Neurofibromatosis

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Abstract

As familial cancer syndromes, the neurofibromatoses exhibit complex phenotypes, comprising a range of tumor and nontumor manifestations. Although the three recognized forms of neurofibromatosis (NF1, NF2, and schwannomatosis) all feature the development of nervous system tumors, their underlying genetic bases are clearly distinct. The most prominent common feature of all three is the appearance of Schwann cell–initiated tumorigenesis of the peripheral nervous system. Recent progress in delineating the molecular function of the *NF1*- and *NF2*-encoded proteins, together with the development and use of manipulable mouse models, has led to important advances in understanding the pathogenesis of many features of neurofibromatosis. An important outcome of the study of neurofibromatosis-associated tumorigenesis has been insight into the more general molecular and cellular bases of nervous system tumors.

INTRODUCTION

Neurofibromatosis: group of inherited familial cancer syndromes characterized by multiple cutaneous and/or internal tumors derived mostly from peripheral nerves

NF1: neurofibromatosis type 1

NF2: neurofibromatosis type 2

Neurofibromatoses are a group of genetic disorders featuring the development of tumors of the nervous system, particularly of the nerve sheath (1–3). Over the past two decades, the organization and mobilization of national and international networks of clinicians and researchers invested in neurofibromatosis (NF) have facilitated significant advances in understanding the genetic and molecular pathogenesis of NF and in developing diagnostic criteria, management strategies, animal models, and, recently, clinical trials (2, 3). This effort has also yielded the formation of major NF treatment centers across the United States and in Europe, where both management and clinical research efforts are coordinated. Three major forms of NF are recognized as distinct entities on the basis of their genetic origins and pathogenesis: neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2), and schwannomatosis (3, 4). The genetic bases of NF1 and NF2 are known, and they have both been studied extensively. Schwannomatosis was recognized only recently as a distinct disorder, and much less is known about its genetic or molecular basis (4). This review outlines both commonalities and distinctions among the different forms of NF and describes recent advances in understanding the pathogenesis of individual features of NF.

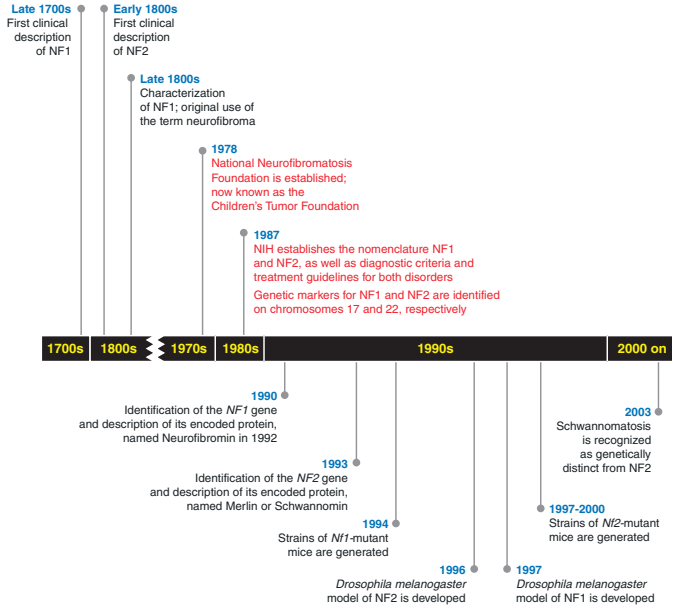

Figure 1

Timeline of historical milestones in neurofibromatosis type 1 and type 2 (NF1 and NF2) research.

General Clinical Features

NF1 was described in the late 1700s, but was first recognized as a disorder and characterized extensively in 1882 by von Recklinghausen (**Figure 1**) (5). NF1 incidence is as high as 1 per 3500 individuals, making it one of the most common genetic disorders among humans (2, 3). Although NF1 is familial, there is a very high incidence of new mutations, likely due to the large size of the *NF1* gene and an unusually high mutation rate associated with the *NF1* locus. The clinical phenotypes associated with NF1 are numerous and include both tumor and nontumor symptoms. The onset and severity of nearly all clinical features of NF1 are age-dependent and extremely variable, even within NF1 families; indeed, the unpredictable expressivity of NF1 is one of the most challenging aspects of NF1 management. The complex diagnostic criteria for NF1 were updated in 1997 (**Figure 2**) (6).

The signature of NF1 is the development of benign neurofibromas, which are mixed tumors composed of all cell types found in the normal peripheral nerve, including Schwann cells (see **Figure 3**) (1, 2). Neurofibromas develop on or around the peripheral nerves, either as encapsulated dermal and subcutaneous masses that pose little clinical threat but can be very disfiguring, or as plexiform neurofibromas that are often congenital and can develop deep within the body near the nerve roots. Plexiform neurofibromas are usually diffuse and tend to expand along large segments of the nerve, often leading to disfigurement and nerve dysfunction (**Figure 4**). Even more insidious, approximately 10% of plexiform neurofibromas progress to malignancy; the

Diagnostic criteria for NF1

An individual having two or more of the following:

Six or more café-au-lait spots:
1.5 cm or larger in individuals past puberty
0.5 cm or larger in individuals before puberty

Two or more neurofibromas of any type, or one or more plexiform neurofibromas

Freckling in the axilla or groin

Optic glioma

Two or more Lisch nodules

A distinct bony lesion, such as dysplasia of the sphenoid bone or dysplasia or thinning of the long bone cortex

A first-degree relative with NF1

Diagnostic criteria for schwannomatosis

An individual over 30 years of age having the following:

Two or more nonintradermal schwannomas, at least one with histological confirmation

No evidence of vestibular tumor on high-quality magnetic resonance imaging scan

No known constitutional *NF2* mutation

or

One pathologically confirmed nonvestibular schwannoma and a first-degree relative meeting the above criteria

Diagnostic criteria for NF2

An individual with the following has definite NF2:

Bilateral vestibular schwannomas **or** family history of NF2 (first-degree relative)

plus

unilateral vestibular schwannoma before age 30 **or** any two of the following:
- — Meningioma
- — Glioma
- — Schwannoma
- — Juvenile posterior subcapsular lenticular opacities

An individual with the following has probable NF2:

Unilateral vestibular schwannoma before age 30 **plus** at least one of the following:
- — Meningioma
- — Glioma
- — Schwannoma
- — Juvenile posterior subcapsular lenticular opacities

or

two or more meningiomas **plus** unilateral vestibular schwannomas before age 30 **or** one of the following:
- — Glioma
- — Schwannoma
- — Juvenile posterior subcapsular lenticular opacities

Figure 2

Current diagnostic criteria for neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2), and schwannomatosis. Adapted from References 4 and 6: MacCollin M, Chiocca EA, Evans DG, Friedman JM, Horvitz R, et al. 2005. Diagnostic criteria for schwannomatosis. *Neurology* 64:1838–45 and Gutmann DH, Aylsworth A, Carey JC, Korf B, Marks J, et al. 1997. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. *JAMA* 278:51–57.

resulting malignant peripheral nerve sheath tumors (MPNSTs) are highly metastatic and essentially incurable. NF1 patients are also predisposed to develop other tumor types, including gliomas, myeloid leukemias, and pheochromocytomas. In each case, loss or mutation of the wild-type *NF1* allele can be detected in the tumor, consistent with the classification of *NF1* as a tumor suppressor gene (7, 8).

Nontumor manifestations of NF1 include abnormal skin pigmentation (café-au-lait spots), learning disabilities, skeletal abnormalities, and visual anomalies (particularly Lisch nodules), any of which can be disfiguring or disabling (1, 2). Increasing evidence suggests that at least some of these phenotypes are due to haplo-insufficiency for *NF1*. Moreover, studies of animal models suggest that *NF1* heterozygosity in

Schwannomatosis: development of multiple schwannomas in patients that neither harbor germ-line mutations in the *NF2* gene nor develop schwannomas of the vestibular nerve

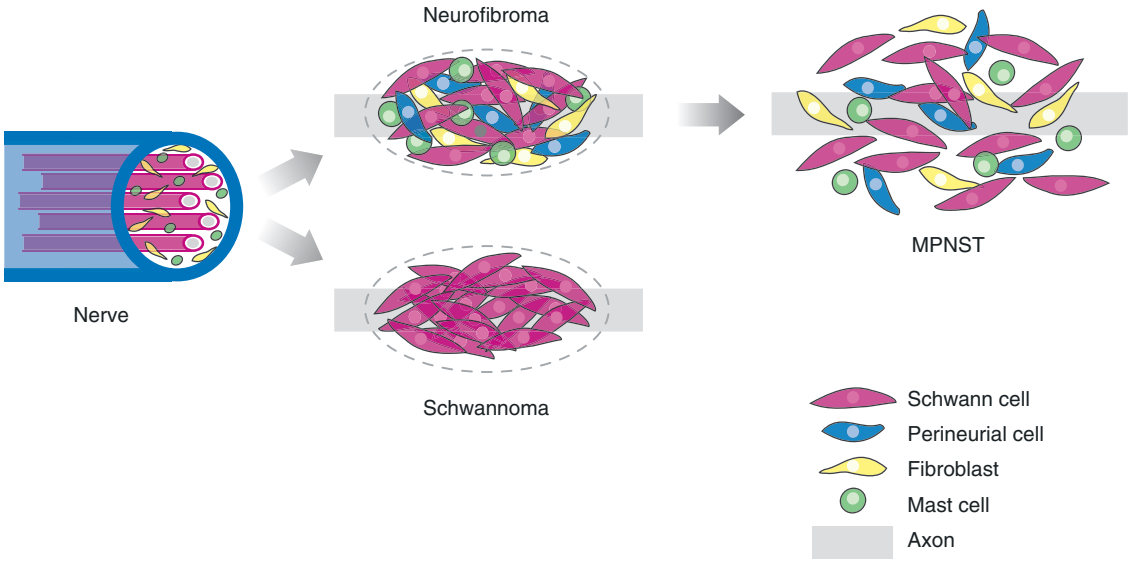

Figure 3

The Schwann cell, which is particularly sensitive to loss of NF1/Neurofibromin and/or NF2/Merlin function, is the cell-of-origin for the neurofibroma, malignant peripheral nerve sheath tumor (MPNST), and schwannoma. Unlike schwannomas, which are comprised of Schwann cells only, neurofibromas contain multiple cell types from the peripheral nerve. Additional mutations promote the progression of neurofibromas to MPNSTs.

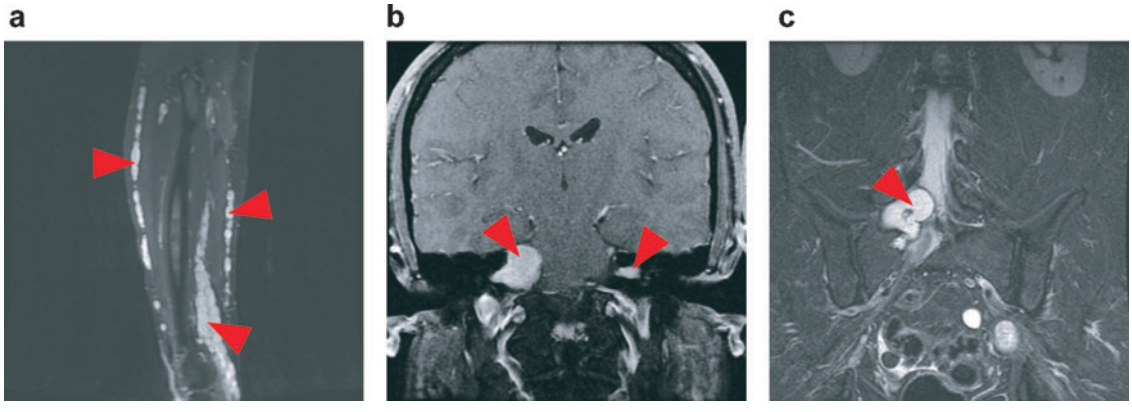

Figure 4

Magnetic resonance imaging of neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2), and schwannomatosis. Coronal images of (*a*) a plexiform neurofibroma of the thigh in an NF1 patient, (*b*) bilateral vestibular schwannomas in an NF2 patient, and (*c*) a spinal schwannoma in a schwannomatosis patient. Arrowheads indicate tumor masses. Images provided by Dr. Scott Plotkin.

surrounding cells plays an obligate role in the formation of neurofibromas and other NF1-associated tumors (see below) (9, 10). Thus, variation in the expression of either the mutant or wild-type *NF1* allele could contribute to the variable expressivity of NF1 symptoms.

NF2, which occurs much less frequently than NF1 (incidence of 1/25,000), was originally described in the 1800s, but it was not

until nearly a century later that NF2 was recognized as a discrete entity (**Figure 1**) (3). The cardinal feature of NF2 is the development of schwannomas on or around the vestibular branches of both auditory nerves (**Figure 4**) (1–3). Loss of hearing is therefore usually the first sign of NF2 and often occurs only when the tumor(s) is relatively large. Many NF2 patients develop additional schwannomas—on other cranial nerves or spinal nerve roots—and meningiomas, benign tumors of the meninges that surround the brain and spinal cord. Although rare, NF2 patients also develop ependymomas and astrocytomas with a much higher frequency than the normal population. Tumors in NF2 patients are uniformly benign and do not progress to malignancy, but they can compress associated nerves, causing considerable pain and nerve dysfunction (schwannomas), or increase cranial pressure, causing a plethora of symptoms including weakness, migraines, and impaired vision, speech, or memory (meningiomas). Finally, many NF2 patients develop posterior subcapsular cataracts, an unusual type of cataract that involves altered cellular architecture rather than crystalline lens opacity. There is variability in the presentation of NF2, but it is not as high as that seen in NF1.

In contrast to neurofibromas, schwannomas are encapsulated tumors composed only of Schwann cells (1, 2). Although most schwannomas in NF2 develop at the boundary between the central and peripheral nervous systems, dermal schwannomas can develop in NF2 and can be confused with cutaneous neurofibromas; histological analysis reveals that these lesions contain only Schwann cells (3). Indeed, the appearance of dermal schwannomas was likely an original source of confusion in distinguishing NF1 and NF2 as distinct disorders.

Schwannomatosis was only recently recognized as a distinct disorder despite recent estimates that its incidence is similar to that of NF2 (1/30,000) (3, 4). This is likely due to the considerable difficulty in distinguishing schwannomatosis from NF2. In fact, schwannomatosis patients develop multiple schwannomas anywhere, except on the vestibular nerve; otherwise, these tumors are indistinguishable from those of NF2 patients (**Figure 4**). Although biallelic *NF2* mutations (and other mutations) are found in schwannomas from schwannomatosis patients, they are not found in normal tissue from the same patient, suggesting that they represent secondary mutations (see below). MacCollin et al. (11) even suggested that the primary genetic mutation that causes schwannomatosis acts by facilitating mutation of the *NF2* locus. The frequency of *NF2* mutation in these tumors suggests that loss of function of the NF2 protein is a key cooperating event in schwannoma formation in these patients. Unraveling the genetic and molecular bases of schwannomatosis will be an important and fascinating endeavor.

Treatment

A particularly poignant consequence of the limited treatment strategies available for NF1 and NF2 is that clinical genetic testing, although now available for both, is not often used in diagnosing NF1 or NF2 (2, 3). In fact, early diagnosis does not influence management; instead, conservative approaches and a strategy of informed vigilance predominate. Treatment of particularly severe tumors involves surgery, often multiple ones. Although benign, the intimate association of neurofibromas and schwannomas with nerves renders them difficult to resect completely, and these tumors often recur. The slow-growing nature of these tumors renders them poor candidates for chemotherapeutic intervention, although radiation therapy is successful in treating some schwannomas. Nevertheless, a key challenge in treating many patients with either disorder is the sheer multiplicity of tumors. Therefore, the development of new therapeutic strategies based on an improved genetic and molecular understanding of NF pathogenesis is an urgent goal of the NF community.

Schwann cell: specialized glial cell that surrounds and electrically insulates the axons in a peripheral nerve

Neurofibroma: benign polyclonal tumor formed by a mixture of Schwann cells, perineurial fibroblasts, and often mast cells, located on or around a peripheral nerve

MPNST: malignant peripheral nerve sheath tumor

Tumor suppressor: any gene/protein whose loss of function results in tumor formation

Schwannoma: benign tumor composed exclusively of Schwann cells that grows on or around a peripheral nerve

GENETICS

The *NF1* and *NF2* Tumor Suppressor Genes

Neurofibromin: protein encoded by the *NF1* tumor suppressor gene; negatively regulates the signaling activity of the small GTPase Ras

Merlin: protein encoded by the *NF2* tumor suppressor gene; inhibits cell proliferation in response to cellular adhesion

The identification of the *NF1* and *NF2* genes in 1990 and 1993, respectively, vaulted NF research to a new level of study (12–16). In addition to raising the possibility of genetic counseling for some NF patients, the identification of the *NF1*- and *NF2*-encoded proteins allowed for studies of the molecular pathogenesis of NF, the generation of animal models that mimic aspects of the human disease, and the possibility of developing targeted therapeutic strategies. Each of these endeavors yielded both expected and unexpected insight into NF. The genetic basis of schwannomatosis has not been elucidated.

Both genes were identified by positional cloning and loss of heterozygosity studies, and can be classified as tumor suppressor genes by genetic criteria (12–16). Thus, NF1 and NF2 patients harbor constitutional heterozygous inactivating mutations at the *NF1* or *NF2* locus, reflecting either inherited or new germline mutations. Somatic mutation of the remaining wild-type allele is presumed to initiate tumor development and can be detected in tumors. In addition, mutations in the *NF1* or *NF2* gene are often found in sporadic versions of the tumors associated with NF1 and NF2. For example, although sporadic neurofibromas are rare, *NF1* mutations have been identified in sporadic MPNSTs and myeloid leukemias (7, 8). Similarly, nearly all sporadic schwannomas and approximately 60% of sporadic meningiomas exhibit biallelic inactivation of *NF2* (17–21). In fact, schwannomas and meningiomas are among the most common brain tumors in humans. In addition to the tumors associated with familial NF2, *NF2* mutations frequently occur in sporadic mesotheliomas, malignant tumors of the lung lining linked to asbestos exposure (17, 21).

The *NF1* gene is extremely large (~350 kb) and encodes a correspondingly large protein, dubbed Neurofibromin (220–280 kDa) (12–14). The large size of the *NF1* gene may help explain the high incidence of mutation at the *NF1* locus; indeed, approximately 50% of NF1 patients represent new mutations without a family history of NF1 (22). Researchers have also proposed that gene conversion involving any of several *NF1* pseudogenes on other chromosomes or intragenic recombination between unusual repeated sequences within the *NF1* gene contributes to a rate of mutation tenfold higher than most other human genes (23, 24). The high mutation rate of the *NF1* gene is evidenced by the recent finding that homozygous mutation of the *Mlh1* mismatch repair gene in humans leads to features of NF1 and to somatic *NF1* mutations (25). Indeed, *Mlh1*-deficiency exacerbates the tumorigenic consequences of heterozygous *Nf1* mutation ($Nf1^{+/-}$) in mouse models (26).

Most *NF1* mutations identified in humans are either small deletions or truncating mutations that completely inactivate the gene (22). Despite the extensive variability in NF1 expressivity, genotype-phenotype correlations have been identified only in patients with particularly severe forms of NF1, who carry large deletions that encompass the entire *NF1* gene plus several adjacent or embedded genes (27). Instead, genetic modifiers that lie outside the *NF1* gene appear to account for a large fraction of the symptomatic variability seen in NF1 (28–30).

The smaller *NF2* gene (110 kb) encodes a 69-kDa protein termed Merlin (also known as Schwannomin) and exhibits a much lower rate of mutation (15, 16). Nevertheless, like NF1, the occurrence of NF2 without family history is common, reflecting a relatively high frequency of new mutation (2). Although there is also considerable variability in NF2 expressivity, there is a strong tendency for disease onset and severity to be similar in NF2 families (31–37). However, neither this apparent genotype-phenotype correlation nor the identity of several missense mutations in the *NF2* gene has yielded clear structure-function insight into the *NF2*-encoded protein.

Evolutionary Conservation

The reduced genome complexity of lower organisms such as the fruit fly *Drosophila melanogaster*, flatworm *Caenorhabditis elegans*, and baker's yeast *Saccharomyces cerevisiae* provides great advantages for studying individual genes. The *NF1* gene is highly conserved evolutionarily, with homologues present in most eukaryotic genomes, including that of the fruit fly and yeast (**Figure 5*a***) (38, 39). As discussed below, studies of fly NF1 have been instrumental in dissecting the major molecular pathways regulated by NF1. Notably, there is no identifiable *NF1* homologue in *C. elegans*, despite conservation of the Ras signaling pathway in which the NF1 protein is thought to function (40).

Interestingly, homologues of *NF2* are restricted to metazoans (multicellular eukaryotes), including the fruit fly and flatworm; there is no *NF2* homologue in yeast (41, 42). This is consistent with a role for the *NF2*-encoded protein, Merlin, in coordinating cell

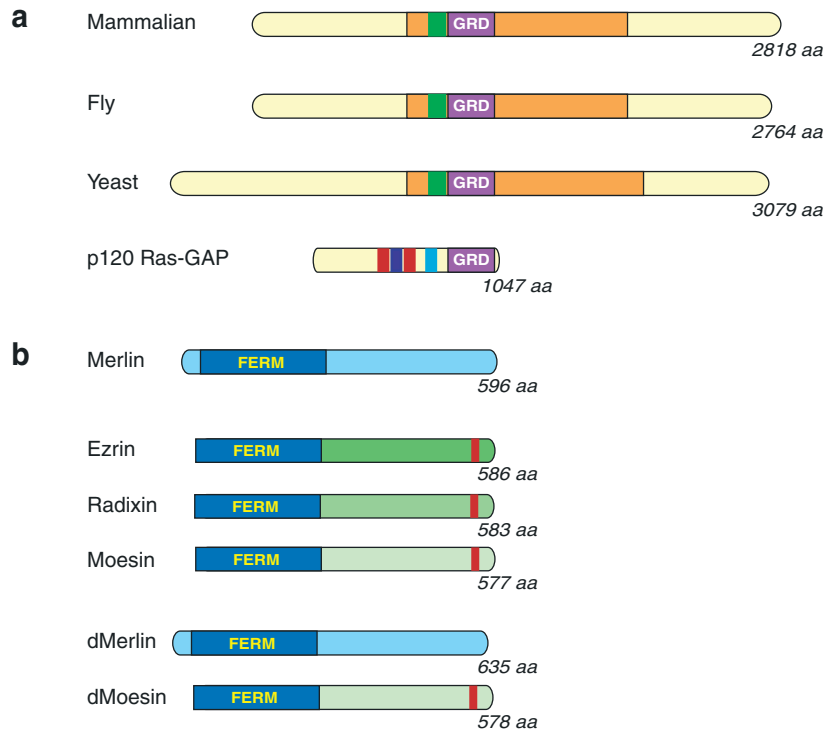

Figure 5

Schematic illustration depicting the organization of the NF1/Neurofibromin and NF2/Merlin proteins and their evolutionary conservation among species. (*a*) The large NF1/Neurofibromin protein is conserved from yeast to mammals. It was recently suggested that, in addition to a central GTPase activating protein–related domain (GRD), an immediately adjacent region of approximately 80 amino acids (aa) (*indicated in green*) controls ubiquitin-mediated degradation of Neurofibromin. Both domains are contained within a region of higher homology (*indicated in orange*) than the flanking parts. This portion encompasses approximately one-third of the protein and is functionally undefined. In comparison, homology with the smaller mammalian p120 GTPase activating proteins for Ras (Ras-GAP) is limited to the GRD. (*b*) NF2/Merlin and the ERM (Ezrin, Radixin, and Moesin) proteins share a highly conserved N-terminal FERM domain (Four-point-one, Ezrin, Radixin, Moesin), whereas the less-conserved C termini of the ERMs contain bona fide actin-binding domains (*red*) not present in Merlin.

proliferation and cell-cell contact, as discussed below. The striking conservation of the *NF2* coding sequence across species suggests conservation of function (**Figure 5*b***). Indeed, somatic inactivation of fly *NF2* (*Mer*) leads to excessive cellular proliferation (43, 44). Studies of fly NF2 have also expanded our understanding of the molecular function of NF2, as discussed below. In fact, the human protein can function reliably in both the mouse and *Drosophila* systems (43). The *C. elegans* homologue *nfm*-1 has been identified, but not extensively studied (45).

Mouse Models of Neurofibromatosis

The mouse *NF1*- and *NF2*-encoded proteins both share 98% amino acid identity with their cognate human proteins (46, 47). The generation and study of mice carrying targeted mutations in the mouse *Nf1* and *Nf2* loci confirmed that both mouse homologues function as tumor suppressor genes and that both the *Nf1*- and *Nf2*-encoded proteins have important roles during embryogenesis (48–52). Although both heterozygous *Nf1*- or *Nf2*-mutant ($Nf1^{+/-}$ or $Nf2^{+/-}$) mice, which should genetically mimic human NF1 and NF2 patients, respectively, are cancer prone, neither spontaneously develops the hallmarks of human NF1 and NF2 (51, 52). However, second-generation mouse models that target *Nf1*- or *Nf2*-deficiency to specific cell types have now modeled most aspects of NF1 and NF2, as described below (52, 53). Collectively, mouse models of NF have been used to address key questions concerning the genetic, molecular, and cellular pathogenesis of NF. These *Nf*-mutant mice are also valuable sources of primary *Nf1*- and *Nf2*-deficient cells for study and vehicles for preclinical testing of targeted therapeutics.

Constitutional inactivation of the mouse *Nf1* gene revealed that, like most tumor suppressors, Nf1 is required for normal embryonic development (48, 49). Homozygous *Nf1*-mutant ($Nf1^{-/-}$) embryos die during midembryogenesis (E13.5, or embryonic day 13.5 of the 19–20 day mouse gestation) owing to a cardiac defect known as double outlet right ventricle. Subsequent studies revealed that this reflects a critical function for Nf1 in the endothelial cells of the endocardial cushion (54). At older ages (~18 months), $Nf1^{+/-}$ mice are prone to developing some of the tumors seen in NF1 patients, notably pheochromocytoma and myeloid leukemia; both exhibit loss of the wild-type *Nf1* allele (49).

Targeted inactivation of the mouse *Nf2* gene revealed a role for Nf2 at an even earlier stage of embryonic development (50). Homozygous *Nf2*-mutant ($Nf2^{-/-}$) embryos fail very early during development (E7.0) without gastrulating. Developmental failure in these embryos appears to be due to a lack of extraembryonic ectoderm, without obvious overproliferation of any cell compartment. Mosaic embryos partially composed of $Nf2^{-/-}$ cells evade this defect but exhibit defects in other tissues, suggesting multiple roles for normal Nf2 function during embryogenesis (I. Saotome & A.I. McClatchey, unpublished observations). $Nf2^{+/-}$ mice predominantly develop osteosarcoma and liver tumors (both hepatocellular carcinoma and cholangiocarcinoma) late in life (12–24 months of age) (50, 52). Notably, these mice also develop a number of other tumor types with lower frequency, including a range of soft tissue sarcomas such as chondrosarcoma, fibrosarcoma, and rhabdomyosarcoma. The frequent loss of the wild-type *Nf2* allele in all these tumor types suggests that *Nf2* loss contributed to their formation. Moreover, the high rate of metastasis exhibited by many of these tumors suggests that *Nf2* loss can also promote metastatic progression (51). Thus, impaired NF2 function may play a broader role in cancer development than originally predicted. Although *NF2* mutations have not been described in human tumors of these types, accumulating evidence suggests that elimination of NF2 function can occur via other mechanisms, including epigenetic regulation of the *NF2* gene or post-translational inactivation of the *NF2*-encoded

protein (55–57). Deregulation of NF2 signaling may also occur at other steps in the pathway (see below).

Genetic Cooperativity

To determine whether inactivation of other tumor suppressor genes might cooperate with loss of *Nf1* or *Nf2* to accelerate tumorigenesis or elicit the hallmarks of NF1 and NF2 in mice, *Nf1*- and *Nf2*-mutant mice were crossed with mice carrying other tumor suppressor gene mutations (51, 58–60). Such studies of genetic cooperativity yielded important insight into both specific and general genetic influences on NF-associated tumorigenesis. In contrast to humans, the *Nf1*, *Nf2*, and *p53* tumor suppressor loci are all genetically linked on the long arm of mouse chromosome 11 (**Figure 6*a***). Compound heterozygotes ($Nf1^{+/-}$;$p53^{+/-}$, $Nf2^{+/-}$;$p53^{+/-}$, and $Nf1^{+/-}$;$Nf2^{+/-}$) were engineered with both the linked (*cis*) and unlinked (*trans*) configuration of mutant alleles (51, 58–60). In each case, the *cis* configuration (linkage of the tumor suppressor mutations) led to a markedly more severe tumor phenotype than that of either the *trans* or singly heterozygous mutant configuration. Thus, $Nf1^{+/-}$;$p53^{+/-}$ *cis* mice predominantly develop aggressive MPNSTs and malignant astrocytomas at 6–12 months of age, most of which exhibit loss of both wild-type *Nf1* and *p53* alleles presumably due to loss of the wild-type chromosome (**Figure 6*b***) (58–60). Consistent with the idea that variable NF1 expressivity is at least partly due to genetic modification, the penetrance of both MPNSTs and astrocytomas in these mice varies greatly depending upon the genetic background (60). In fact, recent studies have identified a locus linked to *Nf1* and *p53* on mouse chromosome 11 that dominantly modifies the susceptibility to both astrocytomas and MPNSTs (61, 62). Importantly, this locus is also imprinted, yielding allele-of-origin effects on tumor susceptibility (61).

Analogous work revealed that inactivation of *p53* also cooperates with *Nf2* loss in

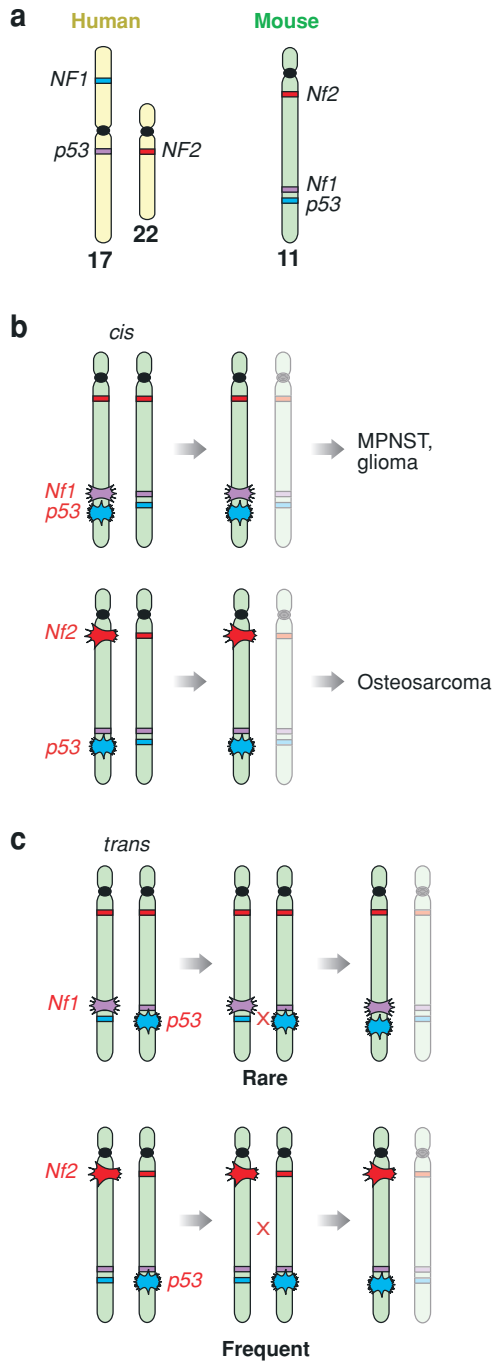

Figure 6

Location of the mouse and human *NF1*, *NF2*, and *p53* tumor suppressor loci. Also depicted are the genetic steps and consequences of cooperativity between mutations in the *Nf1*, *Nf2*, and *p53* tumor suppressor loci (see text). MPNST, malignant peripheral nerve sheath tumor.

Ras-GAP: GTPase activating protein for Ras

tumorigenesis in the mouse; *Nf2*$^{+/-}$;*p53*$^{+/-}$ *cis* mice develop multiple osteosarcomas that also show loss of both wild-type *Nf2* and *p53* alleles by 3–4 months of age (51). Notably, *Nf2*$^{+/-}$;*p53*$^{+/-}$ *trans* mice also develop osteosarcomas earlier than *Nf2*$^{+/-}$ or *p53*$^{+/-}$ mice; surprisingly, these tumors also exhibit loss of both wild-type *Nf2* and *p53* alleles, suggesting a two-step mechanism whereby somatic recombination yielding a *cis* configuration is followed by loss of the wild-type chromosome (**Figure 6*c***). In contrast, tumors that develop in *Nf1*$^{+/-}$;*p53*$^{+/-}$ *trans* mice exhibit loss of either the wild-type *Nf1* or *p53* allele, but not both (58, 59). This can be explained by the much tighter linkage between *Nf1* and *p53* (~4 centimorgans apart) than between *Nf2* and *p53* (~40 centimorgans) (**Figure 6*c***), which dictates the rate of somatic recombination between loci. These data underscore the influence of genetic linkage of cancer predisposing or modifying mutations.

Remarkably, *Nf1*$^{+/-}$;*Nf2*$^{+/-}$ *cis* mice do not develop osteosarcomas but instead develop tumors classified as schwannomas, both immunohistochemically and ultrastructurally, by 6–18 months of age (63; C.-H. Liu & A.I. McClatchey, unpublished observations). The tight linkage of the *Nf1* and *p53* loci to one another strongly argues against a genetic basis for the differing tumor spectra in *Nf2*$^{+/-}$;*Nf1*$^{+/-}$ *cis* and *Nf2*$^{+/-}$;*p53*$^{+/-}$ *cis* mice. Instead, these models support the hypothesis that loss of *Nf1* cooperates with *Nf2* deficiency in tumorigenesis derived from Schwann cells but not osteoblasts, thereby reflecting cell-type-specific cooperativity. Additional studies of *Nf2/p53*- and *Nf1/p53*-mediated tumorigenesis suggest that the timing of *Nf2* or *Nf1* inactivation relative to that of *p53* dictates the tumor phenotype, likely also reflecting context-dependent cooperativity (64, 65).

Co-inactivation of the *Nf1*, *Nf2*, and/or *p53* tumor suppressor genes is facilitated by their linked organization on mouse chromosome 11. Furthermore, Eden et al. (66) have recently shown that genome-wide hypomethylation promotes tumor development in *Nf1*$^{+/-}$;*p53*$^{+/-}$ *cis* mice by increasing the rate of loss of the wild-type chromosome. Thus, the unique configuration of chromosome 11 may elicit genetic interactions not mimicked during human NF-associated tumorigenesis. However, these studies identified context-dependent cooperation between the NF1, NF2, and p53 tumor suppressor pathways, which is important in delineating the molecular pathogenesis of NF1 and NF2. These studies also highlight the complexity of genetic and epigenetic influences on the process of tumorigenesis and suggest that genome architecture and the distinct configurations of the mouse and human genomes have a profound impact on tumorigenesis in both species.

FUNCTIONS OF THE *NF1*- AND *NF2*-ENCODED PROTEINS

NF1/Neurofibromin and Ras Signaling

The central portion of Neurofibromin contains a small domain that bears striking homology to negative regulators of the small GTPase Ras (**Figure 5*a***). So-called GTPase activating proteins for Ras (Ras-GAPs) retard Ras signaling by enhancing its intrinsic GTPase activity, thereby promoting the inactive GDP-bound state (67). This suggests that Neurofibromin functions as a tumor suppressor by negatively regulating mitogenic Ras signaling and that the inability to downregulate Ras is central to NF1-associated tumorigenesis (**Figure 7**). Indeed, elevated levels of Ras-GTP and of Ras pathway signaling have been measured in *NF1*-mutant tumors, and Neurofibromin functions as a Ras-GAP in vivo and in vitro (68). However, this domain represents only a small portion (~12%) of the Neurofibromin protein. Indeed, although multiple studies now suggest that the Ras-GAP function of Neurofibromin is necessary in some settings for its tumor suppressor function, it is unclear whether

the remainder of Neurofibromin performs other critical functions or provides regulatory and/or localization-determining information. In fact, almost nothing is known about the 88% of Neurofibromin that lies outside of the GTPase activating protein–related domain (GRD).

The strongest support for functions of Neurofibromin beyond Ras regulation comes from studies of *Drosophila NF1*. Homozygous inactivation of *Drosophila NF1* yields viable flies, but ones that are abnormally small and exhibit electrophysiological, learning, and circadian defects (39, 69–71). Only the circadian defect was rescued by genetic reduction of Ras signaling (71). The other defects were rescued instead by increasing adenylyl cyclase (AC)–cyclic adenosine-3′,5′-monophosphate (cAMP)-protein kinase A signaling (39, 69, 70). A role for Neurofibromin in regulating AC activity and cAMP levels has also been demonstrated in mammalian cells (72–74). Notably, Schwann cells are one of the few cell types that require cAMP signaling for proliferation; in many cell types, increased cAMP signaling is instead associated with growth arrest and differentiation (75). Dual roles in regulating both Ras signaling and cAMP production could explain why Neurofibromin function is so important for regulating Schwann cell proliferation. Notably, these studies do not rule out the possibility that in some contexts, aberrant Ras activity inhibits AC. In fact, a recent study suggested that fly NF1 can stimulate AC activity in either a Ras-dependent or -independent manner (76).

The activation of Ras downstream of growth factor receptor signaling is well established, although distinct pools of Ras are likely activated by subsets of receptors (77). Moreover, there are more than 20 Ras-GAPs in the human genome (67). Hence, it is unlikely that loss of NF1 results in a generalized activation of Ras signaling; rather, the NF1-regulated pool of Ras is likely to control a specific signaling output. Activation of the Raf/MEK, PI3K/AKT, and Rac pathways are the best-studied consequences of activated

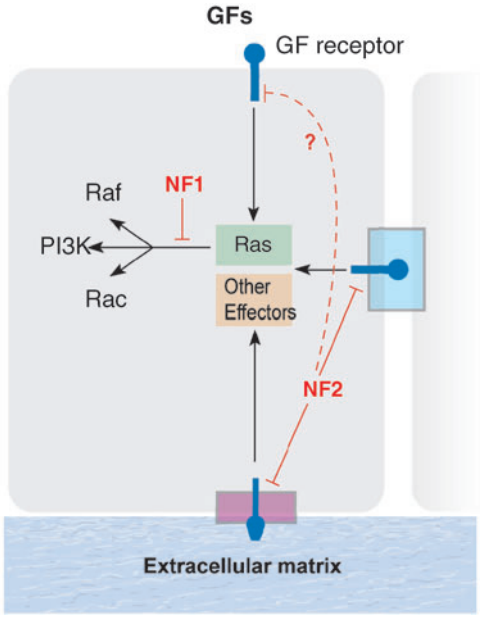

Figure 7

Working model depicting the roles of NF1/Neurofibromin and NF2/Merlin in negatively regulating signaling downstream of growth factor (GF) receptors. Ras signaling output in response to extracellular signals is enhanced in the absence of NF1. NF2 appears to coordinate negative regulation of transmembrane receptor signaling with the formation and maintenance of cell-cell or cell-extracellular matrix adhesive structures. Unlike NF1, loss of NF2 is likely to deregulate the function of multiple receptor effectors, including Ras. PI3K, phosphatidylinositol 3-kinase.

Ras signaling, and all are elevated in *Nf1*$^{-/-}$ cells (**Figure 7**) (68). However, it is not clear which effector pathways are critical for NF pathogenesis. Recent studies suggest that elevated activity of mTOR (mammalian target of rapamycin) is a critical consequence of *Nf1* deficiency (78, 79). These studies clearly established that mTOR is a target of Nf1-regulated Ras signaling, and raised the possibility that the mTOR inhibitor rapamycin might yield therapeutic benefit. Indeed, rapamycin blocks the proliferation of *NF1*$^{-/-}$ primary and tumor cells (78). Rapamycin and its derivatives have been heralded as potential therapeutic agents for several other cancers, which could facilitate an investigation of its efficacy in NF1.

GRD: GTPase activating protein–related domain

Given the extensive evidence suggesting that *Nf1* haplo-insufficiency plays a critical

ERM: Ezrin, Radixin, and Moesin

FERM: Four-point-one, Ezrin, Radixin, Moesin

role in the pathogenesis of several clinical features of NF1, strategies that increase Neurofibromin activity or protein levels could have important clinical benefit not just for NF1, but also for other cancers exhibiting deregulated Ras signaling. Until recently, little was known about how Neurofibromin levels or activity are regulated. Neurofibromin is dynamically regulated by ubiquitin-mediated proteolysis (80). Sequences immediately adjacent to the GRD appear to be required for Neurofibromin degradation, indicating the first clear function for a region of Neurofibromin outside the GRD (**Figure 5*a***). Unlike many other examples of ubiquitin-mediated degradation, this does not appear to be initiated by direct phosphorylation of Neurofibromin (81). Identification of the precise signals that control proteolysis of Neurofibromin may also provide new therapeutic opportunities.

NF2/Merlin and Membrane Organization

The *NF2*-encoded protein, Merlin, is closely related to the ERM (Ezrin, Radixin, and Moesin) proteins, which are thought to organize specific membrane domains by providing regulated linkage between membrane proteins and the actin cytoskeleton (**Figure 5*b***) (41, 82). Early studies established that Merlin, like the ERM proteins, localizes to areas of cortical cytoskeleton remodeling, including cell-cell boundaries and membrane ruffles (83–85). These studies also established that Merlin inhibits the proliferation of both normal and oncogene-transformed cells (86, 87). However, the localization of Merlin to the membrane-cytoskeleton interface is novel among tumor suppressors. From this location, Merlin is poised to modulate the transmission of mitogenic signals from the extracellular environment (**Figure 7**).

Central to the function of Merlin and ERM proteins is their ability to undergo regulated intramolecular self-interaction, which, in turn, controls their localization (41, 83, 88–90). Molecular and structural studies reveal that all four proteins can be roughly superimposed, with a trilobed amino (N)-terminal FERM (Four-point-one, Ezrin, Radixin, Moesin) domain and a carboxyl (C)-terminal domain that can extend across the FERM domain surface, potentially masking the binding sites of other proteins (91, 92). Contrasting models of Merlin and ERM regulation by conformation-dependent changes have been developed and involve reciprocal relationships with members of the Rho family of small GTPases (93). The best-studied mechanism of ERM activation is via activation of the small GTPase RhoA, which induces phosphorylation of the ERM C terminus, challenging N- to C-terminal interaction and effecting translocation to the membrane-cytoskeleton interface. Studies of the single *Drosophila* ERM protein, Moesin, reveal that it can also negatively regulate Rho activity in a feedback regulatory mechanism (94). In contrast, Merlin is regulated by the Rho GTPase Rac1; Rac-induced phosphorylation of Merlin leads to diminished self-association, translocation away from the insoluble compartment, and inactivation of Merlin (57). A reciprocal relationship between Merlin and Rac also appears to exist. Thus, Merlin can negatively regulate Rac-dependent signaling in a feed-forward mechanism. Recent studies have shown that the serine/threonine kinase Pak can mediate Rac-induced phosphorylation of Merlin and that Merlin can bind to and directly inhibit Pak (95–97). Therefore, Rac and Pak may be important therapeutic targets for NF2.

Merlin's molecular function is not well understood. It appears capable of interacting with many different proteins and may associate with several larger molecular complexes. However, it is not yet clear which interactors or complexes are relevant to the pathogenesis of NF2. Indeed, a key question in the field is whether Merlin's association with one particular complex is critical for tumor suppression or whether its association with multiple complexes collectively imparts tumor suppression.

A number of studies suggest that Merlin can control contact-dependent inhibition of proliferation in several cell types (85, 96, 98). Merlin may regulate the poorly understood phenomenon of contact-dependent inhibition of proliferation through interaction with the extracellular matrix (ECM) receptor CD44 in some cell types (98). Alternatively, *Nf2*-deficient cells of several types lack stable cadherin-containing cell junctions known as adherens junctions (AJs) (85). Merlin can localize to AJs and physically associate with AJ components. Merlin may function to stabilize large actin-cytoskeleton-associated membrane signaling complexes such as the AJ or CD44-containing cell-ECM attachment complexes by locally inhibiting Rac-Pak signaling.

Several lines of evidence suggest that Merlin can regulate receptor tyrosine kinase activity and perhaps trafficking. Merlin can reportedly interact physically with several proteins involved in growth factor receptor signaling, including PDZ-domain-containing adaptors such as EBP50/NHE-RF1, that may control the membrane distribution of receptor tyrosine kinases (99–103). The reported localization of Merlin to vesicular structures and/or to lipid rafts further supports a role in the distribution or trafficking of membrane receptors (104, 105). Consistent with this model, recent studies uncovered a role for *Drosophila* Merlin in regulating the abundance and turnover of signaling and adhesion receptors (106). Together with the aforementioned role for Merlin in cell adhesion, these data suggest that Merlin may control contact-dependent inhibition of proliferation by coordinating the turnover of adhesion structures and growth factor receptors (**Figure 7**). This would be consistent with the observation that Merlin both controls AJ stability and prevents Epidermal Growth Factor Receptor internalization specifically in contacting cells (M. Curto & A.I. McClatchey, unpublished observations). Similarly, Merlin could coordinate cell-ECM attachment and proliferation control (82).

PATHOGENESIS OF SPECIFIC LESIONS

Advances in delineating the molecular function of NF1 and NF2, together with the availability and manipulability of *Nf1*- and *Nf2*-mutant strains of mice, have led to a much clearer understanding of the pathogenesis of specific features of NF1 and NF2 as described below.

AJ: adherens junction

Neurofibromatosis Type 1 Pathogenesis

Neurofibromas. Neurofibromas are complex tumors, composed of all of the cell types found in the peripheral nerve, including Schwann cells, neuronal processes, perineurial fibroblasts, and mast cells (**Figure 3**) (1, 2). A key question is whether neurofibroma formation is driven by loss of *NF1* in one or more cell types. Studies of cells cultured from neurofibromas identified *Nf1*-deficiency in Schwann cells (107, 108). However, direct evidence that the Schwann cell is the cell-of-origin for neurofibromas emerged from the manipulation of mouse models. Although $Nf1^{+/-}$ mice do not spontaneously develop neurofibromas or MPNSTs, Cichowski et al. (58) generated chimeric mice composed partly of $Nf1^{-/-}$ cells and found that they developed multiple plexiform neurofibromas derived largely from $Nf1^{-/-}$ cells. Tumor abundance in these mice correlated with the extent of chimerism. This seminal study indicated that the reason why $Nf1^{+/-}$ mice did not develop neurofibromas was that the loss of the wild-type *Nf1* allele was rate-limiting. Although this study was the first to model neurofibromas in the mouse, it did not reveal the cell-of-origin of neurofibromas. Furthermore, these mice did not develop the cutaneous neurofibromas that are the hallmark of NF1.

To test the hypothesis that *Nf1*-deficiency in Schwann cells is required for neurofibroma formation, Zhu et al. (9, 53) generated a strain of mice carrying a conditional *Nf1*-mutant

allele. These mice were mated with transgenic *Krox20-Cre* mice, in which expression of the Cre recombinase is restricted to Schwann cells in the peripheral nervous system (109, 110); Cre-mediated recombination led to *Nf1* inactivation, specifically in these cells. Importantly, these investigators examined both $Nf1^{lox/-}$;*Krox20-Cre* and $Nf1^{lox/lox}$;*Krox20-Cre* mice and found that plexiform neurofibromas developed only in $Nf1^{lox/-}$;*Krox20-Cre* mice (9). This study demonstrated that the Schwann cell is the cell-of-origin for neurofibroma and suggested that *Nf1* inactivation in Schwann cells is sufficient for neurofibroma formation only when combined with *Nf1* heterozygosity in the tumor environment. They went on to suggest that the recruitment of $Nf1^{+/-}$ mast cells to the tumor may play a central role in mediating this effect, consistent with studies indicating that *Nf1* haploinsufficiency leads to hyperproliferation of mast cells (111). Indeed, accumulating evidence suggests that haplo-insufficiency for *Nf1* and for other tumor suppressors plays an unappreciated role in tumorigenesis (112).

MPNSTs. Both genetic and pathological studies indicate that MPNSTs develop as a result of the malignant progression of neurofibromas, a process clearly involving additional genetic mutation (68). Studies of human tumors and animal modeling both indicate that loss of the *p53* tumor suppressor gene is a common event that cooperates with *NF1* loss in MPNST progression (58, 59, 113, 114). In addition, mutations in the *Ink4a* locus, which encodes both the p16/Ink4a and p14ARF tumor suppressors, have also been identified in human MPNSTs (115–117). The established functions of p16/Ink4a as a negative regulator of the cell cycle machinery and p14ARF as an activator of p53 suggest molecular explanations for their role in malignant progression to MPNSTs (118). Finally, some evidence suggests that amplification or increased expression of the Epidermal Growth Factor Receptor, which is expressed at very low levels if at all in primary or neurofibroma-derived Schwann cells, may play a causal role in MPNSTs (119).

Glial tumors. A recent series of papers describes important advances in understanding the pathogenesis of NF1-associated glial tumors through mouse modeling. Approximately 15% of children with NF1 develop optic gliomas, which are low-grade (pilocytic) astrocytomas that develop around the optic nerve and can cause vision abnormalities and/or precocious puberty owing to disturbance of the nearby hypothalamus (1–3). Although rare, adult NF1 patients do develop high-grade astrocytomas more frequently than normal individuals. Although $Nf1^{+/-}$ mice do not develop low- or high-grade astrocytomas, $Nf1^{+/-}$;$p53^{+/-}$ *cis* mice develop high-grade astrocytomas with variable penetrance (60). However, tumors do not develop around the optic nerve in these mice, and tumors that arise elsewhere in the brain do so without progression from low-grade astrocytoma, thereby modeling human secondary glioblastoma. This is consistent with the identification of *p53* mutations in human malignant astrocytomas (120).

The consequences of inactivating *Nf1* specifically in astrocytes in vivo were investigated by crossing conditional $Nf1^{lox/lox}$ mice with **GFAP-Cre* transgenic mice in which expression of the Cre recombinase is restricted to astrocytes beginning at E14.5 (121). The study of these mice revealed that astrocyte-specific loss of Nf1 leads to increased proliferation, but is not sufficient for tumor formation. Subsequent studies revealed that *Nf1*-deficient astrocytes in the context of $Nf1^{+/-}$ surrounding cells do form optic glioma; several lines of evidence suggest that tumorigenesis in these models requires *Nf1* haplo-insufficiency specifically in neurons (10). This is consistent with the observation that neuron-specific inactivation of *Nf1* yields astrogliosis, likely owing to paracrine signaling (53). Importantly, $Nf1^{lox/lox}$;*hGFAP-Cre* mice, in which Cre is expressed earlier in development (E10.5) both in astrocytes and

in neural stem/progenitor cells, develop optic gliomas regardless of the genotype of surrounding cells (122). This may indicate that progenitor cells are the cell-of-origin for optic glioma and are particularly sensitive to loss of *Nf1*; alternatively, *Nf1*-deficiency in early glial/neuronal progenitors may give rise to both $Nf1^{-/-}$ astrocytes and differentiated $Nf1^{-/-}$ neurons that, in turn, provide paracrine signals that promote $Nf1^{-/-}$ astrocytoma formation.

Myeloid leukemias. Although only a small percentage of NF1 children develop juvenile myelomonocytic leukemia (JMML), this represents a 200–500-fold increase in incidence relative to normal children (123). Moreover, JMML is a severe manifestation of NF1, requiring hematopoietic stem cell transplantation. The study of NF1-associated JMML has revealed key insights into the molecular pathogenesis of sporadic JMML and other leukemias. The discovery of loss of the wild-type *NF1* allele, decreased NF1-specific GAP activity, increased Ras-GTP levels, and signaling in primary leukemia cells from NF1 children, suggested that hyperactive Ras signaling is responsible for malignancy in this setting (8, 124–126). Indeed, activating mutations in either *NRAS* or *KRAS2* are found in approximately 25% of sporadic JMMLs (127, 128). This model was further supported by the identification of gain-of-function mutations in the *Ptpn11* gene in Noonan syndrome patients, who are also predisposed to develop JMML (129). *Ptpn11* encodes SHP-2, a phosphatase that positively regulates signaling downstream of growth factor receptors (130). The hypersensitivity of JMML cells to the growth factor granulocyte-macrophage colony stimulating factor (GM-CSF) suggests that the inability to attenuate Ras signaling downstream of the GM-CSF receptor is a critical initiating step in myeloid leukemia (**Figure 8**) (131). This hypothesis was tested using *Nf1*-mutant strains of mice. Although some $Nf1^{+/-}$ mice develop myeloid leukemia, they do so with long latency. To better model NF1-associated leukemia in the mouse, Largaespada et al. (131) transplanted fetal liver cells from $Nf1^{-/-}$ embryos, which are also hypersensitive to GM-CSF, into irradiated recipients (132). This yielded a highly reproducible model of myeloid leukemia that exhibited hyperactive Ras. Importantly, leukemogenesis in these mice was markedly attenuated upon genetic elimination of GM-CSF (133). These mice were used as vehicles for preclinical testing of farnesyl transferase inhibitors, which should block the posttranslational processing and function of Ras (134); although the farnesyl transferase inhibitor used in this study neither inhibited KRAS or NRAS processing nor retarded leukemogenesis, the study highlights the utility of developing

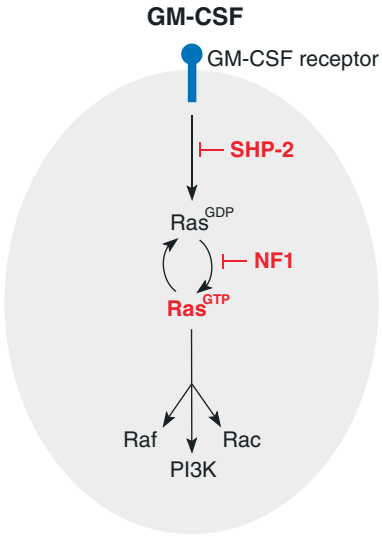

Figure 8

The study of neurofibromatosis type 1–associated leukemia helped uncover the critical role of Ras signaling in sporadic myeloid leukemias. Most sporadic myeloid malignancies exhibit mutations that inactivate *Nf1*, activate *RAS*, or activate *Ptpn11* (which encodes the SHP-2 phosphatase). Each of these mutational events results in increased Ras signaling. The inability to attenuate Ras signaling downstream of the granulocyte-macrophage colony stimulating factor (GM-CSF) receptor appears to be a critical step in leukemogenesis. PI3K, phosphatidylinositol 3-kinase.

genetically defined preclinical mouse models. A more tractable model for preclinical testing was developed through conditional inactivation of *Nf1* in adult hematopoietic cells by crossing $Nf1^{lox/lox}$ mice with mice carrying the interferon-inducible *Mx1-Cre* transgene (135).

Learning and memory. Cognitive defects are a challenging aspect of NF1 management, with nearly half of NF1 children exhibiting some type of learning disability (2, 3). Although the underlying cause of cognitive impairment in NF1 is not known, increasing evidence suggests that it may reflect abnormalities in neuronal networking during development owing to NF1 haploinsufficiency and excess Ras signaling. As for many other aspects of NF pathogenesis, the strongest evidence comes from studies of *Nf1*-mutant mice. Researchers initially appreciated that $Nf1^{+/-}$ mice exhibit marked defects in learning and memory that are rescued by diminishing *KRAS* gene dosage (136, 137). Learning deficits in $Nf1^{+/-}$ mice appear to result from increased Ras signaling in a subset of neurons, which, in turn causes increased production of GABA (λ-amino butyric acid), a neurotransmitter that acts negatively on a second set of neurons that function in long-term potentiation (learning and memory) (137). Importantly, treatment with lovastatin, an inhibitor of the rate-limiting enzyme in cholesterol biosynthesis that inhibits Ras farnesylation and activity, corrected the learning deficits in $Nf1^{+/-}$ mice (138). This study again highlights the utility of *Nf1*-mutant mice in preclinical testing and raises the possibility that lovastatin, a widely prescribed and well-tolerated drug, could be of clinical benefit for cognitive aspects of NF1.

Neurofibromatosis Type 2 Pathogenesis

Schwannomas. Schwannomas are composed entirely of Schwann cells (1–3). In both NF2 and schwannomatosis patients, schwannomas tend to develop at the boundary of the central and peripheral nervous systems rather than at the periphery, which may reflect their origin in a particular subset of Schwann cells (or Schwann cell precursors) or their requirement for a particular microenvironment that promotes $Nf2^{-/-}$ Schwann cell proliferation. $Nf2^{+/-}$ mice do not spontaneously develop schwannomas (51, 52), but targeted inactivation of *Nf2*, specifically in Schwann cells and in a subset of neural crest cells, leads to Schwann cell hyperproliferation and some frank tumors (52). The incomplete penetrance of frank tumors in this model suggests that additional mutations that cooperate with *Nf2* loss may be required. Indeed, mathematical modeling indicates that at least one mutational event in addition to biallelic inactivation of *NF2* is necessary for human schwannoma formation (139).

As for the neurofibroma in NF1, a key goal in the study of NF2-associated tumorigenesis is identifying the cell-of-origin of the schwannoma. During development, neural crest–derived Schwann cell precursors migrate from the dorsal neural tube to positions along developing axon bundles (140). These immature Schwann cells become either myelinating or nonmyelinating Schwann cells that differ in their association with peripheral axons. As they interact with and insulate peripheral axons, maturing Schwann cells assume an exquisite degree of compartmentalization that is coordinated by intra- and intercellular junctions. Studies of cultured $Nf2^{-/-}$ primary or schwannoma-derived Schwann cells reveal actin cytoskeleton defects, increased Rac activity, and loss of contact-dependent inhibition of proliferation, suggesting that the molecular signatures of *Nf2*-deficiency in other cell types are relevant to the study of schwannoma formation (141, 142; W.F. Chan & A.I. McClatchey, unpublished observations).

Meningiomas. In humans, three grades of sporadic meningioma are recognized (grades

I–III); NF2 patients develop almost exclusively benign grade I and II meningiomas (3, 143). To generate a model of *Nf2*-associated meningioma development, Kalamarides et al. (144) injected adenovirus expressing the Cre recombinase into the forebrain of $Nf2^{lox/lox}$ mice. These mice exhibited biallelic inactivation of *Nf2* in arachnoid cells and developed meningiomas histologically similar to those of human NF2 patients. The mice provide a valuable model for studying the cellular origin of meningioma. Moreover, because primary arachnoid cells are extremely difficult to culture and few meningioma cell lines exist, the ability to establish meningioma cell lines from these mice will be a powerful tool for investigating the molecular and cellular basis of meningioma development.

Mesothelioma. Somatic inactivation of *NF2* is frequently observed in sporadic mesothelioma, an aggressive tumor associated with environmental exposure to asbestos (145). Although mesothelioma is not a recognized feature of familial NF2, Baser et al. (146) recently suggested that NF2 patients exposed to asbestos might be at a higher risk of developing malignant mesothelioma. Two groups recently reported that $Nf2^{+/-}$ mice, which do not spontaneously develop mesothelioma, are particularly susceptible to mesothelioma upon exposure to asbestos fibers via peritoneal injection (147, 148). Both studies reported loss of the wild-type *Nf2* allele in primary cultures or cell lines established from the mesothelial tumors. In one study, mutations at the *Ink4a* and *p53* tumor suppressor loci were frequently identified in mesothelioma cells (147); this is consistent with reports of mutations in the corresponding loci in human mesothelioma and suggests that these represent cooperating events (145). As for meningioma, these models, and cells derived from them, represent important tools for delineating the pathogenesis of mesothelioma.

SUMMARY POINTS

1. NF1, NF2, and schwannomatosis are inherited cancer syndromes that feature the development of tumors of the nervous system; however, the genetic bases and pathogenesis of these disorders are distinct.
2. Genetic studies using mice with constitutional *Nf1*- and *Nf2*-mutant alleles suggest that the configurations of the mouse and human genomes strongly influence the profiles of tumors developed by the two species.
3. Conditional inactivation of *Nf1* and *Nf2* alleles has been used to successfully model many of the features of NF1 and NF2.
4. Increased Ras signaling appears responsible for Nf1-associated tumorigenesis.
5. *NF1* haplo-insufficiency makes important contributions to both tumor and nontumor phenotypes in NF1.
6. Despite the limited tumor spectrum exhibited by human NF2 patients, studies of *Nf2*-mutant mice reveal a requirement for Merlin function in many cell types and an unexpectedly wide range of tumors associated with *Nf2*-deficiency.
7. The NF2 tumor suppressor Merlin appears to coordinate cell-cell or cell-ECM adhesion with growth factor receptor signaling.

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9. Homozygous loss of *Nf1* in Schwann cells is sufficient for neurofibroma formation and heterozygous loss of *Nf1* in surrounding non-neoplastic cells is necessary for tumorigenesis.

11. Genetic demonstration that mutations in the *NF2* gene do not cause schwannomatosis, thereby defining it as a third major form of neurofibromatosis.

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