Tumor Suppressor Mutations and Growth Factor Signaling
in the Pathogenesis of NF1-Associated Peripheral Nerve Sheath Tumors

II. The Role of Dysregulated Growth Factor Signaling

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Abstract

Patients with neurofibromatosis type 1 (NF1), one of the most common genetic diseases affecting the nervous system, develop multiple neurofibromas that can transform into aggressive sarcomas known as malignant peripheral nerve sheath tumors (MPNSTs). Studies of human tumors and newly developed transgenic mouse models indicate that Schwann cells are the primary neoplastic cell type in neurofibromas and MPNSTs and that development of these peripheral nerve sheath tumors involves mutations of multiple tumor suppressor genes. However, it is widely held that tumor suppressor mutations alone are not sufficient to induce peripheral nerve sheath tumor formation and that dysregulated growth factor signaling cooperates with these mutations to promote neurofibroma and MPNST tumorigenesis. In Part I of this review, we discussed findings demonstrating that a loss of NF1 tumor suppressor gene function in neoplastic Schwann cells is a key early step in neurofibroma formation and that progression from neurofibroma to MPNST is associated with abnormalities of additional tumor suppressor genes, including p53, INK4A, and p27Kip1. In Part II of this review, we consider evidence that dysregulated signaling by specific growth factors and growth factor receptors promotes the proliferation, migration, and survival of neoplastic Schwann cells in neurofibromas and MPNSTs.

Key Words: Growth factor, Mast cell, Neurofibromatosis, Schwann cell, Tumor progression, Tumorigenesis, Tumor suppressor.

INTRODUCTION

Neurofibromatosis type 1 (NF1) is one of the most common genetic diseases involving the nervous system, affecting approximately 1 in 3,500 infants (1–3). In addition to pigmentary lesions (café-au-lait macules, axillary freckling, and Lisch nodules), bony dysplasias, and learning disabilities, individuals with this autosomal dominant tumor predisposition syndrome may develop several types of neoplasms, including pheochromocytomas, optic gliomas, and juvenile chronic myeloid leukemia. The most distinctive characteristic of NF1 patients, however, is the occurrence of multiple neurofibromas. Neurofibromas are tumors of peripheral nerve and are composed of Schwann cells intermingled with smaller numbers of fibroblasts, mast cells, perineurial cells, and vascular elements. These nerve sheath tumors manifest themselves as fleshy nodules in skin (dermal neurofibromas), circumscribed masses in nerves (intraneural or nodular neurofibromas), or lesions growing diffusely through multiple fascicles of large nerves or nerve plexuses (plexiform neurofibromas). Although benign, neurofibromas cause significant disfigurement, pain, and neurologic deficits. Further, plexiform and, less commonly, intraneural neurofibromas can transform into highly aggressive sarcomas known as malignant peripheral nerve sheath tumors (MPNSTs). Beyond surgery, few therapeutic options are available for patients with MPNSTs; consequently, the prognosis for individuals with these neoplasms is poor. In light of the significant morbidity and mortality neurofibromas and MPNSTs produce in NF1 patients, there is considerable interest in defining the molecular mechanisms underlying the pathogenesis of these tumors, with the hope that this will lead to the development of new, more effective therapies.

As discussed in Part I of this review (4), studies of human tumors and recently developed transgenic mouse models indicate that Schwann cells are the primary neoplastic cell type in neurofibromas and that the pathogenesis of neurofibromas and MPNSTs results, at least in part, from loss-of-function mutations of tumor suppressor genes in Schwann cells. However, a growing body of evidence indicates that dysregulated signaling by growth factors and their receptors acts cooperatively with tumor suppressor mutations to promote peripheral nerve sheath tumor formation. Defining the precise role specific growth factors play in neurofibromas and MPNSTs has recently acquired practical importance as a number of growth factor receptor inhibitors have proved effective in treating other tumor types (e.g., Herceptin [trastuzumab; an erbB2 inhibitory antibody used to treat patients with c-neu overexpressing breast cancers] and Gleevec
[STI-571, imatinib; used to treat chronic myeloid leukemia and gastrointestinal stromal tumors]) and could potentially be adapted to treat peripheral nerve sheath tumors arising in NF1 patients. Our goal in Part II of this review is to examine evidence indicating that specific growth factors and growth factor receptors contribute to neurofibroma and MPNST tumorigenesis, focusing particularly on findings that shed light on the action each of these molecules has on neoplastic Schwann cells.

**EVIDENCE SUGGESTING A ROLE FOR DYSREGULATED GROWTH FACTOR SIGNALING IN NF1-ASSOCIATED PERIPHERAL NERVE SHEATH TUMORS**

Coincident with the search for the NF1 tumor suppressor gene during the 1980s and early 1990s, several lines of investigation began to indicate that neurofibromas and MPNSTs elaborate growth factors that contribute to their own development. For instance, crude extracts of NF1-associated neurofibromas were found to contain an activity that promoted the growth of cultured neurofibroma explants (5), and plasma from NF1 patients (but not normal controls) was demonstrated to stimulate the proliferation of cultured cells derived from neurofibromas (6). Spurred by observations such as these, multiple laboratories initiated studies that culminated in the identification of growth factors and growth factor receptors that are aberrantly expressed in neurofibromas and MPNSTs and promote Schwann cell mitogenesis, migration, and/or survival (see below for details on specific factors). Depending on their cellular source, these growth factors may stimulate neoplastic Schwann cells in an autocrine, paracrine, or endocrine fashion.

The identification of the tumor suppressor genes that are mutated in neurofibromas and MPNSTs and a consideration of the functional requirements of the affected signaling pathways provided additional, albeit indirect, evidence that dysregulated growth factor signaling promotes peripheral nerve sheath tumorigenesis. NF1 patients carry a constitutional mutation of the NF1 tumor suppressor gene, and inactivation of the remaining functional NF1 allele appears to be an essential early event in the development of neurofibromas (4). Neurofibromin, the product of the NF1 locus, facilitates the inactivation of Ras proteins (Fig. 1), a family of small G-proteins that serve as key regulators of mitogenesis, migration, and other cellular responses. Neurofibromin loss in Schwann cells within neurofibromas thus promotes the increased activation of Ras and Ras-activated signaling cascades evident in these neoplasms. Loss-of-function mutations or functional abnormalities of other tumor suppressor genes such as p53, INK4A, and p27\(^{kip1}\) also accumulate as neurofibromas transform into MPNSTs and likely cooperate with NF1 loss to produce the malignant Schwann cell phenotype (4) (see also Reference [7] in this issue).

Although neurofibromin loss interferes with the cell’s ability to inactivate activated Ras, it is clear that complementary mechanisms must exist that serve to activate these G-proteins in the first place. As activating mutations of Ras are rarely, if ever, detected in neurofibromas and MPNSTs, this cannot be the mechanism promoting Ras activation in neoplastic Schwann cells. The identification of Schwann cell growth factors in neurofibromas and MPNSTs provided support for an alternative concept, namely, that dysregulated signaling by growth factors promotes tumorigenesis by stimulating the proliferation, migration, and/or survival of Schwann cells whose cytoplasmic signaling (i.e. Ras-dependent; Fig. 1) and cell cycle regulatory mechanisms are defective because of a loss of tumor suppressor gene function.

**GROWTH FACTORS AND GROWTH FACTOR RECEPTORS IMPLICATED IN PERIPHERAL NERVE SHEATH TUMORIGENESIS**

Over the last three decades, a number of growth factors have been suggested as candidate molecules promoting the development of neurofibromas and MPNSTs. To rigorously establish that these molecules do indeed promote Schwann cell neoplasia, there are criteria that each candidate growth factor

*FIGURE 1. Schematic representation of the regulation of Ras activity in neoplastic Schwann cells. Ras proteins, a group of molecules that includes the neurofibromin-regulatable “classic” (H-Ras, N-Ras, and K-Ras) and “nonclassic” (R-Ras and TC21/Ras2) Ras proteins, are active when associated with GTP, leading to the activation of mitogen-activated protein (MAP) kinase cascades promoting mitogenesis, migration, transformation, and other effects. Neurofibromin inactivates Ras proteins by stimulating an intrinsic GTPase activity present in Ras proteins, resulting in hydrolysis of GTP to GDP; guanine nucleotide exchange factors (GEFs) promote an exchange of GDP for GTP, resulting in reactivation of Ras. Growth factors, commonly acting through membrane tyrosine kinase receptors and associated accessory proteins (e.g. Grb2 and Sos) are also required to stimulate Ras activation. For more detailed descriptions of the Ras proteins, the signaling events they control and the consequences of activating Ras-dependent signaling cascades, see Part I of this review (4).*
should meet. First, the growth factor must be present in the neoplasm in a biologically active form. Second, neoplastic Schwann cells, alone or in combination with other cell types within the peripheral nerve sheath tumor, must express the receptor(s) necessary for responsiveness to the growth factor. Third, blocking signaling by the growth factor or its receptor (e.g. using pharmacologic inhibition [preferably with at least two functionally distinct inhibitors], expression of dominant negative mutants, “knock-down” by RNA interference or inhibition with neutralizing antibodies) should reduce the in vitro proliferation, survival, or migration of neoplastic Schwann cells isolated from human neurofibromas or MPNSTs. Fourth, interference with growth factor signaling should reduce the proliferation, survival, migration, or metastasis of neoplastic human Schwann cells in in vivo model systems (e.g. tumor cells xenografted into nude mice). Finally, overexpression of the candidate growth factor or growth factor receptor in Schwann cells of transgenic mice should induce the formation of neurofibroma- or MPNST-like neoplasms that meet the previous four criteria. Demonstrating that stimulation with exogenous growth factor promotes proliferation, migration, or survival can also be useful, but is not strictly required as we have encountered situations in which levels of endogenously produced growth factor are sufficient to maximally stimulate tumor cells and addition of exogenous factor produces no further increase in the measured parameter (8). The criteria delineated above may require modification under some circumstances (e.g. neoplasms in which activating mutations or overexpression of growth factor receptors result in ligand-independent activation of the receptor).

At present, the criteria outlined above have not been fully satisfied for any of the growth factors discussed in this review. Below, we consider the evidence that is available for several candidate growth factors and growth factor receptors, beginning with the four systems that have been most extensively studied and then proceeding to several more molecules for which intriguing initial studies are available.

Neuregulin-1 and Its ErbB Receptors

In contrast to the other growth factors discussed below, the hypothesis that neuregulin-1 (NRG-1) and its erbB receptors play a role in peripheral nerve sheath tumorigenesis had its origins in studies of animal models rather than observations in human tumors. Beginning nearly 40 years ago, it was noted that rats (9, 10), mice (11, 12), and hamsters (13) transplacentally exposed to the alkylating agent N-ethyl-N-nitrosourea (EtNU) frequently developed malignant peripheral nerve sheath tumors arising in P 0-GGF b 3 mice also uniformly develop preneoplastic lesions in their trigeminal, dorsal root, and sympathetic ganglia (Fig. 2A, B) in contrast to the diffuse hyperplasia seen in the sciatic nerve of these animals, the intraganglionic lesions in P 0-GGF b 3 mice are initially delimited from adjacent nerve (Fig. 2C). About 60% of these animals develop large tumor masses, most commonly in the trigeminal nerve, by 6 to 12 months of age. These tumors are markedly hypercellular neoplasms (Fig. 2D, E) with significant cellular atypia, brisk mitotic activity, and foci of hemorrhage and necrosis. Their behavior is locally aggressive, as demonstrated by invasion of brain parenchyma (Fig. 2F) and other adjacent tissues. As the tumors arising in P 0-GGF b 3 mice also demonstrate immunohistochemical and ultrastructural evidence of schwannian differentiation (Fig. 2G–J), we have classified them as genetically engineered murine (GEM) Grade III peripheral nerve sheath tumors (PNGTs); see Part I of this review for a discussion of the frequent expression of a constitutively activated form of the erbB2 (c-neu) membrane receptor tyrosine kinase carrying a point mutation in sequences encoding the transmembrane domain of this molecule (20–25). Consistent with the hypothesis that the mutant erbB2 kinase confers a proliferative advantage on immature Schwann cells (25), transfection of this oncogene into cultured embryonic Schwann cells immortalized and transformed these glia (26). Furthermore, a kinase-deficient erbB2 dominant negative mutant inhibited the proliferation and anchorage-independent growth of EtNU-induced MPNST cells (27). Mutation of erbB2 occurs very early in the development of EtNU-induced MPNSTs, with cells carrying the mutant allele being evident as soon as 7 days after exposure to the carcinogen (25). It is unclear whether tumor suppressor gene mutations occur in EtNU-induced MPNSTs and, if so, whether they mirror those found in human MPNSTs. Although two loci on rat chromosome 10 (which shares homology with the mouse chromosome carrying the Nf1 and p53 loci) have been associated with susceptibility to EtNU-induced tumorgenesis, these loci are distinct from the Nf1 and p53 genes (28).

When first identified as an oncogene in EtNU-induced MPNSTs, erbB2 was an “orphan” receptor with no known ligand. In the early 1990s, however, it was found that erbB2 serves as a coreceptor that is activated when the related kinases erbB3 and erbB4 bind proteins in the NRG-1 family of growth and differentiation factors. NRG-1 proteins (which include heregulin, neu differentiation factor, glial growth factor [GGF], acetylcholine receptor inducing activity [ARIA], and sensory and motor neuron-derived factor [SMDF]) are a family of structurally diverse polypeptides encoded by alternatively spliced mRNAs transcribed from a single gene (29). NRG-1 factors potently promote the proliferation, survival, and migration of Schwann cells during embryogenesis and early postnatal life (30–34), raising the question of whether they might have similar effects in peripheral nerve sheath tumors. To test this hypothesis, we produced transgenic mice in which expression of the NRG-1 isoform GGFb3 is directed by regulatory elements of the Schwann cell-specific myelin protein zero promoter (P 0-GGF b 3 mice) (35). P 0-GGF b 3 mice develop prominent Schwann cell hyperplasia in their sciatic nerves, an abnormality that culminates in the development of a hypertrophic neuropathy resembling Charcot-Marie-Tooth disease. P 0-GGF b 3 mice also uniformly develop preneoplastic lesions in their trigeminal, dorsal root, and sympathetic ganglia (Fig. 2A, B) in contrast to the diffuse hyperplasia seen in the sciatic nerve of these animals, the intraganglionic lesions in P 0-GGF b 3 mice are initially delimited from adjacent nerve (Fig. 2C). About 60% of these animals develop large tumor masses, most commonly in the trigeminal nerve, by 6 to 12 months of age. These tumors are markedly hypercellular neoplasms (Fig. 2D, E) with significant cellular atypia, brisk mitotic activity, and foci of hemorrhage and necrosis. Their behavior is locally aggressive, as demonstrated by invasion of brain parenchyma (Fig. 2F) and other adjacent tissues. As the tumors arising in P 0-GGF b 3 mice also demonstrate immunohistochemical and ultrastructural evidence of schwannian differentiation (Fig. 2G–J), we have classified them as genetically engineered murine (GEM) Grade III peripheral nerve sheath tumors (PNGTs); see Part I of this review for a discussion
FIGURE 2. MPNST-like neoplasms developing in transgenic mice expressing the NRG-1 isoform GGFβ3 in myelinating Schwann cells. Comparison of the trigeminal ganglion of a wild-type mouse (A) and a P0-GGFβ3 mouse (B), demonstrating the preneoplastic lesions uniformly present in the peripheral ganglia of P0-GGFβ3 mice. (C) A preneoplastic lesion in the trigeminal ganglion of a P0-GGFβ3 mouse, demonstrating the clear delineation of this lesion from adjacent trigeminal nerve (right side of the field). (D, E) Two examples of GEM grade III peripheral nerve sheath tumors developing in the trigeminal nerves of P0-GGFβ3 mice. These neoplasms are markedly hypercellular lesions composed of highly anaplastic cells. Mitotic figures (arrows) are frequent in these tumors. These neoplasms, like their human counterparts, show some variability in their histologic appearance. (F) A third GEM grade III PNST invading and destroying brain parenchyma in a P0-GGFβ3 mouse. (G) Immunoreactivity for S-100β in a GEM grade III PNST developing in P0-GGFβ3 mice. S-100 immunoreactivity is indicated by Cy-3 labeling (red); the section has been counterstained with the nuclear dye bisbenzamide (blue). (H) Immunoreactivity for the basal lamina protein collagen type IV individually invests tumor cells in GEM grade III PNSTs developing in P0-GGFβ3 mice. This section is from the same neoplasm shown in panel (D) and has been lightly counterstained with hematoxylin to highlight tumor cell nuclei. (I, J) Transmission electron micrographs of the tumor shown in D. Individual tumor cells are invested by a basal lamina, with loops of basal lamina material frequently seen extending away from the cells (arrow). (K) Membranous and cytoplasmic immunoreactivity for the NRG-1 receptor erbB3 in a neurofibroma from a human NF1 patient. (L) Immunoreactivity for the NRG-1 receptor erbB4 in a MPNST from a human NF1 patient. This antigen is evident in association with membranes as well as tumor cell nuclei; this latter distribution likely reflects the cleavage and internalization of a cytoplasmic fragment of erbB4, as described in other cell types (71). (A–H), (K), and (L) original magnification: 40 ×; scale bar = 50 μm. (I) original magnification: 10,000 ×; (J) original magnification: 40,000 ×; scale bar = 0.5 μm.
of recent consensus recommendations for the classification of transgenic mouse peripheral nervous system tumor (4). NRG-1 expression is evident in these tumors and they overexpress NRG-1 receptors, suggesting that autoinhibitory or paracrine signaling promotes their proliferation, survival, and other effects. We do not yet know whether the GEM Grade III MPNSTs arising in P2-GGFβ3 mice carry mutations of Nf1, p53, or other tumor suppressor genes, as is seen in human MPNSTs.

There is also evidence that dysregulated NRG-1/erbB signaling contributes to peripheral nerve sheath tumorogenesis in humans. In 1986, Brockes et al reported that a subset of neurofibromas and the single MPNST they examined contained a GGF-like activity (36). We have recently determined that neurofibromas and MPNSTs do indeed express multiple α and β transmembrane precursors from the Class II (GGF) and III (SMDF) NRG-1 subfamilies (M.S. Stonecypher, S.J. Byer and S.L. Carroll, submitted). Like the neoplasms developing in P2-GGFβ3 mice, many human neurofibromas and MPNSTs also express the erbB receptors (erbB2, erbB3, and/or erbB4) mediating NRG-1 responsiveness (Fig. 2K, L). A similar pattern of NRG-1 and erbB expression is present in four human MPNST cell lines (NMS-2, NMS-2PC, Mash-1, and YST-1 cells), and treating these lines with two structurally and functionally distinct erbB inhibitors, PD158780 and PD168393, markedly reduces their proliferation. These observations indicate that activation of erbB kinases, potentially mediated by an NRG-1/erbB autocrine loop, promotes the proliferation of human MPNST cells. It remains to be determined whether NRG-1/erbB signaling has other protooncogenic effects (e.g. increased survival and migration) and whether these erbB inhibitors or other anti-erbB agents (e.g. Herceptin) will prove to be effective for treating NF1-associated neurofibromas and MPNSTs in vivo.

**Epidermal Growth Factor Receptor**

The EGFR receptor (EGFR; also known as erbB1), a membrane receptor tyrosine kinase closely related to the NRG-1 receptors (erbB2, erbB3, and erbB4) has also been implicated in the pathogenesis of neurofibromas and MPNSTs. Examining three human lines derived from NF1-associated MPNSTs (90–8, 88–14, and 88–3 cells) and one isolated from a sporadic MPNST (S-26T cells), DeClue et al found that all four lines expressed EGFR and responded to EGF with increased EGFR and MAP kinase phosphorylation (37). The proliferation of human 88–14 MPNST cells grown in the presence of limiting (0.1%) amounts of fetal calf serum is dependent on exogenous EGF and inhibition of EGF signaling with an anti-EGFR antibody (mAb225) or pharmacologic inhibitors of the EGFR (tyrostatins A-25 and AG-1478) significantly reduces this growth. When maintained in higher (2%) concentrations of fetal calf serum, human 88–14 and 90–8 MPNST cells proliferate independent of exogenous EGF, but their growth is still inhibited by EGFR antagonists, indicating that EGFR contributes to mitogenesis even in the absence of added EGF. EGFR is also expressed in human peripheral nerve sheath tumors in vivo. Although evident only in fibroblasts and perineurial cells in normal human nerves, a small subpopulation of EGFR+/S-100β+ cells is found in human neurofibromas. EGFR expression is present in human MPNSTs as well but is variable, with some neoplasms containing strong EGFR immunoreactivity in nearly all cells and others containing only scattered weakly labeled cells (37). This variable EGFR expression has been suggested to reflect transient EGFR expression associated with specific stages in the pathogenesis of Schwann cell neoplasms. Alternatively, there may be more than one pathway to Schwann cell neoplasia, some of which do not require EGFR expression (37).

Prominent EGFR expression has also been found in 23 of 24 cell lines derived from soft tissue neoplasms (13 malignant Triton tumors [MTTs]), 1 MPNST, 1 leiomyosarcoma, 1 rhabdomyosarcoma, 1 sarcoma not otherwise specified, and 7 with unknown diagnoses) developing in Nf1+/p53 haploinsufficient mice (38) and in vitro transformed Nf1+/p53− Schwann cells isolated from embryonic day 12.5 mouse embryos (37). As with human MPNST cell lines, the growth of these lines derived from tumors arising in Nf1+/p53 haploinsufficient mice is EGF-dependent when maintained in medium with limiting amounts of fetal calf serum (38). The EGFR can be activated by multiple ligands, including EGF, transforming growth factor-α, amphiregulin, heparin-binding EGF, epiregulin, and β-cellulin (39). These mouse tumor lines express amphiregulin, heparin-binding EGF, and epiregulin; they do not, however, express EGF or β-cellulin (38). It is not yet known whether EGFR ligands are expressed in human neurofibromas and MPNSTs and, if so, whether they are required for tumorigenesis. It is conceivable that ligand expression may not be necessary for human peripheral nerve sheath tumorogenesis as ligand-independent activation of EGFR has been observed in other tumor types with high levels of EGFR expression (39). Consistent with this hypothesis, amplification of the EGFR locus, an event thought to be associated with EGFR overexpression, occurs in a subset of MPNSTs, being evident in 26% of human tumors in one series (40). Alternatively, EGFRs expressed by neoplastic Schwann cells in neurofibromas and MPNSTs may be transactivated by other growth factors (e.g. lysophosphatidic acid, cytokines) as described in several other cell types (39).

We have found that EGFR and NRG-1 receptors are coexpressed in some, but not all, human neurofibromas and MPNSTs (M.S. Stonecypher, S.J. Byer and S.L. Carroll, submitted). This observation is intriguing as the EGFR is capable of heterodimerizing and “cross-talking” with NRG-1 receptors (39). It will be interesting to determine whether coexpressed EGF and NRG-1 receptors enhance each other’s signaling capabilities and produce novel responses in neoplastic Schwann cells.

**Hepatocyte Growth Factor and Its c-Met Receptor**

Hepatocyte growth factor (HGF; also known as scatter factor) is a heparin-binding growth factor that is produced by a variety of mesenchymal cell types during development (41, 42) and released from these cells as an inactive zymogen. Following cleavage by a serine protease (the HGF activator [HGFA]), mature HGF promotes the proliferation, migration, and morphogenesis of nearby epithelial cells expressing the c-Met membrane receptor tyrosine kinase. HGF activation of
c-Met is frequently enhanced by CD44, a transmembrane glycoprotein implicated in the activation of several high-affinity growth factor receptors and in cell-cell and cell-matrix adhesion (43). Dysregulated HGF/c-Met signaling, facilitated in many instances by CD44 splice variants carrying variant exons 3 or 6 (CD44v3 or CD44v6), has been implicated in the pathogenesis of a variety of carcinomas where these molecules enhance mitogenesis, invasion, metastasis, and angiogenesis.

Purified neonatal rat Schwann cells also express c-Met, and the mitogenesis of these glia is potently stimulated by HGF (44). Neonatal Schwann cells do not, however, themselves express HGF, suggesting that HGF released from another cell type acts on Schwann cells in developing peripheral nerve (44). In contrast, HGF and c-Met are coexpressed in human neurofibromas (45–47) and MPNSTs (45, 47), with immunoreactivity for both molecules being reportedly greater in MPNSTs than in neurofibromas (45). Based on these observations and the demonstration that HGF is a mitogen for neonatal rat Schwann cells, it was expected that HGF and c-Met would form an autocrine loop driving the proliferation of neoplastic Schwann cells in neurofibromas and/or MPNSTs. Consistent with this hypothesis, Su et al found that HGF, c-Met, IGFA, and CD44 colocalize in distinct regions within MPNSTs in vivo (48). However, these regions of colocalization did not correspond to areas demonstrating increased MIB-1 labeling indices, raising the question of whether HGF/c-Met autocrine signaling has effects other than stimulating mitogenesis. Examining three human MPNST cell lines, these investigators identified one line, ST8814, cells, which coexpressed HGF, c-Met, CD44, and the HGF activator. Ablation of c-Met expression in ST8814 cells with a ribozyme targeting c-Met mRNA had no effect on the proliferation of this line, confirming that this signaling pathway was not promitogenic in at least some MPNST cells. In contrast, knock-down of c-Met expression markedly inhibited ST8814 cell invasion in an in vitro assay (48), leading these authors to suggest that HGF/c-Met signaling promotes metastasis from MPNSTs. Curiously, ST8814 cell invasion promoted by HGF/c-Met signaling was not inhibited by neutralizing anti-CD44v3, antiCD44v6, or anti-pan CD44 antibodies. It is not yet known whether HGF is widely required for MPNST invasion and metastasis in vivo and, if so, why CD44 is not required for these effects.

**Stem Cell Factor and Its Receptor, c-Kit**

Stem cell factor (SCF, Kit ligand), acting through its receptor, the c-Kit membrane tyrosine kinase, promotes mast cell precursor migration into normal tissues and the subsequent maturation, activation, and survival of these cells (49). The prominent population of mast cells present within neurofibromas likewise express the c-Kit receptor (50), and these neoplasms contain SCF (50) that is produced, at least in part, by neoplastic Schwann cells (51, 52). SCF expression is related to the level of neurofibromin activity in Schwann cells, with murine Nf1−/− Schwann cells secreting approximately sixfold more SCF than either wild-type or Nf1+/− Schwann cells (53). Further, Nf1−/− mast cells demonstrate increased proliferation (54, 55), survival (54, 55), and migration (53) in response to SCF. Considered together, these observations indicate that enhanced SCF expression by neoplastic Schwann cells is an important factor promoting mast cell accumulation in neurofibromas.

SCF may have other roles in peripheral nerve sheath tumor formation as well. Despite the fact that MPNSTs contain much smaller numbers of mast cells than are present in neurofibromas, human MPNST cell lines continue to express SCF (51, 52), suggesting that this growth factor acts on additional cell types in MPNSTs. These alternative SCF targets may include the neoplastic Schwann cells themselves. Some, but not all, MPNST cell lines express high levels of c-Kit protein and stimulation with exogenous SCF increases the proliferation of these lines (56). Further, the basal proliferation of c-Kit expressing MPNST cell lines is inhibited by treatment with tyrphostin A9 (56), a pharmacologic inhibitor that targets platelet-derived growth factor (PDGF)-related tyrosine kinases, the group of receptors to which c-Kit belongs. It is not yet clear whether autocrine or paracrine signaling through the SCF/c-Kit signaling pathway promotes the proliferation of only a subset of MPNSTs in vivo or is more widely used.

**Other Growth Factors Potentially Promoting Peripheral Nerve Sheath Tumorigenesis**

**PDGF and Its Receptors**

PDGF BB, acting through its β receptor, is a potent mitogen for neonatal rat Schwann cells (57, 58). PDGFB BB and the PDGF-β receptor are coexpressed in neurofibromas in vivo (59) and by cultured neurofibroma (60) and MPNST (61, 62) cells. MPNSTs and MPNST-derived cell lines also express the PDGF-α receptor. The proliferation of cultured neurofibroma (60) and MPNST cells (61) is potently enhanced by stimulation with PDGF BB, a response that is associated with abnormal activation of calcium-dependent signaling pathways (62). These observations suggest that autocrine or paracrine signaling involving PDGF BB and its β- and/or α-receptors promotes the mitogenesis of neoplastic Schwann cells in neurofibromas and MPNSTs in vivo. This hypothesis has not yet been verified by approaches such as demonstrating that inhibition of PDGF or PDGF receptor action inhibits the basal proliferation of neurofibroma and MPNST cells or by showing that transgenic mice overexpressing PDGF in Schwann cells develop peripheral nerve sheath tumors.

**Midkine**

Midkine is a 13 kDa heparin-binding growth factor that is highly expressed during embryogenesis and is overexpressed in several human tumor types (63). Midkine is also overexpressed in the skin of NF1 patients relative to normal controls and stimulates the proliferation of endothelial cells (64, 65), neurofibroma-derived fibroblastoid-like cells (64, 65), and ST8814 cells (65), an S-100-positive line derived from a human MPNST. Neoplastic Schwann cells likely are a major source of midkine in neurofibromas and MPNSTs in vivo, as this growth factor is expressed at higher levels in Nf1−/− Schwann cells than in Nf1+/− or wild-type Schwann cells.

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Transforming Growth Factor-β

Transforming growth factor-β (TGF-β) has complicated effects on normal Schwann cells, inducing proliferation and phenotypic alterations in some situations (66) and triggering apoptosis in others (67). Our knowledge of TGF-β action in neurofibromas and MPNSTs is intriguing but limited. TGF-β1 and its receptor are expressed in neurofibromas and MPNSTs, with higher levels of expression evident in the latter tumor type (47). This factor has been reported to stimulate DNA synthesis in cells derived from a human neurofibroma (60). However, a second group of investigators found that TGF-β1 inhibited the proliferation of a single cell line derived from an ENU-induced rat MPNST (68). It is currently unclear whether these apparently contradictory findings reflect differences in neurofibroma and MPNST responses to TGF-β1 or other variables in these two experimental systems.

Sex Steroids

It is well known that new neurofibromas frequently appear at puberty and that existing neurofibromas increase in size during this period. Neurofibromas also increase in size and frequency in pregnant women and regress after parturition. These observations suggest that puberty- and pregnancy-associated changes such as alterations in blood levels of sex steroids promote neurofibroma growth. Despite these well-known clinical observations, information supporting this hypothesis is surprisingly sparse. In a series of 59 human neurofibromas, 75% of these neoplasms were found to express progesterone receptors (69), with expression of this steroid receptor being more common in dermal neurofibromas (which occur with increased frequency after puberty) than in plexiform neurofibromas (which are thought to be congenital). Interestingly, the neurofibroma cells expressing progesterone receptors were S-100 negative, indicating that they are not NF1-expressing Schwann cells and that NF1 is not involved in neurofibroma pathogenesis. In contrast, progesterone receptor expression in schwannoma cells is more common in NF1 neurofibromas and MPNSTs (70) and is associated with higher levels of expression evident in the latter tumor type (47). This factor has been reported to stimulate DNA synthesis in cells derived from a human neurofibroma (60). However, a second group of investigators found that TGF-β1 inhibited the proliferation of a single cell line derived from an ENU-induced rat MPNST (68). It is currently unclear whether these apparently contradictory findings reflect differences in neurofibroma and MPNST responses to TGF-β1 or other variables in these two experimental systems.

CONCLUSIONS AND FUTURE DIRECTIONS

As discussed in Part I of this review, considerable evidence has accumulated over the last 15 years indicating that loss of function of the NF1 tumor suppressor gene is a key early step in the pathogenesis of NF1-associated neurofibromas and that subsequent progression from neurofibroma to MPNST is associated with abnormalities of additional tumor suppressor genes, such as p53, INK4A, and p27kip1. It has also become increasingly evident that multiple aberrantly expressed growth factors and/or growth factor receptors contribute to peripheral nerve sheath tumorigenesis by promoting the proliferation, migration, and/or survival of neoplastic Schwann cells. However, several aspects of growth factor action in neurofibromas and MPNSTs remain poorly defined. First, our current understanding likely underestimates the complexity of the functions these factors perform in neurofibromas and MPNSTs. For instance, further study is needed to determine whether specific promitogenic growth factors contribute to tumorigenesis in alternative ways such as promoting survival, intraneural migration, and metastasis. As attested to by the observation that SCF targets both mast cells and neoplastic Schwann cells, it is also likely that several of these factors have important protumorigenic effects on more than one cell type within peripheral nerve sheath tumors. Finally, it must be determined whether each of the growth factors considered in this review is universally required for neurofibroma and/or MPNST pathogenesis or whether there are distinct subtypes of peripheral nerve sheath tumor, each relying on the action of a different growth factor or growth factor receptor for its development.

Answering questions such as these will require investigations that go beyond simply demonstrating that neoplastic Schwann cells express a growth factor and its receptor and that stimulation with that growth factor promotes Schwann cell proliferation. In particular, it will be important to develop new transgenic mouse models in which candidate growth factors or growth factor receptors are overexpressed in Schwann cells; at present, P0-GGFβ3 mice, in which NRG-1 is overexpressed in Schwann cells, represent the only reported model in which growth factor overexpression induces the formation of peripheral nerve sheath tumors. Once developed, transgenic mice overexpressing growth factors in Schwann cells can be crossed to animals carrying targeted mutations of tumor suppressor genes (e.g., Nf1, p53), allowing the hypothesis that dysregulated growth factor expression and tumor suppressor mutations cooperatively promote peripheral nerve sheath tumorigenesis to be directly tested for the first time. Transgenic mouse models in which peripheral nerve sheath tumors result from growth factor overexpression will also serve as extraordinarily useful tools for assessing the therapeutic potential of existing agents that inhibit specific growth factors or growth factor receptors.

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