripheral nerve sheath tumors (MPNST). Taking advantage of the fact that in mice the *Nf-1*, *p53* and also *Nf-2* loci are on the same chromosome and relatively close together, transgenic mice in which both *Nf-1* and *p53* could be deleted was generated [17]. These mice developed MPNST, which is in keeping with the loss of p53 in a large subset of human MPNST, and suggests that a second hit with p53 loss is involved in this malignant conversion of neurofibromas.

### 4.2 Neurofibromatosis 2

Similar to NF-1, the cloning of the *Nf-2* gene on chromosome 22q soon led to it being knocked out in mice. The *Nf-2* (-/-) null mice died in embryo similar to *Nf-1* null mice, with the heterozygotes developing osteosarcomas

and other tumors, but not the expectant schwannomas [29,30]. The Cre-loxP system was used to overcome this embryonic lethality when *Nf-2* was deleted globally, with schwann cell specific Cre recombinase expression leading to development of schwannomas [31]. Meningiomas, which are highly prevalent in NF-2 patients also formed, with meninges specific excision of the *Nf-2* gene using adenoviral delivered Cre into the CSF of NF-2/*lox* mice [32].

## 5. Potential use of transgenic mouse models

The ability to manipulate the mouse genome with a variety of technologies, such as those discussed above, have opened the doors for many research and potential pre-clinical applications. The marked similarity between the mouse and human genome, with syntenic regions mapped between the two species, have allowed us to target specific genes which are causal for human diseases such as gliomas and peripheral nerve tumors in mice. In addition to allowing us to study the temporal, spatial and cellular interactions between these known genetic alterations towards causing the human disease, transgenic mouse models also serve as reagents to decipher novel genetic alterations that may have been not been recognized from human samples. These can then tested in the human samples and if indicated incorporated in subsequent models.

Characterization and better understanding of the molecular and pathological progression of tumors, such as those in the nervous system, is another use of transgenic mouse

Table 3. Mouse models of gliomas: virus infection.

Tumor type	Signal transduction abnormality	Cell cycle arrest disruption	Cell of origin/ Affected cells	References
GBM (Mixed gliomas)	PDGF (v-sis)	Ink4a-ARF (-/-)	Mixed population (nestin+, GFAP+)	Uhrborm et al., 1998 Dai et al., 2001
GBM	K-ras & Akt	Ink4a-ARF (-/-)	GFAP + Nestin +	Holland et al., 2000 L.Uhrborm et al., 2002
Mixed gliomas	EGFRvIII	P53 +/-, Cdk4+	Nestin +	Holland et al, 1998
Mixed gliomas	MTAg		GFAP +	Holland et al., 2000

models, which cannot be readily undertaken in humans. Understanding interactions of tumor cells with other cellular elements such as the vascular system, immunological system, extracellular matrix and other stromal cells, are other areas of research that is facilitated by transgenic mouse models. Having a spontaneous orthotopic occurring model in an immunocompetent mammal, of course lends itself well to pre-clinical trials of established

and novel biological therapies. To augment this vital translational use of transgenic mouse modeling of human diseases, a variety of readouts such as small animal imaging, are also being developed to follow the efficacy of the therapeutic interventions being tested. Although much progress has been made in development of better human disease models using transgenic and other technologies, one must remember that significant epigenetic

differences between mice and humans do exist, and the ultimate test of therapies will still rely on properly designed clinical trials. However, with better models of human diseases such as those being developed for nervous system tumors using transgenic technologies, the chance of promising pre-clinical therapies translating to efficacy in clinical trials should be much improved in the future.

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