

## MOLECULAR, GENETIC, AND CELLULAR PATHOGENESIS OF NEUROFIBROMAS AND SURGICAL IMPLICATIONS

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NEUROFIBROMATOSIS 1 (NF1) IS A common autosomal dominant disease characterized by complex and multicellular neurofibroma tumors. Significant advances have been made in the research of the cellular, genetic, and molecular biology of NF1. The *NF1* gene was identified by positional cloning. The functions of its protein product, neurofibromin, in RAS signaling and in other signal transduction pathways are being elucidated, and the important roles of loss of heterozygosity and haploinsufficiency in tumorigenesis are better understood. The Schwann cell was discovered to be the cell of origin for neurofibromas, but understanding of a more complicated interplay of multiple cell types in tumorigenesis, specifically recruited heterogeneous cell types such as mast cells and fibroblasts, has important implications for surgical therapy of these tumors. This review summarizes the most recent NF1 and neurofibroma literature describing the pathogenesis and treatment of nerve sheath tumors. Understanding the biological underpinnings of tumorigenesis in NF1 has implications for future surgical and medical management of neurofibromas.

**KEY WORDS:** Loss of heterozygosity, Malignant peripheral nerve sheath tumor, Neurofibroma, Plexiform neurofibroma, Spinal neurofibroma

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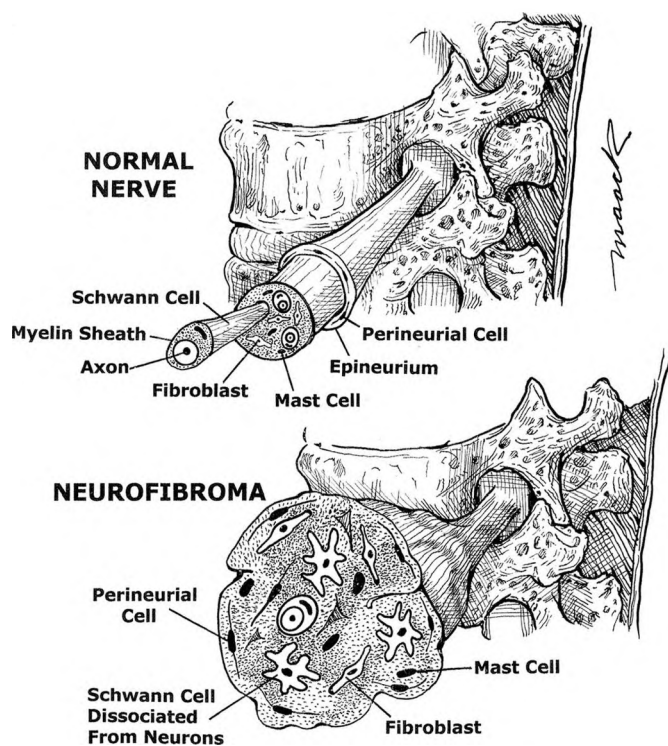
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**N**eurofibromatosis 1 (NF1), or von Recklinghausen's disease, is an autosomal dominant condition occurring in 1 out of 3500 individuals (150). It is the most frequent of the phacomatoses, is clinically heterogeneous, and is characterized by neural crest-derived tumors (20, 194). Neurofibromas, which are complex tumors arising from peripheral nerve sheaths, are the key feature of NF1 (94, 194). The morphological variants include localized or diffuse cutaneous neurofibromas and intraneural neurofibromas of localized or plexiform type (20, 94, 194). Less commonly, patients may develop massive soft tissue or visceral neurofibromas (20, 94, 194). The presence of numerous localized cutaneous neurofibromas or a plexiform neurofibroma is virtually pathognomonic of NF1 (20, 94, 194).

Although neurofibromas consist mostly of Schwann cells and fibroblasts, they also contain other cell types, including perineural cells, mast cells, pericytes, endothelial cells, smooth muscle cells, and cells with intermediate features (*Fig. 1*) (10, 94, 139, 157, 194). Axons pass through neurofibromas, and typ-

ically, the lesions are associated with a recognizable nerve (20). There is also a large amount of extracellular matrix (ECM) with collagen (139).

Recently, significant advances have been made in the understanding of the cellular, genetic, and molecular biology of NF1 and neurofibromas. For example, the *NF1* gene has been identified (26, 187, 196), the functions of the neurofibromin protein and the interaction of that protein with signal transduction pathways are better understood, the Schwann cell was discovered to be the cell of origin of neurofibromas (although it must develop in concert with other cells in the microenvironment to form neurofibromas), inflammatory cells (including the mast cell) are recruited to the tumor environment and are important to neurofibroma tumorigenesis, and several NF1 animal models have improved our understanding of the pathogenesis of neurofibromas and provided biological systems in which to test new therapies. This review summarizes the most recent NF1 literature describing the genetic, cellular, and molecular pathogenesis, cell culture, and animal models of neurofibro-



**FIGURE 1.** Schematic drawing showing a normal nerve and a neurofibroma. The nerve is composed of Schwann cells that surround the axons and a collagen matrix containing fibroblasts and mast cells. It is surrounded by perineurial cells, which serve as a diffusion barrier. In contrast, the neurofibroma consists of greater numbers of Schwann cells, Schwann cells that are dissociated from axons, greater numbers of supporting cells that are less organized than in the normal fascicle, and a breakdown of the surrounding perineurial layer. Concept of cells adapted with permission from McLaughlin and Jacks (124).

mas. Implications for surgical management of neurofibromas are discussed.

## CLINICAL GENETICS

NF1 is an autosomal dominant inherited condition, and all affected individuals are heterozygous for an NF1 mutation (54). Because homozygosity in murine models has been shown to be lethal to embryos (16, 78), it is believed that one functional NF1 allele is necessary for survival (54). In contrast to the typical patient who is heterozygous for the NF1 mutation, some individuals demonstrate NF1 features in a localized pattern, termed "segmental neurofibromatosis" or somatic mosaicism, and this phenotype is likely owing to a postzygotic, somatic mutation of the NF1 gene in an early stage of fetal development (186, 188).

The penetrance of the NF1 mutation is virtually 100% by the age of 10 years (54, 71, 72). A positive family history is identified in approximately half of all NF1 cases (17, 21, 30, 71, 134, 181), whereas half represent new mutations (54). The incidence of new mutations at the NF1 locus ranges from 1 out of

7800 to 1 out of 23,000 (30, 71, 114, 181). Approximately 80% of new NF1 mutations are paternal in origin (49, 79, 101, 178, 185). It is not clear why the mutation rate at the NF1 locus is high (54), but it is possible that a mutation at that locus provides a selective and proliferative advantage in a germ-cell precursor (151).

## MOLECULAR GENETICS

### NF1 Gene Structure and Function

In 1987, genetic linkage analysis of a large number of independent families was used to map the NF1 locus close to the centromere on the long arm of chromosome 17 (7, 162). In 1990, the NF1 gene was identified by positional cloning and it was located at 17q11.2 (26, 187, 196). The NF1 gene is complex, spans more than 350 kb of genomic deoxyribonucleic acid (DNA), and contains 60 exons (111). The NF1 gene produces an 11- to 13-kb messenger ribonucleic acid (mRNA) (10, 117) that is expressed in almost all tissues (196) but is most highly expressed in the brain, spinal cord, and the peripheral nervous system (38, 188). There are alternatively spliced NF1 mRNA isoforms, and, depending on the tissue, they are differentially expressed (171).

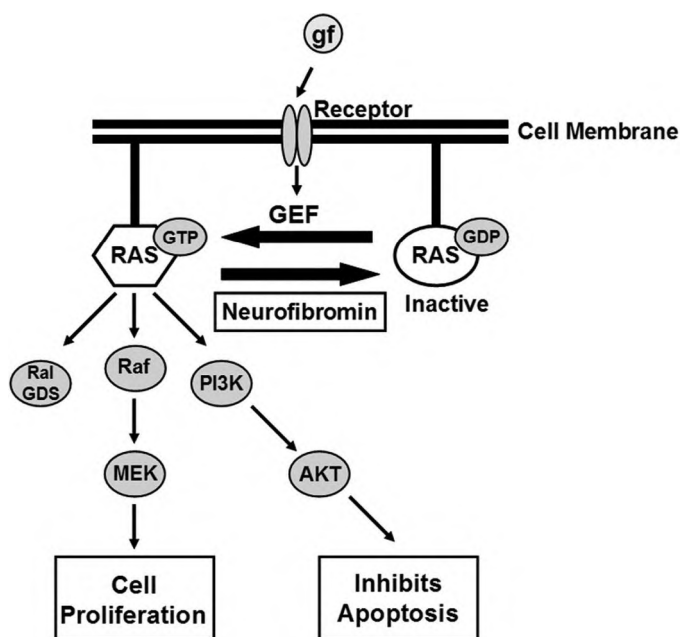
### Neurofibromin Protein

The protein product of the NF1 gene is neurofibromin, which is a large peptide (220 kD) with 2818 amino acids (40, 59, 65). It is most abundant in the nervous system. In adults, it is also found in neurons, oligodendrocytes, and Schwann cells (38, 188). It is also expressed in a variety of other cell types in adults, such as keratinocytes, adrenal medulla, and white blood cells (64, 188). Neurofibromin is ubiquitously expressed during embryonic development and the adult pattern of tissue expression is established after the first week of postnatal life (63, 64). Neurofibromin is reduced or absent in rat and mouse neurofibroma cells (60, 88), and immunohistochemical staining of dermal and plexiform neurofibromas demonstrated that they are composed principally of Schwann cells that lack functional neurofibromin (131).

An important functional region of neurofibromin is its RAS-GTPase activating protein (GAP)-related domain (GRD), although it only occupies a small area of the protein (360 amino acids) (18, 203, 204). The neurofibromin GRD stimulates the intrinsic GTPase of p21-RAS-GTP to hydrolyze GTP to GDP, thereby inactivating p21-RAS (Fig. 2) (1, 6, 15, 118, 182, 203). Thus, the main function of neurofibromin is inactivation of the active RAS-GTP and its signal transduction pathways. This is further corroborated by evidence that in neurofibroma tissue, which has decreased neurofibromin protein, amounts of the active RAS-GTP protein are increased compared with the inactive form (9, 14, 60, 88, 100, 122, 167).

RAS proteins regulate the cell by transducing signals from the plasma membrane to the nucleus by a series of downstream effectors (Fig. 2) (198, 200). The RAS protein is anchored to the cell membrane by farnesyl transferase (FT) (24),





**FIGURE 2.** Illustration depicting the role of neurofibromin in RAS activation and signaling. Growth factors (gf) interact with receptors at the cell surface, activating guanine nucleotide exchange factors (GEF) and resulting in activated RAS. Activated RAS sends intracellular signals through the phosphoinositide 3' kinase (PI3K) pathway to inhibit apoptosis and the raf-MAK (mitogen-activated kinase) pathway to stimulate cell proliferation. It also signals through the Ral GDS pathway, but its function is less well understood. Normally, neurofibromin downregulates RAS through its GAP-related domain, and therefore, in its absence or at decreased levels, signaling is increased through all of these pathways resulting in cell proliferation and inhibited apoptosis.

and this step is necessary in its normal functioning (198). RAS functions as part of a signal transduction pathway that is activated by growth factors and their receptors, including receptor tyrosine kinases such as epidermal growth factor (EGF), nerve growth factor, and platelet-derived growth factor via guanine nucleotide exchange factors (9). Increased RAS-GTP leads to increased signaling through raf kinase, and raf in turn activates a kinase cascade involving MEK kinase and the Erk1 and Erk2 isoforms of mitogen-activated protein (MAP) kinase resulting in cell proliferation (14, 43, 179). Increased RAS-GTP also protects cells from apoptosis by activating protein kinase B/Akt via phosphoinositide 3-OH kinase (PI3-kinase) (43, 85) or by activation of NF- $\kappa$ B (43, 121). Overall, RAS is a key component of many growth factor signaling pathways and in the absence of neurofibromin it is constitutively activated, resulting in increased cell proliferation and survival (188).

### Mutations and Loss of Heterozygosity

Extensive loss of heterozygosity (LOH) testing and sequence analysis indicate that consistent with Knudson's two-hit hypothesis of tumor suppressor gene inactivation (92), one NF1 allele carries a genetic alteration in all cells of an NF1

patient (germline), and loss of the second NF1 allele (somatic) results in functional loss of neurofibromin (48, 83, 158, 163). Both copies of the *NF1* gene are mutated in NF1 tumors (48, 49, 79, 158), but, contrary to the classical Knudson's "two-hit" hypothesis (92), the majority never become malignant.

Studies have correlated LOH or inactivation of both NF1 genes with deletions of the somatic *NF1* gene for benign cutaneous neurofibromas (32, 48, 83, 145, 158, 164, 199), plexiform neurofibromas (37, 91, 145), malignant peripheral nerve sheath tumors (MPNSTs) (104, 127, 145, 172), pheochromocytomas (205), and juvenile myelomonocytic leukemias (169) from NF1 patients. It is significant to note that LOH only occurs in the chromosome that does not carry the germline mutated allele (163, 164). The size of the deletion is variable and may include a limited region of the *NF1* gene or nearly the whole 17q arm with retention of 17p and centromeric markers (163).

LOH represents the second hit of the remaining allele and, in general, it may occur by nondisjunction, interstitial deletions, large somatic deletions, loss of a whole chromosome 17, or somatic recombination (37, 135). When LOH is not present, the second hit is usually a somatic gene mutation or a small intragenic deletion, and, overall, small subtle mutations may occur at a similar frequency to LOH (48, 120, 135). Furthermore, mutations may occur in unsequenced coding or non-coding regions, and methylation or transcription repression could silence *NF1* gene expression without a mutation (39, 53, 152, 155).

Point mutations affecting the correct splicing of the *NF1* gene are a common cause of NF1 (3, 51, 128), and they are responsible for both somatic and germline mutations (163). Most mutations in NF1 patients are thought to result in truncation of the protein product (184), which would result in an inactive protein and decreased amounts of active neurofibromin. Small intragenic deletions and insertions account for a third of all mutations (Table 1) (188). Of the 300 reported mutations of the *NF1* gene (51), only 7% have been observed more than once (194).

Particular mutations are not typically associated with distinct phenotypic expressions of NF1, and several studies have been unable to find strong genotype-phenotype correlations (22, 25, 47, 180, 188). The marked clinical variability between multiple affected relatives with the same germline mutation may be owing to the nature, timing, and location of the "second hit" mutation at the NF1 locus (25, 155, 158, 164, 199). Variants of other unknown genes may also modify the expression of the disease and result in certain phenotypes (199). Somatic mosaicism is another potential cause of interindividual phenotypic variation (25).

### Genetic Differences between Plexiform Neurofibromas and Malignant Peripheral Nerve Sheath Tumors

Although benign and malignant neurofibromas both involve the inactivation of the *NF1* gene and decreased neurofibromin, malignant lesions have additional genetic abnormal-

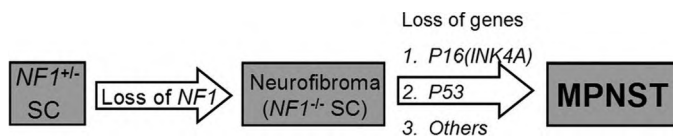
**TABLE 1. Spectrum of *NF1* gene mutations<sup>a</sup>**

| Type of mutations                        | No. of reported independent occurrences |
|--|---|
| Chromosome rearrangements                | 9                                       |
| Deletions of the entire gene             | 38                                      |
| Single- and multi-exon deletion          | 42                                      |
| Small intragenic deletions               | 63                                      |
| Large insertions                         | 3                                       |
| Small insertions                         | 29                                      |
| Direct stop mutation (nonsense)          | 51                                      |
| Amino acid substitution (missense)       | 31                                      |
| Mutation in introns                      | 26                                      |
| Alteration of the 3' untranslated region | 4                                       |
| Nonpolymorphic silent base exchanges     | 4                                       |
| Total                                    | 300                                     |

<sup>a</sup> Originally from the National Neurofibromatosis Foundation International NF1 Genetic Analysis Consortium Database (188, 194).

ities that may be involved in the transformation to malignancy (Fig. 3). MPNSTs demonstrate increased levels of the active RAS protein, RAS-GTP, compared with neurofibromas (60). The loss or mutation of the *P53* gene, located at 17p13.1, has been observed in many NF1-related MPNSTs, but never in benign neurofibromas (13, 58, 82, 93, 103, 108, 116, 127, 133, 145, 183, 202), and abnormalities in *P53* expression in MPNSTs are associated with a poor prognosis (112). The loss of the *P53* gene results in abnormalities in DNA damage-induced cell cycle arrest and apoptosis (183). In animal models, loss of *p53* in addition to *Nf1* is required to generate MPNSTs (29, 192). Overall, loss of *NF1* appears to be an early tumorigenic event in MPNSTs followed later by *P53* loss (140) (Fig. 3).

In addition to LOH for the *P53* gene in MPNSTs, LOH for the *CDKN2A* gene has also been observed (183). Approximately 50% of MPNSTs in NF1 patients have homozygous deletions for exon 2 of the *CDKN2A* (also known as *INK4A* or *INK4A/ARF*) gene, which encodes two distinct tumor suppressors, *P16<sup>INK4A</sup>* and *P14<sup>ARF</sup>* (95, 132), but this deletion is not found in benign neurofibromas (12, 13, 95, 132). Also, mutations have been found at *CDKN2A* in 60% to 75% of MPNSTs



**FIGURE 3.** Genetic changes involved in malignant transformation. Loss of both *NF1* alleles in the Schwann cell (SC) results in neurofibroma formation. Not pictured is the important role of the heterozygote supporting cells in neurofibroma pathogenesis. Malignant transformation requires abnormalities or loss of additional genes including *P53* and/or *P16* (*INK4A*), and there are likely many other undiscovered genetic abnormalities in MPNSTs.

(12, 95). The loss of *P16<sup>INK4A</sup>* contributes to a failure to inactivate the cyclin D/cdk 4 complex that promote retinoblastoma (Rb) phosphorylation, which leads to a release of inhibition of E2F, increasing cellular proliferation (183). Loss of *P14<sup>ARF</sup>* protein stabilizes and activates *p53* pathways by inhibiting the Mdm-2-induced *P53* degradation and transactivational silencing (168). Therefore, both *P16<sup>INK4A</sup>*-cyclin D/cdk4-Rb and *P53* pathways are critical to cell cycle control and tumor surveillance and may be involved in the transformation to malignancy (Fig. 3) (124).

MPNSTs demonstrate a high frequency of microsatellite instability, which is consistent with the idea that additional loci become targets for mutations during the malignant transformation of neurofibromas, and overall, it involves a complex multistep process with multiple locations for genetic defects (183). MPNSTs also have unique chromosomal imbalances (115, 126), including gains in 7, 8q, 15q, and 17q (159, 160). Survival of patients with MPNSTs and gains in 7p15-21 and 17q22-qter is significantly decreased (160). Patients with MPNSTs may also have genetic losses (177) and may have large-scale chromosomal amplifications (126, 159, 160).

## CELLULAR ENVIRONMENT

### Neurofibroma Cellular Characteristics

The multicellular composition of neurofibromas has presented a major challenge in understanding their pathogenesis (94, 157). Neurofibromas are unique among tumors because of the extent of cellular heterogeneity they exhibit; all of the cell types found in normal peripheral nerves, including Schwann, fibroblast, mast, and perineural cells, are found in neurofibromas (Fig. 1) (157, 166). Reciprocal signaling among these cell types is known to occur in normal peripheral nerve sheath (81, 89), and likely occurs in neurofibromas. However, decreased neurofibromin may result in altered responses in neurofibromas. Neurofibromas are also characterized by the close cellular associations between the cell types (207) and by excessive ECM deposition (77, 157, 207).

### Schwann Cell as the Progenitor for Neurofibromas

Although it is well known that the Schwann cell is the cell of origin of schwannomas, it was far more difficult to define the key cell in neurofibroma tumorigenesis because of its cellular heterogeneity (157). The identification of the Schwann cell as the progenitor cell of neurofibroma formation represented a tremendous breakthrough and has resulted in new neurofibroma models focusing specifically on this cell (90, 131, 140, 155, 165, 166, 197). Several observations led to the recognition of the Schwann cell as the primary cell type for neurofibroma formation. For example, Schwann cells in normal nerves are usually found only in association with nerves, but in neurofibromas they are also found free in the ECM (84, 166). Also, Schwann cells seem to be the primary target of growth factors stimulating neurofibroma formation (146), and Schwann cells are the major cell type amplified in neurofibro-

mas (141, 176, 195). Neurofibroma Schwann cells have absent or markedly reduced *NF1* mRNA, whereas fibroblasts from the same neurofibromas consistently expressed some *NF1* mRNA and protein (155). More recently, the loss of both *NF1* alleles, which leads to neurofibroma formation, was found to occur exclusively in the Schwann cell (90, 165). Furthermore, both *NF1* gene copies are inactivated only in the Schwann cell, whereas a wild-type gene is retained in the other neurofibroma cells, including fibroblasts (90, 165).

The neurofibroma Schwann cells have other unique in vitro characteristics that may contribute to tumor formation (166). They seem to have a higher potential to be severely compromised by mutations than other cells, because normal Schwann cells already express less *NF1* mRNA than fibroblasts and translation of mRNA into protein is slow and inefficient (155). The neurofibromin-deficient Schwann cell has been shown to demonstrate increased active RAS, RAS-GTP, but this is not seen in the other neurofibroma cells including the fibroblast (167). The neurofibroma Schwann cell also has altered morphology, delayed senescence, lack of density-limited growth, and a propensity to form proliferative cell aggregates rich in ECM spontaneously (87, 131). Schwann cells that lack functional neurofibromin have a substantial growth advantage compared with those with limited neurofibromin (131). The neurofibroma Schwann cell promotes angiogenesis and invades basement membranes in contrast with normal Schwann cells and fibroblasts from neurofibromas (166). In addition to increased invasiveness, the cell displays a loss of negative autocrine growth control (130).

### Role of the Haploinsufficient Supporting Cells

A growing body of experimental evidence supports the idea that haploinsufficiency of *NF1* in the microenvironment of neurofibromas contributes to its tumorigenicity, and the importance of tumor suppressor gene haploinsufficiency in tumor cell biology is gaining more attention (123). In the heterozygous supporting cells in neurofibromas, half of the normal activity of the neurofibromin protein is lost. The single active *NF1* allele may not generate enough functional protein to rise above the threshold needed to achieve an appropriate biological response (46), which may confer a growth advantage that also contributes to tumorigenesis (74). An example of haploinsufficiency is the neurofibromin-deficient, *NF1*<sup>+/-</sup> Schwann cell, which has decreased but remaining neurofibromin function and aids in tumor formation (131). The *NF1*<sup>+/-</sup> Schwann cells may also contribute to neurofibroma formation with their increased ability to induce angiogenesis and increased invasiveness (87). Recently, it was discovered that neurofibromas contain both *NF1*<sup>-/-</sup> and *NF1*<sup>+/-</sup> Schwann cells and that only a portion of neurofibroma Schwann cells have mutations in both alleles (131, 165). Thus, neurofibromas demonstrate Schwann cell heterogeneity (167).

Although *NF1*<sup>+/-</sup> and *NF1*<sup>-/-</sup> Schwann cells are both necessary in the pathogenesis of neurofibromas, alone they are not sufficient for tumor formation, which demonstrates the

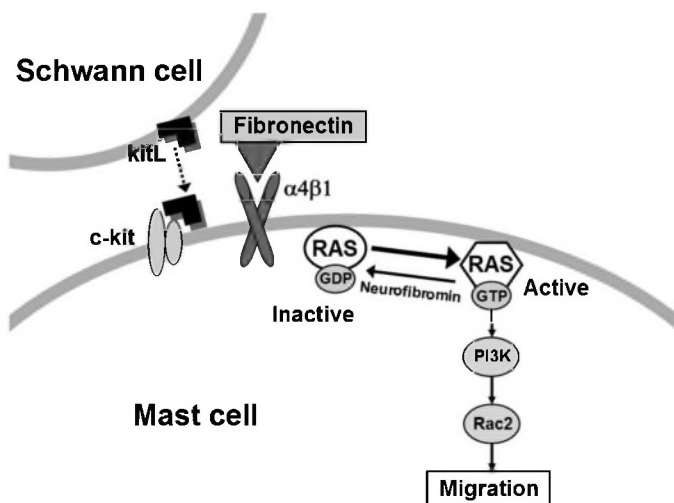
importance of the presence of the other heterozygous supporting cells in the cellular environment (210). In neurofibromas, *NF1*<sup>+/-</sup> fibroblasts demonstrate abnormal responses to cytokines, increased collagen deposition, and increased proliferation (4, 139). *NF1*<sup>+/-</sup> mast cells have increased infiltration to preneoplastic peripheral nerves in comparison with wild-type mast cells (210) and have increased proliferation in vitro and in vivo (74). In a mouse model, the type of supporting cells in mice with *NF1*-deficient Schwann cells (*Nf1*<sup>-/-</sup>) determined whether they developed neurofibromas (210). The mice in which the neighboring cells were heterozygous (*Nf1*<sup>+/-</sup>) developed widespread neurofibromas that closely resembled human tumors, whereas the mice with neighboring cells that were wild-type (*Nf1*<sup>+/+</sup>) only developed infrequent hyperplastic lesions in the cranial nerves (210). This study emphasizes the essential function of *NF1* heterozygous cells in neurofibroma formation and the importance of deciphering their individual roles in tumorigenesis and the mechanism of recruitment to the tumor microenvironment (210).

### Mast Cell Functions and Interactions with Schwann Cells

Recent studies demonstrate that inflammatory cells including mast cells are important to tumor initiation, progression, and angiogenesis (33, 34, 66) and that understanding the mechanisms that control recruitment of these cells is important (207). A recent publication by Yang et al. (207) has expanded the understanding of the contribution of the heterozygous mast cell (*NF1*<sup>+/-</sup>) to neurofibroma formation. The authors demonstrated that mast cells are recruited, migrate to the tumor microenvironment, and are hypermotile because of secretion of kit ligand, a growth factor, by the nullizygous Schwann cell (*NF1*<sup>-/-</sup>) (207). Neurofibromin-deficient Schwann cells secrete five times the normal kit ligand, which serves as a chemoattractant for mast cells expressing c-kit receptor. In general, mast cells have increased survival and proliferation in response to kit ligand (73, 74), and the increased motility and migratory abilities of the mast cells (*NF1*<sup>+/-</sup>) in response to kit ligand are thought to be mediated by increased RAS activity (207). Although multiple RAS effector pathways are altered in *NF1*<sup>+/-</sup> mast cells in response to kit ligand, there is evidence that activation of the Class 1<sub>A</sub>-PI3K-Rac2 pathway, which is enhanced in *NF1*<sup>+/-</sup> mast cells, is directly responsible for the increased migration (207). This pathway has also been shown to be responsible for increased proliferation and survival of mast cells (73). Yang et al. (207) also provided evidence that heterozygous inactivation of *NF1*<sup>+/-</sup> promotes rapid migration of mast cells on  $\alpha$ 4B1 integrins (mast cell surface proteins), in response to the kit ligand, and that this integrin attaches to endothelial cells through VCAM-1. This group notes that kit and  $\alpha$ 4B1 may be targets for future therapies as represented in Figure 4.

This study has demonstrated a unique interaction between the *NF1*<sup>-/-</sup> Schwann cell and the heterozygous mast cell (Fig. 4) (207). The homozygous *NF1* mutant (*NF1*<sup>-/-</sup>) Schwann





**FIGURE 4.** Schematic drawing of the molecular pathways involved in mast cell recruitment to neurofibromas. The neurofibromin Schwann cell secretes kit ligand (kitL), which interacts with the mast cells expressing c-kit receptor. Migration of the mast cell is mediated by the RAS/PI3K/Rac2 signal pathway, which is already upregulated in  $NF1^{+/-}$  mast cells. Endothelial cells are also involved in the mast cell migration by their VCAM-1 receptor interaction with the mast cell  $\alpha4\beta1$  integrin. Modified with permission from Viskochil (189).

cells caused the migration of the mast cells by secretion of the kit ligand (207). This finding demonstrates that the loss of  $NF1$  in Schwann cells results in an increase in growth factor production that initiates an autocrine or paracrine loop, which is important for the progression of tumorigenesis (207). It is possible that many other Schwann cell growth factors also affect the supporting heterozygous cells and are important for tumor formation.

## GROWTH FACTORS AND RECEPTORS

In addition to the influence of cellular environment on tumor formation, many other factors are involved in neurofibroma tumorigenesis. Schwann cell growth is normally regulated through interactions with neurons (154, 201). In the normal adult nerve, Schwann cell proliferation is usually very slow (201). The family of small peptides known as heregulins and neuregulins (including glial growth factor (GGF)), are Schwann cell mitogens (86, 106, 109). Neuregulins stimulate Schwann cell growth in vitro (109) and activate transmembrane tyrosine kinase receptors (erbB2, -3, -4) that are structurally and functionally related to epithelial growth factor receptor (EGF-R) (142).

Neurofibroma Schwann cells do not proliferate on their own in tissue culture but can be stimulated to divide when exposed to specific Schwann cell mitogens including transforming growth factor (TGF)- $\beta$ , acidic and basic fibroblast growth factor, and platelet-derived growth factor (PDGF) (28, 131, 147, 161, 173, 174). Normally, growth factors cooperate to suppress cell death in Schwann cell precursors (55), but

growth factor dysregulation is thought to be involved in the tumorigenesis of neurofibromas (146). It is not known whether the changes in the growth factor expression are the direct result of  $NF1$  gene loss or from secondary genetic events in the tumorigenesis of neurofibromas (119). Inactivation of the  $NF1$  gene may render cells more responsive to humoral factors that are usually expressed in embryogenesis or it may increase growth factor expression.

Many Schwann cell mitogens may contribute to neurofibroma formation: hepatocyte growth factor (HGF) (96, 144), basic fibroblast growth factor (FGF) (55, 146), insulin growth factor 1 (IGF-1) (55, 67), and pigment epithelium-derived growth factor (PEDF) (36). Loss of neurofibromin in  $NF1^{-/-}$  mouse Schwann cells induced and upregulated fibroblast growth factor (FGF-2), PDGF, and midkine (MK) (119). Finally, gene expression and upregulation of proteins in MPNSTs are increased compared with benign neurofibromas including matrix metalloproteinase-13 (MMP-13), platelet-derived growth receptor alpha (PDGFA), HGF, PEDF, MK, and epithelial growth factor (EGF) (70). Overall, these growth factors stimulate Schwann cell proliferation or survival, support invasion by other cell types, or stimulate angiogenesis (28, 55, 161).

In addition to growth factors stimulating neurofibroma formation, specific receptor expression may also have a role. The EGF-R is expressed in cells from mostly malignant neurofibromas, in transformed Schwann cells from  $NF1^{-/-}$  mice, and rarely in benign neurofibromas, but it is not present in normal Schwann cells (41, 110). All cells with EGF-R responded to EGF by activation of downstream signaling pathways including MAP/extracellular signal-regulated kinase, PI3K/AKT (41, 110). Thus, EGF-R expression may play an important role in  $NF1$  tumorigenesis and Schwann cell transformation (41), and it is thought to drive tumor development by providing a mitogenic signal or by serving as a survival factor by avoiding apoptosis (110). Agents that antagonized EGF-R or inhibited the PI3K pathway inhibited growth in MPNST cell lines and the transformed  $NF1^{-/-}$  Schwann cells (41, 110). Another key receptor, the kit tyrosine kinase receptor (c-kit), contributes to Schwann cell proliferation and hyperplasia (5). Expression of kit receptor is increased in  $NF1$ -derived Schwann and neurofibrosarcoma cells, and high levels of kit expression correlated with a decreased expression of neurofibromin (5).

As mentioned above, the kit ligand is also important to neurofibroma formation. Kit ligand transcripts are markedly increased in neurofibromas (69, 156), transformed Schwann cells secrete high concentrations of kit ligand (5, 69), and other neurofibroma cells secrete far lower concentrations (207). The proliferation of the neurofibrosarcoma-derived Schwann cells is also increased by stimulation with kit ligand (5). Additionally, as described above, heterozygous mast cells have increased survival and proliferation and are recruited to the tumor microenvironment in response to the kit ligand, and in return, mast cells can secrete nerve growth factor (NGF) (107) and vascular endothelial growth factor (VEGF) (175), which

are potent stimulants for Schwann cell proliferation, migration, and survival (175).

Other molecules including hedgehogs and their receptors are thought to be involved in neurofibroma pathogenesis (50). Hedgehogs are intercellular signaling molecules that normally regulate growth and patterning during early development (50). The hedgehog receptor patched (PTCH-2) is found in normal and neurofibroma Schwann cells (50). Interestingly, perineural cells in plexiform neurofibromas express Indian hedgehog and Sonic hedgehog (50). These results suggest that PTCH-2 on Schwann cells may mediate paracrine hedgehog signaling from perineural cells in plexiform neurofibromas (50).

### ANIMAL MODELS

Neurofibroma animal models have elucidated some of the roles of the neurofibromin protein in neurofibroma formation and in normal growth and development (98, 99). The models have also provided additional evidence that *NF1* acts as a tumor suppressor gene (78). Additionally, animal models provide genetically accurate models for preclinical testing and can be used to identify novel targets for future therapies (68). The first mammalian *NF1* models have been in the mouse, which provides a biological system more similar to humans than earlier models in *Drosophila* (188). The mouse and human *NF1* genes are highly related, the amino acid sequence of neurofibromin is 98% identical, and there is also significant similarity between the non-coding region of the mRNA (11).

#### Xenograft Mouse Models

Early neurofibroma studies involved a xenograft model for studying neurofibroma formation by injecting human neuro-

fibroma tissue or Schwann cell preparations into immunodeficient mice. This model was useful to study tumor growth and cellular modification (2, 102, 166). One such model involved injection of human neurofibroma cells into sciatic nerves of nude mice; 2 months after engraftment, 70% of tumors survived and proliferated (102). Tumors survived longer and had better proliferation in the sciatic nerve than in the subrenal capsule (102). In another study, neurofibroma cells injected into subcutaneous tissue of nude mice did not produce tumors, suggesting the importance of environment in the development of tumors (166). In a later study, neurofibromin-deficient Schwann cells injected in the sciatic nerve consistently produced persistent neurofibroma-like tumors with diffuse and extensive intraneural growth similar to human neurofibromas (131). This study demonstrated the importance of the neurofibromin-deficient Schwann cell in neurofibroma tumorigenesis (131).

#### Transgenic Mouse Models

The initial *NF1* transgene mouse model (Table 2) was based on a mutation that was initially identified in an NF1 patient (16, 78). It was constructed by inserting a neomycin resistance gene (*neo*) in exon 31, resulting in an interruption of the gene segment that corresponded to a premature truncation *Nf1* mutation (16, 78). Interestingly, these mice that carry a germline mutation and are heterozygous for the *Nf1* mutation (*Nf1*<sup>+/</sup>/*Nf1*<sup>n31</sup>) did not develop the classic phenotype of NF1 disease including neurofibromas, but they appeared to have learning problems (170), and 17 of 40 mice developed tumor types that are typical for humans with NF1, including pheochromocytoma (9 mice), neurofibrosarcoma (one mouse), and myeloid leukemia (seven mice) (78). The wild-type *Nf1* allele was lost in many of these tumors, including all of the phoe-

TABLE 2. *NF1* transgene mice models

| Genotype  | Neurofibromas      | Other lesions                       | Notes   | Ref. no.  |
|---|--------------------|-------------------------------------|---|-----------|
| Heterozygous ( <i>Nf1</i> <sup>+/-</sup> )  | No                 | Pheochromocytoma, leukemia, sarcoma | These tumors are found in NF, many with LOH for wild-type <i>Nf1</i>  | (78)      |
| Homozygous ( <i>Nf1</i> <sup>-/-</sup> )  | No                 | Cardiac abnormalities <sup>a</sup>  | Death at 12-14 days gestation; cells ( <i>Nf1</i> <sup>-/-</sup> ) with increased proliferation                         | (16)      |
| Chimeric ( <i>Nf1</i> <sup>-/-</sup> and <i>Nf1</i> <sup>+/+</sup> )  | Plexiform          | No                                  | Tumors did not have supporting wt cells ( <i>Nf1</i> <sup>+/-</sup> )   | (29)      |
| Conditional (cre/lox) <i>Nf1</i> <sup>lox/-</sup> ; Krox20-cre (wt <i>Nf1</i> <sup>+/+</sup> supporting cells/ <i>Nf1</i> <sup>-/-</sup> Schwann cells)   | Plexiform          | No                                  | Tumors with typical <i>Nf1</i> <sup>-/-</sup> Schwann cells and <i>Nf1</i> <sup>+/-</sup> supporting cells <sup>b</sup> | (210)     |
| Conditional (cre/lox) <i>Nf1</i> <sup>lox/lox</sup> ; Krox20-cre (wt <i>Nf1</i> <sup>+/+</sup> supporting cells/ <i>Nf1</i> <sup>-/-</sup> Schwann cells) | No                 | Hyperplastic nerve lesions          | Demonstrates need for <i>Nf1</i> <sup>+/-</sup> cells in neurofibroma formation   | (210)     |
| Cis-linked <i>Nf1</i> <sup>+/-</sup> : <i>p53</i> <sup>+/-</sup>  | MPNST <sup>c</sup> | No                                  | Most tumors with LOH for both gene loci   | (29, 192) |

<sup>a</sup> Cardiac abnormalities are not typically seen in NF1 patients.

<sup>b</sup> Tumor histologically and molecularly most similar to human neurofibroma.

<sup>c</sup> One-hundred percent of mice harboring null *Nf1* and *p53* alleles in cis synergize to develop MPNST (192).

chromocytomas and myeloid leukemias, supporting the concept that the LOH is a necessary step in tumorigenesis in NF1 (78). Incidentally, heterozygous mice also demonstrated a greater risk of developing several other tumor types not associated with NF1, including lymphoma, adenocarcinoma, hepatoma, and fibrosarcoma, but these tumors are also typically seen in older wild-type mice (78). It is not clear why these heterozygous mice did not develop the typical *Nf1* phenotype, but this model may have had a lower mutation rate for the remaining wild-type *Nf1* allele, or the regulatory mechanism controlling growth and development of target cells in the mouse may be different (78).

In contrast, transgene mice that are homozygous for the *Nf1* mutation (Table 2) die in utero at approximately 12 to 14 days gestation (16, 78). They display cardiac developmental abnormalities and have generalized edema and venous hemorrhages, but they do not have any other grossly apparent developmental defects at the time of fetal death (16, 78). The lethal cardiac defect with a double outlet right ventricle, a ventricular septal defect, and overabundant endocardial cushions that block inflow of blood to the heart results in heart failure during gestation (16, 78). This abnormality is owing to hyperproliferation and the lack of normal apoptosis caused by the absence of the neurofibromin protein that normally modulates epithelial-mesenchymal transformation and proliferation in the developing heart by downregulating RAS activity (78). These findings are unique to mice because humans with NF1 do not normally demonstrate congenital cardiac abnormalities (113), but humans typically have one copy of the wild-type *NF1* gene during gestation. Interestingly, neurons harvested from the peripheral nervous system of these embryos survive in culture without growth factor stimulation (191), hematopoietic stem cells show constitutive MAP kinase activity and increased cell proliferation compared with heterozygote or wild-type cells (14, 100, 209), fibroblasts proliferate at an increased rate, and perineurial cells fail to form fascicles around nerve bundles (153). These examples in cells without any neurofibromin demonstrate its importance for cell growth control and cell death.

Overall, the heterozygous and homozygous mice did not result in a suitable model for human NF1. Therefore, other animal models were established to include the features of NF1, including neurofibromas and neurofibrosarcomas (29). Chimeric mice (*Nf1*<sup>-/-</sup>; *Nf1*<sup>+/+</sup>) (Table 2) were developed by injection of *Nf1*<sup>-/-</sup> homozygous mutant embryonic stem cells at an early developmental stage (blastocysts) to overcome the lethality of the germline homozygous genotype (29). These mice survived to birth and all developed multiple neurofibromas that resemble human plexiform neurofibromas, but they did not develop cutaneous neurofibromas (29). The neurofibromas contained Schwann cells with homozygous mutations for the *Nf1* gene (*Nf1*<sup>-/-</sup>), supporting the concept that LOH is necessary for the development of neurofibromas (29). The neurofibromas differed from human ones, because there was not recruitment of wild-type cells in the lesions and Schwann cells were not found in the lesion and were only associated

with the adjacent trapped nerves (29). The difficulty with this model was that the cell type in which the *Nf1* was deleted could not be controlled and these animals could not be crossed to other mutant mouse strains (123).

### Conditional Mouse Model

Later, a new conditional mouse model was developed that resulted in somatic inactivation of *Nf1* and ablation of neurofibromin function specifically in Schwann cells (210) (Table 2). Zhu et al. (210) developed a mouse model in which a floxed *Nf1* allele was deleted by a Cre transgene under the control of the Schwann cell-specific promoter, *Krox-20*. In this model, Cre activity was confirmed to occur only in the Schwann cells. Thus, only the Schwann cells were homozygous for inactivated *Nf1*<sup>-/-</sup>. When these mice were backcrossed onto an *Nf1*<sup>+/-</sup> background resulting in all non-Schwann cells being haploinsufficient at *Nf1*, the progeny, *Nf1*<sup>flox/+</sup>; *Krox20-cre*, developed plexiform neurofibromas and all had enlarged peripheral nerves. The tumors included all of the typical supporting cells that retain neurofibromin function (*Nf1*<sup>+/-</sup>) and were identical to those seen in humans, exhibiting every discernible molecular and histological feature of the human counterpart, and this model had 100% penetrance in producing neurofibromas. This model gave further evidence that the Schwann cell is the cell of origin of neurofibromas (210).

Interestingly, the same researchers developed another mouse model, *Nf1*<sup>flox/flox</sup>; *Krox20-cre*, that had intact (wild-type) *Nf1* function (*Nf1*<sup>+/+</sup>) in all cells except those that expressed the *Krox20-cre* transgene (the Schwann cells, *Nf1*<sup>-/-</sup>). These mice did not exhibit enlarged peripheral nerves, failed to form frank neurofibromas, and only had hyperplastic lesions in the cranial nerves (210). Mast cell infiltration showed a marked reduction in the hyperplastic lesions compared with the model described above, which had significant mast cell involvement. The difference is that the hyperplastic lesions developed in an environment with *Nf1*<sup>+/+</sup> mast cells, whereas the neurofibromas developed in the context of haploinsufficient *Nf1*<sup>+/-</sup> mast cells. This supports the paracrine model for neurofibromas, and overall, this model provides evidence for the absolute necessity for haploinsufficient supporting cells in neurofibroma formation (210). It also illustrates another example of the importance of the relationship between the *NF1*<sup>-/-</sup> Schwann cell and the *NF1*<sup>+/-</sup> mast cell in neurofibroma formation.

### Animal Model for MPNST

Another mouse model was developed for MPNSTs by generating mice with mutations in both *Nf1* and *p53* genes (29, 192) (Table 2). This model was generated by brother-sister mating of doubly heterozygous mice harboring null *Nf1* and *p53* alleles linked in cis (29, 192). A cis-acting element is a genomic sequence that influences the transcription of a gene on the same DNA strand (188). All of these mice (*Nf1*<sup>+/-</sup>; *p53*<sup>+/-</sup>) developed soft tissue sarcomas and MPNSTs in neural crest-derived tissue that had the concomitant loss of the



normal *Nf1* and *p53* alleles (29, 192). Most of these sarcomas exhibited LOH at both gene loci, and 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 (29, 192) (Fig. 3). This mouse strain can lose both *Nf1* and *p53* in one genetic event. The location of *p53* and *Nf1* on the same chromosome suggests that mechanisms such as large deletions, rearrangements, or chromosomal loss may lead to the somatic inactivation of one allele or both of these genes during tumorigenesis (110).

### TREATMENT APPROACHES

Improvements in the understanding of the cellular, molecular, and genetic biology of NF1 and neurofibroma pathogenesis provide an opportunity to develop novel treatment approaches. Some areas that have been targeted in preclinical or clinical studies include various aspects of mast cell functioning, the RAS signaling pathway, and several growth factors and receptors (Table 3), but almost any molecule involved with neurofibroma tumorigenesis may be a potential target for future therapies. It may even be possible to design therapies that neutralize the effects of haploinsufficiency before the onset of tumorigenesis (210).

Prior to these targeted therapies, plexiform neurofibroma treatment was the subject of several clinical trials. In a randomized noncomparative Phase II trial reviewed in Packer et al. (138), cis-retinoic acid was used for its differentiation effects and interferon- $\alpha$  for its anti-inflammatory and antiangiogenic properties to treat plexiform neurofibromas. Although 5 of the 57 patients had a 10 to 20% reduction in tumor size and 8 patients had improvements in their symptoms, none had a demonstrable radiographic response. Although a majority of the patients with plexiform neurofibromas involved in a Phase 1 clinical trial using thalidomide for its antiangiogenic effect abandoned the study for reasons including toxicity, several patients had reduced tumor size on imaging (4 out of 20) and symptomatic improvement (5 out of 20) (61). Additionally,

chemotherapeutic agents, including vincristine and methotrexate, used in combination are under study for NF1 patients with plexiform neurofibromas (138), because of their promising results in childhood desmoid tumors (97).

### Mast Cell Inhibitors

In 1987 and 1993, Riccardi (148, 149) conducted trials of the mast cell inhibitor ketotifen, a relatively selective, noncompetitive histamine antagonist (H1-receptor) and mast cell stabilizer, based on the concept that mast cells found in neurofibromas and their associated secretion of growth factors may contribute to the itching, pain, and tenderness associated with cutaneous neurofibromas and tumor growth. Patients taking oral ketotifen had improvements in these symptoms in an open-labeled trial and in a double-blinded study; it was also observed that some patients had reduced tumor size.

Others have proposed treatments that reduce the mast cell activity, migration, or numbers within neurofibromas as possible therapeutic strategies (207). Options for treatment include pharmacologic inhibition of PI3K, kit activity, or  $\alpha$ 4B1 adhesion (207). Currently, imatinib mesylate is being tested in tumors in which kit activation is important for tumorigenesis (44). Drugs targeting  $\alpha$ 4B1 activation are being tested in clinical trials for Crohn's disease and multiple sclerosis, because inflammatory cell recruitment by adhesion to  $\alpha$ 4B1 is thought to be important for disease progression (56, 129, 193).

### Targeting RAS and Its Signaling Pathways

The RAS signal transduction pathway has also been targeted for future treatments (198, 206). Anti-RAS therapies are broadly applicable, because RAS is one of the most common oncogenes mutated in human malignancies (198). As mentioned earlier, RAS-GTP has been found to be elevated for both benign and malignant NF1 neurofibromas (60).

One approach is the administration of molecules that inhibit the enzyme FT, which is normally important in anchoring the RAS protein to the cell membrane and is absolutely necessary for RAS function (87, 206). Pre-clinical studies have shown that FT inhibitors can block the growth of tumor cells carrying mutant RAS proteins and those with upregulation of wild-type RAS activity (35, 57, 80, 143, 206). One study found that the FT inhibitors inhibited growth in vitro of a NF1-deficient MPNST cell line (206). Overall, FT inhibitors have been demonstrated to decrease hyperproliferation but do not seem to influence invasion in neurofibromin-deficient Schwann cells (87). FT inhibitors are currently be-

**TABLE 3. Tested molecular targets involved in neurofibroma tumorigenesis**

| Event                    | Targeted molecules                                   | Ref. no.                          |
|--------------------------|--|-----------------------------------|
| Mast cell migration      | $\alpha$ 4B1 <sup>a</sup><br>Kit ligand <sup>a</sup> | (56, 129, 193)<br>(44)            |
| RAS                      | Farnesyl transferase <sup>a</sup><br>Antibody to ras | (138, 198)<br>(8, 42)             |
| RAS-dependent pathways   | MEK<br>PI3K<br>GAP-related domain (transfection)     | (45, 52)<br>(190, 208)<br>(8, 42) |
| Fibrosis                 | [PDGF, FGF, EGF, IAM, TGF-B1] <sup>a</sup>           | (62, 75, 76)                      |
| Malignant transformation | EGF-R  | (41, 110)                         |
| Degradation of ECM       | matrix metalloproteinase                             | (105)                             |
| Cytokines                | [c-kit receptor, PDGF] <sup>a</sup>                  | (125)                             |

<sup>a</sup> Evaluated in humans in clinical trials or in isolated cases.

ing administered to patients with refractory cancer in Phase 1 and 2 clinical trials (198). A Phase 1 clinical study using oral FT inhibitors in 17 patients with plexiform neurofibromas reviewed in Packer et al. (138) was the first study for neurofibromas that targeted a specific molecule. No patient had a radiographic decrease in their tumor size, but the study primarily evaluated safety; a current placebo-controlled crossover design study is examining the same agent (138).

Clinical trials have not yet begun on other methods of targeting the RAS effector pathway (198), including inhibitors that target MEK, which reverts many aspects of cellular transformation (45, 52), and *PI3K*, which induces apoptosis (190, 208). These downstream effectors of the RAS signaling pathway are promising targets for future neurofibroma treatment (198).

Another treatment option involving cells that have increased RAS signaling is the delivery of gene therapy vectors with functional NF1 GRD proteins or with dominant negative RAS, which blocks RAS activity (98, 188). In neurogenic sarcoma cell lines from NF1 patients with elevated RAS-GTP, transfection of the GRD portion of neurofibromin restored GAP function and inhibited proliferation of these cells (8, 42). Injection with neutralizing RAS antibodies also inhibited cell proliferation (8, 42). Another treatment approach involving RAS entails the development of viruses that are able to target and infect cells with activated RAS signaling (31). 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 (31).

### Other Potential Targets for Therapy

Another agent, 5-methyl-1-phenyl-2-(1H)-pyridone (pirfenidone; Marnac, Dallas, TX), is currently being tested in clinical trials for adults with progressive plexiform and spinal neurofibromas (138). Pirfenidone is an antifibrotic tissue growth antagonist that modulates actions of cytokines including PDGF, FGF, EGF, intracellular adhesion molecules (IAM), and TGF- $\beta$ 1 (62, 75, 76). It is designed to inhibit the growth-promoting effects of fibroblasts in plexiform neurofibromas.

EGF-R expression is common in cell lines from human malignant NF1 tumors and results in enhanced growth, and therefore inhibition of this molecule or downstream target may be useful in the treatment of Nf1-related malignancies (41, 110). For example, growth of malignant Nf1 lines and the Nf1<sup>-/-</sup> mouse Schwann cell is stimulated by EGF in vitro and is blocked by agents that antagonize EGF-R and PI3K function (41, 110). Other growth factors and receptors involved in neurofibroma tumorigenesis may also be targeted for future therapies. For example, imatinib mesylate, a rationally designed inhibitor of c-Abl, PDGFR- $\alpha$  receptor and ligand, PDGFR- $\beta$  receptor and ligand, and c-kit tyrosine kinase receptor (19, 23, 137), has been used with success in a patient with metastatic pilocytic astrocytoma (125). This agent may have success treating neurofibromas because, as described above, increased expression of PDGF and c-kit occur in this tumor (136, 137).

## SURGICAL IMPLICATIONS AND FUTURE DIRECTIONS

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. The increased understanding of the pathogenesis of neurofibromas has several important implications for the surgical management of this disease.

Mast cells are recruited to tumor as part of an inflammatory response and subsequently function as active participants in tumor development. This recruitment of supporting cells and the lack of organization of the supporting and Schwann cells that completely encompass and surround the associated nerve root help explain why neurofibromas are phenotypically so different from a surgical management standpoint from schwannomas, which grow peripherally from the nerve sheath and do not infiltrate the nerve or recruit other cell types. Inflammatory cell and fibroblast recruitment are the likely key factors in the adherence of neurofibromas to surrounding tissue and pose the greatest challenge to their successful removal. Also, this mechanism of recruiting cells that infiltrate and encase the nerve explains why nerves involved with neurofibromas are typically not functional (27). The foregoing discussion of heterogenous cellular recruitment to the tumor also poses the possibility that surgery itself, with associated trauma and inflammation, may result in the recruitment of supporting cells to the local environment, leading to increased growth of existing nearby lesions or new formation of neurofibromas (4), but this hypothesis remains to be tested.

Overall, many preclinical studies, as well as a few clinical trials using biological-based therapeutic approaches to target specific genetic or molecular events involved in neurofibroma 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 incidence of spinal or plexiform neurofibromas. It may also be possible to apply new therapeutic agents to the local tumor environment to reduce the incidence of recurrences.

## CONCLUSIONS

Remarkable progress has been made toward understanding the pathogenesis of neurofibromas since the cloning of the *NF1* gene in 1990. The loss of *NF1* function results in decreased neurofibromin and its GAP activity resulting in activation of the RAS pathways and uncontrolled cell proliferation. The development of neurofibromas requires loss or inactivation of



the somatic NF1 allele in the Schwann cell as well as the presence of haploinsufficient supporting Schwann cells, mast cells, perineurial cells, and fibroblasts. It remains to be discovered whether these supporting cells (*NF1*<sup>+/-</sup>) respond to growth factors and signals from the Schwann cell that has already undergone a second hit mutation (*NF1*<sup>-/-</sup>) or whether they respond to another stimulus including trauma or hormonal change to hyperproliferate and secrete factors resulting in an increased chance of a second mutation in the Schwann cell (124). Regardless, both activities are necessary for neurofibroma formation. Additional genetic changes involving genes such as *P53* and *INK2A* are most likely needed for malignant transformation.

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## COMMENTS

This is an informative and excellent in-depth discussion of the metabolic and cellular basis for neurofibromas, especially those associated with neurofibromatosis Type 1 (NF1). The authors emphasize the Schwann cell as the most likely cellular origin of these tumors, even though they are often weakly positive with S-100 stains. Some neuropathologists think a more likely origin is the progenitor cell for the Schwann cell, as well as the perineurial fibroblasts. Just as frequent as NF1 in our experience as surgeons is the solitary, usually globular, and non-plexiform neurofibroma. In these cases, there has been a major change in management in the past decade. These affect a nerve or element of the plexus and have many similarities to solitary schwannomas, except that their capsule is not as well developed. They have two or more entering and exiting fascicles, which are usually tested by nerve action potential recordings. Like the schwannomas, they do not transmit a signal and can usually be sacrificed with minimal or minor functional loss. Usually, the other functional fascicles are worked away, much as in a schwannoma, if the fascicular structure as each pole is worked out. Fascicles are usually somewhat more adherent, especially at the base of the tumor, than a schwannoma, but they usually can be worked away from it.

Thus, in our 1994 publication (1), 99 patients with complete excision of solitary non-von Recklinghausen's disease neurofibromas were available for follow-up; 90% displayed either improved or unchanged motor function and 88% experienced partial or complete resolution of pain. In addition, in another 48 patients with von Recklinghausen's disease who had solitary tumors removed and underwent adequate follow-up, 83% showed improved or unchanged motor function and 74% had partial or complete resolution of their pain syndromes. Even when difficult sites, such as the brachial plexus, are evaluated, the statistics are similar. And so, the neurofibromas previously thought to be unresectable without severe deficit are often resectable with little or

no deficit (2). This is a major change in non-plexiform neurofibroma management.

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1. Donner T, Voorhies R, Kline DG: Nerve sheath tumors of major nerves. *J Neurosurg* 81: 362-373, 1994.
2. Kline DG, Gaha A, Tiel RL, Hudson A: Tumors of peripheral nerve, in Winn R (ed): *Neurological Surgery*. Philadelphia, WB Saunders, 2004.

The key molecular, genetic, and cellular events in neurofibroma tumorigenesis are discussed in detail in this article. The key points of the article include evidence that loss or decreased activity of neurofibromin leads to increased Ras-guanosine triphosphate, which promotes cell proliferation and protects cells from apoptosis. The authors further elucidate the genetic mutations necessary for conversion to malignant peripheral nerve sheath tumors, including p53 gene mutation and loss of heterozygosity for the CDKN2A gene. The authors cogently discuss the cellular microenvironment necessary for neurofibroma genesis. Although NF1-/- Schwann cells are the progenitor cells of the neurofibroma, these tumors are composed of multiple cell types including fibroblasts, mast cells, perineurial cells, and NF1+/- Schwann cells. These other cells, which are haploinsufficient seem to confer a growth advantage contributing to tumorigenesis. In addition, increased growth factor and growth factor receptor expression seem to play a role in NF1 tumorigenesis. The authors discuss a number of biologically-based treatments. These include inhibiting the cellular activity of these supporting cells and targeting of the Ras signaling pathway. I consider the article a must-read for those interested in NF1.

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This is a very informative and complete review of NF1 that could be useful for busy surgeons who may not have the time or know where to look for this information. Articles like this are always helpful and, under Dr. Couldwell's direction, we now have an important reference for review. These types of reports make a subscription to *Neurosurgery* valuable.

I suggest that readers note the discussion regarding the role of the extracellular matrix and non-tumor cells in supporting and promoting neoplastic phenotype. This will be seen as an emerging story in neuro-oncology, as well as in other forms of cancer. *Neurosurgery* should publish more reports like this. They are informative, clear and very valuable for the practicing surgeon.

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