Brain tumors in neurofibromatosis type 1

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Abstract

As a cancer predisposition syndrome, individuals with neurofibromatosis type 1 (NF1) are at increased risk for the development of both benign and malignant tumors. One of the most common locations for these cancers is the central nervous system, where low-grade gliomas predominate in children. During early childhood, gliomas affecting the optic pathway are most frequently encountered, whereas gliomas of the brainstem and other locations are observed in slightly older children. In contrast, the majority of gliomas arising in adults with NF1 are malignant cancers, typically glioblastoma, involving the cerebral hemispheres. Our understanding of the pathogenesis of NF1-associated gliomas has been significantly advanced through the use of genetically engineered mice, yielding new targets for therapeutic drug design and evaluation. In addition, \textit{Nf1} murine glioma models have served as instructive platforms for defining the cell of origin of these tumors, elucidating the critical role of the tumor microenvironment in determining tumor growth and vision loss, and determining how cancer risk factors (sex, germline \textit{NF1} mutation) impact on glioma formation and progression. Moreover, these preclinical models have permitted early phase analysis of promising drugs that reduce tumor growth and attenuate vision loss, as an initial step prior to translation to human clinical trials.

Key Points

- EGFRvIII dPCR assay is rapid and ultrasensitive for the detection of EGFRvIII and EGFR amplified in patient tumors.
- The unique sequence generated due to fusion of exon 1 and 8 is utilized to design primer and probe specific to EGFRvIII.
- The lowest limit of quantification of EGFRvIII detection using dPCR is 0.003%.

Gliomas in Children and Adults with NF1

The vast majority of the brain tumors encountered in individuals with NF1 are histologically classified as gliomas (astrocytomas).\textsuperscript{1-4} However, tumor location, age of onset, symptomatology, and clinical behavior can be quite heterogeneous in this population of at-risk patients. In general, gliomas in children most commonly are localized to the optic pathway and brainstem;\textsuperscript{6,7} however, recent studies have shown that gliomas in other locations are also frequently observed.\textsuperscript{5-14} Lastly, while far less common, high-grade (malignant) gliomas involving the cerebral hemispheres may arise in young adults.\textsuperscript{3,7,9,15}
Optic Pathway Gliomas

In early childhood (mean age, 4.5 y), the most common brain tumor is a glioma of the optic pathway (optic pathway glioma; OPG). These tumors can affect any segment of the optic pathway, including the optic nerves, chiasm, tracts, and radiations (Figure 1A). While neuroimaging is not an element of routine medical screening of children, the proportion of children with NF1 and OPG has been estimated at ~15%. While most OPGs diagnosed in children with NF1 are asymptomatic or nonprogressive, as many as 50% of children with NF1-OPG will experience ophthalmologic (vision loss, proptosis) or endocrinologic (precocious puberty) signs or symptoms. This is in striking contrast to OPGs arising in children without NF1, who generally have a less favorable course.

Currently, children with NF1 are screened annually using age-appropriate visual acuity measures, including Teller, Lea, HOTV, and Snellen acuity cards, for at least the first decade of life. While these tests can provide accurate assessments of vision, they are often limited by patient cooperation, which can be problematic in children with NF1 and concurrent attention or cognitive deficits. For this reason, ocular coherence tomography (OCT) is emerging as an objective measure of visual acuity. OCT provides ultrasound quantification of the retinal fiber nerve layer (RFNL) and ganglion cell layers, but requires sedation (general anesthesia) in young children.

Since routine neuroimaging is not performed and visual assessments can be challenging in children with NF1, risk factors for OPG development and progression have been sought. To date, several risk factors for OPG development have been postulated. First, there is evidence for genotype–phenotype association in NF1-OPG, where individuals with mutations in the 5′ end of the NF1 gene more often develop gliomas than individuals with mutations located elsewhere in the gene. Second, NF1-OPGs are more prevalent in Caucasian children than in those from other races and ethnicities; however, race had no impact on clinical progression. The observation that ethnicity/race modifies glioma risk, which could relate to genomic variations seen in different ethnic groups or races. This notion has been further explored by examining single nucleotide polymorphisms, where variants in the adenylate cyclase-8 (AC8) gene in individuals with NF1 are associated with different risks of low-grade glioma formation. Third, children with NF1 who have co-existing atopic conditions (eczema, asthma) are less likely to harbor an OPG.

With respect to vision loss, three additional risk factors have been described, including involvement of the posterior optic pathway (tracts and radiations), young age at presentation (<2 y), and sex (female). While boys and girls with NF1 have the same incidence of OPGs, females harbor a 3- to 5-fold greater risk of vision loss. At this time, each of these risk factors lacks sufficient sensitivity and specificity to be incorporated into clinical decision making, but their integration into future risk assessment algorithms might help to stratify children into high and low risk groups.

Treatment is typically initiated when there is evidence of progressive vision loss (2-line decrement in visual acuity). While investigational treatments (e.g., MEK and mTOR inhibitors) are being evaluated in clinical trials, the standard first line treatment is carboplatin/vincristine chemotherapy. Those children who fail upfront therapy are usually treated with vinblastine or a combination of irinotecan and bevacizumab. Surgical resection is reserved for uncommon indications, such as atrophy of the eye, and radiation therapy is avoided due to the risk of secondary malignant tumor formation in children with this cancer predisposition syndrome. Unfortunately, in most cases, successful antitumoral treatment does typically not result in improved visual acuity.
**Brainstem Gliomas**

While less common than OPGs, children with NF1 can also develop brainstem gliomas (BSGs; Figure 1B), with a mean age at diagnosis of ~7 years.51–53 These tumors occur in fewer than 10% of individuals with NF1,6,253 are more indolent than those observed in the general population,51 and are usually low-grade gliomas.53 Within the brainstem, they most frequently involve the midbrain and medulla.51,53 Unlike NF1-OPGs, NF1-BSG progression is not influenced by sex53; however, older children more often require treatment.53 While many patients with NF1-BSGs are asymptomatic51–53; some tumors can cause obstructive hydrocephalus,5,51,52 as a result of aqueductal stenosis or lead to other neurologic signs/symptoms, such as headache, nausea/vomiting, cranial neuropathies and ataxia or gait instability.6,7,10,51–54 Due to their indolent behavior, a conservative approach to treatment is typically recommended, with hydrocephalus managed by cerebrospinal fluid (CSF) diversion (e.g., ventricular shunt) and continued tumor growth with chemotherapy or less commonly surgery.6,7,51–53 It should be noted that progression-free survival was 3 years shorter for children with NF1-BSG receiving tumor-directed therapy relative to those who received no treatment or CSF diversion.53

**Gliomas Arising in Other Locations**

It is not uncommon for individuals with NF1 to harbor more than one CNS tumor, particularly those outside of the optic pathway or brainstem. These gliomas are typically located in the temporal lobe, cerebellum, thalamus, basal ganglia or spinal cord.7–10,13 and many are asymptomatic.7,13 Compared to NF1-OPGs and NF1-BSGs, less is known about the natural history of these tumors. When symptomatic or increasing in size on neuroimaging, patients with these tumors are managed with surgical resection, chemotherapy, and/or CSF diversion, depending upon the location and specific clinical indications.7,13 Those tumors involving the thalamus (Figure 1C) tend to have a poor prognosis.8

In addition, rosette-forming glioneuronal tumors have been found to harbor mutations in the *NF1* gene.5 This rare low-grade brain neoplasm most typically arises in the fourth ventricle and cerebellum of young adults, and has been reported to occur in rare individuals with NF1.56

**High-Grade (Malignant) Gliomas**

High-grade (malignant) gliomas are uncommon in children with NF1, but increase in prevalence in early adulthood.3,7,10,15 Most frequently, these tumors arise in the cerebral hemispheres; however, the rarity of these neoplasms has limited our ability to identify clinical patterns. Based on epidemiologic studies, high-grade gliomas are encountered more often than would be predicted, with estimates of >50-fold increased risk relative to the general population.12,14,54,57 Molecular analyses of NF1-associated high-grade gliomas (anaplastic astrocytoma and glioblastoma)15 and anaplastic astrocytomas with piloid features58,59 have revealed mutational and genomic alterations similar to those observed in their sporadic counterparts, including mutations in the *ATRX*, *TP53*, and *CDKN2A* genes, as well as in genes whose proteins function within the phosphoinositide-3 kinase (PI3K) pathway. Importantly, NF1-associated high-grade gliomas lack the IDH and histone H3 mutations commonly observed in sporadic malignant gliomas.15,59,60 Current therapies are similar to those used to treat glioblastoma arising in adults without NF1.

**The NF1 Tumor Suppressor Gene**

The *NF1* gene encodes a tumor suppressor protein (neurofibromin) that largely functions as a negative regulator of cell growth through suppression of RAS activation.51–53 Neurofibromin contains three putative structural domains: (a) a cysteine/serine-rich domain (CSD), (b) a GTPase activating protein (GAP) related domain (GRD), and (c) a domain with homology to the lipid-binding domain of the *Saccharomyces cerevisiae* phosphatidylinositol transfer protein Sec 14p (Sec14p).64-68 While mutations within the Sec14p and CSD have been reported, their functional relevance remains to be elucidated. In contrast, mutations within the GRD are hypothesized to lead to increased RAS activation, resulting in increasing cell growth. Whereas all patients are born with a germline mutation in the *NF1* gene (creating a non-functional NF1 allele), tumor formation requires somatic inactivation of the second NF1 allele (loss of heterozygosity), leading to loss of neurofibromin expression and function.57–71 As such, neurofibromin loss in Schwann cells,71–73 astrocytes,74,75 and myeloid cells76,77 is associated with high levels of activated RAS.73,78 Consistent with this mechanism of tumor growth regulation, increased RAS activation has also been observed in both human74 and mouse75 NF1-associated gliomas.

RAS hyperactivation induces cell growth by inducing activation of downstream signaling intermediates (Figure 2), including Mitogen Activated Protein Kinases (MEK/ERK),79–81 Phosphoinositide-3-Kinase (PI3K),74,81–83 and cyclic AMP (cAMP).84 Additionally, ERK and PI3K/Protein Kinase-B (AKT) phosphorylation both lead to mechanistic target of rapamycin (mTOR) activation.74,87,82 While less is known about the mechanism of cAMP regulation by neurofibromin in neural progenitors and astroglial cells, neurofibromin controls cAMP homeostasis in a RAS-dependent manner through the activation of the atypical protein kinase C-zeta (PKCζ) in neurons.85 Each of these effector molecules serve as logical targets for therapeutic drug design (see below section on “Preclinical drug identification and evaluation”).

**Modeling NF1-Gliomas in Mice**

Since human tumors are not routinely biopsied as part of routine clinical care and have not been successfully maintained as patient-derived xenografts, much of our understanding of the pathobiology of these tumors has resulted from the use of genetically engineered mouse models. Even though there are striking differences between
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humans and rodents in terms of brain structure and organization, these preclinical experimental platforms have revealed important insights into the role of genetic and genomic factors (germline mutation, sex), the tumor microenvironment, and systemic disease in the formation and progression of NF1-associated gliomas.

Optic Gliomas

NF1-OPGs rarely harbor additional somatic mutations, and are genetically characterized by bi-allelic NF1 inactivation. For this reason, murine Nf1-OPGs have been modeled by combining an inactivating germline Nf1 gene mutation with somatic Nf1 loss using conditional Cre-Lox technology. Whereas neither mice heterozygous for a germline mutation in the Nf1 gene (Nf1+/− mice) nor those with bi-allelic Nf1 loss in neuroglial progenitors develop OPGs, the combination of these two genetic events is sufficient to generate optic gliomas in mice. As such, >95% of these mice develop low-grade gliomas involving the prechiasmatic optic nerves and chiasm. These tumors can be visualized by small-animal magnetic resonance imaging (MRI), and are associated with progressive axonal damage, retinal ganglion cell (RGC) death, and reduced visual acuity. Moreover, like other human brain tumors, they contain a small population of CD133+ cancer stem cells, which can generate low-grade gliomas following transplantation into the brainstems of naïve recipients.

Malignant Glioma

In contrast to low-grade gliomas in individuals with NF1, their malignant counterparts harbor additional genetic alterations, including mutations in the TP53, EGFR, and RB1 genes. Based on the frequent cooccurrence of NF1 and TP53 mutations in human NF1-associated malignant glioma, murine glioblastoma models have focused on combining Nf1 and Trp53 (p53) gene loss. In this regard, mice carrying heterozygous germline mutations in Nf1 and Trp53 on the same copy of chromosome 11 (NPt1s mice) develop brain tumors following loss of the wild-type Nf1 and Trp53 genes. The astrocytomas encountered range in tumor grade from low-grade diffuse astrocytoma to high-grade glioblastoma, with 100% tumor penetrance observed in mice.
older than 6 months. These tumors have elongated astrocytic nuclei with irregular contours, increased mitosis and, in some rare cases, necrosis. Mice with these tumors die within 18 weeks of age. Tumor cells from these mice can be serially transplanted for malignant glioma therapeutic studies. Moreover, the addition of PTEN mutation, mimicking the PI3K pathway activation seen in human NF1-GBM, results in a more aggressive malignancy, with a shorter latency to tumor development and attenuated survival. In addition, complementary modeling approaches using the MADM (Mosaic Analysis with Double Markers) viral transduction methods (RCAS/IVTA system, CRISPR/Cas editing, or in utero CRISPR/Cas9 induced Nf1 and Trp53 loss also result in high-grade glioma formation.

**Insights from Nf1 Genetically Engineered Mice**

Using these Nf1 genetically-engineered mouse glioma models, significant insights have been derived regarding the tumor cell of origin, the role of the tumor microenvironment, and the contributions of risk factors (sex, germline genetics, and genomic alterations) to brain tumor disease pathogenesis.

**Cell of Origin**

Brain tumors can arise from different progenitor cell populations that reside in distinct germinal (ventricular) zones. Nf1 optic glioma mice have been instructive for discovering that this type of low-grade glioma originates from specific progenitor cells that line the ventricular surface of the third ventricle (TVZ), rather than from the lateral ventricular subventricular zone (Iv-SVZ). First, Nf1 mutation results in increased proliferation and glial differentiation in stem cells lining the TVZ, rather than the Iv-SVZ. Second, in both human and mouse brain sections, proliferation in the TVZ disappears early in postnatal development. Third, only GFAP⁺, BLBP⁺, and CD133⁺ neural progenitor cells serve as the cells of origin for Nf1 optic glioma. No optic glioma formation is observed in Nf1+/− mice with somatic Nf1 inactivation in astrocytes or NG2⁺ glia. Fourth, Nf1 optic gliomagenesis requires somatic Nf1 loss to occur during late embryonic development, as postnatal loss in the same neuralglial progenitors does not result in tumor development. Lastly, in a second progenitor cell population that can also give rise to optic gliomas in Nf1 genetically engineered mice. These Olig2⁺ oligodendrocyte progenitor cells serve as cells of origin for optic gliomas; however, the latency to tumor formation is nearly twice as long (6 months of age), suggesting that the specific cell of origin partially dictates glioma biology.

In striking contrast, high-grade gliomas appear to originate from stem cell populations within the Iv-SVZ of adult mice. In addition to serving as the cell of origin for these malignant tumors, depletion of these stem cells in established tumors also reduces tumor size and extends mouse survival. Similar to Nf1 optic gliomas, pathologically identical, but molecularly distinct tumors are generated when these molecular changes occur in adult neural stem cells compared to oligodendrocyte progenitors. However, using the MADM experimental platform, simultaneous Nf1 and Trp53 inactivation in neural stem cells or OPCs resulted in the formation of gliomas, which, in both cases, reflected a common cell of origin (oligodendrocyte progenitor cells).

**Tumor Microenvironment**

Studies in Nf1 optic glioma mice have revealed an essential role for non-neoplastic (stromal) cells in tumor development and progression. One of the most important of these non-neoplastic cells, microglia, comprise 30–50% of the cellular content in both sporadic and NF1-associated low-grade gliomas, and are present at higher densities in murine Nf1 optic gliomas than in the optic nerves of normal mice. In Nf1 optic glioma-bearing mice, the microglia harbor a germline mutation in the Nf1 gene, which results in increased proliferation and migration, as well as increased production of key growth factors. In this regard, genetic reduction of the key receptor critical for directed migration of microglia (Cx3cr1), reduces microglia infiltration in Nf1-OPG mice and leads to a delay in glioma formation. Similarly, genetic or pharmacologic inhibition of microglia function attenuates Nf1 optic glioma growth, and microglia were isolated from Nf1 optic glioma-bearing mice and compared to those from nontumor-bearing mice by RNA sequencing, revealing that tumor-associated microglia secrete CC-chemokine ligand 5 (Ccl5). Support for the importance of microglia Ccl5 in tumor maintenance was subsequently provided by demonstrating that the treatment of Nf1 optic glioma mice with Ccl5 neutralizing antibodies and the injection of optic glioma stem cells into Ccl5-deficient mice resulted in reduced tumor growth and an absence of glioma formation, respectively. It is worth noting that high-grade gliomas produce their own Ccl5, thus reducing their dependence on stromal cells for this growth factor. Other chemokines, like Cxcl12, may also be important for dictating the formation and growth of these tumors. While less well explored, it is likely that microglia in the tumor microenvironment also govern the growth and spread of NF1-malignant glioma.

Microglia are not the only important nonneoplastic cells in the glioma microenvironment. Recently, T cells were found to be both mouse and human NF1-LGGs. In murine Nf1-OPGs, these lymphocytes prime microglia to produce Ccl5 through the elaboration of paracrine factors (Figure 3). Additionally, Ccl5 levels in these mouse tumors correlate with the abundance of T cells and microglia. Importantly, glioma stem cells from murine Nf1 optic gliomas do not form low-grade gliomas following injection into the brains of mice lacking T cells, where the microglia fail to produce Ccl5. Taken together, these findings support a model in which a supportive neuroimmune axis is established to foster Nf1 glioma development and progression; however, the factors that
attract and activate T cells in the setting of glioma remain to be fully elucidated.

**Risk Factors**

Another use for *Nf1* optic glioma mice is an opportunity to define the etiologies for risk factors operative in patients with NF1 (Figure 4), including sex, germline genetics, the presence of additional genomic alterations, and background genomic variation.

**Sex**

As mentioned above, girls with NF1 more frequently lose vision from their OPG than boys. Using *Nf1*-OPG mice, this sexual dimorphism reflects in part differences in gonadal sex hormones. In this regard, only female *Nf1* optic glioma mice exhibit increased RGC death and RNFL thinning sufficient to result in reduced visual acuity. In mice, this is due to estrogen receptor-beta (ERβ) activation in microglia, which leads to the production of neurotoxins.

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**Figure 3.** Tumor microenvironment is involved in glioma progression. Glioma cells produce chemokines that attract both T lymphocytes (from blood vessels) and microglia. In response to paracrine factors released by T cells ("priming"), microglia produce growth factors, like CCL5, which increase tumor cell proliferation or survival.

**Figure 4.** Risk factors for glioma formation. Research over the past 20 years has revealed numerous factors that can alter the risk of NF1-associated glioma formation and progression. As discussed in the text, these include the germline *NF1* gene mutation, patient age, patient sex, background genomics (ethnicity/race), co-existing atopic conditions (eczema, asthma), tumor location within the neuroaxis, the presence of additional somatic mutations in the tumor, and prior tumor treatment (radiation therapy).
| Trial | Study number | Status | Sponsor | Phase | Ages | Disease status | Primary outcome |
|-------|--------------|--------|---------|-------|------|----------------|----------------|
| Selumetinib | NCT03871257 | Not yet recruiting | NCI | III | 2–21 y | Progressive | Event-free survival and visual acuity |
| Selumetinib | NCT03326388 | Not yet recruiting | Great Ormond Street Hospital for Children NHS Foundation Trust | II/II | 3–18 y | Progressive, relapsed | Maximum tolerated dose, objective response rate |
| Trametinib | NCT03363217 | Recruiting | St. Justine's Hospital | II | 1 month to 25 y | Recurrent, refractory | Objective response rate |
| MEK162 | NCT02285439 | Recruiting | Children's Hospital Los Angeles | II | 1–18 y | Recurrent, refractory, progressive | Objective response rate |
| Vinblastine +/- Bevacizumab | NCT02840409 | Recruiting | The Hospital for Sick Children | II | 6 months to 18 y | Unresectable or progressive | Response rate |
| Selumetinib | NCT01089101 | Recruiting | NCI | II | 3–21 y | Recurrent, refractory | Response rate |
| Trametinib | NCT02465060 | Recruiting | NCI | II | >18 y | Advanced refractory | Response rate |
| RAD0001 (everolimus) | NCT01588651 | Active, not recruiting | University of Alabama at Birmingham | II | 1–21 y | Chemotherapy refractory, radiologically progressive | Objective response rate |
| Lenalidomide | NCT01553149 | Active, not recruiting | NCI | II | <21 y | Recurrent, refractory, or progressive | Objective response rate |
| TAK-580 | NCT03429803 | Active, not recruiting | Karen D. Wright, MD | II | <18 y | Refractory | Dose limiting toxicity and progression free survival |
| Pomalidomide | NCT02415153 | Active, not recruiting | NCI | I | 3–20 y | Recurrent, progressive, or refractory | Maximum tolerated dose |
| Sorafenib | NCT01338857 | Completed | NYU Langone Health | II | >2 y | Recurrent or progressive | Sorafenib ineffective for the treatment of recurrent or progressive PLGA |

NCI = National Cancer Institute; NYU = New York University.
that induce axonal injury in female NF1 optic glioma mice. Pharmacologic inhibition of the estrogen receptor (ERβ) or estrogen depletion by surgical or chemical ovariectomy reverses the RNFL thinning and RGC loss. Future work will be required to define the mechanisms underlying estrogen reprogramming of microglia and the paracrine factors responsible for microglia-mediated axonal damage. In addition, there could be sex chromosome effects that mediate sexually dimorphic differences in glioma penetrance or progression.127–129

Germline Genetics

Several lines of evidence have raised the intriguing possibility that not all germline NF1 gene mutations are functionally equivalent. First, population-based studies have revealed that a subgroup of patients with specific mutations (e.g., Arg1809 missense mutations) lack the signature nerve sheath tumors that characterize NF1 (neurofibromas).130–133 Second, human induced pluripotent stem cells with different NF1 gene mutations exhibit different levels of neurofibronin and dopamine, supporting the notion of mutational specificity.134 Third, using NF1 mutant mice in which the germline Nf1 knockout allele is replaced with actual NF1 patient mutations, the identical somatic NF1 loss has differential effects on the ability of these mice with different germline NF1 gene mutations to develop optic gliomas.96,135 For example, mice with an Arg1276Pro mutation develop optic gliomas similar to those with an artificial knockout germline Nf1 allele, but with fewer infiltrating microglia, whereas those with an Arg681X germline Nf1 gene mutation form optic gliomas with larger volumes and proliferative rates.135 In addition, mice with a Gly848Arg germline Nf1 gene mutation fail to form optic gliomas,135 while those harboring a Cys383X germline Nf1 mutation develop optic gliomas with reduced penetrance. Moreover, the glioma stem cells from mouse optic gliomas with different germline NF1 mutations exhibit different levels of microglia and T cell infiltration owing to mutation-specific differences in chemokine production.96

Secondary Genomic Alterations

While most NF1-OPG harbor no additional genomic alterations, some patient tumors harbor co-existing mutations, including heterozygous PTEN deletion or KIAA1549:BRAF duplication.87 When modeled in mice, the differential effects of these alterations were confirmed: While coexpression of the KIAA1549:BRAF fusion gene did not further increase Nf1 optic glioma growth in mice, heterozygous Pten inactivation dramatically increased tumor volume, proliferation and microglia infiltration.136 Similarly, in the context of Nf1/Trp53-driven high grade gliomagenesis, the addition of somatic heterozygous Pten loss in NPCis mice leads to the development of more aggressive gliomas with near complete penetrance.100 As additional genetic alterations

| Advantages | Disadvantages |
|------------|--------------|
| - Human cells and tissues | - Requires somatic reprogramming |
| - Patient-relevant genomic background | - Some mutations cannot be engineered in isogenic lines |
| - Actual patient mutations | - Only engraft in immunocompromised rodents |
| - Amenable to high throughput screening | - Pharmacodynamics and pharmacokinetics are different from humans |
| - Can control modifying factors | - Structural and anatomic differences from humans |
| - Genetic engineering efficient and rapid | - Short lifespan |
| - Enables rapid preclinical drug testing | - 66% genetic homology |
| - Pharmacodynamics and pharmacokinetics more similar to humans | - Gene targeting more challenging |
| - Anatomy and behavior more similar to humans | - Requires significant research and veterinary infrastructure |
| - 80% genetic homology | - Longer gestation periods |
| - Limited tissue-specific tools (e.g., Cre transgenic strains) |

Figure 5. Preclinical models of NF1-glioma. Several preclinical models have been developed for NF1-associated low-grade gliomas, including human induced pluripotent stem cells (hiPSCs), genetically engineered mice, and genetically engineered swine. Each of these platforms has limitations and advantages.
are identified through large-scale sequencing efforts, opportunities may arise to understand how these co-existing mutations influence glioma biology.

Genomic Modifiers

Since individuals with NF1 within the same family can exhibit different clinical features and phenotypic severity, it is likely that other factors influence glioma penetrance, including genomic modifiers. While challenging to study in people, NPcis mice have been used to identify potential modifier genes. For example, susceptibility to astrocytoma has been linked to genes on mouse chromosome 11, such that mice that inherit the NPcis mutation from their mothers have an increased risk for astrocytoma. Additionally sexually dimorphic differences in glioma susceptibility are linked to loci on mouse chromosome 19, with the Arlm1 gene representing one potential modifier of astrocytoma resistance in males. Finally, the Scram1 locus on mouse chromosome 5 affects the incidence and latency of spinal cord astrocytoma development, but does not affect the overall latency of astrocytomas.

Preclinical Drug Discovery and Evaluation

In addition to risk assessment, one of the most widely exploited applications for mouse models has been in drug discovery and preclinical testing. For example, NF1 optic glioma mice have been used as preclinical platforms to evaluate the efficacy of RAS pathway inhibitors. As such, successful trials using MEK (PD0325901), PI3K (NPV-BKM120), and mTOR (rapamycin) inhibitors have demonstrated decreased tumor cell proliferation and tumor size. Similarly, increasing cAMP levels in NF1-OPG using an inhibitor of the enzyme responsible for cAMP degradation (phosphodiesterase-4 inhibitor; Rolipram) reduces tumor proliferation and volume. Unfortunately, some of these therapies in mice require drug doses that are not easily tolerated in children, and tumor growth suppression with these pathway-targeted therapies requires continual drug exposure. In addition, the cancer stem cells from NF1-OPG acquire adaptive responses that promote resistance to mTOR and MEK inhibition which may further limit their efficacy. For this reason, additional molecularly targeted or combinational therapies are needed.

While tumor-directed therapies have been the mainstay of treatment, therapies aimed at reducing stromal cell support of tumor growth represent another opportunity. In this regard, suppressing microglia function with minocycline or inhibiting the JNK pathway activated in microglia have resulted in reduced tumor growth. Future strategies that aim to disrupt this immune axis may offer new avenues for pursuit.

In addition to tumor- and stroma-directed therapies, recent studies have also focused on identifying therapies that block further visual decline or result in improved visual acuity. Using NF1-OPG mice, treatment with Lovastatin, a HMG CoA reductase inhibitor that blocks RAS activity, resulted in preservation of RGC numbers two months after the cessation of therapy. Interestingly, tumor proliferation returned to pretreatment levels, suggesting neuroprotective and neurorestorative strategies might emerge as future adjuvant approaches for NF1-OPG.

Future Directions

With the insights provided by basic and preclinical translational research, several clinical trials have been designed to identify more effective therapies for NF1-OPG (Table 1). Most of these studies use chemotherapy agents to halt tumor progression. However, emerging therapies are being considered that target cells and signals in the tumor microenvironment, as well as focused on restoring vision loss. Other approaches, including gene editing (NCT02465060), are in early stages of development.

While mouse models have proven to be extremely useful tools for studying NF1-glioma, successful preclinical experiments in rodents do not always translate well into effective treatments for patients. For this reason, additional models are currently being developed (Figure 5). Genetically engineered minipigs have recently been generated that better recapitulate the full spectrum of NF1-associated features, including café au lait macules, neurofibromas, and OPG. Given the greater similarities between swine and people with respect to brain structure and function, as well as pharmacokinetic and pharmacodynamics profiles, these animals may be better suited for preclinical drug testing than mice. In addition to these animal models, researchers are also working to establish human induced pluripotent stem cell models of NF1-tumors. Take together, the implementation of these complementary model systems are likely provide unprecedented insights into the pathobiology of these tumors and result in the development of more effective treatments for patients with NF1-associated glioma.

Keywords

brain tumor | brainstem | glioma | NF1 | optic pathway | RAS

Funding

D.H.G. is funded by a Research Program Award grant from the National Institutes of Health (1-R35-NS07211-01).

Acknowledgments

The authors would like to thank the members of the Gutmann Laboratory for their helpful comments and suggestions. D.H.G. is supported by a Research Program Award grant from the National Institutes of Health (1-R35-NS07211-01).
Conflict of interest statement. The authors have no relevant conflicts to disclose.

Authorship statement: A.C. wrote the initial draft of the manuscript and designed some of the figures. D.H.G. edited the manuscript and finalized the figures.

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