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Review

Merlin: The wizard requires protein stability to function as a tumor suppressor

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ABSTRACT

Neurofibromatosis type 2 (NF2), characterized by tumors of the nervous system, is a result of functional loss of the *NF2* gene. The *NF2* gene encodes Merlin (moesin–ezrin–radixin-like protein), an ERM (Ezrin, Radixin, Moesin) protein family member. Merlin functions as a tumor suppressor through impacting mechanisms related to proliferation, apoptosis, survival, motility, adhesion, and invasion. Several studies have summarized the tumor intrinsic mutations in Merlin. Given the fact that tumor cells are not in isolation, but rather in an intricate, mutually sustaining synergy with their surrounding stroma, the dialog between the tumor cells and the stroma can potentially impact the molecular homeostasis and promote evolution of the malignant phenotype. This review summarizes the epigenetic modifications, transcript stability, and post-translational modifications that impact Merlin. We have reviewed the role of extrinsic factors originating from the tumor milieu that influence the availability of Merlin inside the cell. Information regarding Merlin regulation could lead to novel therapeutics by stabilizing Merlin protein in tumors that have reduced Merlin protein expression without displaying any *NF2* genetic alterations.

Contents

1. Introduction . . . 400
2. Merlin functions as a tumor suppressor . . . 401
3. Epigenetic modifications . . . 401
4. mRNA stability . . . 401
5. Post-translational regulation of Merlin . . . 402
6. Discussion . . . 403
Abbreviations . . . 404
Acknowledgements . . . 404
References . . . 404

1. Introduction

Neurofibromatosis type 2 (NF2) is an autosomal dominant disease affecting 1 in 30,000 children and young adults [1]. This disease is caused by a loss of heterozygosity of the *NF2* (neurofibromatosis-2) gene located on chromosome 22q12 [2]. *NF2* is a classical tumor suppressor gene that is frequently inactivated or its expression lost as a result of mutations in tumors of the nervous system such as schwannomas, meningiomas, and ependymomas [3–7]. While infrequent, loss of *NF2* function due to mutations has been documented in other non-nervous system cancers such as mesotheliomas [8–10], colorectal cancer [11], prostate cancer [12], melanoma and thyroid cancer [13]. The presence of mutations in non-nervous system tumors accompanied by loss of *NF2* function suggests that this tumor suppressor functions in a broad range of tissue types.

Merlin, also known as schwannomin or neurofibromin 2 was first discovered in 1993 as the protein encoded by the *NF2* gene [14,15]. Merlin is a member of the Band 4.1 family of cytoskeletal linker proteins that include the ERM (Ezrin, Radixin, Moesin) proteins [16].

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Traditionally, proteins of this family function to process signals from the extracellular matrix and transmit these signals downstream to proteins inside the cell. Loss of Merlin function lends cells of the nervous system to unchecked proliferation and motility which results in the formation of the non-malignant tumors that NF2 patients present. Loss of Merlin is embryonic lethal in mice, indicating that Merlin is a critical molecule expressed during normal embryonic development [17,18]. Moreover, heterozygous Merlin knockout mice (*Nf2*+/−) develop a series of highly metastatic tumors including hepatocellular carcinomas, fibrosarcomas, and osteosarcomas [19]. Cumulatively, this suggests that Merlin may function to inhibit tumor growth and progression in a variety of cell types. This review will focus on Merlin's tumor suppressor function with a large portion devoted to the various mechanisms by which Merlin is regulated.

2. Merlin functions as a tumor suppressor

Merlin has been shown to inhibit tumor growth and suppress malignant activity of cancer cells through multiple mechanisms. There are three main mechanisms by which Merlin acts to inhibit tumor growth: contact-dependent growth inhibition, decreased proliferation, and increased apoptosis. Merlin initially was discovered to reverse the Ras-induced phenotype and restore contact-inhibition of growth [20]. Morrison et al. showed that Merlin disrupts the Ras and Rac signaling pathways leading to contact-dependent growth inhibition [21]. Evidence suggests that Merlin can suppress Ras-induced transformation through several mechanisms including binding to RalGDS (Ral guanine nucleotide dissociation stimulator) [22], binding to and inhibiting p21-activated kinase [23,24], and inhibiting Rac/Cdc42 [25]. More recently, Yi et al. demonstrated that Merlin complexes with the tight junction-associated protein, Angiomotin, and functions to suppress cell growth by inhibiting Rac1 and Ras-MAPK signaling [26].

Several studies have revealed the ability of Merlin to negatively regulate cell growth and proliferation [27–29]. Kim et al. showed that Merlin can induce apoptosis upon over-expression, in part by causing degradation of Mdm2 leading to the increased stability and overall tumor suppressor function of p53 [30]. Others have shown that Merlin can inhibit cell cycle progression through suppression of PAK1-mediated expression of Cyclin D1 [31]. Merlin's function as a tumor suppressor by negative regulation of cell proliferation and induction of apoptosis is conserved in *Drosophila* [32]. Merlin can also reduce cell proliferation by binding to the cytoplasmic tail of the CD44 receptor. This binding inhibits the interaction of hyaluronic acid (HA) with CD44 and suppresses downstream signaling events [28,33]. More recent studies have focused on previously undefined roles for Merlin such as nuclear translocation to inhibit the E3 ubiquitin ligase CRL4 (DCAF1) resulting in decreased proliferation in schwannomas [34,35]. In addition, Merlin has been shown to sequester EGFR in mouse embryonic fibroblasts (MEFs) and halt downstream signaling [36,37] resulting in decreased cell proliferation. Based on this information, a clinical trial was developed to study the effect of erlotinib (a small molecule inhibitor of EGFR) in patients with progressive vestibular schwannoma. The study concluded that there was no tumor response using this inhibitor alone suggesting that Merlin's tumor suppressor effect is not mediated solely through EGFR [38]. James et al. showed that constitutively active mammalian target of rapamycin complex 1 (mTORC1) in Merlin-deficient meningioma cells led to increased cell growth [39]. NF2 patient tumors as well as *Nf2*-deficient MEFs displayed elevated mTORC1 signaling. Re-introduction of Merlin suppressed mTORC1 signaling. This group also found that Merlin inhibits mTOR signaling through a novel PI3-Kinase/AKT-independent mechanism which may lead to combination therapies including rapamycin or other mTOR inhibitors. Although Merlin has been shown to inhibit several signaling pathways that are important for tumor growth and progression, it is likely that future studies will reveal many more aspects of Merlin's activity. Regulation studies on Merlin have largely been focused on mutational events. However, there are other important regulatory mechanisms determining Merlin's availability in a given cell. These include epigenetic alterations, transcript stability, and post-translational modifications. There is a significant body of literature that details the various mutations associated with loss of Merlin [40,41]. This review focuses on aspects of Merlin regulation that include epigenetics, transcript stability and post-translational modifications that are influenced by the dialog between the tumor cells and their microenvironment.

3. Epigenetic modifications

Although mutations in the *NF2* gene cause tumors of the nervous system, there are likely multiple mechanisms that account for the inactivation of the Merlin protein. Promoter methylation has been shown to cause silencing of several tumor suppressor genes including *E-cadherin* [42], *p16* [43], and *VHL* [44]. Although there are likely several reasons for compromised expression of Merlin, promoter methylation is the only epigenetic modification that has been associated with changes in Merlin expression. Thus far there is no available literature on promoter deacetylation with regard to regulation of Merlin. Kino and colleagues found that nearly 60% of tumors from schwannoma patients displayed methylation of the *NF2* promoter at three different sites within a CpG island. They also noted that Merlin mRNA expression was consistent with the methylation status [45]. Another study confirmed *NF2* promoter methylation as a frequent event in schwannomas although at a much lower rate [46]. More recently, it was determined that there were a significant number of sporadic vestibular schwannoma patients that did not exhibit methylation of wild-type *NF2* (>40%) [47]. Given the discrepancy regarding *NF2* methylation in schwannomas, several studies aimed to determine the methylation status in other tumors of the nervous system such as meningiomas and ependymomas. One study confirmed that *NF2* methylation was a rare event (1 of 21) in meningioma patients [48], with another study only detecting methylation at one CpG site in 1 out of 12 tumor samples [49]. An independent study determined that *NF2* methylation occurred in less than 10% of ependymoma cases analyzed [50]. It appears that promoter methylation may be important for *NF2* regulation, but further investigations are required to confidently make this assertion.

4. mRNA stability

Transcript stability is another important aspect that controls tumor suppressor protein expression. Tumor suppressors such as p53 [51] and p21 [52] are known to be regulated at the mRNA level by other proteins and miRNAs. Evidence is inconclusive as to whether Merlin mRNA stability plays a role in tumor progression and also which mechanisms (miRNA signaling or mutational events) are important for Merlin transcript stability. Since there are no studies yet concerning regulation of Merlin mRNA by miRNAs, this section will be centered on whether mutational events are necessary for stability of Merlin mRNA. Hoang-Xuan et al. found that Merlin transcript levels are not altered in gliomas [53], while Jacoby et al. determined that different mutations resulted in varying degrees of Merlin mRNA expression in NF2 and schwannomatosis [54]. Another study in meningiomas showed that tumors from patients harboring *NF2* mutations had 10-fold lower Merlin transcript levels [55]. Deguen et al. showed that 11 of 18 human malignant mesothelioma (HMM) cell lines exhibited decreased Merlin transcript levels, 4 of which displayed no detectable mutations [56]. With the limited available literature focusing on Merlin transcript stability, it appears that a reduction in Merlin mRNA levels is associated with mutations of the *NF2* gene. Moreover, in the absence of mutations, Merlin transcript stability is unaltered. Three independent studies confirmed the absence of mutations in the *NF2* gene in breast cancer [57–59]. Kanai et al. reported