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Malignant Peripheral Nerve Sheath Tumors: From Epigenome to Bedside

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Abstract

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas typically developing in the context of neurofibromatosis type 1 (NF-1). With the exception of surgical resection, these tumors are resistant to all current therapies, and unresectable, recurrent, or metastatic tumors are considered incurable. Preclinical studies have identified several novel candidate molecular targets for therapeutic intervention, but, to date, targeted therapies have proven ineffective. Recent studies have identified recurrent mutations in polycomb repressive complex 2 (PRC2) core components, EED and SUZ12 in MPNST. These mutations result in global loss of the histone H3 lysine 27 trimethylation epigenetic mark, normally deposited by PRC2, and subsequent gain in acetylation at this residue. This altered chromatin state has been shown to promote MPNST malignancy; however, acetylation at this residue sensitizes MPNSTs to BRD4 and BET inhibition. Interestingly, the catalytic component of PRC2, enhancer of zeste homolog 2 (EZH2), is not mutated in MPNST, hinting that a non-canonical, PRC2-independent function of EZH2 may play a role in this cancer. This review examines the pathobiology of MPNST, the contribution of PRC2 subunits to this process, and the prospects for PRC2-related therapies for this cancer.

Introduction

Neurofibromatosis type 1 (NF-1) is an autosomal dominant cancer predisposition syndrome afflicting approximately one in 3,500 individuals worldwide (1), making it one of the most common genetic disorders. NF-1 patients exhibit a wide variety of symptoms, including skeletal malformities such as scoliosis (2) and tibial dysplasia (3), cognitive and behavioral impairments (4), and neoplasms that range from benign pigmented lesions to aggressive sarcomas known as malignant peripheral nerve sheath tumors (MPNSTs).

NF-1 arises through germline loss of function mutations in the neurofibromin 1 gene (*NF1*), first identified in 1990 (5–7). Its protein product, neurofibromin 1 (NF1), is 2,818 amino acids in length and possesses multiple functions, including Ras GTPase activating protein (GAP) activity (6,8,9), regulation of cyclic AMP levels (10–12), and microtubule binding (13). Among these roles, the Ras GAP activity is thought to be most pertinent to NF-1

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associated neoplasia. NF1 promotes the hydrolysis of Ras-bound GTP to GDP (8,9), thus transitioning Ras to its inactive state. Consequently, loss of function mutations in NF1 result in hyperactive Ras signaling, promoting aberrant cellular proliferation.

All NF-1 patients are either *NF1* heterozygous or mosaic for an *NF1* mutation, since homozygous germline mutations are embryonically lethal (14,15). It is unclear to what degree NF1 heterozygosity itself drives aspects of this disorder. However, germline mutations in the NF1 gene predispose patients to neoplasia in accordance with the Knudson two-hit hypothesis (16). In this regard, all neurofibromas that typify NF-1 result from loss of heterozygosity of *NF1*.

The most common NF-1-associated neoplasm is the café-au-lait macule. These are regions of hyperproliferative melanocytes that manifest clinically as areas of increased skin pigmentation (17). NF-1 patients also frequently develop dermal and cutaneous benign neurofibromas that arise from NF1 nullizygous progenitors termed skin derived precursor cells (18). Larger, more aggressive tumors, called plexiform neurofibromas (PNs) and MPNSTs, initially develop from *NF1* loss of heterozygosity in Schwann precursor cells (SPCs) (19). Even the more severe skeletal malformities, like tibial dysplasia and pseudarthrosis, are associated with biallelic *NF1* inactivation and aberrant osteoclast bone resorption (2,20).

Genotype-Phenotype Correlations in NF-1

While all NF1 germline loss of function mutations are fully penetrant (21,22) and result in NF-1, disease presentation is highly variable (23). Symptomatology and disease severity do not seem to correlate with any specific mutations, except in a few specific examples. The in-frame deletion c.2970–2972delAAT and missense mutations at this codon result in a relatively attenuated NF-1 phenotype (24). Patients with mutations affecting p.Arg1809 show a similar mild phenotype (25). Recently, Koczkowska *et al.* identified a set of missense mutations in *NF1* codons 844–848 that correlate with more severe NF-1 manifestations. These patients present with a higher incidence of PNs as well as other NF-1-associated malignancies (26). *NF1* microdeletion syndrome, in which the chromosomal locus 17q11.2 shows a 1.0–1.4 Mb deletion, is rare, but consistently severe clinically. These patients exhibit facial dysmorphism, scoliosis, and ADHD. They also suffer a higher risk of developing MPNSTs and other NF-1 associated neoplasms (27,28). Determining the precise genetic driver of each symptom in NF1 microdeletion syndrome is complicated by the fact that 14 protein coding genes and 4 microRNA genes are contained within the most common, 1.4 Mb deletion. Three of these genes are contained within an intron of NF1 on the antisense strand: EVI2A, EVI2B, and OMGP (7,29–31).

The difficulty in establishing genotype-phenotype correlations is in part attributable to *NF1*'s large size. The gene contains 60 exons and encodes a protein of 2,818 amino acids with multiple distinct functional domains (5,7). Several different *NF1* splice variants are found in different tissues, and some of these variants have differential localization and function (32). The impact of various *NF1* mutations on different isoforms and their respective functions is poorly understood.

Beyond its canonical RAS GAP activity, some isoforms of NF1 contain a tubulin binding domain and a nuclear localization signal. NF1 has been shown to associate with the microtubule-chromosome junction during cell division (33–36). Consistent with these observations, Koliou *et al.* showed that NF1 depletion in glioblastoma cells by siRNA disrupted proper chromosome congression (chromosomal alignment during metaphase) (33). This NF1 function may help to explain the frequent aneuploidy observed in NF-1-associated neoplasias (33,37–42). Interestingly, the tissues most affected in NF-1 are those which express the NF1 isoform that contains a nuclear localization signal, suggesting that nuclear NF1 functions may be particularly relevant for NF-1 associated tumorigenesis (32).

Some NF-1 patients develop symptoms in only one portion of the body, a condition termed segmental NF-1 (23,43,44). This subset of disease is caused by a de novo somatic *NF1* mutation occurring early in embryonic development, rather than germline mutation. The resulting mosaicism leads to a phenotype in which only cells and tissues in the affected lineage manifest NF-1 phenotypes (44). These individuals will not pass on the mutation to their offspring unless it is present in the germline. This occurs in a small minority of patients with segmental disease.

NF-1 Associated Neoplasia

Individuals with NF-1 have a 60% lifetime risk of developing cancer (45) and are 4 times more likely to develop cancer compared to the general population (46,47). Glioblastoma, paraganglioma and pheochromocytoma, breast cancer, gastrointestinal stromal tumors, and MPNSTs all develop frequently in the context of NF-1 (46,48–51). While NF-1 patients exhibit a moderate predisposition to cancer generally, their likelihood of developing malignant neoplasms of the nervous system, such as malignant glioblastoma and MPNST, are 40-fold and 1000-fold higher, respectively than that of the general population (46). Indeed, MPNSTs develop in 8–13% of NF-1 patients (52) and represent the leading cause of death in NF-1. 50% of all MPNSTs develop in the context of NF-1, and MPNSTs constitute 10% of all malignant sarcomas overall (53). NF-1 patients are also highly predisposed to develop non-malignant tumors, such as dermal neurofibromas, PNs, and atypical neurofibromas (ANFs) (46); the latter two are precursor lesions to MPNST.

Dermal neurofibromas are benign growths that can develop in a cutaneous or subcutaneous setting. Each develops from a skin derived precursor cell that has somatically lost its functional allele of *NF1* (17,18). These neurofibromas tend to be numerous and can cause itching and pain for patients. Treatment of these growths involves surgical removal or other local treatments (54). Dermal neurofibromas can also cause significant cosmetic concerns for NF-1 patients because these growths can develop on the face or on other exposed skin. Dermal neurofibromas typically develop during adolescence, exacerbating their psychological impact.

PNs, while still benign, are much larger tumors of the peripheral nerves that have the potential to cause disfigurement and can impose pressure on and disrupt the function of surrounding tissues and organs. These growths develop after loss of NF1 heterozygosity in an SPC (19) (Figure 1). Paracrine signaling within the tumor then occurs, recruiting

fibroblasts (55), mast cells (55–58), macrophages (59), and Schwann cells (SCs) (58), thus creating a heterogenous mass of cells. Proliferation of neoplastic SCs is dependent upon growth factor signaling from surrounding cells (55–59); the relative contributions of cell autonomous versus paracrine signaling factors in these tumors have yet to be fully elucidated.

The presence of non-neoplastic cells promotes neoplastic SC growth in PN through the generation of extracellular matrix components by fibroblasts (55,60) and provision of growth factors by mast cells (55). Secretion of KIT ligand combined with aberrant expression of KIT by NF1-null SCs may create an autocrine loop promoting SC proliferation (58). SC secretion of KIT ligand also recruits mast cells to the growing tumor (57). In turn, mast cells stimulate fibroblast recruitment, proliferation, and extracellular matrix production through TGF beta secretion (55). The increasingly fibrotic environment is conducive to further tumor growth (55,60). Thus, cell-cell interactions in PN create a self-perpetuating cycle fueling tumor expansion. Notably, accumulation of mast cells in peripheral nerves occurs after nerve injury, and PN formation is anecdotally associated with prior nerve injuries to the area in which PN arise (59,61–63).

Macrophages may also play a dual role in both inhibiting and promoting neurofibroma formation. Prior to neurofibroma development, macrophages suppress tumorigenesis through secretion of tumor necrosis factor. Conversely, in established neurofibromas, depletion of macrophages by dual c-KIT and FMS kinase inhibition induced tumor regression in a genetically engineered mouse model of neurofibroma formation (59). These results, however, are difficult to disentangle from other cell-cell or autocrine interactions within neurofibromas, since many of these interactions are mediated by KIT (56–59).

PN typically arise in early childhood and grow throughout adolescence. PN growth does not generally continue into adulthood, except in the context of malignant progression. Circulating steroid hormones have been implicated in this phenomenon. There have been reports of PN growth during pregnancy followed by postpartum PN regression. These observations prompted studies demonstrating that circulating progesterone and estrogen may stimulate PN growth. While there are conflicting and inconsistent results regarding the PN cell types targeted by these hormones, a unifying finding in the literature is that high doses of progesterone may stimulate neurofibroma growth, and thus caution should be taken when administering this hormone to NF-1 patients (64–67).

In spite of the aforementioned findings, the discontinuation of PN growth in adulthood is still not completely understood. Some PNs continue to grow into adulthood, developing distinct nodular sub-lesions (68), which protrude from, or are found adjacent to PN. Such lesions are histologically dissimilar to the PN (69,70). These nodular lesions display regions of hypercellularity, possess hyperchromatic nuclei relative to the associated PN (69,70) and manifest increased FDG uptake (68). Given their atypical histology, such growths are termed atypical neurofibromas. ANF is thought to represent a premalignant stage of MPNST, through loss of the tumor suppressor p16^{INK4A} that occurs in ANF but not PN (70,71). Among patients with ANFs, surgical resection is largely successful when possible, and most patients do not develop recurrent disease (69). However, a subset of patients with ANF

experience local recurrence and/or development of MPNST following ANF resection, lending further support to the hypothesis that ANFs represent precursor lesions of MPNST.

ANF and MPNST both exhibit loss of additional tumor suppressors beyond *NF1* alone. This similarity, along with their histologic likeness, can render MPNST difficult to differentiate from ANF based on histologic criteria. MPNSTs, however, require wide margins of surgical resection (72,73), readily metastasize, and exhibit a significantly higher rate of recurrence (74,75). They also impart worse prognosis, with five-year survival rates of around 45%, based on a meta-analysis of over 1,800 patients (76). In a recent study, MPNST local recurrence rates ranged from 25% to 37%, depending on tumor grade (75). Of 9 patients who were treated with amputation for MPNST, 3 developed recurrent tumors at the amputation site (75). This finding is in stark contrast to a study that showed 100% disease specific survival at 200 months after surgical resection in patients with ANF (74). Moreover, even though the majority of the tumors in this study had positive resection margin, recurrent disease was still rare (74).

MPNST is typified by the loss of additional tumor suppressor loci in an *NF1* null SC (71,77,78). Deletion of *p16^{INK4A}* is the most common cooperating mutation with *NF1* loss, occurring in about 75% of cases (71,77,79), though *TP53* mutations are also common, occurring in 40% of MPNSTs (79). Genetically engineered mouse models with mutations in *NF1* and either *TP53* (80,81) or *CDKN2A* (82,83) generate tumors resembling human MPNSTs.

Despite compelling evidence that loss of function mutations in *TP53* and *CDKN2A* contribute to MPNST tumorigenesis, the fact that these mutations occur in ANF and low grade MPNST means that defects in these genes cannot differentiate between non-malignant and malignant tumors. Recently, recurrent mutations in PRC2 components *SUZ12* and *EED* have been identified in MPNST, and loss of the PRC2 product, histone H3 lysine 27 trimethylation (H3K27me3), is associated with progression from PN to MPNST (Figure 1). These mutations occur in 55% and 30% of MPNSTs respectively, and the mutations are typically mutually exclusive ($p = 0.042$) (Figure 2). These mutation frequencies were established in a meta-analysis of next generation sequencing studies of MPNST (79,84–87). The frequency of PRC2 mutations in MPNST has established H3K27me3 as a potentially useful biomarker to diagnose MPNST, and to distinguish this tumor from ANF and PN (84). The aforementioned clinical entities are summarized in Table 1.

Loss of Polycomb Repressive Complex 2 in MPNST

Gene expression is regulated in part regulated through post-translational modifications at the lysine 27 residue of histone H3 (H3K27). Acetylation at this residue (H3K27Ac) and consequent localization of bromodomain and extra-terminal domain (BET) proteins is associated with active transcription (88–91). Conversely, trimethylation at this residue (H3K27me3) compacts chromatin and represses transcription (92,93). PRC2 and *KDM6A/KDM6B* are respectively responsible for depositing and removing H3K27me3 (92–96). *P300/CBP* and the NuRD complex are responsible, respectively, for depositing and

removing the acetyl mark (97,98). Together, these enzymes help to regulate transcription (99).

PRC2 consists of the core components embryonic ectoderm development protein (EED), enhancer of zeste homologue 2 (EZH2), suppressor of zeste 12 homologue (SUZ12), retinoblastoma binding protein 4/7 (RBBP4/7), and several other accessory components. PRC2 mutations and aberrant H3K27me3 levels are characteristic of several different cancers (100). PRC2 was initially thought to play a general oncogenic role since many tumors exhibit copy number gains and gain of function mutations in the catalytic subunit EZH2 (101–106). However, PRC2 is frequently inactivated in MPNST, and loss of H3K27me3 is considered a predictor of poor outcome and an oncogenic driver in other cancers as well (Figure 3). For example, Wei *et al.* correlated loss of H3K27me3 with poor prognosis in breast, ovarian, and pancreatic cancers (107). PRC2 mutations and loss of H3K27me3 are also observed in acute lymphoblastic leukemias and myelodysplastic syndromes (108–111). Furthermore, mutations of lysine 27 to methionine in histone H3 lead to a global decrease in H3K27me3 through PRC2 sequestration and inhibition (112), and represent a key oncogenic driver in pediatric glioblastoma (112–117). Some of these mutations occur in conjunction with *NF1* mutations (115).

In MPNST, PRC2 is inactivated through loss of function mutations in SUZ12 and EED. Together, the PRC2 core components SUZ12 or EED are mutated in about 85% of MPNSTs, and these mutations are associated with more aggressive and more frequent tumors in the case of NF1 microdeletion syndrome (27,28,79). The result of these mutations is a global H3K27me3 loss (84–86). Lack of H3K27me3 allows for aberrant deposition of acetyl groups at loci normally silenced by PRC2 (84). Genetic and epigenetic aberrations in MPNST are summarized in Table 2. As previously mentioned, H3K27Ac recruits bromodomain extra-terminal domain (BET) proteins, specifically BRD4, to chromatin, which in turn promotes RNA polymerase II-mediated transcription (91). In this regard, De Raedt *et al.* demonstrated that SUZ12 null cell lines are more sensitive to the BRD4 inhibitor JQ1 (85). Moreover, reintroduction of ectopic SUZ12 into SUZ12 mutant MPNST cell lines was sufficient to reestablish H3K27me3 levels, deplete H3K27Ac, and reduce MPNST proliferation in cell culture (84). De Raedt *et al.* attributed these effects to down-regulation of a RAS transcriptional signature observed after SUZ12 reconstitution or JQ1 treatment. This paper identified a general inverse relationship between PRC2 activity and enrichment of RAS transcriptional signatures, though the precise mechanism of this interplay remains unclear (85). Patel *et al.* observed increased BRD4 protein level levels in mouse MPNSTs compared to their benign precursors, further supporting the notion that aberrant BRD4 expression is a pathogenic driver in MPNST (118). Alternatively, increased BRD4 protein levels in MPNST could represent a by-product of increased transcriptional demand imposed on rapidly proliferating cells.

Despite the association of loss of PRC2 subunits with MPNST pathogenesis, loss of function mutations are not evenly distributed among PRC2 core components: nearly all PRC2 mutations in MPNST occur in SUZ12 and EED. By contrast, some MPNSTs exhibit overexpression of the PRC2 catalytic component, EZH2 (119). Moreover, EZH2 inhibition or depletion impair proliferation and promote apoptosis in cultured or xenografted MPNST

cells (119,120). These data suggest that EZH2 may possess oncogenic functions in MPNST outside the context of PRC2, a phenomenon observed in other cancers. In many contexts, EZH2 expression negatively correlates with H3K27me3 levels but positively correlates with cancer cell proliferation and poor disease prognosis (121–123). There are also numerous examples of specific non-canonical EZH2 targets and interactions that drive tumor development and metastasis. For example, EZH2 functions as a transcriptional activator independent of its histone methyltransferase activity in breast cancer, and it can also hyperactivate Wnt signaling through interaction with PCNA associated factor and β -catenin (124–126). Yan *et al.* showed that overexpression of both EZH2 and catalytically inactive EZH2 conferred a growth advantage to Nasal-type Natural Killer/T-cell lymphoma cells in vitro (127). Phosphorylation of EZH2 at threonine 367 directs EZH2 to the cytoplasm and drives a metastatic phenotype in breast cancer mediated by EZH2 interactions with the cytoskeleton (128,129). In prostate cancer and glioblastoma, phosphorylation of EZH2 at serine 21 by AKT causes EZH2 to methylate the androgen receptor and STAT3 to drive disease progression. In prostate cancer, this occurs independently of other PRC2 components (130,131). Although the two aforementioned roles for EZH2 are not directly related to its canonical activity in PRC2, phosphorylation of EZH2 at this residue by AKT can also reduce H3K27me3 levels by diminishing EZH2's affinity for histone H3 (132). Altogether, these data imply potential non-canonical roles for EZH2 in MPNST pathogenesis. Loss of other PRC2 core components could result in higher levels of unbound EZH2 that could promiscuously interact with other binding partners to potentiate disease progression.

To date, studies of PRC2 in MPNST have been unable to elucidate the precise mechanisms through which loss of SUZ12 and EED promotes MPNST malignancy. Two models through which PRC2 component loss contributes to MPNST pathogenesis emerge: PRC2 loss of function results in loss of the H3K27me3 mark and derepression of PRC2 target genes; and loss of SUZ12 or EED result in increased levels of unbound EZH2 that could participate in other, as-yet undefined oncogenic activities (Figure 4). These models are not mutually exclusive and could both function in MPNST. Additional work is needed to understand the precise mechanisms through which loss of SUZ12 and EED drive MPNST behavior.

Current and Future Therapies for MPNST

Currently, the only effective therapy for MPNST is complete surgical resection to achieve negative margins (72,73). MPNSTs rapidly develop resistance to chemotherapy, and there is little data to indicate that such treatments improve patient outcome when employed in combination with surgery. Ill-defined margins in MPNST are a barrier to successful surgical resection (133,134), as tumor location and/or metastatic disease frequently are. There are currently no effective treatment options for patients with recurrent or metastatic disease or inoperable tumors. These individuals are encouraged to enroll in clinical trials (73).

Efforts to target MPNST pharmacologically have been met with little success to date. Two phase II clinical trials aimed at treating MPNST via tyrosine kinase (TK) inhibition have proven unsuccessful in recent years. In one trial, epidermal growth factor receptor inhibition with erlotinib failed to induce any clinical responses (135) despite encouraging preclinical

data supporting this intervention. Another TK inhibitor, sorafenib, with activity against vascular endothelial growth factor receptor and platelet derived growth factor receptor, was tested in combination with the standard chemotherapy agent dacarbazine, also with limited success (136). A recent publication described a novel therapeutic strategy, in which YAP/TAZ signaling and PDGFR were targeted simultaneously to inhibit MPNST growth (137). The only clinical trial to date that has achieved promising results deployed doxorubicin, etoposide, and ifosfamide against chemotherapy-naive MPNSTs (138), with many patients exhibiting stable disease, and some even achieving partial responses.

Current clinical trials are largely focused on testing targeted therapies that have shown efficacy in other sarcomas. Many of these trials are evaluating mTOR inhibitors, TK inhibitors, or combination of these treatments. There is preclinical evidence suggesting that such interventions could be effective in MPNST (139–147), but such data has been poorly predictive of success against MPNST in the past. A candidate therapeutic approach that has been of great recent interest is the application of BET/BRD4 inhibitors. As previously noted, PRC2 core components are frequently mutated in MPNST, preventing PRC2-mediated deposition of the H3K27me3 repressive mark on chromatin, with a concomitant gain in acetylation at this site. Unfortunately, a phase II clinical trial involving the BET inhibitor CPI-0610 in MPNST was recently withdrawn due poor enrollment.

Encouragingly, there has been some success in targeted therapy for inoperable PN. A phase I clinical trial of the MEK inhibitor selumetinib achieved partial responses in 17/24 patients, and all patients experienced some decrease in tumor volume. Responses were maintained in 15/17 patients, with no patients exhibiting progressive disease (148). This trial is an important step forward in NF-1 treatment, since prevention of MPNST development may represent an effective means of reducing MPNST-associated mortality in NF-1. Selumetinib is currently in phase II clinical trials for PN.

Major therapeutic progress has been made in treatment of cutaneous melanoma and other cancers using immune checkpoint inhibitors. For example, interaction between programmed cell death protein 1 (PD-1) and its ligand, PD-L1, expressed by many neoplastic cells, leads to suppression of anti-tumoral T-cell immune responses. Blockade of these receptors or other immune checkpoint mediators (*e.g.* CTLA-4) can reactivate this response. Two recently published studies have attempted to characterize the MPNST immune microenvironment. One of these studies found that MPNSTs exhibit slightly increased PD-L1 expression compared to benign nerve tissue, significant CD8+ staining, and no PD-1 expression. It found no correlation between PD-L1 or CD8 staining and disease state or patient survival (149). It described the majority of tumor samples as non-inflamed, characterized by low neoantigen levels and limited response to PD pathway blockade (149,150). Haworth *et al.* report no difference in PD-L1 expression between benign and malignant NF-1 lesions (151). Both studies hypothesized that immunotherapy would only have limited utility in MPNST treatment, though inconsistencies between their findings underscores the need for further research in this area.

Considerations for Future Research

Many groups have developed genetically engineered mouse models of MPNST, which develop neoplasms that are histologically similar to human MPNST (152). Mo *et al.* (153) and Chau *et al.* (154) described a mouse model in which Nf1 null skin derived neurofibroma precursor cells identified by Le *et al.*, 2009 (18) gave rise to PNs when orthotopically implanted in a nerve. When *Nf1* and *Tp53* null skin derived precursor cells were implanted into a nerve, they produced MPNSTs. Such models provide isogenic systems to examine the transition from benign PN to MPNST. Recently, Wu *et al.* developed a genetically engineered mouse model, in which *Lats1/2* deficient mice rapidly develop tumors resembling MPNSTs (137). Additionally, Li *et al.* developed a series of immortalized SC lines from healthy individuals and from NF-1 patients. Furthermore, *NF1* null and heterozygous immortalized cell lines were established from the same individual, providing isogenic cells for study (155). The availability of immortalized SC lines will expedite the process of understanding genetic and epigenetic aberrations that result from loss of NF1, and those that occur during MPNST evolution. This system will also aid in understanding MPNST pathogenesis by providing a platform on which to perform genetic manipulations of genes frequently mutated in MPNST. Crucially, potential interventions must be considered within the context of an *NF1* heterozygous individual, since therapies that target hyperactive Ras signaling could exert deleterious effects in individuals with germline *NF1* haploinsufficiency. *NF1* heterozygous immortalized SC lines will be helpful in studying this narrowed therapeutic window (155).

Despite recent advances in understanding MPNSTs, progress is still hindered by lack of comprehensive genetic data on many MPNST cell lines and lack of robust, reliable NF1 antibodies. Additional steps should be taken toward establishing large databases of patient *NF1* genotypes and outcomes in order to gain additional, detailed insights into genotype-phenotype correlations and how these might be leveraged to allow more effective MPNST treatment. Two patient registries have been established in recent years; more information is needed to understand the variability in NF-1 presentation (156,157).

While MPNST treatment is a critical area for study, the importance of potentially preventing MPNST altogether in NF-1 patients should not be overlooked. Currently available data indicate that premalignant NF-1 associated neoplasms likely respond better to pharmacological intervention than MPNSTs, and they more amenable to surgical cure. Drugs to treat PNs, and delay or even prevent progression to MPNST, may prove to be valuable therapies for individuals with NF-1. The MEK inhibitor selumetinib has showed promise in this context (148).

The outlook for patients with MPNST still remains guarded, but new findings regarding epigenetic aberrations in these tumors have provided a foundation on which new clinical trials may be built. Future studies should also elucidate in detail how loss of PRC2 components EED and SUZ12 contribute to malignancy, especially in the context of recent studies identifying EZH2 as a potential therapeutic target.

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Implications

Identification of mutations in the PRC2 components EED and SUZ12 in the majority of MPNSTs may imply noncanonical oncogenic activities of the intact component, EZH2, and provide new opportunities for therapeutic intervention.

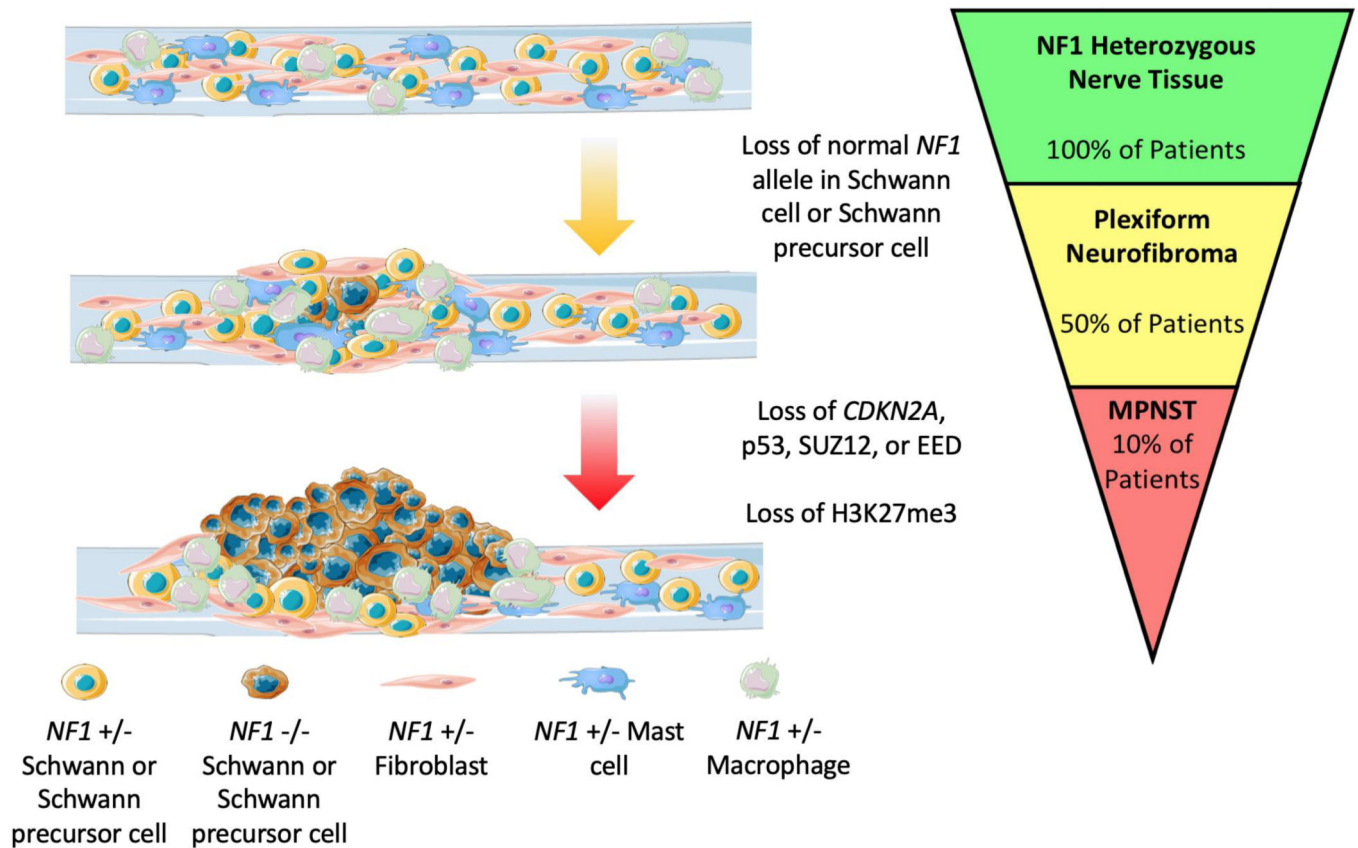


Figure 1. MPNST Pathogenesis.

Neoplastic growth in the nerves of an NF-1 patient is initiated after loss of the normal *NF1* allele in an SPC. The ensuing paracrine signaling recruits fibroblasts, macrophages, and mast cells to the growing tumor. These tumors are termed plexiform neurofibromas (PNs), due to their heterogeneous composition. They develop in 50% of NF-1 patients. Typically, PNs only grow through adolescence. Further tumor growth in adulthood is observed to the context of ANF and MPNSTs. Upon loss of additional tumor suppressors (p53, *CDKN2A*, *SUZ12*, *EED*) in an *NF1* null SC or SPC, SCs can transform into MPNST. This occurs in about 10% of NF-1 patients. These tumors metastasize readily and recur frequently after removal. High grade tumors are characterized by loss of H3K27me3. This figure was created in part from Servier Medical Art.

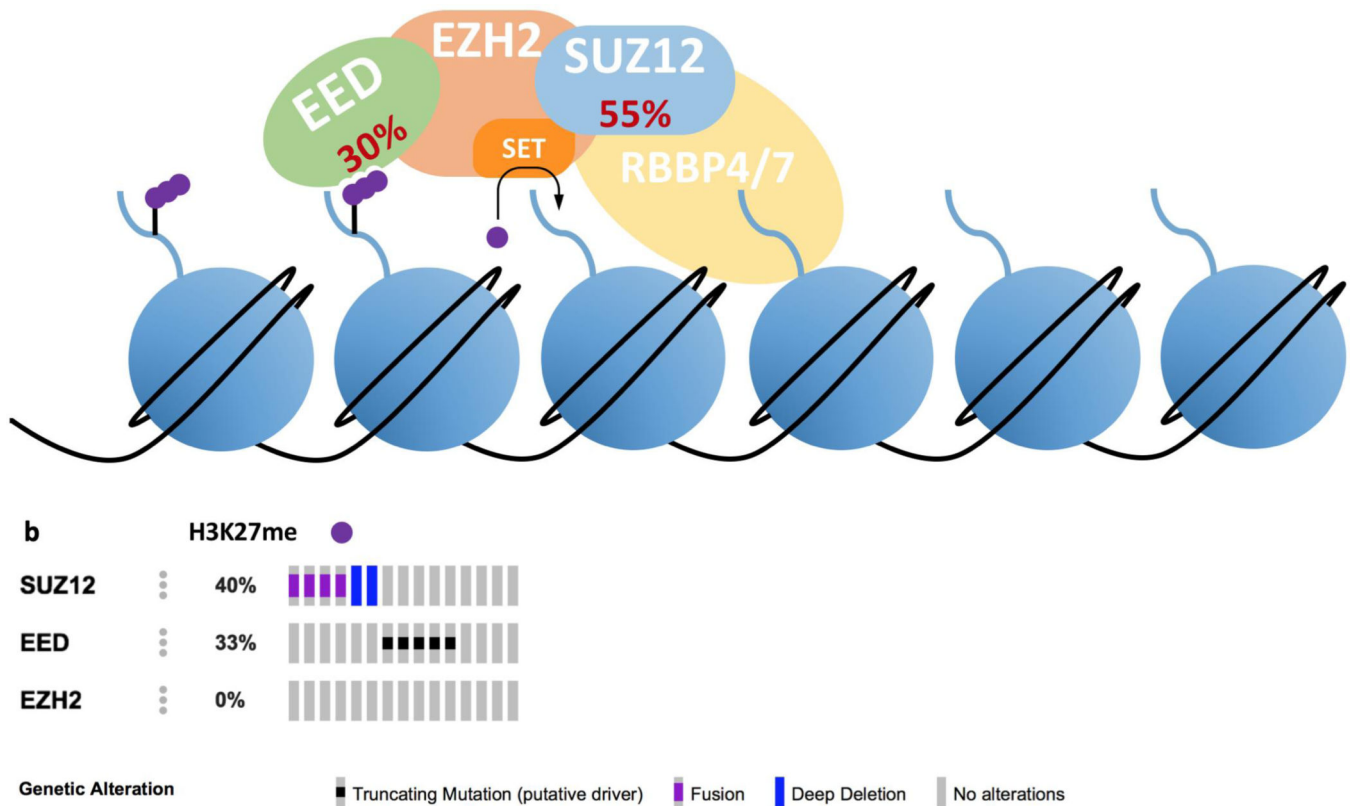


Figure 2. PRC2 Function and Mutations in MPNST.

(a) PRC2 consists of four core components: EED, EZH2, SUZ12 and RBBP4/7 (158). There are also several PRC2 accessory polypeptides, not be discussed here. PRC2 trimethylates lysine 27 of histone H3 (158). EED (embryonic ectoderm development) recognizes trimethylated histone H3 lysine 27 (H3K27me3), allosterically activating the enzymatic activity of the entire complex (159,160). EZH2 is the catalytic component of PRC2, trimethylating H3K27 via its SET domain (158). SUZ12 is necessary for the catalytic activity of PRC2 and may regulate PRC2 activity through interactions with noncoding RNAs (161,162). RBBP4/7 recognizes unmodified histone H3, while active chromatin marks like H3K4me3 and H3K36me3 allosterically inhibit PRC2 activity (163). Loss of function mutation frequencies of PRC2 components in MPNST are denoted in red. (b) Publicly available data on PRC2 mutations in MPNSTs (cbioportal.org). Mutations in SUZ12 and EED occurred in 11 of 15 samples. Mutations in these two PRC2 core components are mutually exclusive ($p = 0.042$).

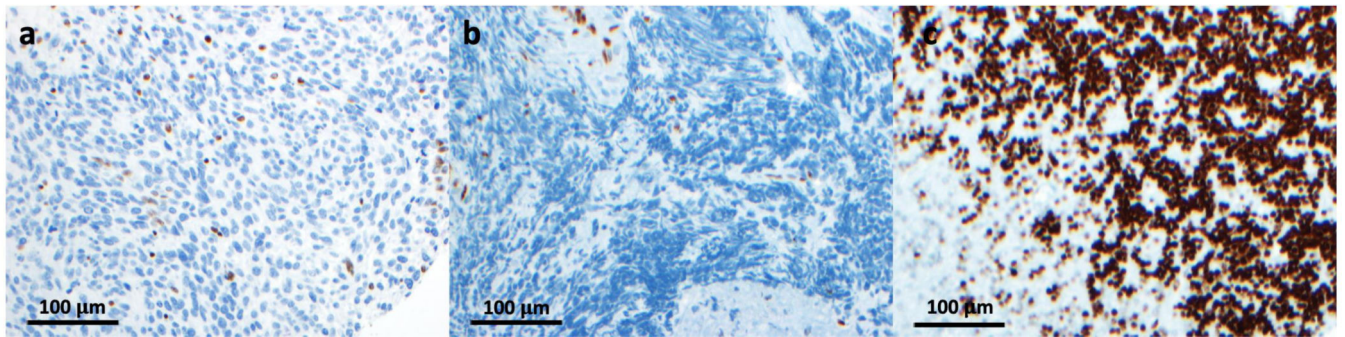


Figure 3. H3K27me3 Staining in MPNST.

MPNSTs frequently exhibit global loss of H3K27me3. **(a,b)** Immunohistochemical staining for H3K27me3 in MPNST tissue sections. Cells showing positive staining have been identified as inflammatory cells and endothelium (164). **(c)** H3K27me3 staining of granular neurons in human cerebellum, which exhibit high levels of H3K27me3, and serve as a positive control (117). Recent studies have highlighted the loss of this chromatin mark as an effective means of differentiating high grade MPNST from low grade MPNST and premalignant lesions. Images were provided by Drs. Sriram Veneti and Drew Pratt.

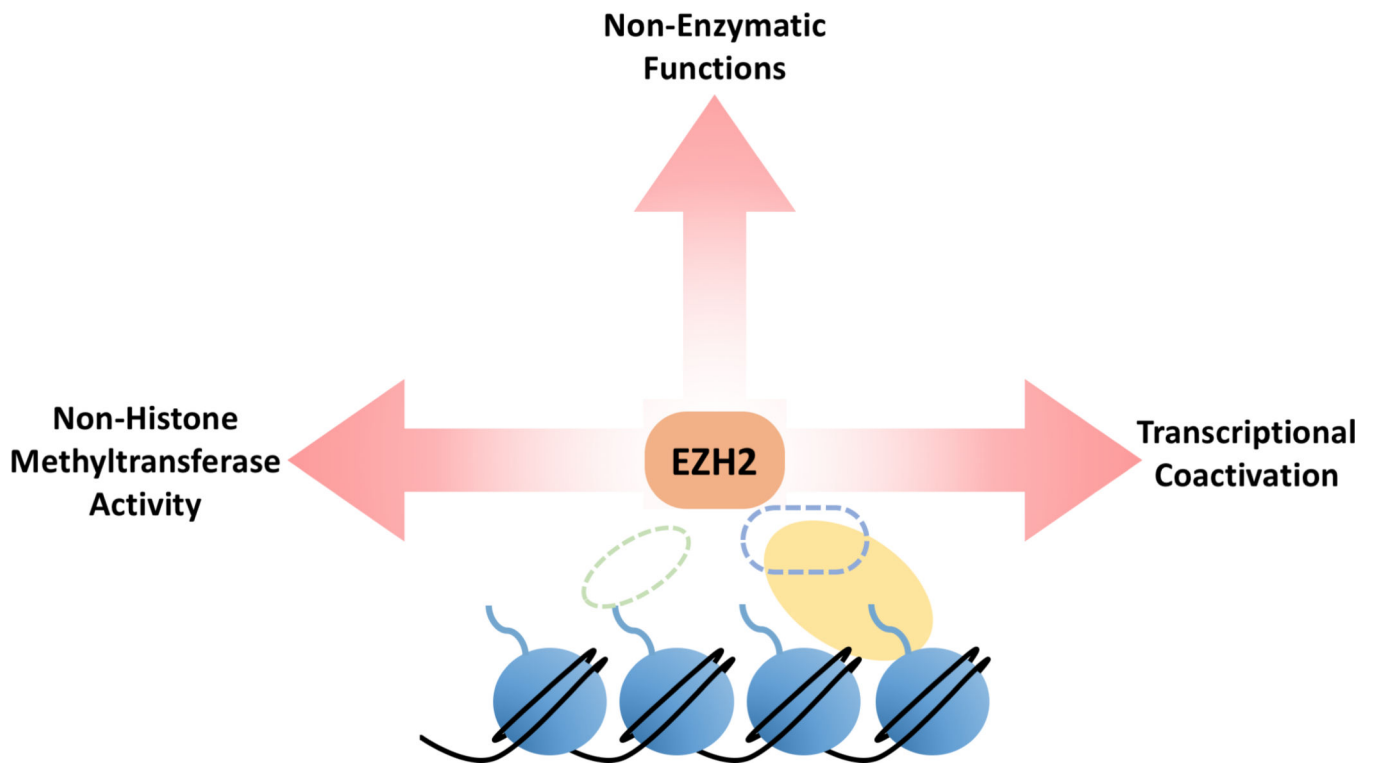


Figure 4. Known Non-Canonical Roles of EZH2.

While SUZ12 or EED are mutated in approximately 85% of MPNSTs, EZH2 loss of function has not been observed. Overexpression of EZH2 has been reported in MPNSTs. EZH2 inhibition and depletion have also proven efficacious in slowing MPNST growth in preclinical models despite PRC2 already being genetically inactivated. This suggests that EZH2 may assume pathogenic functions in MPNST in the absence of other PRC2 core components. Non-canonical, pathogenic roles for EZH2 have been demonstrated in numerous other contexts. For example, overexpression of catalytically inactive EZH2 in natural killer/T-cell lymphoma conferred a growth advantage to these cells. Phosphorylated EZH2 has been shown to contribute to glioblastoma, breast cancer, and prostate cancer tumorigenicity through divergent mechanisms. The efficacy of EZH2 inhibition and depletion in slowing MPNST growth in preclinical models, as well as the clearly established non-canonical roles of EZH2 in other contexts represent an understudied avenue for potential MPNST therapies.

Table 1:

Clinical entities discussed in this review.

Abbreviation	Term and Description
NF-1	<ul style="list-style-type: none"> • Neurofibromatosis Type 1 • Disease results from heterozygous germline loss of function mutations in <i>NF1</i>
NF1	<ul style="list-style-type: none"> • Neurofibromin 1 • 2,818 amino acid protein • Functions include RAS GAP, cyclic AMP regulation, and microtubule binding • Tumor suppressor
SPC	<ul style="list-style-type: none"> • Schwann precursor cell • Cell type in the developmental lineage of Schwann cells • Plexiform neurofibromas, atypical neurofibromas, and malignant peripheral nerve sheath tumors arise from this lineage
SC	<ul style="list-style-type: none"> • Schwann cell • Supports peripheral nerves • NF-1 associated neurofibromas can rise from this cell type
PN	<ul style="list-style-type: none"> • Plexiform neurofibroma • Large benign neurofibroma variant • Heterogenous cellular composition • Can cause pain, disfigurement, local tissue dysfunction
ANF	<ul style="list-style-type: none"> • Atypical neurofibroma • Nonmalignant tumor • Thought to develop from plexiform neurofibromas • Has mutations in tumor suppressors in addition to <i>NF1</i>
MPNST	<ul style="list-style-type: none"> • Malignant peripheral nerve sheath tumor • Malignant sarcoma with metastatic potential • Develop from nonmalignant neurofibromas in some NF-1 patients • Can arise sporadically in individuals without NF-1
NF-1 Microdeletion Syndrome	<ul style="list-style-type: none"> • NF-1 variant • Entire <i>NF1</i> gene deleted • Other genes in region are lost, including SUZ12 • More frequent and numerous plexiform neurofibromas • Higher MPNST risk

Table 2:

Selected genes and chromatin elements discussed in this review.

Abbreviation	Protein	Description
PRC2	Polycomb repressive complex 2	<ul style="list-style-type: none"> • Chromatin modifying complex responsible for laying down H3K27me3 • Contains SUZ12, EED, EZH2, and RBBP4/7 • Frequently disrupted in MPNST
SUZ12	Suppressor of zeste 12 homologue	<ul style="list-style-type: none"> • Core component of PRC2 • Frequently mutated in MPNST • Deleted in NF-1 microdeletion syndrome
EED	Embryonic ectoderm development protein	<ul style="list-style-type: none"> • Core PRC2 component • Frequently mutated in MPNST
EZH2	Enhancer of zeste homologue 2	<ul style="list-style-type: none"> • Core, catalytic PRC2 component • Mutations not observed in MPNST • Increased expression in a subset of MPNSTs
CDKN2A	Cyclin dependent kinase inhibitor 2A	<ul style="list-style-type: none"> • Gene encoding tumor suppressors p16^{INK4A} and p14^{ARF} • p14^{ARF} and p16^{INK4A} transcripts transcribed in different reading frames • Often lost or silenced in MPNST
p16^{INK4A}	Cyclin dependent kinase 4 inhibitor A	<ul style="list-style-type: none"> • A protein encoded by <i>CDKN2A</i> • Absent in approximately 75% of MPNSTs
H3K27me3	Histone H3 lysine 27 trimethylation	<ul style="list-style-type: none"> • Epigenetic mark deposited by PRC2 • Typically lost along with mutations PRC2 core components