Pathogenesis of Plexiform Neurofibroma: Tumor-Stromal/ Hematopoietic Interactions in Tumor Progression

Karl Staser,[1,2] Feng-Chun Yang,[2,3] and D. Wade Clapp[1,2,4]

[1]Department of Biochemistry, [2]Herman B. Wells Center for Pediatric Research, Department of Pediatrics, [3]Department of Anatomy and Cell Biology, and [4]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana 46202; email: dclapp@iupu.edu

Annu. Rev. Pathol. Mech. Dis. 2012. 7:469–95

First published online as a Review in Advance on November 7, 2011

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org

This article's doi: 10.1146/annurev-pathol-011811-132441

1553-4006/12/0228-0469$20.00

Keywords

neurofibromatosis, NF1, mast cell, SCF, c-kit

Abstract

Neurofibromatosis type 1 (NF1) is a genetic disease that results from either heritable or spontaneous autosomal dominant mutations in the *NF1* gene. A second-hit mutation precedes the predominant NF1 neoplasms, which include myeloid leukemia, optic glioma, and plexiform neurofibroma. Despite this requisite *NF1* loss of heterozygosity in the tumor cell of origin, nontumorigenic cells contribute to both generalized and specific disease manifestations. In mouse models of plexiform neurofibroma formation, *Nf1* haploinsufficient mast cells promote inflammation, accelerating tumor formation and growth. These recruited mast cells, hematopoietic effector cells long known to permeate neurofibroma tissue, mediate key mitogenic signals that contribute to vascular ingrowth, collagen deposition, and tumor growth. Thus, the plexiform neurofibroma microenvironment involves a tumor/stromal interaction with the hematopoietic system that depends, at the molecular level, on a stem cell factor/c-kit-mediated signaling axis. These observations parallel findings in other NF1 disease manifestations and are clearly relevant to medical management of these neurofibromas.

OVERVIEW: NF1, NEUROFIBROMAS, AND MAST CELLS

NF1

Neurofibromatosis type 1 (NF1, also known as von Recklinghausen's disease) is a genetic disorder caused by autosomal dominant mutations in the *NF1* gene, which encodes neurofibromin, a protein that accelerates the intrinsic hydrolysis of $p21^{ras}$ (Ras) from its guanosine triphosphate (GTP)- to guanosine diphosphate (GDP)-bound conformation. This disease afflicts approximately 1 in 3,500 persons worldwide in a pandemic fashion, and it is the most common genetic disorder with a predisposition to cancer (1). NF1 manifests with both nontumorigenic and tumorigenic maladies, including learning disabilities, skeletal dysplasia, nonhealing fractures (pseudarthrosis), myeloid leukemia, and tumors such as optic glioma and the namesake neurofibroma. The disease's hallmarks include hyperpigmented areas of the skin (café-au-lait macules) and hamartomas on the iris (Lisch nodules), which are important diagnostic criteria and may be observed in the infancy or childhood of afflicted individuals (2, 3). Because prominent NF1 symptoms arise from neural crest–derived tissue (e.g., glia, Schwann cells, melanocytes), some reports have characterized NF1 as a disorder of the neural crest. However, NF1 pathologies arise in organs derived from all embryonic germ layers, and we should consider NF1 to be not only a tumor predisposition syndrome but also a systemic developmental disorder (4).

Neurofibromas

NF1-like cutaneous tumor syndromes appeared in the literature during the eighteenth century (5–7), and in the 1880s, Friedrich von Recklinghausen (8) published seminal observations detailing cutaneous tumors composed of both neuronal and fibroblastic tissue. NF1's pathognomonic neurofibromas are slowly progressing, heterogeneous, solid tumors composed of Schwann cells, fibroblasts, vascular cells, and invading hematopoietic cells, which are predominantly degranulating mast cells (**Figure 1**) (9–14). Cutaneous and subcutaneous neurofibromas derive from small peripheral nerve branches during adolescence or adulthood and are found in nearly all individuals with NF1 (15). By comparison, plexiform neurofibromas afflict half (or fewer) of individuals with NF1 and develop from cranial and large peripheral nerve sheaths; they may initiate during gestation or early infancy from abnormally differentiated nonmyelinating Schwann cells or their less differentiated precursors (16, 17).

Plexiform neurofibromas typically cause lifelong disfigurement, disability, and mortality. In many cases, plexiform neurofibromas compress cranial nerves and/or peripheral nerve roots at the vertebral column and create an array of morbidities, including paresthesias, paralysis, drooling, sleeplessness, respiratory and gastrointestinal distress, blindness, and loss of bowel and bladder control (18, 19). A plexiform neurofibroma can also transform into a malignant peripheral nerve sheath tumor, a highly morbid, metastatic cancer that afflicts up to 10% of NF1 patients in their lifetime (20, 21).

Treatment of plexiform neurofibromas consists primarily of symptom management and/or surgical resection. In many cases, the tumor's close involvement with vital nerve tissue, vasculature, or other viscera complicates surgery (18, 19, 22). Currently, the tumors have no medical therapy or cure, although several molecularly targeted compounds are in preclinical or clinical testing (23–27). Problematically, nerve sheaths and heavily collagenized areas may diminish drug bioavailability, complicating direct pharmacological inhibition of the tumor mass. Therefore, therapeutic strategies that target components of the tumor microenvironment, including vascular cells and infiltrating mast cells, may prove viable alternatives (28). In this review, we discuss insights into the interactions among the tumor, stroma, and pathogenic mast cells that may be relevant for therapy.

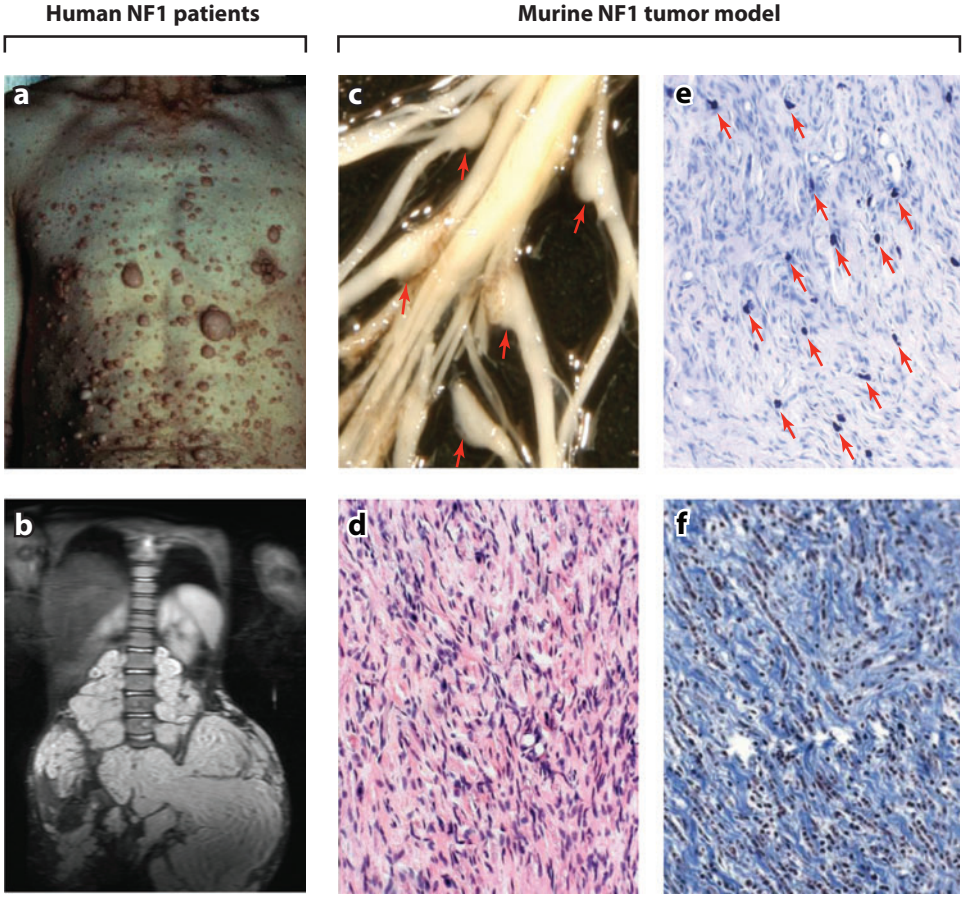

Figure 1

Cutaneous neurofibromas, plexiform neurofibromas, and histology. Example of cutaneous neurofibromas covering the chest and abdomen of a patient with neurofibromatosis type 1 (NF1). Magnetic resonance images of a large plexiform neurofibroma (*indicated by arrows*) compressing (*a*) the spinal column and (*b*) the abdominal viscera. Mouse models of NF1-associated plexiform neurofibroma development exhibit (*c*) enlarged dorsal root ganglia (*red arrows*), which are histologically composed of wavy Schwann cells, numerous fibroblasts, and a mast cell infiltrate (*d*). (*e*) Alcian blue deeply stains mast cell granules, and (*f*) trichrome stain highlights the abundant collagen typical of neurofibromas. Images of human patients reproduced with permission from the Children's Tumor Foundation (**http://www.ctf.org**).

Mast Cells

Mast cells are cytoplasmic granule–containing hematopoietic cells that are thought to arise from common myeloid progenitors prior to granulocyte/monocyte lineage commitment (29). Mast cell precursors migrate from the bone marrow into the vasculature and enter dermal tissue, where they mature into immune effector cells. Mast cells help to fight pathogens, increase resistance to certain venoms and toxins, and may perform other immunomodulatory functions, both pro- and anti-inflammatory (30–33). Although mast cells are known predominantly as the mediators of allergy and allergic asthma via immunoglobulin E/Fcε receptor I pathways, they depend on stem cell factor (SCF) signaling at the c-kit receptor tyrosine kinase for their generation and, in some contexts, pathophysiological activation

(34–37). Indeed, mice that have marked loss of c-kit signaling because of spontaneous mutations affecting both copies of the c-kit receptor tyrosine kinase gene (*W*, or white spotting locus, mutants) exhibit profoundly reduced numbers of tissue-resident mast cells (35).

There is evidence that the proinflammatory activities of mast cells and other immune effector cells sustain tumor microenvironments in various disease models (reviewed in References 38–40). In this inflammatory microenvironment hypothesis, tumorigenic cells recruit and co-opt the functions of nontumorigenic hematopoietic cells via unchecked mitogenic and chemotactic signals. These recruited cells, in turn, coordinate vascular ingrowth; collagen deposition; and the pathological inflammation promoting extracellular matrix remodeling, tumor expansion, invasion, and metastasis. Specifically, mast cells can synthesize and secrete matrix metalloproteinases, various cytokines (e.g., interleukin-6 and tumor necrosis factor α), and multiple mitogens [e.g., nerve growth factor, vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF)] (32, 33) with putative roles in tumor initiation, maintenance, and growth.

Mast cells have been associated with NF1 since 1911, when Greggio (14) first noted "les cellules granuleuses" in neurofibroma tissue. Decades later, several investigators confirmed their presence by using traditional histology and electron microscopy (9–13). By the 1980s, mast cells were widely recognized inflammatory effectors and hallmark histological features (albeit of unknown significance) of the neurofibroma. Riccardi (41) hypothesized that mast cells may critically contribute to neurofibroma formation and proposed that mast cell degranulation explained his clinical observations of coincident pruritus and cutaneous neurofibroma formation. A small human study with ketotifen, a second-generation H1 antihistamine that is also reported to have mast cell–stabilizer activities, reduced pruritus and/or slowed neurofibroma growth (42), but a subsequent multiphase trial confirmed only antipruritic and analgesic effects, not neurofibroma reduction (43). Thus, although ketotifen may provide symptomatic improvement, its role in changing the overall disease appears to be limited.

As discussed in this review, recent biochemical, transplantation, and pharmacological studies have implicated a preponderant role for SCF-mediated mast cell gains in function in orchestrating the neurofibroma microenvironment. This SCF/c-kit coordination of mast cell inflammation and tumor growth may inform a novel approach to NF1 therapeutics.

NF1 GENETICS

NF1 Mutations

Discovery of the *NF1* gene and its cloning have been fundamental to diagnosing the associated disease, to the evolving understanding of NF1 pathogenic mechanisms, and to the ongoing development of novel therapeutics. Notably, the genetic identification of *NF1* was a prerequisite for the generation of accurate NF1 disease models in mice, biological tools that have greatly accelerated research into experimental therapeutics. A century after von Recklinghausen's seminal case reports (8), genetic linkage studies in NF1-afflicted families identified the pericentromeric region of chromosome 17 as the genomic region harboring the gene responsible for the disease (44, 45). Further studies in patients with translocations of chromosome 17 (46–49) facilitated the identification and full-length sequencing of the *NF1* gene (50), which spans 350 kb of human chromosome 17 (17q11.2) and encodes 59 exons that produce a 2,818–amino acid protein (46, 51–53). Note that human neurofibromin and its mouse homolog share 98% identity at the protein level (54).

Approximately half of *NF1* mutations in humans arise spontaneously (55); the majority of mutations lead to premature truncation of the protein neurofibromin (56, 57). When *NF1* mutations occur postmeiotically, individuals may exhibit segmental NF1 with manifestations confined either regionally or to a subset

of normally affected cell types (e.g., only pigmentation defects) (58). Different *NF1* frameshift and point mutations do not necessarily correlate with phenotypic severity, although some studies have shown that microdeletions that encompass the entire *NF1* locus (which account for less than 10% of mutations) are associated with earlier onset and more profound disease manifestations (59, 60). Phenotypic variation tends to be high even within families, and pedigree analyses indicate that although *NF1* mutations are fully penetrant, variation in genes independent of the *NF1* locus critically modulates time to onset and course of the disease (61, 62). Parallel to the human data, different *Nf1*-mutant mouse strains exhibit both varied expression levels of *Nf1* and variable susceptibility to different NF1-like disease manifestations (63). Overall, with the exception of the documented severity associated with *NF1* locus–encompassing microdeletions and a uniquely mild phenotype associated with a 3-bp deletion in exon 17 (64), particular genetic mutations or genomic variations that may correlate to specific disease outcomes are largely unknown.

NF1 encodes neurofibromin, a protein that functions, at least in part, as a Ras GTP-activating protein (GAP) (65–69). Neurofibromin and other Ras-GAPs exponentially accelerate the intrinsic hydrolysis of Ras-GTP to its inactive GDP-bound conformation (70). In response to multiple mitogenic stimuli, active Ras-GTP orchestrates diverse protein signaling networks, including mitogen-activated protein kinase (MAPK)- and Akt-directed pathways (71–75). Thus, by accelerating the conversion of Ras-GTP to Ras-GDP, neurofibromin negatively regulates Ras-dependent signaling cascades and, generally, downregulates mitogenic events across diverse protein networks. In cases of *NF1* heterozygosity or nullizygosity, as observed in somatic cells and in tumor cells of individuals with NF1, respectively, downstream Ras-mediated phosphorylation and transcriptional events can increase in duration and total output. Such global upregulation of Ras-dependent activity in *NF1*/*Nf1*-disrupted tissue typically leads to cellular gains in function, including enhanced proliferation, migration, and survival in multiple cell types (reviewed in References 76–80). Notably, the specific Ras effectors potentiated by loss of *NF1* may vary by cell and receptor type, and biochemical consequences in one cell-receptor system may or may not be observed in another.

NF1 Gene Dosage

Although *NF1* is classified as a classical Knudson tumor-suppressor gene, multiple studies have shown that *NF1* heterozygosity critically modulates cell fate and function by altering Ras-dependent biochemical pathways in distinct cell types (reviewed in Reference 79). Moreover, physiological Ras activity regulates embryogenesis, early development, and normal tissue maintenance. Therefore, neurofibromin may be viewed not only as a tumor suppressor but also as a regulator of histiogenesis, cellular maintenance, and repair (4). Accordingly, NF1 is a disorder of both tumor predisposition and developmental dysplasia.

Although somatic cells in an individual with NF1 are heterozygous for *NF1*, loss of heterozygosity (LOH) in different cell types typically precedes hallmark hyperplastic, dysplastic, and neoplastic disease manifestations. LOH has been demonstrated in human tissue samples and confirmed in NF1 mouse models of certain NF1 pathologies via multiple molecular techniques, which coincides with *NF1*'s designation as a tumor-suppressor gene. For example, LOH permits neurofibroma formation in Schwann cells or their precursors (17, 81–83), precedes cutaneous neurofibroma formation in skin-derived glial precursors (15), initiates pheochromocytoma in chromaffin cells (84), produces hyperpigmentation (e.g., café-au-lait macules) in melanocytes (58, 85), induces myelomonocytic leukemia in myeloid progenitor cells (86), and permits astrocytoma formation in glia (87). Recent studies have also revealed LOH in tissues extracted from tibial pseudarthrosis (88), although involved cell types and their exact genetic requirements have

Table 1 The cell of origin and known heterotypic interactions in multiple neurofibromatosis type 1 (NF1) symptoms

Neoplasia	Cells of origin	Other cells
Plexiform neurofibromas	*Nf1*$^{-/-}$ Schwann cells or their precursors	*Nf1*$^{+/-}$ mast cells
CNS gliomas	*Nf1*$^{-/-}$ astrocytes	*Nf1*$^{+/-}$ microglia
Dermal neurofibromas	*Nf1*$^{-/-}$ skin-derived glial precursors	*Nf1*$^{+/-}$ background/unclear[a]
Pheochromocytomas	*Nf1*$^{-/-}$ chromaffin cells	Unclear
Early CNS gliomas	*Nf1*$^{-/-}$ astrocytes	None[b]
Early glial tumors	*Nf1*$^{-/-}$ glia	None
Myeloid leukemia	*Nf1*$^{-/-}$ myeloid cells	None
Other symptoms		
Café-au-lait macules	*Nf1*$^{-/-}$ melanocytes	Unclear
Pseudarthroses	*Nf1*$^{-/-}$ or *Nf1*$^{+/-}$ connective tissue	Unclear
Osteoporosis	*Nf1*$^{+/-}$ osteoblasts	Unknown[c]
	Nf1$^{+/-}$ osteoclasts	
Cognitive deficits	*Nf1*$^{+/-}$ neurons	Unknown
Vascular disease	*Nf1*$^{+/-}$ endothelia	Unknown
	Nf1$^{+/-}$ vascular smooth muscle	

[a]Unclear indicates that the data were derived from mouse and/or human samples with a heterozygous *Nf1/NF1* background but that a firm conclusion has not been made as to whether the condition requires this background.
[b]None indicates that these symptoms can emerge despite wild-type supporting cells.
[c]Unknown indicates that although *Nf1* haploinsufficiency appears to be sufficient for pathogenesis, the possibility of undetected cooperating events cannot be ruled out. These data derive principally from studies in both mice and humans.
Abbreviation: CNS, central nervous system.

not yet been clearly documented (**Table 1**) (89, 90).

Although it is unknown why some tissues are more susceptible to *NF1* LOH and subsequent disease manifestation, a general hypothesis suggests that normal regulation of Ras signaling in certain cell types, for unclear reasons, depends more heavily on neurofibromin activity and/or expression levels than on the activity of other Ras-GAPs. Similarly, identical cell types located within discrete regions of an organ may demonstrate an increased dependency on *NF1* expression and its Ras-GAP activity. Recent studies have shown that astrocytes and neuroglial stem cells in the optic nerve and brain stem express more neurofibromin and more readily manifest phenotypic consequences in its absence when compared with astrocytes from the frontal cortex (91, 92). These observations help explain the NF1-associated predisposition of astrocytoma to arise as optic glioma and not within the forebrain. In other NF1 pathologies, the mechanisms behind regional and tissue type–specific reliance on functional neurofibromin is largely unknown, although insights in this area could impel future discovery of spatially and/or temporally targeted molecular therapies.

Individuals with NF1 also have an increased prevalence of multiple generalized manifestations that do not appear to require cell-specific biallelic inactivation of *NF1*, implicating a systemic gene-dosage effect. These pathologies include skeletal and mesenchymal dysplasia (e.g., short stature, osteoporosis, and soft tissue malformation), disorders of neurocognitive development (e.g., retardation, spatial/visual coordination, and autism), and vascular pathologies (e.g., fistulae, infarcts, and aneurysms). Corresponding to these findings, biochemical and animal studies have shown various abnormalities of function in *Nf1/NF1* heterozygous osteoblasts and osteoclasts (93–95), GABA neurons (96), endothelial cells (ECs)

(97, 98), and smooth muscle cells (99). These studies and others have additionally demonstrated increased risk for NF1-associated bone, neuronal, and vascular disease in *Nf1* heterozygous mice. Thus, *NF1* heterozygosity alone alters Ras-dependent pathways to a degree sufficient for the pathological alteration of normal developmental and homeostatic processes in multiple organ systems.

Although *NF1* heterozygosity may predispose NF1 patients to generalized deficiencies, *NF1* heterozygous cells appear to also critically modulate neurofibroma growth and maintenance. In mice, *Nf1* haploinsufficient mast cells and fibroblasts, major constituents of the heterogeneous plexiform neurofibroma, demonstrate multiple gain-in-function phenotypes that include enhanced proliferation, survival, migration, and cytokine production in response to specific stimuli (100, 101). These data parallel findings in *Nf1* haploinsufficient microglia (102), which critically modulate the inflammatory microenvironment of NF1-associated optic glioma (103–105). In some mouse models of plexiform neurofibroma and optic glioma formation, tumorigenesis requires *Nf1* haploinsufficiency in nontumorigenic cells. Specifically, hematopoietic stem cell transplantation studies in the $Nf1^{flox/flox}$;*Krox20Cre* and $Nf1^{flox/flox}$;*P0aCre* models (discussed in detail in the section titled The Stem Cell Factor/c-kit Axis in Plexiform Neurofibroma Formation and Its Therapeutic Implications, below) have shown that neurofibroma genesis requires *Nf1* haploinsufficiency and c-kit-mediated signaling in the hematopoietic compartment (24).

Thus, heterotypic interactions between the tumor, the hematopoietic system, and other stromal components critically promote and sustain the neurofibroma microenvironment. Moreover, *Nf1/NF1* gene dosage (normal, heterozygous, and nullizygous) in discrete cell lineages differentially modulates cell fate, function, and in some mouse models, disease outcome. These mouse models may illuminate the pathogenic mechanisms underpinning human genetic disease. Although several NF1-associated diseases have been successfully modeled in mice, this review focuses predominantly on plexiform neurofibroma formation while making parallel observations in models of optic glioma formation. From the ongoing insights derived from human data and these mouse models, investigators can seek out novel molecular therapeutics targeting specific and essential interactions that permit disease pathogenesis.

NF1 MOUSE TUMOR MODELS

Nf1 Traditional Knockout

Nf1 knockout mice harbor a disruptive neomycin (*neo*) cassette in *Nf1* exon 31 ($Nf1^{+/n31}$) (106, 107), an exon site homologous to a hot spot of human *NF1* mutations (108). The encoded *neo* cassette induces protein instability and degradation, which lead to a reduction of approximately 50% in total neurofibromin protein level. $Nf1^{+/n31}$ (i.e., $Nf1^{+/-}$) mice have a shortened life span and occasionally develop pheochromocytoma and leukemia, diseases found in increased incidence in humans with NF1. Other investigations have shown these mice to have neuronal deficiencies (96), increased risk for vascular pathology (109), and increased susceptibility to osteoporosis (95). However, $Nf1^{+/-}$ mice do not develop the pathognomic neurofibroma or hallmark café-au-lait macules and, by themselves, are not used as models of NF1-associated tumor formation.

The failure of $Nf1^{+/-}$ mice to develop neurofibromas—which individuals with NF1 develop nearly universally—was initially puzzling because humans, like the heterozygous mice, are born essentially $NF1^{+/-}$. This phenotypic disparity may be ascribed to inherent differences between humans and mice: Mice have shorter life spans, different exposures and responses to toxins and carcinogens, and perhaps different susceptibilities to mutations in the normal *Nf1* allele (106). Therefore, the chance of an *Nf1* second hit and concomitant neoplasia may be lower for a $Nf1^{+/-}$ mouse than for a human born heterozygous at the *NF1* locus.

Despite the absence of neurofibromas in the *Nf1*$^{+/-}$ mice, extracted leukemic myelocytes and pheochromocytoma tissue exhibit *Nf1* LOH, providing strong evidence that tumorigenesis requires a second hit in these tissues and supporting the classification of *Nf1* as a tumor suppressor gene (106). Thus, many or all cell types and tissues with forced biallelic *Nf1* inactivation could demonstrate aberrant and/or neoplastic phenotypes. However, mice born *Nf1*$^{-/-}$ die at embryonic day 13.5 secondary to defects in developing structures of the heart (107). In parallel with these findings, humans are never born nullizygous for *NF1*; presumably, they die in utero. Due to the developmental requirement for at least one allele of *NF1/Nf1*, the investigation of biallelic *Nf1* inactivation in adult mice must be performed using chimeric or conditional knockout models.

Indeed, chimeric *Nf1*$^{-/-}$ mice (mice with mixed *Nf1*$^{+/+}$ and *Nf1*$^{-/-}$ somatic cells) provided direct evidence that neurofibroma genesis requires biallelic inactivation of *Nf1*. To create this model, investigators injected *Nf1*$^{-/-}$ embryonic stem cells into wild-type (WT) blastocysts during embryogenesis (81). Moderately chimeric animals developed several hallmark symptoms of NF1, including neuromotor defects, myelodysplasia, and neurofibromas in the tongue and limb and along the dorsal root ganglia. By contrast, high *Nf1*$^{-/-}$ chimerism was lethal, whereas low chimerism produced no immediately observable phenotype. In this study, *Nf1*$^{-/-}$ embryonic stem cells also carried a β-galactosidase transgene, and tumor histology revealed widespread β-galactosidase expression. Conclusively, then, the neurofibromas arose specifically from the cell lineages developmentally derived from *Nf1*$^{-/-}$ embryonic stem cells.

Observations from this chimeric model, vis-à-vis findings from the *Nf1* heterozygous mouse, validate biallelic *Nf1* inactivation as a condition that engenders and, most likely, is required for tumorigenesis. Despite these critical insights, tumor formation in the chimera depends upon an undefined quantity of *Nf1*$^{-/-}$ cells (of all lineages) admixed with *Nf1*$^{+/+}$ cells (also of all lineages). These genetic doses do not mimic human NF1 genetics, in which, presumably, only the tumorigenic cells are essentially *NF1*$^{-/-}$ and the surrounding ECs, fibroblasts, pericytes, and hematopoietic cells—namely the microenvironment—are essentially *NF1*$^{+/-}$. Although observations from the *Nf1*$^{-/-}$ chimera have advanced the mechanistic understanding of neurofibroma pathogenesis, they do not allow delineation of the specific genetic conditions and heterotypic interactions that underpin tumor formation.

Nf1 Conditional Knockout

Cre-loxP site-specific recombination technology permits tissue- and condition-specific gene disruption, allowing researchers to circumvent the embryonic lethality of traditional knockout constructs and to investigate protein function in specific cell lineages. To these ends, investigators engineered a conditional *Nf1* knockout construct targeted for disruption in a subset of Schwann cells (76, 82, 110). First, they generated a mouse harboring flanking *loxP* (*flox*) sites at *Nf1*'s exons 31 and 32 (*Nf1*$^{flox/flox}$). *LoxP* is a 34-bp recognition sequence for the bacteriophage-derived protein Cre recombinase (Cre), which mediates excision and recombination at these sites (111). The investigators then crossed this *Nf1*$^{flox/flox}$ mouse with a mouse carrying the *Krox20Cre* construct, which places *Cre* downstream of the promoter element for *Krox20*. *Krox20* is an endogenous mammalian gene that directs peripheral nerve myelination (112), and it robustly expresses in approximately 10% of Schwann cells and their precursors (82). In the *Nf1*$^{flox/flox}$;*Krox20Cre* mouse, then, a subset of Schwann cells harbor biallelic *Nf1* disruption, while the bulk of somatic cells remain functionally WT.

However, *Nf1*$^{flox/flox}$;*Krox20Cre* mice do not develop neurofibromas. Therefore, biallelic *Nf1* inactivation in only a subset of Schwann cells and/or Schwann cell precursors is not sufficient to engender tumorigenesis. By contrast, plexiform neurofibromas readily and reliably form in *Nf1*^{flox};*Krox20Cre* mice

that additionally carry the *Nf1* knockout allele (*Nf1*$^{flox/-}$;*Krox20Cre*). In such animals, a subset of Schwann cells are *Nf1* deficient, whereas other somatic cells are phenotypically *Nf1*$^{+/-}$ (i.e., *Nf1*$^{flox/-}$ but without *Cre*-mediated recombination). At approximately one year of age, the *Nf1*$^{flox/-}$;*Krox20Cre* mouse develops dorsal root ganglia tumors that grossly and histologically resemble plexiform neurofibromas. Histological examination of these tumors reveals wavy, dysplastic Schwann cells, abundant fibroblasts, collagenization, and an infiltration of degranulating mast cells. As observed in the *Nf1*$^{flox/-}$;*Krox20Cre* model, then, plexiform neurofibroma genesis requires biallelic *Nf1* inactivation in Schwann cells and heterotypic interactions with an *Nf1* haploinsufficient stroma, which includes the fibroblasts, vascular cells, and mast cells long known to constitute the neurofibroma microenvironment.

Notably, widespread *Nf1* deletion in glial precursor cells during embryogenesis (at approximately embryonic day 12.5), as driven by the *Nf1*$^{flox/flox}$;*DhhCre* model, permits plexiform neurofibroma genesis despite WT levels of neurofibromin in the surrounding cells of the microenvironment (17). These *Nf1*$^{flox/flox}$;*DhhCre*-derived plexiform neurofibromas exhibit dysplastic Schwann cell–like cells, aberrant collagenization, and an abundant infiltration of mast cells, just as the neurofibromas observed in the *Nf1*$^{flox/-}$;*Krox20Cre* model do. Thus, early and widespread biallelic inactivation of *Nf1* circumvents the requirement for an *Nf1* haploinsufficient microenvironment in plexiform neurofibroma formation (17). This model gives important insights into potential tumor cells of origin and provides a model for sporadic neurofibroma formation as observed in individuals without genetic NF1.

NF1 Models as Parallels to Models of Astrocytoma Formation

A mouse model of NF1-associated optic nerve glioma formation demonstrates a similar requirement for *Nf1* haploinsufficiency in the tumor microenvironment (113). To generate this model, *Nf1*$^{flox/flox}$ mice were intercrossed with a transgenic mouse carrying *Cre* downstream of the promoter element for glial fibrillary acidic protein (*GFAP*) gene, which drives *Cre* expression in astrocytes. Although both *Nf1*$^{+/-}$ and *Nf1*$^{flox/flox}$;*GFAPCre* mice demonstrate increased numbers of astrocytes (102, 114), *Nf1*$^{flox/flox}$;*GFAPCre* mice do not develop optic nerve gliomas. These findings directly parallel those in *Nf1*$^{flox/flox}$;*Krox20Cre* mice, which never develop plexiform neurofibromas (113, 114). As another direct parallel to the *Krox20Cre* tumor model, additional haploinsufficiency in the cellular background permits optic glioma formation. At approximately one year of age, these *Nf1*$^{flox/-}$;*GFAPCre* mice reliably demonstrate tumors of the optic chiasm that are histologically consistent with low-grade gliomas infiltrated by inflammatory microglia (113).

In another intriguing parallel, biallelic inactivation in neuroglial progenitor cells induces optic glioma formation independent of the *Nf1* genetic dose in surrounding cells (87), just as in the *Nf1*$^{flox/flox}$;*DhhCre* model of plexiform neurofibroma genesis. These mutant mice carry a transgenic construct that expresses *Cre* under control of the human GFAP promoter (*hGFAPCre*), which expresses in mature astrocytes as well as in the more primitive radial glial cell population. In this model, *Nf1*$^{flox/-}$;*hGFAPCre* and *Nf1*$^{flox/flox}$;*hGFAPCre* mice manifest similar disease phenotypes compared with their *hGFAPCre*-negative littermates; these phenotypes include increased numbers of astrocytes, increased proliferation of glial progenitor cells during brain development, hyperplasia of the optic nerve, and shortened life spans (87).

Thus, multiple mouse models in two separate NF1-associated pathologies demonstrate different *Nf1* dosage requirements for tumorigenesis that seemingly depend on the cell-specific developmental timing of the gene's deletion. As a general observation, widespread biallelic inactivation of *Nf1* in precursors of the putative tumor cell appears to bypass the requirement for haploinsufficiency of cells in the tumor microenvironment. Mechanistically, it is

unclear whether *Nf1*-inactivated precursor cells harbor an intrinsic proclivity toward neoplasm, whether they respond more aggressively to recruited nontumorigenic cells, or whether they are simply better at recruiting these cells. Above all, given the consistent observation of invading mast cells and microglia, all these models suggest pathogenic roles for hematopoietic effectors co-opted by tumorigenic cells.

SCHWANN CELLS, *Nf1* HAPLOINSUFFICIENT MAST CELLS, AND THE TUMOR MICROENVIRONMENT HYPOTHESIS

The *Nf1*$^{flox/-}$;*Krox20Cre* model, among others, approximates the genetic conditions expected in humans with NF1, wherein all body cells are *NF1* heterozygous and only a small fraction of Schwann cells and Schwann cell precursors experience biallelic *NF1* inactivation. Thus, heterozygous nontumorigenic cells, including vascular cells, fibroblasts, and mast cells, probably contribute to tumor pathogenesis and maintenance in the human patient. Here, we focus on cell culture– and transplantation-based studies that demonstrate the phenotypic consequence of biochemical deregulation in nontumorigenic cells, as well as the critical importance of heterotypic interactions between the tumorigenic *Nf1*$^{-/-}$ Schwann cell, the recruited *Nf1*$^{+/-}$ mast cell, and other mitogenic events within the tumor stroma.

Schwann Cells

That the Schwann cell is the tumor cell of origin in NF1-associated peripheral nerve tumors had long been a matter of controversy; von Recklinghausen himself believed the tumors to be of a primary fibroblastic or connective tissue origin (see, for example, Reference 115 for an early and detailed discussion of this debate). Today, preponderant molecular evidence implicates a Schwann cell or Schwann cell precursor cell as the tumor cell of origin. As foundational evidence, investigators detected *NF1* LOH in neurofibroma-derived Schwann cells but not fibroblasts (116–118), and Schwann cells cultured from neurofibromas demonstrate angiogenic and invasive phenotypes in tissue culture (119). Importantly, all characterized mouse models of plexiform neurofibroma formation depend upon biallelic *Nf1* inactivation using Cre drivers restricted primarily to neural crest– and Schwann cell–directed cell lineages (15–17, 24, 82, 83).

However, questions still exist as to the precise stage of Schwann cell differentiation that gives rise to the tumor (reviewed in Reference 120). Plexiform neurofibromas probably develop congenitally, and thus, neural crest stem cells or other progenitor cells may generate tumors during embryogenesis, although investigators have largely agreed that *Nf1*-deficient fetal neural crest stem cells cannot directly form a plexiform neurofibroma (83, 120). Moreover, aggregated observations from studies of different mouse tumor models (*Nf1*$^{flox/-}$;*Wnt1Cre*, *Nf1*$^{flox/-}$;*P0aCre*, *Nf1*$^{flox/-}$;*Krox20Cre*, and *Nf1*$^{flox/-}$;*3.9PeriostinCre*) implicate differentiated glia, including mature nonmyelinating Schwann cells (i.e., Remak bundles), as the probable tumor cells of origin (16, 24, 82, 83). However, given the observation from the *Nf1*$^{flox/flox}$;*DhhCre* model, which expresses Cre at the late boundary cap cell stage, as well as the fact that both *P0aCre* and *Krox20Cre* express in Schwann cell precursors, tumorigenic events in these precursor cells or their progeny cannot be ruled out (17). Finally, Le et al. (15) have created dermal and plexiform neurofibromas by autologous transplantation of neural crest–like stem cells derived from skin [skin-derived precursors (SKPs)] of adult *Nf1*$^{flox/-}$ mice carrying a tamoxifen-inducible *Cre* transgene. This study has shown that adult-type neural precursor cells can form tumors, and it has demonstrated the importance of localized microenvironment cues whereby an SKP can develop into a dermal neurofibroma or a plexiform neurofibroma, depending on its engrafted location. Intriguingly, SKP-derived tumor formation is greatly accelerated in pregnant

females versus males and nonpregnant females, which suggests that hormonal cues, as well as genetic and spatial cues, dictate tumor fate.

On the basis of these studies, firm conclusions cannot yet be made as to the exact tumor cell of origin, but these models and the multiple molecular studies in human tissues almost certainly rule out a fibroblast-derived neoplasia, as some investigators once postulated. Probably, then, biallelic *Nf1* inactivation at multiple stages of Schwann cell differentiation can initiate tumorigenesis with potentially disparate requirements for cooperative cues, whether genetic or environmental.

Because a Schwann cell or Schwann cell–like cell is neurofibroma's tumorigenic cell, $Nf1^{-/-}$ cultured primary Schwann cells have provided critical insights into putative mechanisms of neurofibroma pathophysiology. An initial study demonstrated that $Nf1^{-/-}$ Schwann cells induce EC and fibroblastoid cell proliferation by secreting PDGF, fibroblast growth factor 2 (FGF-2), and midkine (121). These factors may potentially drive vascularization and collagenization of the growing tumor, which suggests a mechanism by which $Nf1^{-/-}$ Schwann cells orchestrate the nascent tumor microenvironment. A subsequent study found that Schwann cells derived from $Nf1^{-/-}$ mouse embryos proliferate more quickly than do WT cells, display an irregular morphology in cell culture, and secrete approximately six times as much soluble SCF as do $Nf1^{+/-}$ and WT Schwann cells (122). Conditioned media from such $Nf1^{-/-}$ Schwann cells promotes the chemotaxis of primary cultured mast cells, doing so at twice the rate for $Nf1^{+/-}$ mast cells versus WT mast cells. Recombinant SCF reproduces this chemotactic effect, and genetic disruption of the mast cell's c-kit receptor (via $Nf1^{+/-}$;*W* mutations) or addition of c-kit receptor–blocking antibodies abolishes Schwann cell–mediated chemotaxis. These data implicate a direct link between the $Nf1^{-/-}$ Schwann cell and the mast cell that is mediated mechanistically through the SCF/c-kit signaling axis. As further evidence of this signaling pathway's importance in humans with NF1, previous and subsequent studies have shown that neurofibroma tissues express SCF messenger RNA, that human neurofibroma–derived Schwann cells secrete high levels of SCF, and that serum from individuals with NF1 contains increased levels of SCF compared with controls (122–125). When considered with the well-established histological phenotype of mast cell invasion and degranulation in tumor tissue (as well as the mast cell's dependency on c-kit for its cytopoiesis), these studies roundly implicate an SCF/c-kit signaling axis as a potential pathogenic mechanism.

Nf1 Haploinsufficient Mast Cell Phenotype

SCF regulates mast cell cytopoiesis, proliferation, survival, and cytokine synthesis, functions that *Nf1* haploinsufficiency potentiates. In fact, the study of SCF-stimulated $Nf1^{+/-}$ mast cells provided foundational evidence that haploinsufficiency of the *Nf1* tumor suppressor could modulate multilineage cell fate and function both in tissue culture and in vivo (100). In these experiments, $Nf1^{+/-}$ mice were intercrossed with mice that were naturally mutated at the c-kit receptor tyrosine kinase (W^{41}). This naturally occurring mutation at the *W* (or white spotting) locus reduces c-kit receptor tyrosine kinase activity by approximately 85%, leading to albinism and tissues that are largely devoid of mast cells (34, 126–129). Ingram et al. (100) found that $Nf1^{+/-};W^{41}/W^{41}$ intercrossed mice exhibit increased numbers of dermal and peritoneal mast cells compared with their W^{41}/W^{41} counterparts. Likewise, $Nf1^{+/-}$ mice demonstrate increased numbers of dermal and peritoneal mast cells. Mast cells cultured from $Nf1^{+/-}$ and $Nf1^{+/-};W^{41}/W^{41}$ bone marrow progenitor cells display several SCF-mediated gains in function, including proliferation at approximately twice the rate of WT and W^{41}/W^{41} mast cells, respectively. $Nf1^{+/-}$ and $Nf1^{+/-};W^{41}/W^{41}$ bone marrow cells also form increased numbers of mast cell colonies ex vivo (100). In vivo, $Nf1^{+/-}$ mast cells and mast cell precursors demonstrate an exquisite hypersensitivity to low doses of soluble SCF, as measured

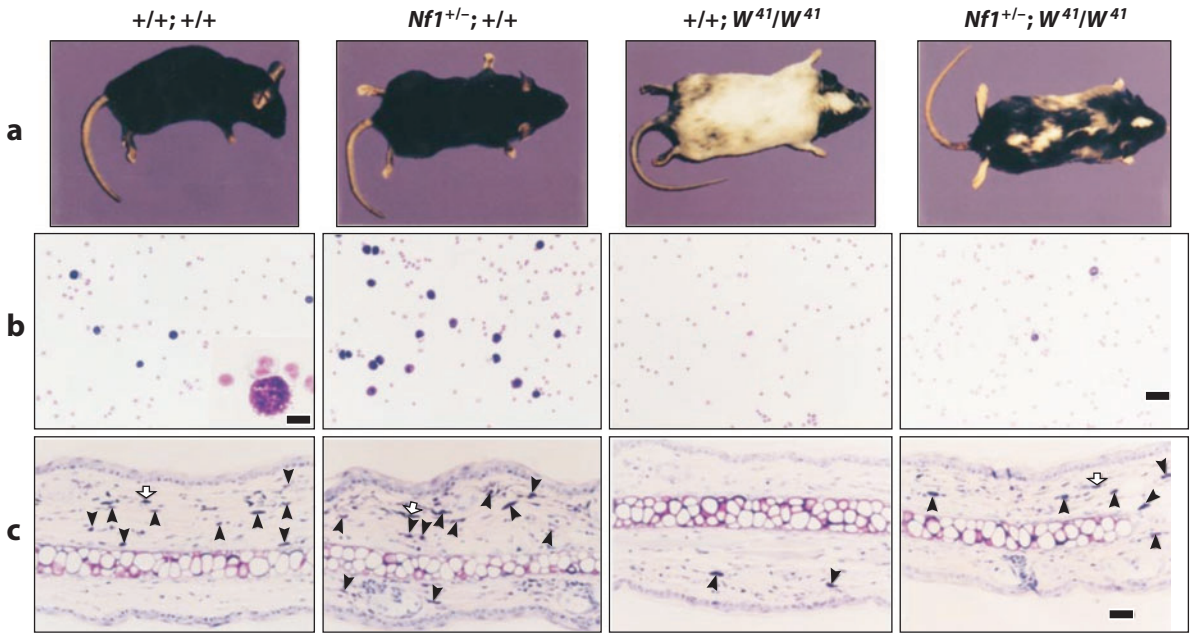

Figure 2

Effect of haploinsufficiency of *Nf1* on coat color and total numbers of cutaneous and peritoneal mast cells. (*a*) Coat-color pattern of a representative mouse from each of the following genotypes: +/+;+/+, $Nf1^{+/-}$;+/+, +/+;W^{41}/W^{41}, and $Nf1^{+/-}$;W^{41}/W^{41}. Haploinsufficiency at *Nf1* partially corrects the coat-color deficiency in mice homozygous for the W^{41} allele in a C57BL/6 genetic background. (*b*) Representative cytospins from peritoneal lavage fluid stained for mast cells from individual mice of the four *Nf1* and *W* genotypes. Peritoneal cells were stained with toluidine blue to quantify the total number of mast cells per peritoneal lavage. A higher magnification of a representative mast cell of the wild-type mouse is shown at left (original magnification, 3,200×). Scale bar at far left, 10 μm. Scale bar at far right, 30 μm. (*c*) Representative ear biopsies stained for cutaneous mast cells from individual mice of the four *Nf1* and *W* genotypes. Specimens were stained with hematoxylin and eosin to assess routine histology and with Giemsa to identify mast cells. Ear biopsies were stained with Fontana-Masson to differentiate melanin-containing cells from mast cells. Cutaneous mast cells (Giemsa positive, Fontana-Masson negative) were quantitated in a blinded fashion by counting the distal 5 mm of the ears. Black arrows indicate Giemsa-positive mast cells, and white arrows indicate Fontana-Masson melanin–containing cells. Scale bar, 35 μm. Reproduced from Reference 100.

by mast cell proliferation and recruitment through the use of SCF-loaded micro-osmotic pumps implanted in dermal tissue (130). Moreover, $Nf1^{+/-}$;W^{41}/W^{41} animals have a black mottling of their normally predominant albino coat, which shows that *Nf1* haploinsufficiency restores c-kit-impaired melanocyte function (100), a neural crest–derived lineage that expresses neurofibromin and is relevant to human NF1 (e.g., café-au-lait macules) (**Figure 2**) (131). Biochemical inquiries further demonstrated increased latency and potency of GTP-bound Ras in SCF-stimulated $Nf1^{+/-}$ mast cells. Taken together, these data indicate that *Nf1* haploinsufficiency restores c-kit-dependent kinase activity to a biologically relevant degree, alters mast cell cytopoiesis and functional potential, and modulates fate and function in multiple cell lineages in live animals.

Nf1 Haploinsufficient Biochemistry in the Stem Cell Factor–Stimulated Mast Cell

Subsequent studies have detailed the biochemical mechanisms modulating SCF-mediated gains in function in the *Nf1*

haploinsufficient mast cell. Principally, these alterations arise from deregulated signaling events in Ras-dependent networks. In response to ligand binding at diverse cell-surface receptors, Ras activates to its GTP-bound state and promotes phosphorylation in downstream protein networks, including those orchestrated by MAPKs and phosphatidylinositol 3-kinase (PI3K) (72–75). Neurofibromin, which contains a highly conserved GAP-related domain with homology to the yeast gene products *IRA1* and *IRA2*, markedly accelerates the intrinsic hydrolysis of active GTP-bound Ras to its GDP-bound state (47, 52, 65, 68, 76, 132). Generally, loss-of-function mutations in genes encoding Ras-GAPs promote cell growth, proliferation, migration, and survival (40). In myeloid progenitor cells, microglia, and mast cells, loss of one or both alleles of *Nf1* leads to increased duration of Ras-GTP and the activity of specific effectors within Raf/Mek/Erk, PI3K/Rac/Pak/p38 (where Pak refers to p21-activated kinase), and PI3K/Akt cascades (86, 100, 105, 122, 133–138).

Cell-culture and in vivo studies of genetically disrupted mast cells indicate that the Raf/Mek/Erk pathway may primarily modulate SCF-mediated proliferation and that the PI3K/Rac2/Pak/p38 pathway controls F-actin dynamics and cellular motility (130, 135, 136, 138–140). However, biochemical investigations have also shown that the PI3K-dependent pathway reinforces the classical Raf/Mek/Erk cascade through the activity of the Paks (130, 136). Thus, PI3K directly modulates SCF-mediated proliferation. In this schema, PI3K-activated Rac2 induces Pak1 to phosphorylate Mek at serine 298, as well as Raf1 at serine 338, which potentiates Raf1's phosphorylation of Mek at serines 217 and 222. These activities potentiate phosphorylation of the extracellular signal–regulated kinases, Erk1 and Erk2. Erk1/2 phosphorylate cytoplasmic targets (e.g., $p90^{rsk}$), translocate to the nucleus, and activate multiple mitogenic transcription factors (e.g., c-Fos, Elk1, C/EBP), although Erk-dependent transcriptional events and gene products are largely undocumented for the SCF-stimulated $Nf1^{+/-}$ mast cell. Ultimately, Erk activity appears to regulate SCF-mediated cell-cycle progression, events that are potentiated in the $Nf1^{+/-}$ mast cell. Furthermore, these studies have suggested that SCF-mediated hyperproliferation in the $Nf1^{+/-}$ mast cell directly relies on PI3K/Rac2/Pak1's aberrant potentiation of Erk1/2.

In addition to promoting proliferation through reinforcement of Raf/Mek/Erk, SCF-dependent hyperactivation of PI3K critically orchestrates $Nf1^{+/-}$ mast cell migration and survival (130, 136, 137). Genetic and chemical inhibitor (PD98059, SB203580, LY294002)-based studies have described a PI3K-dependent activation of Rac2 that controls SCF-mediated mast cell chemotaxis as well as *Nf1*-dependent gain in motility. Specifically, a Rac2/Pak1/p38 signaling pathway induces F-actin rearrangement and cell motility, although the precise downstream effectors remain unknown (136). These cytoskeletal observations directly correlate with a genetic study showing that $Nf1^{+/-}$ mast cells require PI3K activity for $Nf1^{-/-}$ Schwann cell–conditioned, media-dependent degranulation (140). Moreover, SCF-induced PI3K/Rac2 activity promotes Akt's modulation of Bcl-2 family proteins (e.g., BAD/Bcl-XL), thereby increasing mast cell survival through suppression of proapoptotic pathways (139). Taken together, these biochemical data explain SCF-mediated gains in function in the $Nf1^{+/-}$ mast cell, and they illuminate potential molecular therapeutic targets within Ras-dependent pathways. **Figure 3** summarizes these pathways.

The Tumor Microenvironment

$Nf1^{-/-}$ Schwann cells potently recruit normal and $Nf1^{+/-}$ mast cells via SCF-mediated activation of Ras pathways, but what role do these recruited mast cells play in the nascent and mature plexiform neurofibroma microenvironment? The plexiform neurofibroma microenvironment includes (but is perhaps not restricted to) tumorigenic Schwann cells and/or their

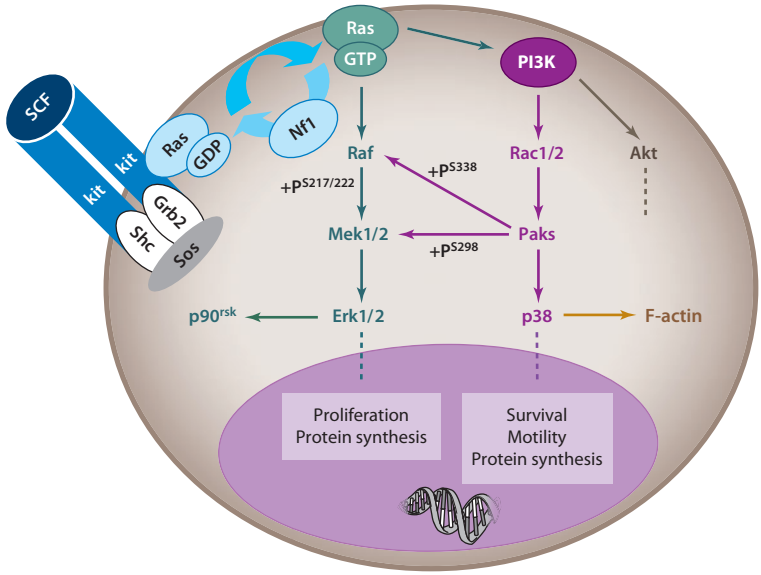

Figure 3

Hyperactive SCF/c-kit pathways in the *Nf1*$^{+/-}$ mast cell. Kit-ligand (SCF) binding at the c-kit receptor tyrosine kinase induces receptor dimerization, activates Ras to its GTP-bound conformation, and induces Ras/Raf/Mek/Erk and PI3K/Rac/Pak/p38 signaling pathways. Although Mek/Erk signals may principally mediate mast cell proliferation, PI3K mediates survival, motility, and through its Pak-dependent cross talk with Raf/Mek, proliferation. Nf1 accelerates the intrinsic hydrolysis of Ras-GTP to inactive Ras-GDP and serves, at least in part, to negatively regulate Mek/Erk- and PI3K-directed pathways. Although SCF/c-kit interactions initiate other molecular events (e.g., Akt/mTOR), this schematic highlights only those known to be hyperactivated in the *Nf1*$^{+/-}$ mast cell. Dashed lines indicate multiple downstream effectors that are not fully detailed. Abbreviations: Erk, extracellular signal–regulated kinase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; Pak, p21-activated kinase; SCF, stem cell factor. Modified from Reference 79.

precursors, mast cells, other hematopoietic cells, pericytes, smooth muscle cells, ECs (i.e., vasculature), fibroblasts, and the extracellular matrix, including a large amount of collagen. Indeed, fibroblasts and fibroblast-secreted collagen are major constituents, and extracellular matrix material typically accounts for approximately half of neurofibromas' dry weight (141). Given that SCF-stimulated mast cells can synthesize and secrete pleiotropic cytokines and growth factors, the mast cell could promote growth and activity in the tumor microenvironment through multiple hypothetical mechanisms, including nerve growth factor stimulation of Schwann cells, VEGF stimulation of vascular cells, and transforming growth factor β (TGF-β) stimulation of fibroblasts.

Fibroblasts

Accordingly, Yang et al. (101) have experimentally demonstrated that SCF-stimulated *Nf1*$^{+/-}$ mast cells modulate multiple fibroblast functions through secreted TGF-β. TGF-β induces fibroblasts to migrate, proliferate, and synthesize collagen, which are well-documented and critical biological processes, especially during embryogenesis and inflammatory wound healing. Not only do SCF-stimulated mast cells secrete TGF-β, *Nf1*$^{+/-}$ mast cells generate 2.5-fold more TGF-β compared with WT mast cells. In response to mast cell–conditioned media, WT and *Nf1*$^{+/-}$ fibroblasts proliferate, migrate, produce collagen, and promote extracellular matrix remodeling; *Nf1*$^{+/-}$ mast cell/*Nf1*$^{+/-}$ fibroblast

interactions demonstrate the greatest biological activity. Yang et al's study directly demonstrated TGF-β dependency by disrupting the fibroblast gain-in-function phenotype through the addition of TGF-β blocking antibody to the *Nf1*$^{+/-}$ mast cell–conditioned media. Intriguingly, TGF-β-stimulated fibroblasts derived from human neurofibroma samples demonstrated gains in function similar to those in murine *Nf1*$^{+/-}$ cultured fibroblasts, and reintroduction of the NF1 GAP-related domain restored normal activity. Mechanistically, TGF-β induces hyperactivity of the nonreceptor tyrosine kinase c-abl, and genetic or pharmacological suppression of c-abl corrects multiple fibroblast gain-in-function phenotypes. Correspondingly, imatinib mesylate (Gleevec®), a U.S. Food and Drug Administration–approved inhibitor of c-abl, suppresses collagen production and fibroblast migration/invasion in mice when implanted subcutaneously with TGF-β and *Nf1*$^{+/-}$ mast cell–conditioned media. This study, in conjunction with previous studies of Schwann cells and mast cells, illuminates critical microenvironment interactions whereby the *Nf1*$^{-/-}$ Schwann cell recruits the *Nf1*$^{+/-}$ mast cell, which, in turn, promotes fibroblast proliferation and collagenization.

Vascular Cells

Neurofibromas, like other human tumors, require the ingrowth of new vessels for their expansion and metastasis (40, 142). An initial study found that *Nf1*$^{-/-}$ Schwann cells directly promote angiogenesis when engrafted on chorioallantoic membranes of postfertilization chicken eggs, potentially through aberrant Ras-mediated expression of VEGF (143). An in vivo murine study corroborated these findings by demonstrating that *Nf1*$^{-/-}$ Schwann cell–conditioned media implanted into mice induce angiogenesis; *Nf1*$^{+/-}$ mice showed a greater angiogenic response than did WT mice (97). Likewise, ECs cultured from *Nf1*$^{+/-}$ mice and humans with NF1 exhibit a heightened migratory and proliferative response to neurofibroma-derived and recombinant growth factors, including VEGF and basic fibroblast growth factor (bFGF). Both proliferation and migration in patient-derived and *Nf1*$^{+/-}$ ECs appear to require aberrant Mek/Erk activity, which is an interesting contrast to *Nf1*$^{+/-}$ mast cells, in which Mek/Erk are largely dispensable for SCF-mediated migration. A concomitant study from the same laboratory found enhanced proliferation and migration in *Nf1*$^{+/-}$ vascular smooth muscle cells (VSMCs) in response to PDGF/BB. Intriguingly, *Nf1*$^{+/-}$ fibroblasts secrete fivefold-higher concentrations of PDGF/BB than their WT counterparts, which suggests that the haploinsufficient fibroblast directly participates in promoting the pathogenic microenvironment. As in ECs, this PDGF/BB-mediated potentiation of VSMC function requires hyperactivity in the Raf/Mek/Erk pathway. The investigators detected no *Nf1* haploinsufficient enhancement of Akt activity in either ECs or VSMCs—a surprising finding, given Ras's well-documented modulation of PI3K in the mast cell and other cell types. Thus, *Nf1* haploinsufficiency appears to modify biochemistry in a cell type–specific manner.

Other Lineages

Macrophages and pericytes may also modulate plexiform neurofibroma genesis and maintenance, but their roles remain largely undocumented. Although mast cells make up the majority of hematopoietic cells in the neurofibroma, macrophages account for approximately 10% to 15% of CD45^{+} cells, at least in mouse models (24). Also, in some cancers, tumor-associated macrophages mediate key angiogenic and mitogenic signals that promote tumor vascularization, remodeling, and growth (144). Therefore, macrophages are a reasonable target for future investigations of pathogenic mechanisms in the neurofibroma microenvironment. Likewise, pericytes, which are less differentiated cells that support vascular and connective tissue growth, may modulate

events in the microenvironment, including angiogenesis. Intriguingly, intracranial tumors in *Nf1*$^{+/-}$;*Trp53*$^{+/-}$ mice secrete stromal-derived factor 1, which recruits endothelial precursor cells from the bone marrow to the tumor site (145). These recruited precursors can differentiate into either ECs or pericytes, although this phenomenon has not been directly studied in plexiform neurofibroma formation.

In sum, complex heterotypic interactions underlie neurofibroma genesis and maintenance. As a global model, tumorigenic cells secrete SCF, recruiting and/or activating inflammatory mast cells, which then promote fibroblast proliferation and collagenization. Tumorigenic cells and hyperactive fibroblasts can promote neoangiogenesis, potentially through their stimulation of ECs and VSMCs via VEGF, bFGF, and PDGF/BB. Moreover, other cell types, including macrophages and pericytes, may modulate angiogenic and mitogenic events in the microenvironment, although their roles are relatively unknown. **Figure 4** presents the microenvironment model, including both known and hypothetical interactions.

Parallels to the Optic Glioma Microenvironment

Mouse models of NF1-associated optic glioma formation have demonstrated the importance of heterotypic interactions between the tumorigenic astrocyte and gliomagen-elaborating microglia, hematopoietic effectors derived from monocyte precursor cells (reviewed in Reference 146). Neoplastic and preneoplastic astrocytes secrete multiple factors that attract and expand microglia populations (146). Similar to *Nf1*$^{+/-}$ mast cells, *Nf1*$^{+/-}$ microglia demonstrate multiple gains in function, including enhanced macrophage colony–stimulating factor–mediated proliferation and production of hyaluronidase (105). This secreted hyaluronidase drives aberrant *Nf1*$^{-/-}$ astrocyte proliferation and correlates with glioma formation in vivo, both in mouse models and in humans with NF1. Mechanistically, *Nf1*$^{+/-}$ microglial gain in function appears to depend upon the aberrant activity of c-Jun/NH_2 kinase and not upon hyperactive Mek/Erk, p38, or PI3K/Akt, which reinforces the notion that *Nf1* haploinsufficiency modifies signaling in a cell type–specific manner (104). *Nf1*$^{+/-}$ microglia also express threefold more CXCL12 than do WT microglia, which, intriguingly, promotes astrocyte growth only in the context of *Nf1* deficiency (CXCL12 induces apoptosis in normal astrocytes). This finding is associated with aberrantly low cyclic AMP (cAMP) levels in the CXCL12-stimulated *Nf1*-deficient astrocyte (147), and forced suppression of cAMP can induce NF1-associated glioma formation in brain regions that typically are not susceptible to this tumor (148). Thus, optic gliomagenesis demonstrates a direct parallel to neurofibroma genesis, whereby tumorigenic cells co-opt inflammatory cells that, in turn, enable tumor growth and maintenance through secreted mitogens.

THE STEM CELL FACTOR/c-kit AXIS IN PLEXIFORM NEUROFIBROMA FORMATION AND ITS THERAPEUTIC IMPLICATIONS

Tumorigenesis Requires an *Nf1*$^{+/-}$ Hematopoietic System

Given the requirement for an *Nf1* haploinsufficient cellular background for plexiform neurofibroma formation in multiple mouse models, in combination with the various studies showing interactions between *Nf1* disrupted Schwann cells, mast cells, fibroblasts, and ECs, the question arises as to which haploinsufficient cell types specifically engender tumorigenesis. To address this question, Yang et al. (24) transplanted hematopoietic stem cells from *Nf1*$^{+/-}$ mice into lethally irradiated *Nf1*$^{flox/flox}$;*Krox20Cre* mice, a genetic status that, as discussed above, does not lead to tumor formation. This *Nf1*$^{+/-}$ hematopoietic stem cell transplant into *Nf1*$^{flox/flox}$;*Krox20Cre* mice created animals in which 5% to 10%

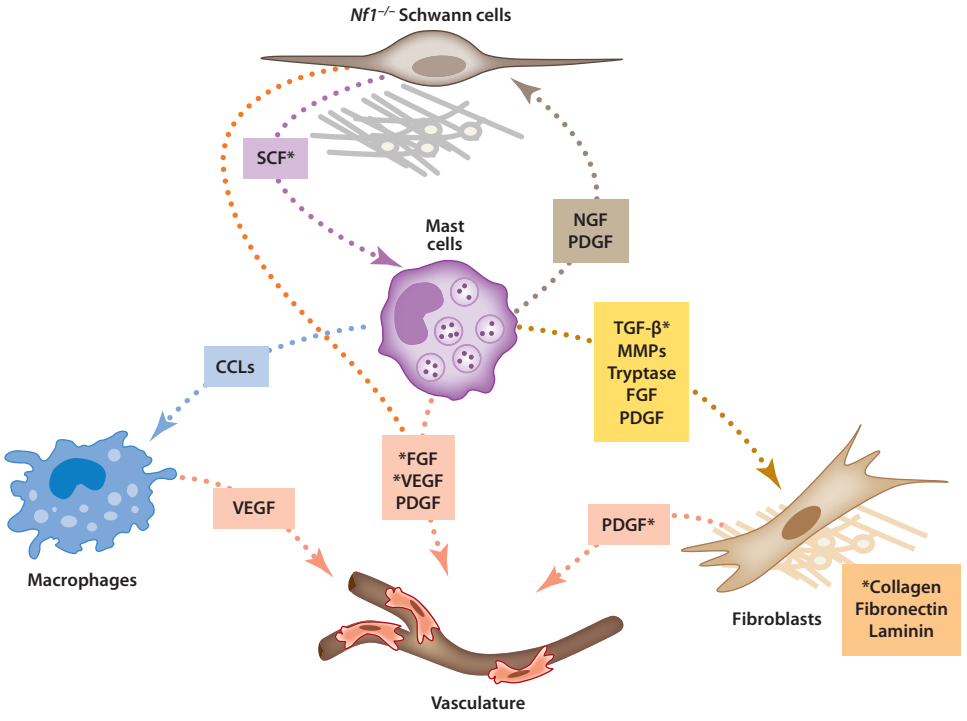

Figure 4

Hypothetical tumor/stromal/hematopoietic interactions in the neurofibroma microenvironment. In this tumor microenvironment model, loss of heterozygosity in Schwann cells or their precursor leads to aberrant (i.e., increased) production and secretion of stem cell factor (SCF), the ligand for the c-kit receptor tyrosine kinase. SCF induces maturation, proliferation, and recruitment of mast cells from bone marrow progenitor cells and can have effects on native differentiated mast cells at the site. Such SCF-activated mast cells generate and release multiple inflammatory cytokines and growth factors that act on macrophages, vasculature, fibroblasts, and the tumorigenic Schwann cells. Transforming growth factor β (TGF-β)-stimulated fibroblasts, in turn, aberrantly proliferate and produce collagen, increasing tumor bulk and pressure. Schwann cells, fibroblasts, and macrophages may directly contribute to vascularization through various growth factors. Asterisks denote heterotypic interactions with experimental validation.
Abbreviations: CCL, CC chemokine ligand; FGF, fibroblast growth factor; MMP, matrix metalloproteinase; NGF, nerve growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor. Modified from Reference 80.

of their Schwann cells and Schwann cell precursors were biallelically inactivated for *Nf1*, their hematopoietic system was haploinsufficient for *Nf1*, and all their other cells were phenotypically normal (i.e., $Nf1^{flox/flox}$). Approximately six months after transplantation, the $Nf1^{flox/flox}$;*Krox20Cre* mice reconstituted with an $Nf1^{+/}$ hematopoietic system developed enlargements along their dorsal root ganglia that histologically resembled plexiform neurofibromas. The tumor formation and increased mortality were directly comparable to the symptoms observed in the reliably tumorigenic $Nf1^{flox/-}$;*Krox20Cre* mice. **Figure 5** presents the schematics for hematopoietic stem cell transplantation into tumor model mice.

These transplant experiments indicate that *Nf1* haploinsufficiency in the hematopoietic system permits tumorigenesis in *Nf1* disrupted Schwann cells. However, these observations do not exclude the possibility that $Nf1^{+/-}$ fibroblasts, ECs, and/or other stromal cells could

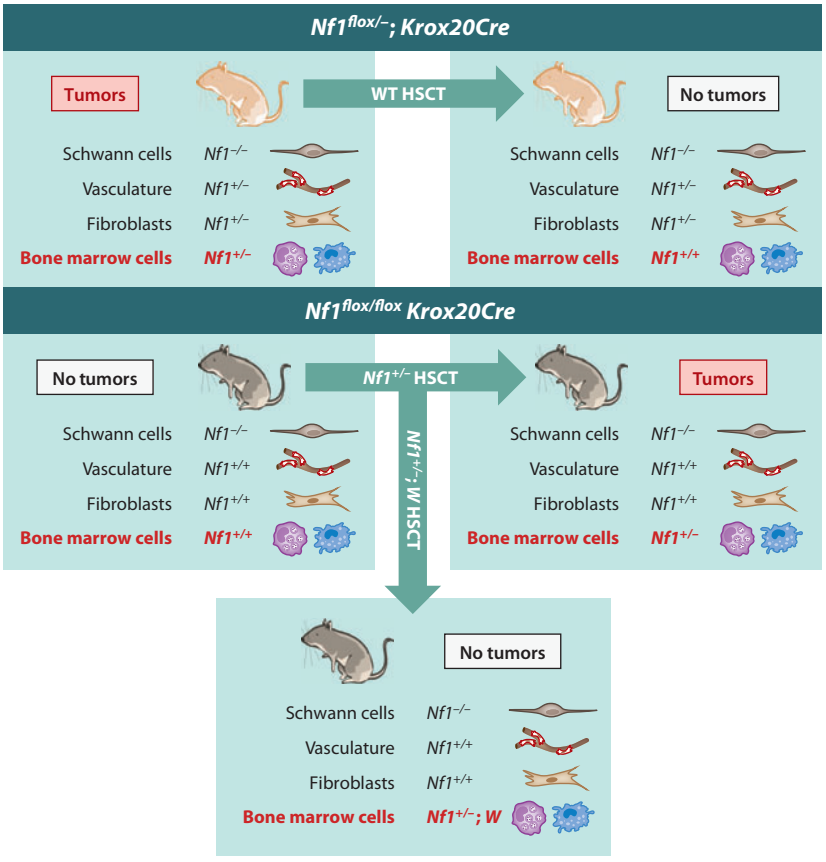

Figure 5

Transplant experiments in the *Krox20Cre* tumor model. (*Left*) The genetic status of Schwann cells, vascular cells, fibroblasts, and bone marrow cells in two different mice genotypes: the $Nf1^{flox/-}$;*Krox20Cre* tumor model and the $Nf1^{flox/flox}$;*Krox20Cre* model, which did not develop tumors. The arrows indicate the genotype of the hematopoietic stem cells transplanted into these two different genotypes of mice. *W* represents either W^{41} or W^{v} c-kit receptor tyrosine kinase–deficient mutations. (*Right and bottom*) The resultant genetic status of each cell lineage, posttransplant. As indicated, only those mice with $Nf1^{-/-}$ Schwann cells and c-kit-competent $Nf1^{+/-}$ bone marrow developed tumors. Abbreviations: HSCT, hematopoietic stem cell transplant; WT, wild type. Modified from Reference 80.

permit tumorigenesis despite a genetically normal hematopoietic system. To test this hypothesis, Yang et al. (24) additionally transplanted WT hematopoietic stem cells into lethally irradiated $Nf1^{flox/-}$;*Krox20Cre* mice, creating mice with *Nf1* disrupted Schwann cells, a WT hematopoietic system, and all other cells phenotypically $Nf1^{+/-}$. These mice never developed tumors. Therefore, an $Nf1^{+/-}$ hematopoietic system is both required and sufficient for tumorigenesis in the *Krox20Cre* model.

Similar investigations have revealed that the *P0aCre* tumor model requires an $Nf1^{+/-}$ hematopoietic system and that an $Nf1^{+/-}$ hematopoietic system increases the penetrance and severity of tumors in the *PeriostinCre* model. Thus, in multiple murine plexiform neurofibroma models with clear relevance to the human genetic disease, pathogenesis hinges on the inflammatory cues granted by an *Nf1* haploinsufficient hematopoietic system. We reiterate, however, that the early glia-driven *DhhCre*

model does not require an *Nf1* haploinsufficient hematopoietic system, and we emphasize that different mouse models may distinctly inform the varied constellation of NF1-associated pathologies observed in humans with or without heritable NF1.

Tumorigenesis Requires High-Functioning c-kit in an *Nf1*$^{+/-}$ Hematopoietic System

The *Nf1*$^{+/-}$ hematopoietic stem cell transplant experiments do not rule out possible contributions from other *Nf1*$^{+/-}$ hematopoietic cells, such as macrophages and endothelial precursor cells. To test the hypothesis that tumor formation requires *Nf1*$^{+/-}$ mast cells, Yang et al. (24) and colleagues also transplanted *Nf1*$^{+/-}$ hematopoietic stem cells carrying one of two naturally occurring c-kit receptor tyrosine kinase mutations, either W^{41}/W^{41} or W^{v}/W^{v}. These mutations reduce kinase activity by 85% and 92%–95%, respectively, which leads to loss of coat color and failed mast cell cytopoiesis, as discussed above. In these transplant experiments, *Nf1*$^{flox/flox}$;*Krox20Cre* mice reconstituted with an *Nf1*$^{+/-}$;W^{41}/W^{41} or *Nf1*$^{+/-}$;W^{v}/W^{v} hematopoietic system protected against dorsal root ganglia hyperplasia and mast cell invasion, as compared with the *Nf1*$^{flox/flox}$;*Krox20Cre* mice reconstituted with an *Nf1*$^{+/-}$-only hematopoietic system. Notably, because c-kit-mutated hematopoietic stem cells do not necessarily engraft as efficiently as WT hematopoietic stem cells, the authors used Southern blot analysis of myeloid colonies grown from bone marrow progenitor cells in *Nf1*$^{+/-}$;W^{41}/W^{41} or *Nf1*$^{+/-}$;W^{v}/W^{v} recipients to assay engraftment efficiency. In the transplant recipients, approximately 95% of the hematopoietic progenitors originated from the *Nf1*$^{+/-}$;*W* donor stem cells, indicating successful engraftment. These data indicate that plexiform neurofibroma formation requires an *Nf1* haploinsufficient and c-kit-competent hematopoietic system and, in light of the other studies discussed above, roundly implicate the SCF/c-kit-dependent mast cell as a principal pathogenic effector underpinning neurofibroma genesis.

Pharmacological Modulation of the Stem Cell Factor/c-kit Axis

Imatinib mesylate (Gleevec) inhibits multiple receptor and nonreceptor tyrosine kinases, including c-kit, PDGF-β, and bcr/abl and carries U.S. Food and Drug Administration approval for use in children and adults (149). Imatinib mesylate is principally used to treat chronic myelogenous leukemia, other hematological cancers, and some solid tumors. Given the findings that plexiform neurofibroma genesis and/or maintenance requires c-kit-mediated signals, Yang et al. (24) used imatinib mesylate to treat *Nf1*$^{flox/-}$;*Krox20Cre* mice with preexisting tumors, as measured by fluoridated deoxyglucose signals in positron emission tomography (FDG-PET). After a three-month course of treatment, volumetric analyses on FDG-PET demonstrated reduced tumor volume and metabolic activity, and histology of the dorsal root ganglia showed fewer mast cells, more orderly patterned Schwann cells, increased numbers of apoptotic cells, and diminished cellular proliferation, compared with the placebo cohort. In fact, the placebo cohort showed a small increase in glucose metabolism in affected areas, which suggests that tumor growth occurred throughout the three-month treatment course.

Imatinib mesylate also successfully reduced a progressively growing plexiform neurofibroma in a pediatric patient with hallmark symptoms of NF1. By six months of age, the patient had developed a histologically identified neurofibroma involving the left floor of her mouth, tongue, mastoid bone, carotid artery, and jugular vein. As the tumor compressed her airway, she showed symptoms of drooling, insomnia, and anorexia. The tumor's intimate involvement of vasculature and nerve tissue precluded surgical resection. Given the absence of treatment options and the results from the study of imatinib mesylate in the mouse plexiform neurofibroma model, clinicians discussed the risks and potential benefits

of this experimental therapeutic with her pediatrician and her parents, leading to initiation of medical therapy. Following three months of treatment with 350 mg m^{-2} per dose of imatinib mesylate, the tumor diminished in size to approximately one-third of its pretreatment volume, as assessed by magnetic resonance imaging. Importantly, the patient's symptoms of drooling, insomnia, and anorexia resolved, and imatinib mesylate produced no apparent side effects. On the basis of these findings, a phase II trial of NF1 patients with plexiform neurofibromas was recently completed and has been submitted for publication.

How Does Imatinib Mesylate Treatment Reduce a Plexiform Neurofibroma?

A neurofibroma's maintenance depends upon continual Schwann cell proliferation and/or survival, mast cell invasion, angiogenesis, and fibroblast proliferation and secretion of collagen. A simplistic hypothesis suggests that imatinib disrupts SCF/c-kit-mediated mast cell cytopoiesis and inflammation, thereby interrupting a key link between the tumorigenic Schwann cell and the mitogenic cues required to sustain itself and its microenvironment. However, cooperating mechanisms of action probably exist. For example, imatinib's well-characterized inhibitory action on the c-abl nonreceptor tyrosine kinase may additionally disrupt TGF-β-mediated signaling in the biochemically deregulated fibroblasts that constitute the tumor stroma, as has been suggested experimentally in studies of mouse- and patient-derived cells. Further, imatinib can inhibit signaling at the PDGF receptor, which experiments have shown is expressed by neurofibroma-derived Schwann cells and responds to imatinib treatment in tumor xenograft models (27). Similarly, vascular cells in the tumor microenvironment may also depend on PDGF receptor signaling (99). Accordingly, imatinib may operate on several cell-receptor systems that are important for tumor growth, although the data from transplantation-based and other studies compellingly suggest that disruption of the SCF/c-kit signaling axis may be of primary importance.

Future Targeted Therapies

In addition to imatinib mesylate, then, novel agents targeting the c-kit receptor tyrosine kinase and other relevant receptors and kinases may be viable therapeutics for the medical treatment of plexiform neurofibromas. A deeper understanding of the multiple mechanisms that doubtlessly underlie heterotypic tumor interactions, as well as the discovery of associations between genomic variation and disease course, will permit treatments tailored to the specific way in which a plexiform neurofibroma manifests within the individual patient. In a medical era that is increasingly cognizant of the potentials of personalized medicine and receptor-specific therapeutics, we view the results from imatinib as a first step. Indeed, singular or combined use of novel antiangiogenic drugs (e.g., bevacizumab), targeted anti-inflammatory agents (e.g., infliximab), and other c-kit antibodies (e.g., sunitinib) could prove beneficial, perhaps in a case-specific manner. Moreover, pharmacological-grade, small-molecule kinase inhibitors, which typically modulate intracellular effectors involved in multiple ligand-receptor systems, may be efficacious, given the observed hyperactivity of MAPK and PI3K pathways in *Nf1*-deficient cell types. Notably, accurate mouse models of tumorigenesis are currently facilitating the high-throughput testing of multiple experimental therapeutic agents.

CONCLUSION

Neurofibromin-deficient glial cells do not grow in isolation. Rather, they send mitogenic signals that interact with and co-opt ECs, fibroblasts, and hematopoietic cells, orchestrating the tumor microenvironment. As experimentally demonstrated, SCF-recruited and activated mast cells buttress nascent tumors and support mature microenvironments by promoting

fibroblast expansion and collagen synthesis, and Schwann cells and fibroblasts secrete factors that directly contribute to vascular ingrowth. The SCF-stimulated mast cell may also release factors that promote the recruitment and/or growth of ECs (e.g., VEGF, PDGF, bFGF), other hematopoietic cells (e.g., interleukin-6, tumor necrosis factor α, MCP-1), and Schwann cells (e.g., NGF, PDGF), but the importance of these signals remains largely unexamined. Thus, broadly, although the complex and cooperating events in tumor genesis are not fully understood, they potentially include multiple ligand-receptor interactions across diverse cell types and the modulating influences of variation in other genes. Regardless, genetic dosage of *NF1* is central to neurofibroma pathogenesis; there is a requirement for *NF1* nullizygosity in the tumorigenic cell and, in several disease models, for *Nf1* heterozygosity in the hematopoietic system. Whether or not this requirement for *Nf1* heterozygosity has special relevance for the tumor's medical treatment in humans is uncertain, although these insights have pointed to a particular importance of the SCF/c-kit signaling axis in modulating the disease course. Above all, continued exploration of the genomic variation among individual phenotypes and a deeper delineation of heterotypic interactions underlying tumorigenesis will propel the development and application of targeted, effective medical therapies.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported, in part, by National Institutes of Health/National Cancer Institute grants RO1 CA074177-11A1/D and P50 NS052606-04 (to D.W.C.). K.S. was additionally supported by a predoctoral fellowship from National Institutes of Health grant T32 CA111198.

LITERATURE CITED

1. Friedman J, Gutmann DH, Maccollin M, Riccardi VM. 1999. *Neurofibromatosis: Phenotype, Natural History, and Pathogenesis.* Baltimore: Johns Hopkins Univ. Press. 381 pp.
2. Lubs ML, Bauer MS, Formas ME, Djokic B. 1991. Lisch nodules in neurofibromatosis type 1. *N. Engl. J. Med.* 324:1264–66
3. Riccardi VM. 1991. Neurofibromatosis: past, present, and future. *N. Engl. J. Med.* 324:1283–85
4. Riccardi VM. 2010. Neurofibromatosis type 1 is a disorder of dysplasia: the importance of distinguishing features, consequences, and complications. *Birth Defects Res. A Clin. Mol. Teratol.* 88:9–14
5. Akenside M. 1768. Observations on cancers. *Med. Trans. Coll. Phys. Lond.* 1:64–92
6. Tilesius von Tilenau W. 1793. *Historia pathologica singularis Cutis Turpitudinis*. Leipzig, Ger.: Crusius
7. Morse RP. 1999. Neurofibromatosis type 1. *Arch. Neurol.* 56:364–65
8. von Recklinghausen F. 1882. *Über die multiplen Fibrome der Haut und ihre Beziehung zu multiplen Neuromen*. Berlin: Hirschwald
9. Gamble HJ, Goldby S. 1961. Mast cells in peripheral nerve trunks. *Nature* 189:766–67
10. Baroni C. 1964. On the relationship of mast cells to various soft tissue tumours. *Br. J. Cancer* 18:686–91
11. Pineda A. 1965. Mast cells—their presence and ultrastructural characteristics in peripheral nerve tumors. *Arch. Neurol.* 13:372–82
12. Olsson Y. 1971. Mast cells in human peripheral nerve. *Acta Neurol. Scand.* 47:357–68
13. Isaacson P. 1976. Mast cells in benign nerve sheath tumours. *J. Pathol.* 119:193–96

14. Greggio H. 1911. Les cellules granuleuses (Mastzellen) dans les tissus normaux et dans certaines maladies chirurgicales. *Arch. Med. Exp.* 23:323–75
15. Le LQ, Shipman T, Burns DK, Parada LF. 2009. Cell of origin and microenvironment contribution for NF1-associated dermal neurofibromas. *Cell Stem Cell* 4:453–63
16. Zheng H, Chang L, Patel N, Yang J, Lowe L, et al. 2008. Induction of abnormal proliferation by nonmyelinating Schwann cells triggers neurofibroma formation. *Cancer Cell* 13:117–28
17. Wu J, Williams JP, Rizvi TA, Kordich JJ, Witte D, et al. 2008. Plexiform and dermal neurofibromas and pigmentation are caused by Nf1 loss in desert hedgehog–expressing cells. *Cancer Cell* 13:105–16
18. Raffensperger J, Cohen R. 1972. Plexiform neurofibromas in childhood. *J. Pediatr. Surg.* 7:144–51
19. Serletis D, Parkin P, Bouffet E, Shroff M, Drake JM, Rutka JT. 2007. Massive plexiform neurofibromas in childhood: natural history and management issues. *J. Neurosurg.* 106:363–67
20. McCaughan JA, Holloway SM, Davidson R, Lam WW. 2007. Further evidence of the increased risk for malignant peripheral nerve sheath tumour from a Scottish cohort of patients with neurofibromatosis type 1. *J. Med. Genet.* 44:463–66
21. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. 2002. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. *J. Med. Genet.* 39:311–14
22. Cavallaro G, Basile U, Polistena A, Giustini S, Arena R, et al. 2010. Surgical management of abdominal manifestations of type 1 neurofibromatosis: experience of a single center. *Am. Surg.* 76:389–96
23. Wu J, Dombi E, Jousma E, Scott Dunn R, Lindquist D, et al. 2011. Preclincial testing of Sorafenib and RAD001 in the *Nf*$^{flox/flox}$;*DhhCre* mouse model of plexiform neurofibroma using magnetic resonance imaging. *Pediatr. Blood Cancer*. In press
24. Yang FC, Ingram DA, Chen S, Zhu Y, Yuan J, et al. 2008. Nf1-dependent tumors require a microenvironment containing *Nf1*$^{+/-}$and c-kit-dependent bone marrow. *Cell* 135:437–48
25. Jakacki RI, Dombi E, Potter DM, Goldman S, Allen JC, et al. 2011. Phase I trial of PEGylated interferon-α-2b in young patients with plexiform neurofibromas. *Neurology* 76:265–72
26. Widemann BC, Salzer WL, Arceci RJ, Blaney SM, Fox E, et al. 2006. Phase I trial and pharmacokinetic study of the farnesyltransferase inhibitor tipifarnib in children with refractory solid tumors or neurofibromatosis type I and plexiform neurofibromas. *J. Clin. Oncol.* 24:507–16
27. Demestre M, Herzberg J, Holtkamp N, Hagel C, Reuss D, et al. 2010. Imatinib mesylate (Glivec) inhibits Schwann cell viability and reduces the size of human plexiform neurofibroma in a xenograft model. *J. Neurooncol.* 98:11–19
28. Packer RJ, Rosser T. 2002. Therapy for plexiform neurofibromas in children with neurofibromatosis 1: an overview. *J. Child Neurol.* 17:638–41
29. Franco CB, Chen CC, Drukker M, Weissman IL, Galli SJ. 2010. Distinguishing mast cell and granulocyte differentiation at the single-cell level. *Cell Stem Cell* 6:361–68
30. Metz M, Piliponsky AM, Chen CC, Lammel V, Abrink M, et al. 2006. Mast cells can enhance resistance to snake and honeybee venoms. *Science* 313:526–30
31. Kalesnikoff J, Galli SJ. 2008. New developments in mast cell biology. *Nat. Immunol.* 9:1215–23
32. Galli SJ, Grimbaldeston M, Tsai M. 2008. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat. Rev. Immunol.* 8:478–86
33. Galli SJ, Tsai M. 2008. Mast cells: versatile regulators of inflammation, tissue remodeling, host defense and homeostasis. *J. Dermatol. Sci.* 49:7–19
34. Chabot B, Stephenson DA, Chapman VM, Besmer P, Bernstein A. 1988. The proto-oncogene *c-kit* encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. *Nature* 335:88–89
35. Galli SJ, Tsai M, Wershil BK. 1993. The c-kit receptor, stem cell factor, and mast cells. What each is teaching us about the others. *Am. J. Pathol.* 142:965–74
36. Tsai M, Takeishi T, Thompson H, Langley KE, Zsebo KM, et al. 1991. Induction of mast cell proliferation, maturation, and heparin synthesis by the rat c-kit ligand, stem cell factor. *Proc. Natl. Acad. Sci. USA* 88:6382–86
37. Taylor AM, Galli SJ, Coleman JW. 1995. Stem-cell factor, the kit ligand, induces direct degranulation of rat peritoneal mast cells in vitro and in vivo: dependence of the in vitro effect on period of culture and comparisons of stem-cell factor with other mast cell–activating agents. *Immunology* 86:427–33

38. Coussens LM, Werb Z. 2002. Inflammation and cancer. *Nature* 420:860–67
39. Coussens LM, Werb Z. 2001. Inflammatory cells and cancer: Think different! *J. Exp. Med.* 193:F23–26
40. Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. *Cell* 100:57–70
41. Riccardi VM. 1981. Cutaneous manifestation of neurofibromatosis: cellular interaction, pigmentation, and mast cells. *Birth Defects Orig. Artic. Ser.* 17:129–45
42. Riccardi VM. 1987. Mast-cell stabilization to decrease neurofibroma growth. Preliminary experience with ketotifen. *Arch. Dermatol.* 123:1011–16
43. Riccardi VM. 1993. A controlled multiphase trial of ketotifen to minimize neurofibroma-associated pain and itching. *Arch. Dermatol.* 129:577–81
44. Barker D, Wright E, Nguyen K, Cannon L, Fain P, et al. 1987. Gene for von Recklinghausen neurofibromatosis is in the pericentromeric region of chromosome 17. *Science* 236:1100–2
45. Seizinger BR, Rouleau GA, Ozelius LJ, Lane AH, Faryniarz AG, et al. 1987. Genetic linkage of von Recklinghausen neurofibromatosis to the nerve growth factor receptor gene. *Cell* 49:589–94
46. Fountain JW, Wallace MR, Bruce MA, Seizinger BR, Menon AG, et al. 1989. Physical mapping of a translocation breakpoint in neurofibromatosis. *Science* 244:1085–87
47. Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, et al. 1990. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science* 249:181–86
48. Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, et al. 1990. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell* 62:187–92
49. Cawthon RM, Weiss R, Xu GF, Viskochil D, Culver M, et al. 1990. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. *Cell* 62:193–201
50. Marchuk DA, Saulino AM, Tavakkol R, Swaroop M, Wallace MR, et al. 1991. cDNA cloning of the type 1 neurofibromatosis gene: complete sequence of the NF1 gene product. *Genomics* 11:931–40
51. Ledbetter DH, Rich DC, O'Connell P, Leppert M, Carey JC. 1989. Precise localization of *NF1* to 17q11.2 by balanced translocation. *Am. J. Hum. Genet.* 44:20–24
52. Fountain JW, Wallace MR, Brereton AM, O'Connell P, White RL, et al. 1989. Physical mapping of the von Recklinghausen neurofibromatosis region on chromosome 17. *Am. J. Hum. Genet.* 44:58–67
53. Li Y, O'Connell P, Breidenbach HH, Cawthon R, Stevens J, et al. 1995. Genomic organization of the neurofibromatosis 1 gene (*NF1*). *Genomics* 25:9–18
54. Shilyansky C, Lee YS, Silva AJ. 2010. Molecular and cellular mechanisms of learning disabilities: a focus on NF1. *Annu. Rev. Neurosci.* 33:221–43
55. Sergeyev AS. 1975. On the mutation rate of neurofibromatosis. *Humangenetik* 28:129–38
56. Park VM, Pivnick EK. 1998. Neurofibromatosis type 1 (NF1): a protein truncation assay yielding identification of mutations in 73% of patients. *J. Med. Genet.* 35:813–20
57. Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, et al. 2000. Exhaustive mutation analysis of the *NF1* gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. *Hum. Mutat.* 15:541–55
58. Maertens O, De Schepper S, Vandesompele J, Brems H, Heyns I, et al. 2007. Molecular dissection of isolated disease features in mosaic neurofibromatosis type 1. *Am. J. Hum. Genet.* 81:243–51
59. Praxedes LA, Pereira FM, Mazzeu JF, Costa SS, Bertola DR, et al. 2010. An illustrative case of neurofibromatosis type 1 and NF1 microdeletion. *Mol. Syndromol.* 1:133–35
60. Dorschner MO, Sybert VP, Weaver M, Pletcher BA, Stephens K. 2000. *NF1* microdeletion breakpoints are clustered at flanking repetitive sequences. *Hum. Mol. Genet.* 9:35–46
61. Wiest V, Eisenbarth I, Schmegner C, Krone W, Assum G. 2003. Somatic *NF1* mutation spectra in a family with neurofibromatosis type 1: toward a theory of genetic modifiers. *Hum. Mutat.* 22:423–27
62. Sabbagh A, Pasmant E, Laurendeau I, Parfait B, Barbarot S, et al. 2009. Unravelling the genetic basis of variable clinical expression in neurofibromatosis 1. *Hum. Mol. Genet.* 18:2768–78
63. Hawes JJ, Tuskan RG, Reilly KM. 2007. Nf1 expression is dependent on strain background: implications for tumor suppressor haploinsufficiency studies. *Neurogenetics* 8:121–30
64. Upadhyaya M, Huson SM, Davies M, Thomas N, Chuzhanova N, et al. 2007. An absence of cutaneous neurofibromas associated with a 3-bp inframe deletion in exon 17 of the *NF1* gene (c.2970–2972 delAAT): evidence of a clinically significant NF1 genotype-phenotype correlation. *Am. J. Hum. Genet.* 80:140–51

65. Xu GF, O'Connell P, Viskochil D, Cawthon R, Robertson M, et al. 1990. The neurofibromatosis type 1 gene encodes a protein related to GAP. *Cell* 62:599–608
66. Gutmann DH, Collins FS. 1993. The neurofibromatosis type 1 gene and its protein product, neurofibromin. *Neuron* 10:335–43
67. Gutmann DH, Boguski M, Marchuk D, Wigler M, Collins FS, Ballester R. 1993. Analysis of the neurofibromatosis type 1 (NF1) GAP-related domain by site-directed mutagenesis. *Oncogene* 8:761–69
68. Martin GA, Viskochil D, Bollag G, McCabe PC, Crosier WJ, et al. 1990. The GAP-related domain of the neurofibromatosis type 1 gene product interacts with Ras p21. *Cell* 63:843–49
69. DeClue JE, Papageorge AG, Fletcher JA, Diehl SR, Ratner N, et al. 1992. Abnormal regulation of mammalian $p21^{ras}$ contributes to malignant tumor growth in von Recklinghausen (type 1) neurofibromatosis. *Cell* 69:265–73
70. McCormick F. 1989. Ras GTPase activating protein: signal transmitter and signal terminator. *Cell* 56:5–8
71. Vigil D, Cherfils J, Rossman KL, Der CJ. 2010. Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? *Nat. Rev. Cancer* 10:842–57
72. Bourne HR, Sanders DA, McCormick F. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348:125–32
73. Bourne HR, Sanders DA, McCormick F. 1991. The GTPase superfamily: conserved structure and molecular mechanism. *Nature* 349:117–27
74. Hall A. 1990. The cellular functions of small GTP-binding proteins. *Science* 249:635–40
75. Hall A. 1992. Signal transduction through small GTPases—a tale of two GAPs. *Cell* 69:389–91
76. Le LQ, Parada LF. 2007. Tumor microenvironment and neurofibromatosis type I: connecting the GAPs. *Oncogene* 26:4609–16
77. Dasgupta B, Gutmann DH. 2003. Neurofibromatosis 1: closing the GAP between mice and men. *Curr. Opin. Genet. Dev.* 13:20–27
78. Costa RM, Silva AJ. 2003. Mouse models of neurofibromatosis type I: bridging the GAP. *Trends Mol. Med.* 9:19–23
79. Staser K, Yang FC, Clapp DW. 2010. Plexiform neurofibroma genesis: questions of *Nf1* gene dose and hyperactive mast cells. *Curr. Opin. Hematol.* 17:287–93
80. Staser K, Yang FC, Clapp DW. 2010. Mast cells and the neurofibroma microenvironment. *Blood* 116:157–64
81. Cichowski K, Shih TS, Schmitt E, Santiago S, Reilly K, et al. 1999. Mouse models of tumor development in neurofibromatosis type 1. *Science* 286:2172–76
82. Zhu Y, Ghosh P, Charnay P, Burns DK, Parada LF. 2002. Neurofibromas in NF1: Schwann cell origin and role of tumor environment. *Science* 296:920–22
83. Joseph NM, Mosher JT, Buchstaller J, Snider P, McKeever PE, et al. 2008. The loss of *Nf1* transiently promotes self-renewal but not tumorigenesis by neural crest stem cells. *Cancer Cell* 13:129–40
84. Zinnamosca L, Petramala L, Cotesta D, Marinelli C, Schina M, et al. 2010. Neurofibromatosis type 1 (NF1) and pheochromocytoma: prevalence, clinical and cardiovascular aspects. *Arch. Dermatol. Res.* 303:317–25
85. De Schepper S, Maertens O, Callens T, Naeyaert JM, Lambert J, Messiaen L. 2008. Somatic mutation analysis in NF1 café au lait spots reveals two *NF1* hits in the melanocytes. *J. Investig. Dermatol.* 128:1050–53
86. Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, et al. 1996. Loss of *NF1* results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. *Nat. Genet.* 12:144–48
87. Zhu Y, Harada T, Liu L, Lush ME, Guignard F, et al. 2005. Inactivation of *NF1* in CNS causes increased glial progenitor proliferation and optic glioma formation. *Development* 132:5577–88
88. Stevenson DA, Zhou H, Ashrafi S, Messiaen LM, Carey JC, et al. 2006. Double inactivation of *NF1* in tibial pseudarthrosis. *Am. J. Hum. Genet.* 79:143–48
89. Sakamoto A, Yoshida T, Yamamoto H, Oda Y, Tsuneyoshi M, Iwamoto Y. 2007. Congenital pseudarthrosis of the tibia: analysis of the histology and the *NF1* gene. *J. Orthop. Sci.* 12:361–65
90. Kolanczyk M, Kuhnisch J, Kossler N, Osswald M, Stumpp S, et al. 2008. Modelling neurofibromatosis type 1 tibial dysplasia and its treatment with lovastatin. *BMC Med.* 6:21

91. Yeh TH, Lee DY, Gianino SM, Gutmann DH. 2009. Microarray analyses reveal regional astrocyte heterogeneity with implications for neurofibromatosis type 1 (NF1)-regulated glial proliferation. *Glia* 57:1239–49
92. Lee DY, Yeh TH, Emnett RJ, White CR, Gutmann DH. 2010. Neurofibromatosis-1 regulates neuroglial progenitor proliferation and glial differentiation in a brain region–specific manner. *Genes Dev.* 24:2317–29
93. Li H, Liu Y, Zhang Q, Jing Y, Chen S, et al. 2009. Ras dependent paracrine secretion of osteopontin by *Nf1*$^{+/-}$ osteoblasts promote osteoclast activation in a neurofibromatosis type I murine model. *Pediatr. Res.* 65:613–18
94. Yan J, Chen S, Zhang Y, Li X, Li Y, et al. 2008. Rac1 mediates the osteoclast gains-in-function induced by haploinsufficiency of *Nf1*. *Hum. Mol. Genet.* 17:936–48
95. Yang FC, Chen S, Robling AG, Yu X, Nebesio TD, et al. 2006. Hyperactivation of p21^{ras} and PI3K cooperate to alter murine and human neurofibromatosis type 1–haploinsufficient osteoclast functions. *J. Clin. Investig.* 116:2880–91
96. Cui Y, Costa RM, Murphy GG, Elgersma Y, Zhu Y, et al. 2008. Neurofibromin regulation of ERK signaling modulates GABA release and learning. *Cell* 135:549–60
97. Munchhof AM, Li F, White HA, Mead LE, Krier TR, et al. 2006. Neurofibroma-associated growth factors activate a distinct signaling network to alter the function of neurofibromin-deficient endothelial cells. *Hum. Mol. Genet.* 15:1858–69
98. Lasater EA, Bessler WK, Mead LE, Horn WE, Clapp DW, et al. 2008. *Nf1*$^{+/-}$ mice have increased neointima formation via hyperactivation of a Gleevec sensitive molecular pathway. *Hum. Mol. Genet.* 17:2336–44
99. Li F, Munchhof AM, White HA, Mead LE, Krier TR, et al. 2006. Neurofibromin is a novel regulator of RAS-induced signals in primary vascular smooth muscle cells. *Hum. Mol. Genet.* 15:1921–30
100. Ingram DA, Yang FC, Travers JB, Wenning MJ, Hiatt K, et al. 2000. Genetic and biochemical evidence that haploinsufficiency of the *Nf1* tumor suppressor gene modulates melanocyte and mast cell fates in vivo. *J. Exp. Med.* 191:181–88
101. Yang FC, Chen S, Clegg T, Li X, Morgan T, et al. 2006. *Nf1*$^{+/-}$ mast cells induce neurofibroma like phenotypes through secreted TGF-β signaling. *Hum. Mol. Genet.* 15:2421–37
102. Bajenaru ML, Donahoe J, Corral T, Reilly KM, Brophy S, et al. 2001. Neurofibromatosis 1 (NF1) heterozygosity results in a cell-autonomous growth advantage for astrocytes. *Glia* 33:314–23
103. Simmons GW, Pong WW, Emnett RJ, White CR, Gianino SM, et al. 2010. Neurofibromatosis-1 heterozygosity increases microglia in a spatially and temporally restricted pattern relevant to mouse optic glioma formation and growth. *J. Neuropathol. Exp. Neurol.* 70:51–62
104. Daginakatte GC, Gianino SM, Zhao NW, Parsadanian AS, Gutmann DH. 2008. Increased c-Jun-NH_2-kinase signaling in neurofibromatosis-1 heterozygous microglia drives microglia activation and promotes optic glioma proliferation. *Cancer Res.* 68:10358–66
105. Daginakatte GC, Gutmann DH. 2007. Neurofibromatosis-1 (*Nf1*) heterozygous brain microglia elaborate paracrine factors that promote *Nf1*-deficient astrocyte and glioma growth. *Hum. Mol. Genet.* 16:1098–112
106. Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. 1994. Tumour predisposition in mice heterozygous for a targeted mutation in *Nf1*. *Nat. Genet.* 7:353–61
107. Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, et al. 1994. Targeted disruption of the neurofibromatosis type 1 gene leads to developmental abnormalities in heart and various neural crest–derived tissues. *Genes. Dev.* 8:1019–29
108. Valero MC, Velasco E, Moreno F, Hernandez-Chico C. 1994. Characterization of four mutations in the neurofibromatosis type 1 gene by denaturing gradient gel electrophoresis (DGGE). *Hum. Mol. Genet.* 3:639–41
109. Lasater EA, Li F, Bessler WK, Estes ML, Vemula S, et al. 2010. Genetic and cellular evidence of vascular inflammation in neurofibromin-deficient mice and humans. *J. Clin. Investig.* 120:859–70
110. Zhu Y, Romero MI, Ghosh P, Ye Z, Charnay P, et al. 2001. Ablation of NF1 function in neurons induces abnormal development of cerebral cortex and reactive gliosis in the brain. *Genes. Dev.* 15:859–76

111. Sauer B, Henderson N. 1988. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proc. Natl. Acad. Sci. USA* 85:5166–70
112. Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi AB, et al. 1994. Krox-20 controls myelination in the peripheral nervous system. *Nature* 371:796–99
113. Bajenaru ML, Hernandez MR, Perry A, Zhu Y, Parada LF, et al. 2003. Optic nerve glioma in mice requires astrocyte *Nf1* gene inactivation and *Nf1* brain heterozygosity. *Cancer Res.* 63:8573–77
114. Bajenaru ML, Zhu Y, Hedrick NM, Donahoe J, Parada LF, Gutmann DH. 2002. Astrocyte-specific inactivation of the neurofibromatosis 1 gene (*NF1*) is insufficient for astrocytoma formation. *Mol. Cell. Biol.* 22:5100–13
115. Bailey P, Herrmann JD. 1938. The role of the cells of Schwann in the formation of tumors of the peripheral nerves. *Am. J. Pathol.* 14:1–38
116. Kluwe L, Friedrich R, Mautner VF. 1999. Loss of NF1 allele in Schwann cells but not in fibroblasts derived from an NF1-associated neurofibroma. *Genes. Chromosomes Cancer* 24:283–85
117. Rutkowski JL, Wu K, Gutmann DH, Boyer PJ, Legius E. 2000. Genetic and cellular defects contributing to benign tumor formation in neurofibromatosis type 1. *Hum. Mol. Genet.* 9:1059–66
118. Maertens O, Brems H, Vandesompele J, De Raedt T, Heyns I, et al. 2006. Comprehensive *NF1* screening on cultured Schwann cells from neurofibromas. *Hum. Mutat.* 27:1030–40
119. Sheela S, Riccardi VM, Ratner N. 1990. Angiogenic and invasive properties of neurofibroma Schwann cells. *J. Cell Biol.* 111:645–53
120. Carroll SL, Ratner N. 2008. How does the Schwann cell lineage form tumors in NF1? *Glia* 56:1590–605
121. Mashour GA, Ratner N, Khan GA, Wang HL, Martuza RL, Kurtz A. 2001. The angiogenic factor midkine is aberrantly expressed in *NF1*-deficient Schwann cells and is a mitogen for neurofibroma-derived cells. *Oncogene* 20:97–105
122. Yang FC, Ingram DA, Chen S, Hingtgen CM, Ratner N, et al. 2003. Neurofibromin-deficient Schwann cells secrete a potent migratory stimulus for $Nf1^{+/-}$ mast cells. *J. Clin. Investig.* 112:1851–61
123. Hirota S, Nomura S, Asada H, Ito A, Morii E, Kitamura Y. 1993. Possible involvement of c-kit receptor and its ligand in increase of mast cells in neurofibroma tissues. *Arch. Pathol. Lab. Med.* 117:996–99
124. Ryan JJ, Klein KA, Neuberger TJ, Leftwich JA, Westin EH, et al. 1994. Role for the stem cell factor/KIT complex in Schwann cell neoplasia and mast cell proliferation associated with neurofibromatosis. *J. Neurosci. Res.* 37:415–32
125. Mashour GA, Driever PH, Hartmann M, Drissel SN, Zhang T, et al. 2004. Circulating growth factor levels are associated with tumorigenesis in neurofibromatosis type 1. *Clin. Cancer Res.* 10:5677–83
126. Huang E, Nocka K, Beier DR, Chu TY, Buck J, et al. 1990. The hematopoietic growth factor KL is encoded by the SI locus and is the ligand of the c-kit receptor, the gene product of the W locus. *Cell* 63:225–33
127. Zsebo KM, Williams DA, Geissler EN, Broudy VC, Martin FH, et al. 1990. Stem cell factor is encoded at the SI locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell* 63:213–24
128. Nocka K, Tan JC, Chiu E, Chu TY, Ray P, et al. 1990. Molecular bases of dominant negative and loss of function mutations at the murine c-kit/white spotting locus: W^{37}, W^{v}, W^{41} and W. *EMBO J.* 9:1805–13
129. Tan JC, Nocka K, Ray P, Traktman P, Besmer P. 1990. The dominant W^{42} spotting phenotype results from a missense mutation in the c-kit receptor kinase. *Science* 247:209–12
130. Ingram DA, Hiatt K, King AJ, Fisher L, Shivakumar R, et al. 2001. Hyperactivation of $p21^{ras}$ and the hematopoietic-specific Rho GTPase, Rac2, cooperate to alter the proliferation of neurofibromin-deficient mast cells in vivo and in vitro. *J. Exp. Med.* 194:57–69
131. Malhotra R, Ratner N. 1994. Localization of neurofibromin to keratinocytes and melanocytes in developing rat and human skin. *J. Investig. Dermatol.* 102:812–18
132. Ballester R, Marchuk D, Boguski M, Saulino A, Letcher R, et al. 1990. The *NF1* locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. *Cell* 63:851–59
133. Zhang YY, Vik TA, Ryder JW, Srour EF, Jacks T, et al. 1998. *Nf1* regulates hematopoietic progenitor cell growth and Ras signaling in response to multiple cytokines. *J. Exp. Med.* 187:1893–902
134. Ingram DA, Zhang L, McCarthy J, Wenning MJ, Fisher L, et al. 2002. Lymphoproliferative defects in mice lacking the expression of neurofibromin: functional and biochemical consequences of *Nf1* deficiency in T-cell development and function. *Blood* 100:3656–62

135. Khalaf WF, Yang FC, Chen S, White H, Bessler W, et al. 2007. K-ras is critical for modulating multiple c-kit-mediated cellular functions in wild-type and *Nf1*$^{+/-}$ mast cells. *J. Immunol.* 178:2527–34
136. McDaniel AS, Allen JD, Park SJ, Jaffer ZM, Michels EG, et al. 2008. Pak1 regulates multiple c-Kit mediated Ras-MAPK gain-in-function phenotypes in *Nf1*$^{+/-}$ mast cells. *Blood* 112:4646–54
137. Hiatt K, Ingram DA, Huddleston H, Spandau DF, Kapur R, Clapp DW. 2004. Loss of the *Nf1* tumor suppressor gene decreases fas antigen expression in myeloid cells. *Am. J. Pathol.* 164:1471–79
138. Hiatt KK, Ingram DA, Zhang Y, Bollag G, Clapp DW. 2001. Neurofibromin GTPase-activating protein–related domains restore normal growth in *Nf1*$^{+/-}$ cells. *J. Biol. Chem.* 276:7240–5
139. Yang FC, Kapur R, King AJ, Tao W, Kim C, et al. 2000. Rac2 stimulates Akt activation affecting BAD/Bcl-XL expression while mediating survival and actin function in primary mast cells. *Immunity* 12:557–68
140. Chen S, Burgin S, McDaniel A, Li X, Yuan J, et al. 2010. *Nf1*$^{+/-}$ Schwann cell–conditioned medium modulates mast cell degranulation by c-Kit-mediated hyperactivation of phosphatidylinositol 3-kinase. *Am. J. Pathol.* 177:3125–32
141. Jaakkola S, Peltonen J, Riccardi V, Chu ML, Uitto J. 1989. Type 1 neurofibromatosis: selective expression of extracellular matrix genes by Schwann cells, perineurial cells, and fibroblasts in mixed cultures. *J. Clin. Investig.* 84:253–61
142. Arbiser JL, Flynn E, Barnhill RL. 1998. Analysis of vascularity of human neurofibromas. *J. Am. Acad. Dermatol.* 38:950–54
143. Kim HA, Ling B, Ratner N. 1997. *Nf1*-deficient mouse Schwann cells are angiogenic and invasive and can be induced to hyperproliferate: reversion of some phenotypes by an inhibitor of farnesyl protein transferase. *Mol. Cell. Biol.* 17:862–72
144. Lewis CE, Pollard JW. 2006. Distinct role of macrophages in different tumor microenvironments. *Cancer Res.* 66:605–12
145. Aghi M, Cohen KS, Klein RJ, Scadden DT, Chiocca EA. 2006. Tumor stromal–derived factor 1 recruits vascular progenitors to mitotic neovasculature, where microenvironment influences their differentiated phenotypes. *Cancer Res.* 66:9054–64
146. Pong WW, Gutmann DH. 2011. The ecology of brain tumors: lessons learned from neurofibromatosis 1. *Oncogene* 30:1135–46
147. Warrington NM, Woerner BM, Daginakatte GC, Dasgupta B, Perry A, et al. 2007. Spatiotemporal differences in CXCL12 expression and cyclic AMP underlie the unique pattern of optic glioma growth in neurofibromatosis type 1. *Cancer Res.* 67:8588–95
148. Warrington NM, Gianino SM, Jackson E, Goldhoff P, Garbow JR, et al. 2010. Cyclic AMP suppression is sufficient to induce gliomagenesis in a mouse model of neurofibromatosis 1. *Cancer Res.* 70:5717–27
149. Druker BJ. 2008. Translation of the Philadelphia chromosome into therapy for CML. *Blood* 112:4808–17

Annual Review of Pathology: Mechanisms of Disease

Volume 7, 2012

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Pathology: Mechanisms of Disease* articles may be found at http://pathol.annualreviews.org