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Review Article

# The promise of signal transduction in genetically driven sarcomas of the nerve

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Abstract.

Neurofibromatosis type 1 (NF1) is an autosomal dominant tumor predisposition syndrome. Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft tissue sarcomas arising from peripheral nerve sheaths, and the most commonly lethal feature associated with NF1. The hallmark of NF1 and NF1-related MPNST is the loss of neurofibromin expression. Loss of neurofibromin is considered a tumor-promoting event, and leads to constitutive activation of RAS and its downstream effectors.

However, RAS activation alone is not sufficient for MPNST formation, and additional tumor suppressors and signaling pathways have been implicated in tumorigenesis of MPNST. Taking advantage of the rapid development of novel therapeutics targeting key molecular pathways across all cancer types, the best-in-class modulators of these pathways can be assessed in pre-clinical models and translated into clinical trials for patients with MPNST. Here, we describe the genetic changes and molecular pathways that drive MPNST formation and highlight the promise of signal transduction to identify therapies that may treat these tumors more effectively.

## 1. Introduction

Malignant peripheral nerve sheath tumors (MPNST) are nerve-associated neoplasms of Schwann cell lineage origin (Carroll, 2012), and are classified as aggressive soft tissue sarcomas, characterized by high risk of local recurrence and distant metastases (Widemann, 2009). Half of all MPNST are seen in patients with neurofibromatosis type 1 (NF1) and in these patients, typically arise from benign plexiform neurofibromas (PNF) (Evans et al., 2002). *NF1* loss in Schwann cells results in enhanced proliferation and recruitment of other *NF1*$^{+/-}$ cell types that interact to drive neurofibroma pathogenesis (Carroll, 2016). Plexiform neurofibromas (PNF) themselves can be a major cause of morbidity for these patients due to their distortion of normal nerve anatomy and associated neurologic dysfunction and pain. Transformation of neurofibromas to MPNST requires accumulation of additional mutations in cell cycle regulatory elements. Histologically, there is marked pleomorphism, mitosis and invasion of adjacent tissues (Kourea et al., 1999a). Like many soft tissue sarcomas, complete resection with wide margins is mainstay of therapy for MPNST. Yet, compared with other soft tissue sarcomas, MPNST have the highest risk of sarcoma-specific death (Kattan et al., 2002). Surgical resection is not always feasible in patients with MPNST often due to large tumor size, location within large nerve sheath bundles and PNF, and the presence of metastatic disease (Fletcher and McKee, 1985). Resection, when feasible, is unfortunately, often associated with the cost of sacrificing nerve function. Thus inter-disciplinary involvement for clinical decision-making, including the medical and surgical oncologists, radiation oncologist, and neurologist, is critical for management of these patients. The overall prognosis is poor, especially in those tumors that cannot be fully resected (Scaife and Pisters, 2003). There is clearly a desperate need for more effective treatments for patients with MPNST.

The hallmark of NF1 and NF1-related MPNST is the loss of neurofibromin expression. Loss of neurofibromin is considered a tumor-promoting event, and leads to constitutive activation of RAS and its downstream effectors (Basu et al., 1992). However, RAS activation alone is not sufficient for MPNST formation (Kluwe et al., 1999), and additional tumor suppressors and signaling pathways have been implicated in tumorigenesis of MPNST (Kourea et al., 1999a; Rutkowski et al., 2000; Sherman et al., 2000; Nielsen et al., 1999; Kourea et al., 1999b). Taking advantage of the rapid development of novel therapeutics targeting key molecular pathways across all cancer types, the best-in-class modulators of these pathways can be assessed in pre-clinical models and translated into clinical trials for patients with MPNST. Here, we describe the genetic changes and molecular pathways

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that drive MPNST formation and highlight the promise of signal transduction to identify therapies that may treat these tumors more effectively.

### 1.1. Loss of NF1 as a mechanism of RAS pathway activation leading to tumor predisposition

The *NF1* gene is altered *via* a multitude of germline events including missense and nonsense mutations, deletions and insertions, in patients with NF1. This syndrome, inherited in an autosomal dominant pattern, has a widely heterogeneous clinical phenotype, including cutaneous neurofibromas, the characteristic café-au-lait spots, cognitive impairment, and both benign and malignant tumors of the central and peripheral nervous system (Friedman and Birch, 1997). Somatic inactivating mutations in the unaffected allele occur in neurofibromas, suggestive of a second-hit event that is responsible for tumor formation (Serra et al., 1997). The resulting loss of functional NF1 protein leads to disruption of the physiologic regulation of RAS signaling. Among the tumors observed in these patients are optic pathway gliomas, juvenile myelomonocytic leukemia (JMML), gastrointestinal stromal tumors (GIST), rhabdomyosarcoma (RMS), and malignant peripheral nerve sheath tumors (MPNST) (Friedman and Birch, 1997).

The incidence of MPNST is many fold higher in patients with NF1 compared with the general population (up to 10% *versus* 0.001%) (Ducatman et al., 1986), and the risk is even higher in patients with an NF1 micro-deletion (De Raedt et al., 2003; Pasmant et al., 2009; Pasmant et al., 2010). In patients with NF1, these malignancies most often arise within preexisting PNF, which are centrally located and extensive benign nerve sheath tumors (Ferner and Gutmann, 2002). Tumors from patients with NF1 who develop MPNST have loss of neurofibromin and elevated levels of RAS activity (Basu et al., 1992; Guha et al., 1996; DeClue et al., 1992).

The *NF1* gene product neurofibromin is essential for physiologic negative regulation of RAS proteins in the cell (Basu et al., 1992; DeClue et al., 1992). As loss of NF1 RAS GTP-ase activating protein (RAS-GAP) function results in constitutive RAS activity (Basu et al., 1992; Bollag et al., 1996; Hattori et al., 1992), cellular consequences of loss of NF1 result in activation of multiple RAS effector pathways. More than ten families of RAS effectors have been identified, the function of three of which, the RAF kinases, the PI3-kinases, and RAL-GDS, have been associated with RAS transformation in model systems (Fig. 1) (Cox and Der, 2002). It has been shown that the transcriptional response to NF1 deficiency closely resembles the ensemble of feedback regulating genes that are induced by oncogenic RAF (Courtois-Cox et al., 2006), suggesting a critical role for RAF-MEK-ERK signaling in tumors in which NF1 is lost. The role of extracellular signal-regulated kinase (ERK) signaling in cancers has been extensively studied, and small molecule drugs that target this pathway have been effective anticancer therapies, particularly in melanoma.

### 1.2. RAF-MEK-ERK signaling

ERK signaling is hyper-activated in up to 30% of human cancers – genetic alterations responsible for activation of the pathway include mutation or amplification of receptor tyrosine kinases (RTKs), mutations in RAS, mutations in BRAF, and loss of NF1 (Pratilas and Solit, 2010; Nissan et al., 2013). Much of our understanding regarding the predictive role of ERK pathway activating mutations in determining sensitivity of cancers to small molecule inhibitors comes from work done in models of melanoma. In melanoma, about half of all tumors and cell lines harbor mutations in BRAF, and another 15–20% are characterized by activating mutations in RAS. Prior to the recognition of BRAF mutations in cancer, it was widely hypothesized that all tumors with elevated levels of phosphorylated ERK should be susceptible to small molecules targeting the node immediately upstream, the mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK) protein kinase. The role of the BRAF V600E mutation in predicting sensitivity to MEK inhibitors is now well-established: tumor cells with mutations in BRAF are selectively sensitive to MEK inhibitors, while those with RTK activation are not, and those with mutations in RAS are defined by variable or intermediate sensitivity (Solit et al., 2006). Further preclinical studies then defined the MEK-ERK dependence of BRAF mutations in non-melanoma cancers (Pratilas et al., 2008; Leboeuf et al., 2008); the role of loss of phosphatase and tensin homolog (PTEN) in conditioning the response to MEK and RAF inhibitors in melanoma cells with BRAF V600E mutations (Xing et al., 2012); and later, the effects of loss of NF1 in melanoma tumors that harbor both wild-type (WT) NRAS and WT BRAF (Nissan et al., 2014). In a study of those cell lines that lack mutations in RAS and RAF (about one third), the vast majority were MEK-ERK signaling dependent, suggestive of a lineage dependency and/or other driver mutations responsible for ERK activation. Genetic characterization of potential drivers of ERK signaling in this cohort demonstrated that a subset of them harbored gene deletions or truncating mutations in the *NF1* gene (Nissan et al., 2014). In melanoma cell lines with loss of NF1 expression, high levels of GTP-bound RAS and sensitivity to MEK inhibition were noted. The MEK inhibitor trametinib impaired the adaptive response of cells to ERK inhibition, leading to sustained suppression of ERK signaling and significant antitumor effects (Nissan et al., 2014). These findings, although studied more extensively in models of melanoma, now can be applied to the study of other tumors, including those with loss of NF1 such as PNF and MPNST.

An abundance of data suggests that ERK signaling is a critical downstream effector of activated RAS in MPNST. Mattingly et al. showed constitutive activity of ERK1/2 in malignant schwannoma cell lines from patients with neurofibromatosis, compared to those from patients with intact NF1 (Mattingly et al., 2006). Immunohistochemical (IHC) analysis demonstrated phospho-MEK and phospho-ERK positivity in a high percentage (93% and 81%, respectively) of MPNST (Endo et al., 2013a).

### 1.3. Loss of NF1 as a driver of other malignancies

Somatic loss-of-function mutations in *NF1* have now been identified in a number of tumors, including melanoma, brain tumors, lung and ovarian cancers (Cancer Genome Atlas Research, N., 2011; Cancer Genome Atlas Research, N., 2014; Cancer Genome Atlas Research, N., 2008). The extent to which tumors characterized by loss of NF1 are exclusively, or predominantly, dependent on ERK signaling, depends on many other variables including the histologic context as well as other coexisting mutations.

In a large series of primary and metastatic melanoma samples studied by integrated genomic analysis, somatic mutations in *NF1* were detected in approximately 14% of samples (45 out of 318). Mutations in *NF1* were most often associated with loss-of-function (LOF) events, high mutation prevalence (39 mutations/Mb), and more often co-occurred with non-hotspot mutations in BRAF. Alterations in *NF1* commonly co-occurred with mutations in *CDKN2A*, *TP53*, and *RB1* (Cancer Genome Atlas, N., 2015). Mutations in the NF1 tumor suppressor were shown to cooperate with BRAF mutations in melanomagenesis by preventing oncogene-induced senescence, promoting melanocyte proliferation, and enhancing melanoma development (Maertens et al., 2013). Several groups have suggested that NF1 mutations in melanoma are a marker for insensitivity to clinically available RAF inhibitors, but sensitivity to small molecule inhibitors of MEK kinase (Nissan et al., 2014; Maertens et al., 2013; Whittaker et al., 2013). A similar study of whole exome sequencing (WES) yielded inactivating mutations in NF1 in 28 of 213 (13%) human melanoma samples, the highest mutation frequency observed after activating mutations in BRAF and NRAS; this study, however, did not identify loss of NF1 as a predictor of sensitivity to MEK inhibition (Krauthammer et al., 2015). In addition to loss of NF1 observed as a somatic event, melanoma has also been described as a