Neurofibromatosis Type 1 and tumorigenesis: molecular mechanisms and therapeutic implications

OREN N. GOTTFRIED, M.D.,¹ DAVID H. VISKOCHIL, M.D., PH.D.,²
AND WILLIAM T. COULDWELL, M.D., PH.D.¹

¹Department of Neurosurgery and ²Department of Pediatrics, Division of Genetics, University of Utah, Salt Lake City, Utah

Neurofibromatosis Type 1 (NF1) is a common autosomal dominant disease characterized by complex and multicellular neurofibroma tumors, and less frequently by malignant peripheral nerve sheath tumors (MPNSTs) and optic nerve gliomas. Significant advances have been made in elucidating the cellular, genetic, and molecular biology involved in tumor formation in NF1. Neurofibromatosis Type 1 is caused by germline mutations of the *NF1* tumor suppressor gene, which generally result in decreased intracellular neurofibromin protein levels, leading to increased cascade Ras signaling to its downstream effectors. Multiple key pathways are involved with the development of tumors in NF1, including Ras/mitogen-activated protein kinase (MAPK) and Akt/mammalian target of rapamycin (mTOR). Interestingly, recent studies demonstrate that multiple other developmental syndromes (in addition to NF1) share phenotypic features resulting from germline mutations in genes responsible for components of the Ras/MAPK pathway. In general, a somatic loss of the second *NF1* allele, also referred to as loss of heterozygosity, in the progenitor cell, either the Schwann cell or its precursor, combined with haploinsufficiency in multiple supporting cells is required for tumor formation. Importantly, a complex series of interactions with these other cell types in neurofibroma tumorigenesis is mediated by abnormal expression of growth factors and their receptors and modification of gene expression, a key example of which is the process of recruitment and involvement of the *NF1*^{+/-} heterozygous mast cell. In general, for malignant transformation to occur, there must be accumulation of additional mutations of multiple genes including *INK4A/ARF* and *P53*, with resulting abnormalities of their respective signal cascades. Further, abnormalities of the *NF1* gene and molecular cascade described above have been implicated in the tumorigenesis of NF1 and some sporadically occurring gliomas, and thus, these treatment options may have wider applicability. Finally, increased knowledge of molecular and cellular mechanisms involved with NF1 tumorigenesis has led to multiple preclinical and clinical studies of targeted therapy, including the mTOR inhibitor rapamycin, which is demonstrating promising preclinical results for treatment of MPNSTs and gliomas. (DOI: 10.3171/2009.11.FOCUS09221)

KEY WORDS • optic nerve glioma • loss of heterozygosity • malignant peripheral nerve sheath tumor • neurofibroma • plexiform neurofibroma

NEUROFIBROMATOSIS Type 1 is a common autosomal dominant disease and is characterized by neural crest-derived tumors.^{18,101} The key feature of NF1, neurofibromas, are complex tumors arising from peripheral nerve sheaths.^{59,101} Neurofibromas consist primarily of Schwann cells, fibroblasts, and a large amount of extracellular matrix with collagen surrounding an axon, but they also contain many other cell types including perineural cells, mast cells, pericytes, and endothelial cells.^{9,59,80,85,101} Malignant tumors can arise in either childhood or adulthood, with MPNSTs being most common.¹⁶ In addition to the lesions associated with the

peripheral nervous system, patients with NF1 are also at risk for CNS tumors, specifically gliomas.^{2,42} In childhood, these gliomas are primarily located in the optic pathway and less frequently in the hypothalamus and brainstem; adults are more likely to develop higher-grade gliomas.²

Our group has previously published an overview of the genetics and pathogenesis of neurofibromas in NF1.³⁸ In the present paper, we update the relevant information related to the genetic alterations that predispose individuals to this tumor and also discuss the mechanisms involved in the tumorigenesis of MPNSTs and optic nerve gliomas, with attention on implications for targeted therapies (not previously presented).

Genetics

As background information, we include here a brief summary of the genetics of this disease. In this auto-

Abbreviations used in this paper: GAP = GTPase activating protein; GDP = guanosine diphosphate; GRD = GAP-related domain; GTP = guanosine triphosphate; MPAK = mitogen-activated protein kinase; MPNST = malignant peripheral nerve sheath tumor; mTOR = mammalian target of rapamycin; NF1 = neurofibromatosis Type 1.

Fig. 1. Illustration depicting the signal transduction pathways involved in NF1 tumorigenesis. Growth factors (gf) interact with receptors at the cell surface, activating guanine nucleotide exchange factors and resulting in activated Ras. Activated Ras sends intracellular signals activating the phosphoinositol 3' kinase (PI3K), AKT, and mTOR pathway. Activated Ras also activates the Raf-MEK (mitogen-activated kinase)/MEK pathway to stimulate ERK (extracellular signal-related kinase), which can enter the nucleus and promote transcription. Normally, neurofibromin downregulates Ras through its GAP-related domain, and therefore in its absence or at decreased levels in NF1, signaling is increased through all of these pathways resulting in cell proliferation and inhibited apoptosis. Adapted by permission from Macmillan Publishers Ltd: **Oncogene** 26: 4609–4616, copyright 2007.

somal dominant–inherited condition, in which homozygosity is lethal to embryos,^{15,46} all affected individuals are heterozygous for an *NF1* mutation.³⁵ One *NF1* allele carries a genetic alteration in all cells of a patient with NF1, and therefore, a loss of the second *NF1* allele (loss of heterozygosity, LOH) results in complete functional loss of neurofibromin^{31,52,86,87} and risk of tumor formation.^{31,32,47,86} Point mutations affecting the correct splicing of the *NF1* gene are a common cause of NF1,^{4,33,73} responsible for both somatic and germline mutations.⁸⁷ Many mutations in the *NF1* gene result in truncation of the protein product, neurofibromin.⁹⁶ Approximately half of cases represent new mutations,³⁵ and a mutation at a given locus may provide a selective and proliferative advantage in a germ-cell precursor.⁸⁴

Positional cloning identified the *NF1* gene at 17q11.2.^{19,97,103} It produces an mRNA^{9,67} that is expressed in almost all tissues¹⁰³ but most highly in brain, spinal cord, and the peripheral nervous system.^{24,98} Neurofibro-

min, the protein product of the *NF1* gene, is found in neurons, oligodendrocytes, and Schwann cells in adults,^{24,98} and is also expressed in other cell types, such as keratinocytes, adrenal medulla, and white blood cells.^{40,98} Neurofibromin is reduced or absent in neurofibroma cells,^{39,55} which are composed principally of Schwann cells without functional neurofibromin.⁷⁷

Signal Transduction Pathways

Although it only occupies a small area of the protein (360 amino acids), the Ras-GTPase activating protein (GAP)-related domain (GRD) is an important functional region of neurofibromin^{17,106,107} that stimulates the intrinsic GTPase of p21-Ras-GTP to hydrolyze GTP to GDP, inactivating p21-Ras (Fig. 1).^{1,6,14,68,93,106} Inactivation of the active Ras-GTP is the main function of neurofibromin.

The functional domain of neurofibromin, Ras, acts as

Fig. 2. Drawing demonstrating the common Ras/MAPK pathways responsible for multiple developmental syndromes, including NF1. In addition to similar molecular origins, these Ras-related diseases, including Noonan syndrome, LEOPARD syndrome, hereditary gingival fibromatosis 1, capillary malformation–arteriovenous malformation (CM-AVM) syndrome, Costello syndrome, autoimmune lymphoproliferative syndrome (ALPS), cardio-facio-cutaneous (CFC) syndrome, and Legius syndrome, have considerable phenotypic overlap. Thus, targeted molecular treatment may be effective for multiple diseases. Figure reproduced with permission from Tidyman and Rauen.⁹²

part of a signal transduction pathway that is activated by growth factors and their receptors.⁸ Increased Ras-GTP leads to increased signaling through Raf kinase, which activates a kinase cascade involving MEK kinase and the Erk1 and Erk2 isoforms of MAPK resulting in cell proliferation.^{13,27,91} Increased Ras-GTP also protects cells from apoptosis by activating mTOR.^{10,51} Studies confirm that neurofibromin negatively regulates this mTOR pathway with loss of neurofibromin expression in established human neurofibroma cell lines associated with high levels of mTOR activity.^{10,51} The mTOR pathway is constitutively activated in both NF1-deficient primary cells and human tumor, is dependent on Ras and PI3K activation, and is mediated by the phosphorylation and inactivation of the TSC2-encoded protein tuberlin by AKT.⁵¹ Overall, Ras is a key component of many growth factor signaling pathways, and in the absence of neurofibromin it is consti-

tutively activated, resulting in increased cell proliferation and survival.⁹⁸

The Ras/MAPK pathway is critical to normal development by its regulation of cell proliferation, differentiation, motility, growth, apoptosis, and cell senescence.⁹² Interestingly, there are multiple developmental syndromes in addition to NF1 that form tumors by abnormalities in the Ras pathway, and they are referred to as the “RASopathies” or “neuro-cardio-facio-cutaneous syndromes.”^{26,92} These diseases include Noonan syndrome, LEOPARD syndrome, Costello syndrome, capillary malformation–arteriovenous malformation, and cardio-facio-cutaneous syndrome; these syndromes and their relation to the Ras/MAPK pathway are reviewed in detail in Tidyman and Rauen (Fig. 2).⁹² They are related by germline mutations in genes that encode protein components of the Ras/MAPK pathway, and these mutations may result in

increased signal transduction.⁹² Each syndrome exhibits unique phenotypic features; however, there are overlapping clinical features including characteristic facial features, cardiac defects, skin abnormalities, developmental delay, and a risk of malignancy.⁹² Interestingly, there are several NF1-related pathways that are significant in the pathogenesis of other developmental syndromes, including mTOR, which contributes to tuberous sclerosis.^{30,44,74,75} Denayer and colleagues²⁶ noted that these syndromes with common molecular pathways demonstrated the important roles for evolutionarily conserved pathways not only in oncogenesis, but also in cognition, growth, and development.

Malignant Peripheral Nerve Sheath Tumors

Additional molecular cascades may be involved in tumorigenesis for malignant NF1 tumors.¹² For example, Ral overactivation is a novel cell signaling abnormality in MPNSTs.¹² Of note, overactivation of Ras and many of its downstream effectors occurred in only a fraction of MPNST cell lines, but Ral was overactivated in all MPNST cells and tumor samples.¹² Silencing Ral or inhibiting it with a dominant-negative Ral caused a significant reduction in proliferation, invasiveness, and in vivo tumorigenicity of MPNST cells.¹² Also, neurofibromin is shown to regulate Ral activation, and NF1-GRD treatment caused a significant decrease in proliferation, invasiveness, and cell cycle progression, and increased cell death.¹² The mTOR pathway has also been shown to have an important role in NF1-associated malignancies including MPNST.⁵⁰

Among the genetic abnormalities previously reported to be involved in the transformation to malignancy in NF1,³⁹ the loss or mutation of the *P53* gene in NF1-related MPNSTs (but never in benign neurofibromas)^{11,37,49,58,63,64,66,72,78,82,95,105} is associated with a poor prognosis.⁶⁵ The loss of the *P53* gene results in abnormalities in DNA damage–induced cell cycle arrest and apoptosis.⁹⁵ Approximately half of MPNSTs in patients with NF1 show homozygous deletions at the *CDKN2A* (also known as *INK4A* or *INK4A/ARF*) gene, which contributes to increasing cellular proliferation.⁹⁵ Also, MPNSTs demonstrate a high frequency of microsatellite instability, and thus, additional loci become targets for mutations during the malignant transformation.

Gliomas

There is evidence that the *NF1* gene is involved in the tumorigenesis of both NF1-related and sporadically occurring gliomas.^{70,79,112} For example, a genomic analysis of human glioblastoma multiforme identified that the *NF1* gene was mutated in 15% of tumors.⁷⁹ As in other NF1 tumors, biallelic *NF1* gene inactivation is observed in NF1-associated gliomas and the heterozygous supporting cell or astrocyte is also important for tumorigenesis.⁵ Of note, McGillicuddy et al.⁷⁰ found that *NF1* function is inactivated in sporadic gliomas, and it occurs by both proteasomal degradation and genetic loss. For example, neurofibromin protein destabilization is triggered by the

hyperactivation of protein kinase C (PKC) and thus, these tumors may be sensitive to PKC inhibitors. They found that complete genetic loss of *NF1* occurs when p53 is inactivated and allows sensitivity to mTOR inhibitors.⁷⁰ Yeh et al.¹¹² found that there is variability in *NF1* gene expression in distinct populations of glia and increased glial cell proliferation in the optic nerve and brainstem but not in other areas after *NF1* inactivation in vitro and in vivo, and thus differences in *NF1* gene expression might contribute to the regional localization of brain tumors. Recent studies also demonstrated that the mTOR pathway is involved in tumorigenesis of gliomas in NF1,^{16,42,50,51,70} and its inhibitor rapamycin is effective in decreasing tumor proliferation in an animal model.⁴²

Neurofibromas

Neurofibromas are unique among tumors because they exhibit extensive cellular heterogeneity.^{85,89} Reciprocal signaling among the many cell types is known to occur in normal peripheral nerve sheath and in neurofibromas,^{48,56} and decreased neurofibromin in neurofibromas may result in altered responses. Despite this heterogeneity, the Schwann cell lineage has been recognized as the primary cell type for neurofibroma formation. This cell type is the major one amplified in neurofibromas and is the primary target of growth factors stimulating neurofibroma formation.^{81,90,102} In addition, the loss of both *NF1* alleles occurs exclusively in the Schwann cell, whereas a wild-type gene is retained in the other neurofibroma cells, including fibroblasts.^{57,88} This Schwann cell, which lacks functional neurofibromin, has a substantial growth advantage⁷⁷ and a loss of negative autocrine growth control.⁷⁶

The stage of differentiation of the Schwann cell as the progenitor cell for NF1 tumors is still the subject of some debate.^{53,61} In 2009, Le and coworkers⁶¹ proposed that the neural crest stem cell is the cell of origin for cutaneous neurofibromas after identifying a population of stem/progenitor cells residing in the dermis (called skin-derived precursors) that, through loss of *NF1*, form neurofibromas. Interestingly, in another study, Joseph et al.⁵³ showed that stem cells did not persist postnatally in the location of future neurofibromas or MPNSTs and proposed that the cell of origin is a more differentiated glial cell. Finally, Zheng and coworkers¹¹³ noted that plexiform neurofibroma results from *NF1* deficiency in progenitor cells of peripheral nerves. These cells did not cause hyperproliferation or tumorigenesis in the early postnatal period and peripheral nerve development appeared largely normal except for abnormal Remak bundles, the nonmyelinated axon-Schwann cell unit, identified in postnatal mutant nerves. Subsequent degeneration of abnormal Remak bundles occurred concurrently with expansion of nonmyelinating Schwann cells. These findings led the authors to suggest that abnormally differentiated Remak bundles might be the cell of origin for plexiform neurofibroma.

Haploinsufficiency of the *NF1* gene in the microenvironment of neurofibromas contributes to its tumorigenicity.⁷¹ The single active *NF1* allele in the heterozygous supporting cell does not generate enough functional protein

to achieve an appropriate biological response,²⁹ and this confers a growth advantage that contributes to tumorigenesis.⁴⁵ The neurofibromin-deficient *NF1*^{+/-} Schwann cell and other heterozygous supporting cells are absolutely necessary for tumor formation and are critically involved in the pathogenesis of neurofibromas.^{77,114} Also, inflammatory cells, including mast cells, are important to tumor initiation, progression, and angiogenesis.^{22,23,41} Neurofibromin-deficient Schwann cells secrete increased kit ligand, which serves as a chemoattractant for mast cells expressing c-kit receptor. Also, heterozygous inactivation of *NF1*^{+/-} promotes migration of mast cells on $\alpha 4\beta 1$ integrins (mast cell surface proteins), in response to the kit ligand. Overall, the loss of *NF1* in Schwann cells results in an increase in growth factor production that initiates a paracrine loop, and it is important for tumor initiation and progression.¹⁰⁹

Normally, growth factors cooperate to suppress cell death in Schwann cell precursors,³⁶ but growth factor dysregulation is thought to be involved in tumorigenesis.⁸³ Changes in growth factor expression may be the direct result of *NF1* gene loss or from secondary genetic events.⁶⁹ Abnormal growth factor receptor expression also has a role in tumorigenesis, progression, and malignant transformation.

Preclinical Models

The development of animal models for preclinical testing in NF1 is key to identifying novel targets for future therapies.⁴³ Early xenograft models for studying neurofibroma formation produced tumors by injecting human neurofibroma tissue or Schwann cell preparations into the sciatic nerve of immunodeficient mice.^{3,62,89} Subsequently, *Nf1* transgene mouse models were developed based on a mutation identified in patients with NF1.^{15,46} It was hoped that transgene mouse models would prove to be a suitable model for human NF1, but mice heterozygous for the *Nf1* mutation (*Nf1*^{+/-}/*Nf1*ⁿ³¹) do not develop the classic phenotype of NF1 disease including neurofibromas,⁴⁶ and mice that are homozygous for the *Nf1* mutation die in utero.^{15,46} Later, chimeric mice (*Nf1*^{-/-}:*Nf1*^{+/+}) were developed by injection of *Nf1*^{-/-} homozygous mutant embryonic stem cells at an early developmental stage (blastocysts) to overcome the lethality of the germline homozygous genotype with some success.²⁰ Although the mice developed multiple neurofibromas resembling human plexiform neurofibromas, the cell type in which the *Nf1* was deleted could not be controlled.⁷¹ Next, a conditional mouse model was developed that resulted in somatic inactivation of *Nf1* and ablation of neurofibromin function specifically in Schwann cells.¹¹⁴ This animal model, *Nf1*^{fllox/-};Krox20-cre, provided evidence for the necessity for haploinsufficient supporting cells in neurofibroma formation,¹¹⁴ and all mice developed plexiform neurofibromas with all of the typical supporting cells (*Nf1*^{+/-}).

A mouse model was developed for MPNSTs by generating mice with mutations in both *Nf1* and *p53* genes.^{20,100} These mice (*Nf1*^{+/-}:*p53*^{+/-}) developed soft-tissue sarcomas and MPNSTs in neural crest-derived tissue.^{20,100} This model demonstrates that a mutation in the *p53* gene in

addition to that in *Nf1* is required for malignant transformation of cells of neural crest origin.^{20,100}

There are also *NF1*-deficient animal models of NF1 that produce gliomas.^{5,42} In contrast to astrocyte-restricted *NF1* conditional knockout mice, heterozygous mice lacking *NF1* in astrocytes develop optic nerve gliomas. Thus, NF1 glioma formation requires additional cellular or genetic conditions. This mouse model demonstrates that *NF1*^{+/-} cells contribute to the pathogenesis of gliomas in NF1 and provides a tool for the preclinical evaluation of potential therapeutic interventions.

Targeted Treatment

Targeted therapy will have a great impact for patients with numerous neurofibromas, very massive plexiform neurofibromas, or MPNSTs for which surgery and radiation have not been widely successful,^{54,94} and as an adjunct or primary treatment of gliomas. Currently, the principal mode of therapy for spinal neurofibromas or plexiform neurofibromas is surgery. Although isolated single-nerve root neurofibromas can be resected without significant morbidity, patients with multiple spinal neurofibromas often require multiple surgeries during their lifetime. Also, patients with large plexiform neurofibromas represent a surgical challenge; have a high recurrence rate if tumors are subtotally resected, intense adherence, and invasion into local tissue; and have a risk of malignant transformation.^{54,94} Many preclinical studies, as well as a few clinical trials using biological-based therapeutic approaches to target specific genetic or molecular events involved in NF1 tumorigenesis are in progress. In the future, these medical therapies may be useful for treatment of residual tumors, or as a measure to reduce tumor size prior to surgery, or even to decrease the development of tumors. It may also be possible to apply new therapeutic agents to the local tumor environment to reduce the incidence of recurrences.

Preclinical or clinical studies have targeted various aspects of mast cell functioning, the Ras signaling pathways, rapamycin, and growth factors and receptors, but future therapies may be targeted at almost any molecule involved in tumorigenesis (Fig. 1). A number of strategies were discussed in our previous review. Proposed treatments that reduce the mast cell activity, migration, or numbers within neurofibromas and mast cell stabilizers have been considered as possible therapeutic strategies.^{109,110} Options for treatment include pharmacological inhibition of PI3K, kit activity, or $\alpha 4\beta 1$ adhesion.^{109,110} The Ras signal transduction pathways have also been targeted for treatments.^{104,108} Anti-Ras therapies are an ideal target, because RAS is one of the most common oncogenes mutated in human malignancies,¹⁰⁴ and Ras-GTP is elevated for both benign and malignant NF1 neurofibromas.³⁹ Treatment options include the delivery of gene therapy vectors with functional NF1 GRD proteins or with dominant negative Ras, which blocks Ras activity.^{60,98} Other options include the injection of neutralizing Ras antibodies, which inhibits cell proliferation,^{7,25} or viruses that target and infect cells with activated Ras signaling.²¹ For example, the human reovirus requires an activated Ras

signaling pathway for infection of cultured cells and has been shown to cause tumor regression in mouse models.²¹ The downstream effectors of the Ras signaling pathway,¹⁰⁴ including agents that inhibit MEK^{28,34} and PI3K,^{99,111} are promising targets for future neurofibroma treatment.

Newer studies are focusing on the mTOR pathway and its inhibitor rapamycin.^{10,16,42,50,51,70} Importantly, tumor cell lines derived from patients with NF1, and a genetically engineered cell system that requires *Nf1*-deficiency for transformation, are highly sensitive to rapamycin. For example, Bhola et al.¹⁰ developed and characterized a human NF1-MPNST explant grown subcutaneously in mice to evaluate the effect of rapamycin. Rapamycin significantly inhibited human NF1-MPNST mTOR pathway activation and growth without systemic toxicities. It was effective at reducing NF1-MPNST proliferation and angiogenesis, but did not increase apoptosis. Rapamycin effectively decreased activation downstream of mTOR, but there was increased AKT activation. This study therefore demonstrated the therapeutic potential and limits of rapamycin in NF1-associated and possibly sporadic MPNST. Johannessen et al.⁵⁰ demonstrated that rapamycin suppresses the growth of aggressive NF1-associated malignancies in a murine model. Interestingly, they demonstrated that rapamycin does not function via mechanisms generally assumed to mediate tumor suppression, including inhibition of HIF-1 α and indirect suppression of AKT, but does suppress the mTOR target Cyclin D1. Finally, in a genetically engineered mouse model of low-grade optic glioma resulting from inactivation of the *NF1* gene, pharmacological mTOR inhibition in vivo led to decreased tumor cell proliferation in a dose-dependent fashion associated with a decrease in tumor volume.⁴² It is important to realize that rapamycin is an effective treatment in other developmental syndromes with abnormalities of the mTOR pathway, including tuberous sclerosis, where it may be efficacious in the management of cognitive or developmental disorders and epilepsy and in preventing or limiting tumor development.^{30,44,74,75}

Overall, these medical therapies targeting specific genetic or molecular events involved in NF1 tumorigenesis may be useful for treatment of residual tumors, to reduce tumor size prior to surgery, to decrease the incidence of tumor formation, or to apply to the local tumor environment at the time of resection.

Conclusions

Remarkable progress has been made toward understanding the pathogenesis of neurofibromas since the cloning of the *NF1* gene in 1990. Neurofibromatosis Type 1 is caused by germline mutations of the *NF1* tumor suppressor gene, which generally results in decreased intracellular neurofibromin protein, leading to increased cascade Ras signaling to its downstream effectors, including Ras/MAPK and Akt/mTOR. In general, a somatic loss of the second *NF1* allele in the progenitor cell, either the Schwann cell or its precursor, combined with haploinsufficiency in multiple supporting cells is required for tumor formation. Importantly, there is a complex series of interactions with these other cell types, such as *NF1*^{+/-}

heterozygous mast cells, in neurofibroma tumorigenesis. In general, for malignant transformation to occur there must be accumulation of additional mutations of multiple genes including *INK4A/ARF* and *P53*. Increased knowledge of molecular and cellular mechanisms involved with NF1 tumorigenesis has led to more accurate animal models of NF1 tumors to evaluate new agents for targeted molecular therapy, including the mTOR inhibitor rapamycin, which are demonstrating promising preclinical results for treatment of MPNSTs and gliomas.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: WT Couldwell, ON Gottfried, DH Viskochil. Drafting the article: ON Gottfried. Critically revising the article: WT Couldwell, ON Gottfried, DH Viskochil. Reviewed final version of the manuscript and approved it for submission: WT Couldwell, ON Gottfried. Study supervision: WT Couldwell, DH Viskochil.

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References

- Ahmadian MR, Wiesmüller L, Lautwein A, Bischoff FR, Wittinghofer A: Structural differences in the minimal catalytic domains of the GTPase-activating proteins p120GAP and neurofibromin. **J Biol Chem** 271:16409–16415, 1996
- Albers AC, Gutmann DH: Gliomas in patients with neurofibromatosis type 1. **Expert Rev Neurother** 9:535–539, 2009
- Appenzeller O, Kornfeld M, Atkinson R, Snyder RD: Neurofibromatosis xenografts. Contribution to pathogenesis. **J Neurol Sci** 74:69–77, 1986
- Ars E, Serra E, García J, Kruyer H, Gaona A, Lázaro C, et al: Mutations affecting mRNA splicing are the most common molecular defects in patients with neurofibromatosis type 1. **Hum Mol Genet** 9:237–247, 2000
- Bajenaru ML, Hernandez MR, Perry A, Zhu Y, Parada LF, Garbow JR, et al: Optic nerve glioma in mice requires astrocyte *Nf1* gene inactivation and *Nf1* brain heterozygosity. **Cancer Res** 63:8573–8577, 2003
- Ballester R, Marchuk D, Boguski M, Saulino A, Letcher R, Wigler M, et al: The *NF1* locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. **Cell** 63:851–859, 1990
- Basu TN, Gutmann DH, Fletcher JA, Glover TW, Collins FS, Downward J: Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. **Nature** 356:713–715, 1992
- Bernards A: Neurofibromatosis type 1 and Ras-mediated signaling: filling in the GAPs. **Biochim Biophys Acta** 1242:43–59, 1995
- Bernards A, Haase VH, Murthy AE, Menon A, Hannigan GE, Gusella JF: Complete human *NF1* cDNA sequence: two alternatively spliced mRNAs and absence of expression in a neuroblastoma line. **DNA Cell Biol** 11:727–734, 1992
- Bhola P, Banerjee S, Mukherjee J, Balasubramaniam A, Arun V, Karim Z, et al: Preclinical in vivo evaluation of rapamycin in human malignant peripheral nerve sheath explant xenograft. **Int J Cancer** [epub ahead of print], 2009
- Birindelli S, Perrone F, Oggionni M, Lavarino C, Pasini B,

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- Vergani B, et al: Rb and TP53 pathway alterations in sporadic and NF1-related malignant peripheral nerve sheath tumors. **Lab Invest** **81**:833–844, 2001
12. Bodempudi V, Yamoutpoor F, Pan W, Dudek AZ, Esfandyari T, Piedra M, et al: Ral overactivation in malignant peripheral nerve sheath tumors. **Mol Cell Biol** **29**:3964–3974, 2009
13. Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, Thompson P, et al: Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. **Nat Genet** **12**:144–148, 1996
14. Bollag G, McCormick F: Differential regulation of rasGAP and neurofibromatosis gene product activities. **Nature** **351**:576–579, 1991
15. Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Reid SW, et al: Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. **Genes Dev** **8**:1019–1029, 1994
16. Brems H, Beert E, de Ravel T, Legius E: Mechanisms in the pathogenesis of malignant tumours in neurofibromatosis type 1. **Lancet Oncol** **10**:508–515, 2009
17. Buchberg AM, Cleveland LS, Jenkins NA, Copeland NG: Sequence homology shared by neurofibromatosis type-1 gene and IRA-1 and IRA-2 negative regulators of the RAS cyclic AMP pathway. **Nature** **347**:291–294, 1990
18. Burger PC, Scheithauer BW, Vogel FS: **Surgical Pathology of the Nervous System and Its Coverings**, ed 4. New York: Churchill Livingstone, 2002
19. Cawthon RM, Weiss R, Xu GF, Viskochil D, Culver M, Stevens J, et al: A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. **Cell** **62**:193–201, 1990
20. Cichowski K, Shih TS, Schmitt E, Santiago S, Reilly K, McLaughlin ME, et al: Mouse models of tumor development in neurofibromatosis type 1. **Science** **286**:2172–2176, 1999
21. Coffey MC, Strong JE, Forsyth PA, Lee PW: Reovirus therapy of tumors with activated Ras pathway. **Science** **282**:1332–1334, 1998
22. Coussens LM, Werb Z: Inflammation and cancer. **Nature** **420**:860–867, 2002
23. Coussens LM, Werb Z: Inflammatory cells and cancer: think different! **J Exp Med** **193**:F23–F26, 2001
24. Daston MM, Scrabble H, Nordlund M, Sturbaum AK, Nissen LM, Ratner N: The protein product of the neurofibromatosis type 1 gene is expressed at highest abundance in neurons, Schwann cells, and oligodendrocytes. **Neuron** **8**:415–428, 1992
25. DeClue JE, Papageorge AG, Fletcher JA, Diehl SR, Ratner N, Vass WC, et al: Abnormal regulation of mammalian p21ras contributes to malignant tumor growth in von Recklinghausen (type 1) neurofibromatosis. **Cell** **69**:265–273, 1992
26. Denayer E, de Ravel T, Legius E: Clinical and molecular aspects of RAS related disorders. **J Med Genet** **45**:695–703, 2008
27. Downward J: Ras signalling and apoptosis. **Curr Opin Genet Dev** **8**:49–54, 1998
28. Dudley DT, Pang L, Decker SJ, Bridges AJ, Saltiel AR: A synthetic inhibitor of the mitogen-activated protein kinase cascade. **Proc Natl Acad Sci U S A** **92**:7686–7689, 1995
29. Dunn NR, Winnier GE, Hargett LK, Schrick JJ, Fogo AB, Hogan BL: Haploinsufficient phenotypes in Bmp4 heterozygous null mice and modification by mutations in Gli3 and Alx4. **Dev Biol** **188**:235–247, 1997
30. Ehninger D, de Vries PJ, Silva AJ: From mTOR to cognition: molecular and cellular mechanisms of cognitive impairments in tuberous sclerosis. **J Intellect Disabil Res** **53**:838–851, 2009
31. Eisenbarth I, Beyer K, Krone W, Assum G: Toward a survey of somatic mutation of the NF1 gene in benign neurofibromas of patients with neurofibromatosis type 1. **Am J Hum Genet** **66**:393–401, 2000
32. Elyakim S, Lerer I, Zlotogora J, Sagi M, Gelman-Kohan Z, Merin S, et al: Neurofibromatosis type I (NF1) in Israeli families: linkage analysis as a diagnostic tool. **Am J Med Genet** **53**:325–334, 1994
33. Fahsold R, Hoffmeyer S, Mischung C, Gille C, Ehlers C, Kücküceylan N, et al: Minor lesion mutational spectrum of the entire NF1 gene does not explain its high mutability but points to a functional domain upstream of the GAP-related domain. **Am J Hum Genet** **66**:790–818, 2000
34. Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, et al: Identification of a novel inhibitor of mitogen-activated protein kinase kinase. **J Biol Chem** **273**:18623–18632, 1998
35. Friedman JM: Clinical genetics, in Friedman JM, Gutmann DH, MacCollin M, Riccardi VM (eds): **Neurofibromatosis: Phenotype, Natural History, and Pathogenesis**, ed 3. Baltimore: Johns Hopkins University Press, 1999, pp 110–118
36. Gavrilovic J, Brennan A, Mirsky R, Jessen KR: Fibroblast growth factors and insulin growth factors combine to promote survival of rat Schwann cell precursors without induction of DNA synthesis. **Eur J Neurosci** **7**:77–85, 1995
37. Glover TW, Stein CK, Legius E, Andersen LB, Brereton A, Johnson S: Molecular and cytogenetic analysis of tumors in von Recklinghausen neurofibromatosis. **Genes Chromosomes Cancer** **3**:62–70, 1991
38. Gottfried ON, Viskochil DH, Fuets DW, Couldwell WT: Molecular, genetic, and cellular pathogenesis of neurofibromas and surgical implications. **Neurosurgery** **58**:1–16, 2006
39. Guha A, Lau N, Huvar I, Gutmann D, Provias J, Pawson T, et al: Ras-GTP levels are elevated in human NF1 peripheral nerve tumors. **Oncogene** **12**:507–513, 1996
40. Gutmann DH, Geist RT, Wright DE, Snider WD: Expression of the neurofibromatosis 1 (NF1) isoforms in developing and adult rat tissues. **Cell Growth Differ** **6**:315–323, 1995
41. Hanahan D, Weinberg RA: The hallmarks of cancer. **Cell** **100**:57–70, 2000
42. Hegedus B, Banerjee D, Yeh TH, Rothermich S, Perry A, Rubin JB, et al: Preclinical cancer therapy in a mouse model of neurofibromatosis-1 optic glioma. **Cancer Res** **68**:1520–1528, 2008
43. Hesselager G, Holland EC: Using mice to decipher the molecular genetics of brain tumors. **Neurosurgery** **53**:685–695, 2003
44. Huang J, Wu S, Wu CL, Manning BD: Signaling events downstream of mammalian target of rapamycin complex 2 are attenuated in cells and tumors deficient for the tuberous sclerosis complex tumor suppressors. **Cancer Res** **69**:6107–6114, 2009
45. Ingram DA, Yang FC, Travers JB, Wenning MJ, Hiatt K, New S, et al: Genetic and biochemical evidence that haploinsufficiency of the Nf1 tumor suppressor gene modulates melanocyte and mast cell fates in vivo. **J Exp Med** **191**:181–188, 2000
46. Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA: Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. **Nat Genet** **7**:353–361, 1994
47. Jadayel D, Fain P, Upadhyaya M, Ponder MA, Huson SM, Carey J, et al: Paternal origin of new mutations in von Recklinghausen neurofibromatosis. **Nature** **343**:558–559, 1990
48. Jessen KR, Mirsky R: Origin and early development of Schwann cells. **Microsc Res Tech** **41**:393–402, 1998
49. Jhanwar SC, Chen Q, Li FP, Brennan MF, Woodruff JM: Cytogenetic analysis of soft tissue sarcomas. Recurrent chromosome abnormalities in malignant peripheral nerve sheath tumors (MPNST). **Cancer Genet Cytogenet** **78**:138–144, 1994

50. Johannessen CM, Johnson BW, Williams SM, Chan AW, Reczek EE, Lynch RC, et al: TORC1 is essential for NF1-associated malignancies. **Curr Biol** 18:56–62, 2008
51. Johannessen CM, Reczek EE, James MF, Brems H, Legius E, Cichowski K: The NF1 tumor suppressor critically regulates TSC2 and mTOR. **Proc Natl Acad Sci U S A** 102:8573–8578, 2005
52. John AM, Ruggieri M, Ferner R, Upadhyaya M: A search for evidence of somatic mutations in the NF1 gene. **J Med Genet** 37:44–49, 2000
53. Joseph NM, Mosher JT, Buchstaller J, Snider P, McKeever PE, Lim M, et al: The loss of Nf1 transiently promotes self-renewal but not tumorigenesis by neural crest stem cells. **Cancer Cell** 13:129–140, 2008
54. Katz D, Lazar A, Lev D: Malignant peripheral nerve sheath tumour (MPNST): the clinical implications of cellular signaling pathways. **Expert Rev Mol Med** 11:e30, 2009
55. Kim HA, Rosenbaum T, Marchionni MA, Ratner N, DeClue JE: Schwann cells from neurofibromin deficient mice exhibit activation of p21ras, inhibition of cell proliferation and morphological changes. **Oncogene** 11:325–335, 1995
56. Kioussi C, Gruss P: Making of a Schwann. **Trends Genet** 12:84–86, 1996
57. Kluwe L, Friedrich R, Mautner VF: Loss of NF1 allele in Schwann cells but not in fibroblasts derived from an NF1-associated neurofibroma. **Genes Chromosomes Cancer** 24:283–285, 1999
58. Koga T, Iwasaki H, Ishiguro M, Matsuzaki A, Kikuchi M: Losses in chromosomes 17, 19, and 22q in neurofibromatosis type 1 and sporadic neurofibromas: a comparative genomic hybridization analysis. **Cancer Genet Cytogenet** 136:113–120, 2002
59. Korf BR: Neurofibromas and malignant tumors of the peripheral nerve sheath, in Friedman JM, Gutmann DH, MacCollin M (eds): **Neurofibromatosis: Phenotype, Natural History, and Pathogenesis**, ed 3. Baltimore: Johns Hopkins University Press, 1999, pp 142–161
60. Lakkis MM, Epstein JA: Neurofibromin modulation of ras activity is required for normal endocardial-mesenchymal transformation in the developing heart. **Development** 125:4359–4367, 1998
61. Le LQ, Shipman T, Burns DK, Parada LF: Cell of origin and microenvironment contribution for NF1-associated dermal neurofibromas. **Cell Stem Cell** 4:453–463, 2009
62. Lee JK, Sobel RA, Chiocca EA, Kim TS, Martuza RL: Growth of human acoustic neuromas, neurofibromas and schwannomas in the subrenal capsule and sciatic nerve of the nude mouse. **J Neurooncol** 14:101–112, 1992
63. Legius E, Dierick H, Wu R, Hall BK, Marynen P, Cassiman JJ, et al: TP53 mutations are frequent in malignant NF1 tumors. **Genes Chromosomes Cancer** 10:250–255, 1994
64. Leroy K, Dumas V, Martin-Garcia N, Falzone MC, Voisin MC, Wechsler J, et al: Malignant peripheral nerve sheath tumors associated with neurofibromatosis type 1: a clinicopathologic and molecular study of 17 patients. **Arch Dermatol** 137:908–913, 2001
65. Liapis H, Marley EF, Lin Y, Dehner LP: p53 and Ki-67 proliferating cell nuclear antigen in benign and malignant peripheral nerve sheath tumors in children. **Pediatr Dev Pathol** 2:377–384, 1999
66. Luria D, Avigad S, Cohen IJ, Stark B, Weitz R, Zaizov R: p53 mutation as the second event in juvenile chronic myelogenous leukemia in a patient with neurofibromatosis type 1. **Cancer** 80:2013–2018, 1997
67. Marchuk DA, Tavakkol R, Wallace MR, Brownstein BH, Taillon-Miller P, Fong CT, et al: A yeast artificial chromosome contig encompassing the type 1 neurofibromatosis gene. **Genomics** 13:672–680, 1992
68. Martin GA, Viskochil D, Bollag G, McCabe PC, Crosier WJ, Haubruck H, et al: The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p21. **Cell** 63:843–849, 1990
69. Mashour GA, Ratner N, Khan GA, Wang HL, Martuza RL, Kurtz A: The angiogenic factor midkine is aberrantly expressed in NF1-deficient Schwann cells and is a mitogen for neurofibroma-derived cells. **Oncogene** 20:97–105, 2001
70. McGillicuddy LT, Fromm JA, Hollstein PE, Kubek S, Beroukhim R, De Raedt T, et al: Proteasomal and genetic inactivation of the NF1 tumor suppressor in gliomagenesis. **Cancer Cell** 16:44–54, 2009
71. McLaughlin ME, Jacks T: Thinking beyond the tumor cell: NF1 haploinsufficiency in the tumor environment. **Cancer Cell** 1:408–410, 2002
72. Menon AG, Anderson KM, Riccardi VM, Chung RY, Whaley JM, Yandell DW, et al: Chromosome 17p deletions and p53 gene mutations associated with the formation of malignant neurofibrosarcomas in von Recklinghausen neurofibromatosis. **Proc Natl Acad Sci U S A** 87:5435–5439, 1990
73. Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, et al: Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. **Hum Mutat** 15:541–555, 2000
74. Mi R, Ma J, Zhang D, Li L, Zhang H: Efficacy of combined inhibition of mTOR and ERK/MAPK pathways in treating a tuberous sclerosis complex cell model. **J Genet Genomics** 36:355–361, 2009
75. Mozaffari M, Hoogeveen-Westerveld M, Kwiatkowski D, Sampson J, Ekong R, Povey S, et al: Identification of a region required for TSC1 stability by functional analysis of TSC1 missense mutations found in individuals with tuberous sclerosis complex. **BMC Med Genet** 10:88, 2009
76. Muir D: Differences in proliferation and invasion by normal, transformed and NF1 Schwann cell cultures are influenced by matrix metalloproteinase expression. **Clin Exp Metastasis** 13:303–314, 1995
77. Muir D, Neubauer D, Lim IT, Yachnis AT, Wallace MR: Tumorigenic properties of neurofibromin-deficient neurofibroma Schwann cells. **Am J Pathol** 158:501–513, 2001
78. Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hostetter R, Cleary K, et al: Mutations in the p53 gene occur in diverse human tumour types. **Nature** 342:705–708, 1989
79. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al: An integrated genomic analysis of human glioblastoma multiforme. **Science** 321:1807–1812, 2008
80. Peltonen J, Jaakkola S, Lebowitz M, Renvall S, Risteli L, Virtanen I, et al: Cellular differentiation and expression of matrix genes in type 1 neurofibromatosis. **Lab Invest** 59:760–771, 1988
81. Pineda A: Electron microscopy of the lemmocyte in peripheral nerve tumors (neurolemmomas). **J Neurosurg** 25:35–44, 1966
82. Rasmussen SA, Overman J, Thomson SA, Colman SD, Abernathy CR, Trimpert RE, et al: Chromosome 17 loss-of-heterozygosity studies in benign and malignant tumors in neurofibromatosis type 1. **Genes Chromosomes Cancer** 28:425–431, 2000
83. Ratner N, Lieberman MA, Riccardi VM, Hong DM: Mitogen accumulation in von Recklinghausen neurofibromatosis. **Ann Neurol** 27:298–303, 1990
84. Rodenhiser DI, Andrews JD, Mancini DN, Jung JH, Singh SM: Homonucleotide tracts, short repeats and CpG/CpNpG motifs are frequent sites for heterogeneous mutations in the neurofibromatosis type 1 (NF1) tumour-suppressor gene. **Mutat Res** 373:185–195, 1997
85. Sanguinetti C, Greco F, de Palma L, Specchia N, Toesca A, Nori S: The ultrastructure of schwannoma and neurofibroma of the peripheral nerves. **Ital J Orthop Traumatol** 17:237–246, 1991

86. Sawada S, Florell S, Purandare SM, Ota M, Stephens K, Viskochil D: Identification of NF1 mutations in both alleles of a dermal neurofibroma. **Nat Genet** **14**:110–112, 1996
87. Serra E, Ars E, Ravella A, Sánchez A, Puig S, Rosenbaum T, et al: Somatic NF1 mutational spectrum in benign neurofibromas: mRNA splice defects are common among point mutations. **Hum Genet** **108**:416–429, 2001
88. Serra E, Rosenbaum T, Winner U, Aledo R, Ars E, Estivill X, et al: Schwann cells harbor the somatic NF1 mutation in neurofibromas: evidence of two different Schwann cell subpopulations. **Hum Mol Genet** **9**:3055–3064, 2000
89. Sheela S, Riccardi VM, Ratner N: Angiogenic and invasive properties of neurofibroma Schwann cells. **J Cell Biol** **111**:645–653, 1990
90. Stefansson K, Wollmann R, Jerkovic M: S-100 protein in soft-tissue tumors derived from Schwann cells and melanocytes. **Am J Pathol** **106**:261–268, 1982
91. Sternberg PW, Alberola-Ila J: Conspiracy theory: RAS and RAF do not act alone. **Cell** **95**:447–450, 1998
92. Tidyman WE, Rauen KA: The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. **Curr Opin Genet Dev** **19**:230–236, 2009
93. Trahey M, McCormick F: A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. **Science** **238**:542–545, 1987
94. Tucker T, Friedman JM, Friedrich RE, Wenzel R, Fünsterer C, Mautner VF: Longitudinal study of neurofibromatosis 1 associated plexiform neurofibromas. **J Med Genet** **46**:81–85, 2009
95. Upadhyaya M, Han S, Consoli C, Majounie E, Horan M, Thomas NS, et al: Characterization of the somatic mutational spectrum of the neurofibromatosis type 1 (NF1) gene in neurofibromatosis patients with benign and malignant tumors. **Hum Mutat** **23**:134–146, 2004
96. Upadhyaya M, Maynard J, Osborn M, Huson SM, Ponder M, Ponder BA, et al: Characterisation of germline mutations in the neurofibromatosis type 1 (NF1) gene. **J Med Genet** **32**:706–710, 1995
97. Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, Wolff RK, et al: Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. **Cell** **62**:187–192, 1990
98. Viskochil DH: The structure and function of the NF1 gene: molecular pathophysiology, in Friedman JM, Gutmann DH, MacCollin M, Riccardi VM (eds): **Neurofibromatosis: Phenotype, Natural History, and Pathogenesis**, ed 3. Baltimore: Johns Hopkins University Press, 1999, pp 119–141
99. Vlahos CJ, Matter WF, Hui KY, Brown RF: A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). **J Biol Chem** **269**:5241–5248, 1994
100. Vogel KS, Klesse LJ, Velasco-Miguel S, Meyers K, Rushing EJ, Parada LF: Mouse tumor model for neurofibromatosis type 1. **Science** **286**:2176–2179, 1999
101. von Deimling A, Foster R, Krone W: Familial tumour syndromes involving the nervous system, in Kleihues P, Cavenee WK (eds): **Pathology and Genetics: Tumours of the Nervous System**. Lyon: IARC Press, 2000, pp 216–218
102. Waggener JD: Ultrastructure of benign peripheral nerve sheath tumors. **Cancer** **19**:699–709, 1966
103. Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, Saulino AM, et al: Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. **Science** **249**:181–186, 1990
104. Weiss B, Bollag G, Shannon K: Hyperactive Ras as a therapeutic target in neurofibromatosis type 1. **Am J Med Genet** **89**:14–22, 1999
105. Woodruff JM: Pathology of tumors of the peripheral nerve sheath in type 1 neurofibromatosis. **Am J Med Genet** **89**:23–30, 1999
106. Xu GF, Lin B, Tanaka K, Dunn D, Wood D, Gesteland R, et al: The catalytic domain of the neurofibromatosis type 1 gene product stimulates ras GTPase and complements ira mutants of *S. cerevisiae*. **Cell** **63**:835–841, 1990
107. Xu GF, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, et al: The neurofibromatosis type 1 gene encodes a protein related to GAP. **Cell** **62**:599–608, 1990
108. Yan N, Ricca C, Fletcher J, Glover T, Seizinger BR, Manne V: Farnesyltransferase inhibitors block the neurofibromatosis type I (NF1) malignant phenotype. **Cancer Res** **55**:3569–3575, 1995
109. Yang FC, Ingram DA, Chen S, Hingtgen CM, Ratner N, Monk KR, et al: Neurofibromin-deficient Schwann cells secrete a potent migratory stimulus for Nf1+/- mast cells. **J Clin Invest** **112**:1851–1861, 2003
110. Yang FC, Ingram DA, Chen S, Zhu Y, Yuan J, Li X, et al: Nf1-dependent tumors require a microenvironment containing Nf1+/- and c-kit-dependent bone marrow. **Cell** **135**:437–448, 2008
111. Yao R, Cooper GM: Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. **Science** **267**:2003–2006, 1995
112. Yeh TH, Lee Y, Gianino SM, Gutmann DH: Microarray analyses reveal regional astrocyte heterogeneity with implications for neurofibromatosis type 1 (NF1)-regulated glial proliferation. **Glia** **57**:1239–1249, 2009
113. Zheng H, Chang L, Patel N, Yang J, Lowe L, Burns DK, et al: Induction of abnormal proliferation by nonmyelinating schwann cells triggers neurofibroma formation. **Cancer Cell** **13**:117–128, 2008
114. Zhu Y, Ghosh P, Charnay P, Burns DK, Parada LF: Neurofibromas in NF1: Schwann cell origin and role of tumor environment. **Science** **296**:920–922, 2002

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Address correspondence to: William T. Couldwell, M.D., Ph.D., Department of Neurosurgery, University of Utah Medical Center, 175 North Medical Drive East, Suite 3B409, Salt Lake City, Utah 84132. email: neuropub@hsc.utah.edu.