Epigenetic mechanisms drive the progression of neurofibromas to malignant peripheral nerve sheath tumors

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Received: 18 March 16 Accepted: 01 August 16 Published: 14 November 16

Abstract

Thinking Outside the Box: The polycomb repressive complex 2 (PRC2) is a histone methyltransferase complex known to repress gene expression. There is a large body of experimental evidence that supports its role in promoting tumorigenicity by suppressing tumor suppressor genes. Here, we discuss the surprising findings that, in neurofibromas, it may have a completely different role as a tumor suppressor; mutations of PRC2 lead to conversion of benign neurofibromas into malignant peripheral nerve sheath tumors (MPNSTs) by de-repressing and thereby activating genes driving cell growth and development. These findings have potentially powerful clinical applications in both diagnosing and treating MPNSTs.

Hypothesis: PRC2 loss drives malignant transformation of neurofibromas.

Key Words: Epigenetics, MPNST, neurofibroma, PRC2

INTRODUCTION

Neurofibromas are benign tumors of the peripheral nerve sheath. Their origin is heterogeneous and includes schwann cells, neurons, fibroblasts, perineurial cells, and mast cells.[26] These tumors commonly arise in patients with neurofibromatosis type 1 (NF1). NF1 is a hereditary disorder characterized by a mutation in the gene encoding neurofibromin, a Ras GTPase activating protein (Ras GAP) common in the nervous system. Neurofibromas may also arise sporadically.

NF1 patients develop neurofibromas following a second hit mutation to the NF1 gene; this second hit is essential for neurofibroma development, showing that neurofibromin is a tumor suppressor.[10] Loss of neurofibromin allows the Ras pathway to promote cell growth and proliferation unchecked, leading to tumor growth.[13]

Once a patient has developed a neurofibroma, its progression over time is variable. Many stop growing, some continue to grow, and very few may develop into malignant peripheral nerve sheath tumors (MPNSTs).[11,26] For example, one case series on plexiform neurofibromas reported that of 44 tumors followed, 36 remained stable while only 8 grew in size.[20]

The neurofibromas that do develop into MPNSTs have poor prognoses. MPNSTs are classified as sarcomas (malignant mesenchymal tumors) and have 5-year survival rates as low as 35%.[27] An NF1 patient’s lifetime risk of developing an MPNST is 8–13%.[8]
There are several genetic factors that drive the progression of neurofibromas to malignancy. Many studies have shown that MPNSTs harbor mutations in cell cycle regulators including CDKN2A/B and p53; these mutations are rarely found in benign neurofibromas, demonstrating that loss of these regulators is a key event in the development of malignancy.\cite{2,15,19} PTEN, a tumor suppressor in the Ras pathway, is also commonly mutated in MPNSTs. In addition, MPNSTs have been reported to harbor mutations that result in overactivation of growth factor receptors and ligands, further contributing to their development.\cite{17}

Genetic factors are also implicated in the growth arrest observed in many neurofibromas. One study showed that loss of neurofibromin initially triggers the activation of Ras, however, this subsequently triggers a global negative feedback signaling program resulting in “oncogene induced senescence.” This program involves many genes (Sprouty, HDM2, FOXO) that converge on the p53 and Rb pathways to arrest the cell cycle [Figure 1]. In addition, this study looked at benign neurofibromas from patients and found that all expressed SA-B-Gal, a mark of senescence.\cite{5} Similarly, another study found that benign neurofibromas expressed Sprouty genes while MPNSTs did not.\cite{10}

**EPIGENETICS OF MALIGNANT PERIPHERAL NERVE SHEATH TUMORS**

Epigenetic mechanisms have also been shown to play a significant role in the progression of neurofibromas to MPNSTs. These revolve around the polycomb repressive complex 2 (PRC2). PRC2 is a protein complex that methylates lysine 27 on the tail of histone 3 (H3K27), resulting in transcriptional repression. PRC2 is generally regarded as an oncogene as it is up-regulated in many cancers,\cite{3,16,22} but surprisingly it has been shown to be tumor suppressive in MPNSTs.

In one study, 70% of NF-1 associated MPNSTs (and over 90% of radiotherapy-associated and sporadic MPNSTs) harbored loss-of-function mutations in PRC2.\cite{12} Similarly, another study showed PRC2 mutations in all 16 of its MPNST samples.\cite{23} In both studies, few or no neurofibromas harbored the mutation. As a result of PRC2 loss in these MPNSTs, numerous genes were de-repressed (i.e., activated), including many genes associated with growth and development.

PRC2 mutations may also further activate the Ras pathway, already amplified by the loss of neurofibromin [Figure 2]. One study performed an experiment ablating PRC2 in NF1-mutant glioblastoma multiforme (GBM) cells, and noted an enhanced Ras signature in the gene expression profile. This suggests that PRC2 loss potentiates the oncogenicity of the second hit mutation to neurofibromin.\cite{6}

PRC2 mutations are not sufficient for malignancy alone; rather they cooperate with mutations in cell cycle regulators. Indeed, the MPNSTs in the previously described studies harbored CDKN2A mutations in addition to PRC2 mutations.\cite{12} In another study, mice with NF1/P53/PRC2 mutations developed MPNSTs earlier than mice with only NF1/P53 mutations.\cite{6} These findings support the hypothesis that loss of PRC2 cooperates with loss of cell cycle regulators in MPNST development.

Another intriguing relationship between PRC2 and NF1 is that the gene for SUZ12, a component of the PRC2 complex, is 560 kb telomeric to the NF1 gene on chromosome 17q, and they are often codeleted in NF1.\cite{1,6} NF1 patients may harbor a truncating mutation (point mutation, splice mutation, small deletion, insertion, or duplication) or, more rarely, a microdeletion. It is in this latter class of patients that SUZ12 may be codeleted with NF1, and these patients have a higher lifetime risk of developing MPNSTs.\cite{7} However, even in non-microdeletion patients, 53% of MPNSTs harbor PRC2 mutations, supporting the hypothesis that PRC2 plays a critical role in MPNST development.\cite{6}

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**Figure 1:** Ras-driven oncogene induced senescence. Ras overactivation drives the Raf/MEK/ERK pathway to induce (1) Sprouty and RasGAPs, which suppress Ras, and (2) p16 & Rb, which lead to senescence. Suppression of the PI3K/AKT pathway induces p53, which also leads to senescence.

**Figure 2:** PRC2 loss and NF1 loss cooperate to increase the Ras gene signature and drive malignant growth.
While most studies in this area show that PRC2 is tumor suppressive in MPNSTs, there is one study worth noting that takes the opposite view. This study showed that EZH2, a PRC2 component, is up-regulated in MPNSTs. They propose that, in this context, EZH2 promotes a nuclear transport receptor that drives MPNST development. This suggests that individual components of PRC2 may act through different pathways to have conflicting effects on MPNST development.

There are relatively few studies on the epigenetic mechanisms driving growth arrest in neurofibromas, but PRC2 may be involved. One study found that in benign neurofibromas, JMJD3—a protein that demethylates H3K27, thus activating genes previously repressed by PRC2—was up-regulated. Consequently, the Ink4a/Arf locus was activated, inducing senescence. Accordingly, transgenic mice made to develop neurofibromas showed reduced levels of H3K27me3 at the Ink4a/Arf locus in the benign tumors.

These findings have paved the way for more detailed models of how MPNSTs may develop from neurofibromas. One model posits that, if the first or second hit to the NF1 gene is a deletion that encompasses SUZ12, then a third hit to the remaining SUZ12 gene drives MPNST development. Further studies in this direction will elucidate our model of MPNST development.

**AVENUES FOR DIAGNOSIS AND TREATMENT**

The role of PRC2 in the development of MPNSTs presents new avenues for diagnosis and treatment. One diagnostic method makes use of the mark that PRC2 leaves—tri-methylated H3K27 (H3K27me3). A recent study showed that loss of H3K27me3 could be a highly specific diagnostic test for distinguishing MPNSTs from histological mimics. Furthermore, high-grade MPNSTs showed more complete H3K27me3 loss than lower-grade tumors, hence, this test may be used to grade MPNSTs as well. Other studies have also shown H3K27me3 loss in MPNSTs.

It follows that a therapeutic avenue is the reintroduction of wild type PRC2 in MPNSTs, in order to re-methylate the appropriate genes and suppress their transcription. Indeed, this strategy was shown to partially restore the histone methylation signature of the genome and decrease cell growth.

Another potential therapy that rescues the effects of PRC2 loss involves bromodomain inhibitors such as JQ1. When PRC2 is lost and H3K27 methylation cannot be maintained, H3K27 becomes acetylated (H3K27Ac). H3K27Ac recruits bromodomain proteins, which play a role in transcriptional activation. JQ1 interferes with those bromodomain proteins, thus suppressing the genes that PRC2 normally represses. Furthermore, JQ1 was shown to cooperate with an MEK inhibitor, thus inhibiting the Ras pathway in addition to PRC2 targets to trigger tumor regression.

Lastly, the opposing study noted in the previous section was followed up with another by the same authors exploring the therapeutic implications. They showed that EZH2 inhibitors effectively down-regulated a nuclear transport protein, leading to reduced proliferation and eventual apoptosis of MPNST cells. It is possible that targeting different PRC2 components through multiple pathways may cooperatively inhibit MPNST development.

**NEXT STEPS**

Further study of the epigenetic mechanisms regulating the development and progression of neurofibromas could lead to improved diagnosis and treatment of these tumors. In addition to treating MPNSTs, it will be interesting to see if these strategies could arrest the progression of neurofibromas into MPNSTs in the first place. Many neurofibromas spontaneously stop growing, and perhaps epigenetic mechanisms drive this arrest. PRC2 seems a good place to search for answers to these questions.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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