

Review

# The Role of Merlin/NF2 Loss in Meningioma Biology

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**Abstract:** Mutations in the *neurofibromin 2* (*NF2*) gene were among the first genetic alterations implicated in meningioma tumorigenesis, based on analysis of neurofibromatosis type 2 (*NF2*) patients who not only develop vestibular schwannomas but later have a high incidence of meningiomas. The *NF2* gene product, merlin, is a tumor suppressor that is thought to link the actin cytoskeleton with plasma membrane proteins and mediate contact-dependent inhibition of proliferation. However, the early recognition of the crucial role of *NF2* mutations in the pathogenesis of the majority of meningiomas has not yet translated into useful clinical insights, due to the complexity of merlin's many interacting partners and signaling pathways. Next-generation sequencing studies and increasingly sophisticated *NF2*-deletion-based *in vitro* and *in vivo* models have helped elucidate the consequences of merlin loss in meningioma pathogenesis. In this review, we seek to summarize recent findings and provide future directions toward potential therapeutics for this tumor.

**Keywords:** meningioma; merlin; neurofibromin 2; next-generation sequencing; DREAM complex

## 1. Introduction

Meningiomas are the most common type of primary intracranial tumors, currently classified by the World Health Organization (WHO) as benign (grade I), atypical (grade II), or malignant (grade III). Over the years, our understanding of the molecular underpinnings of these tumors has been greatly accelerated by advancements in next-generation sequencing (NGS) technology. It has been thought for some time that the protein encoded by the neurofibromin 2 (*NF2*) gene is a key to understand these brain tumors. The *NF2* gene resides on the long arm of chromosome 22 (chr22q) and encodes a 69 kDa protein called merlin (moesin-ezrin-radixin-like protein), which is a part of the Band 4.1 FERM gene family [1]. Merlin is a cytoskeleton scaffolding protein that links actin filaments, transmembrane receptors, and intracellular signaling molecules to regulate several essential pathways controlling proliferation and survival. These include the hippo pathway, mammalian target of rapamycin (mTOR)/PI3K/AKT pathway, and receptor tyrosine kinases (RTKs) [2,3]. In this review, we summarize the role of *NF2* loss in meningioma pathogenesis and its impact on meningioma biology based on the known functions of merlin.

## 2. Evidence Linking NF2 and Meningiomas—Inherited Disorders

### 2.1. Neurofibromatosis Type 2 (NF2)

The first indication that meningiomas may have a genetic contribution came from neurofibromatosis type 2 (NF2) [4,5]. NF2 is a rare autosomal dominant tumor syndrome with an estimated birth incidence of 1 in 33,000 [6], resulting from biallelic inactivation of *NF2*. Development of bilateral vestibular schwannomas is a pathognomonic feature present in approximately 60% of cases [7]. However, considerable heterogeneity in clinical presentation has led to the development of additional diagnostic criteria [8] (Table 1). Other lesions encountered in NF2 include non-vestibular schwannomas, meningiomas, ependymomas, and congenital cataracts.

**Table 1.** Manchester clinical diagnostic criteria for neurofibromatosis type 2 (NF2).

Diagnostic Criteria	Additional Findings Needed
Bilateral vestibular schwannomas	None.
Family history of NF2	Unilateral vestibular schwannoma, OR at least two of: meningioma, schwannoma, glioma, neurofibroma, cataract.
Unilateral vestibular schwannoma	At least two of: meningioma, schwannoma, glioma, neurofibroma, cataract.
Multiple meningiomas	Unilateral vestibular schwannoma, OR at least two of: schwannoma, glioma neurofibroma, cataract.

The *NF2* mutational spectrum in NF2 is vast; most variations do not recur. Nonsense (39%) and frameshift (27%) mutations in the *NF2* gene are most frequent in NF2 patients, with splice site (25%) and non-truncating mutations (7%) making up a smaller fraction of mutations [9]. Nonsense and frameshift mutations that truncate the protein are associated with a more severe disease phenotype, including increased frequency of multiple and recurring meningiomas [10–14]. Somatic mosaicism occurs in up to 33% of NF2 patients, which results in a milder phenotype and lower risk of transmission to offspring [15–17]. Mutations may only be detectable in the tumor tissue, emphasizing the importance of analyzing surgical specimens.

Intracranial meningiomas affect about half of NF2 patients [8,10,18,19], and spinal meningiomas are seen in approximately 20% of the patients [20]. Over half of these patients have multiple meningiomas that exhibit heterogeneous behavior and an asynchronous growth pattern [21]. While the majority of syndromic meningiomas remain stable in size or grow minimally over time, few tumors, including tumors that appear *de novo*, grow more rapidly and therefore are more frequently resected [22]. Furthermore, several distinct histological subtypes were seen in patients who underwent resection of multiple meningiomas, suggesting that *NF2* inactivation is an early tumorigenic event that occurs prior to commitment to a specific histopathologic subtype. Subsequent studies in sporadic meningiomas demonstrated that up to 60% of these cases exhibit inactivation of *NF2* by somatic mutation, epigenetic inactivation, or allelic loss of chr22q [23–26]. These findings suggest that *NF2* loss is a critical event in the development of a subpopulation of meningiomas.

### 2.2. Schwannomatosis

Schwannomatosis is characterized by the development of multiple schwannomas in the absence of other NF2-defining lesions such as bilateral vestibular schwannomas or ependymomas [27]. It is a rarer disorder than NF2, with an estimated incidence of 1/40,000 to 1/70,000 [28]. Germline mutations in SWItch/Sucose Non-Fermentable (SWI/SNF)-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (*SMARCB1*) or leucine-zipper-like transcriptional regulator 1 (*LZTR1*) predispose to the disorder [29,30]. Both genes are located on chr22q in proximity to *NF2*. In the presence of *SMARCB1* or *LZTR1* mutations, there is also biallelic *NF2* loss of function, via acquired somatic mutations and loss of heterozygosity (LOH) [31]. Multiple meningiomas occur in 5%

of schwannomatosis cases [32], but only in association with *SMARCB1* mutations [33–35], hinting at a potential interaction between *NF2* and *SMARCB1* in meningioma pathogenesis, later validated in NGS studies.

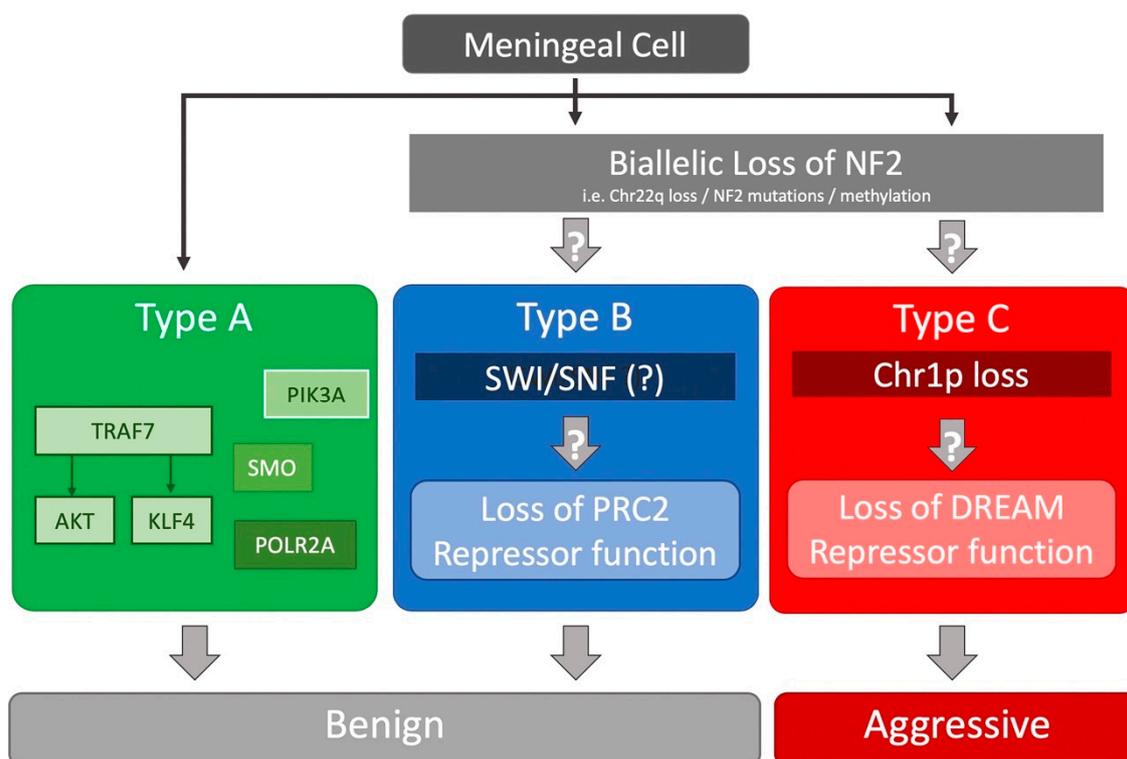
### 3. Insights from NGS Studies

Over the past decade, several groups have leveraged the wide availability and reduced cost of NGS to characterize meningiomas, providing additional insights into the mutation landscape of meningiomas from early genetic studies (Table 2).

**Table 2.** Summary of next-generation sequencing (NGS) studies.

Study	Tumor Type (n)	Genetic Alterations	Key Findings
Clark <i>et al.</i> (2013) [36]	WHO I/II (243/57)	<i>NF2</i> /chr22q loss <i>TRAF7/KLF4</i> <i>TRAF7/AKT1</i> <i>SMO</i>	Mutually exclusive non- <i>NF2</i> driver mutations. <i>NF2</i> tumors are more aggressive. Non- <i>NF2</i> tumors are benign and localize to medial skull base.
Brastianos <i>et al.</i> (2013) [37]	WHO I/II/III (47/15/3)	<i>NF2</i> /chr22q loss <i>SMO</i> <i>AKT1</i>	As above.
Reuss <i>et al.</i> (2013) [38]	Secretory (30)	<i>TRAF7/KLF4</i>	All secretory meningiomas carried the <i>KLF4</i> K409Q mutation.
Clark <i>et al.</i> (2016) [39]	WHO I/II/III/? (552/214/7/2)	<i>POLR2A</i> <i>SMARCB1</i>	Identification of <i>POLR2A</i> driver mutation. <i>SMARCB1</i> and <i>NF2</i> mutations co-occur. <i>NF2</i> gene rearrangements common in radiation-induced tumors.
Agnihotri <i>et al.</i> (2017) [40]	Radiation-induced (31)	<i>NF2</i> /chr22q loss	Non- <i>NF2</i> driver mutations not observed. <i>NF2</i> /chr22q loss and genomic disruptions occur early in progression and remain consistent over time.
Bi <i>et al.</i> (2017) [41]	WHO I/II/III (75/113/21)	<i>NF2</i> /chr22q loss Genomic instability	<i>NF2</i> is the sole driver mutation in atypical meningiomas and occurs in conjunction with genomic instability or <i>SMARCB1</i> mutations.
Harmanci <i>et al.</i> (2017) [42]	WHO I/II/III/? (548/211/7/9)	<i>NF2</i> /genomic instability <i>NF2/SMARCB1</i>	Transcriptional signatures identified a sole subgroup with recurring tumors, characterized by DREAM target genes activation.
Patel <i>et al.</i> (2019) [43]	WHO I/II/III (119/33/5)	Loss of PRC2 or DREAM complex repression	

Several whole-exome sequencing (WES) studies identified recurrent somatic mutations in *NF2*, TNF receptor-associated factor 7 (*TRAF7*), Kruppel-like factor 4 (*KLF4*), v-akt murine thymoma viral oncogene homolog 1 (*AKT1*), smoothened, frizzled family receptor (*SMO*), and RNA polymerase II subunit A (*POLR2A*) in benign (WHO grade I) tumors [36,37,39]. Interestingly, a significant proportion (6.4%) of *NF2*-mutated tumors harbored recurrent mutations in *SMARCB1* [39]. While *KLF4* or *AKT1* mutations almost always co-occurred with *TRAF7* mutations, they did not occur together; *SMO*-mutated meningiomas all harbored the activating L412F mutation [36]. All secretory meningiomas carried *TRAF7/KLF4* mutations [36,38]. One study of 18 radiation-induced meningiomas found that nearly all had chr22q LOH, with a majority also having *NF2* loss via fusion events [40]. All of these studies have found that chr22q/*NF2* loss does not co-occur with *TRAF7*, *AKT1*, *KLF4*, *SMO*, or *POLR2A* mutations, which were all found in benign meningiomas (Figure 1) [36–40].



**Figure 1.** Summary of the mutational landscape of meningiomas based on several next-generation sequencing studies.

On the other hand, a different mutational landscape is found in high-grade (WHO grade II and III) meningiomas, which demonstrate higher recurrence and shorter survival compared to benign meningiomas [26,44–46]. Two recent NGS studies of high-grade meningiomas found more chromosomal abnormalities compared to benign meningiomas, similar to other aggressive cancers [41,42]. Most high-grade meningiomas were characterized by *NF2* loss, without any other significantly recurring somatic mutations, in contrast to benign meningiomas [41,42].

Bi *et al.* examined 36 paired samples of meningiomas undergoing malignant progression and found *NF2* loss in 73% of the cases in both low- and high-grade samples from the same patient, emphasizing that *NF2* loss is an early event in meningioma progression. Losses of chromosome arms 1p, 6q, 14q, 3p, 10q, 18q, and 19q were additionally seen in these tumors. Interestingly, recurrent tumors from the same patient demonstrated 75% overlap of arm-level somatic copy number variations (CNV), suggesting that these chromosomal losses are again an early and essential feature in high-grade meningiomas.

Harmanci *et al.* found that the majority of atypical meningiomas had *NF2* loss and fell into two categories: those with significant chromosomal losses (“CNV-high”) and those with *SMARCB1* mutations (“CNV-low”). However, they did not find any differences in the transcriptional profiles of these two groups, finding all of these tumors significantly enriched for cell cycle processes, including upregulation of E2F and FOXM1 networks. Additionally, they found *NF2* mutant tumors demonstrated increased methylation of polycomb repressive complex 2 (PRC2) target genes, driven by the upregulation of EZH2, the catalytic subunit. Vasudevan *et al.* sought targetable pathways in high-grade meningiomas and found that elevated *FOXM1* expression is associated with poor clinical outcomes [47].

Recently, we undertook a large-scale RNA-seq based study of 160 tumors of all grades to further refine our understanding of meningioma biology [43] (Figure 1). Using only transcriptional profiling, we found three types of meningioma. Subsequent analysis of their genomic landscape revealed one type (type A) was composed of only benign meningiomas with mutations in *TRAF7*,

*AKT1*, *KLF4*, *SMO*. The other two types had chr22q/*NF2* loss (type B and C) but no other known mutations. Interestingly, these two types had very different molecular and clinical characteristics, arguing that merlin inactivation sets the stage for additional tumorigenic events that have dramatically different consequences. While type A and B tumors did not recur after complete resection, type C tumors (a mixture of WHO grade I, II, and III tumors) behaved “aggressively”, recurring frequently despite complete resection. An important outcome of this was that the RNA-seq based classification system could identify WHO grade I meningiomas that behaved aggressively despite their benign histopathologic features.

Type B (a mixture of grade I and II tumors) meningiomas were characterized by chr22q/*NF2* loss and co-occurrence of *SMARCB1* mutations [43]. The atypical meningiomas that Harmanci et al. referred to as “CNV-low” likely represent type B tumors [42]. Gene set enrichment analysis (GSEA) suggests that type B tumors are characterized by loss or dysfunction of the repressive PRC2 complex, which is responsible for H3K27 di- and trimethylation and subsequent chromatin silencing. In type B tumors, co-immunoprecipitation of EZH1, a core subunit of the PRC2 complex, did not pull down the other critical PRC2 subunits such as EED and SUZ12, suggesting a failed assembly of the PRC2 complex. In addition, a portion of type B tumors harbored *SMARCB1* mutations. *SMARCB1*, whose gene is also encoded on chr22q, is a critical component of the SWI/SNF complex. The SWI/SNF and PRC2 chromatin remodeling complexes have a close and complex interconnectivity in regulating the chromatin state [48,49]. How these complexes are dysfunctional in type B meningiomas remains unknown, which highlights the need for further studies to understand the complex biology underlying these tumors. It will be interesting to understand how the di- and trimethylation profiles in the three types are altered.

On the other hand, type C meningiomas, also with *NF2* loss, had a significant burden of chromosomal gains/losses, most commonly loss of chr22q and chr1p together, as well as significantly shorter recurrence-free survival, despite over half of these tumors being WHO grade I [43]. The “CNV-high” atypical meningiomas reported by Harmanci *et al.* likely correspond to these tumors [42]. GSEA showed that the target genes of the DREAM complex, including *FOXM1* and *MYBL2*, were significantly enriched in type C tumors, when compared to the other two types. The DREAM complex is a highly conserved master regulator of the cell cycle which can alternate between a repressive form that inhibits cell cycle gene expression and an activated form that promotes the progression of the cell cycle, on the basis of the interaction of the MuvB core with RB-like proteins (e.g., E2F2) or FOXM1 with MYBL2, respectively [50]. These findings provide further clarity to the observation that increase in the cell cycle-focused E2F2 transcriptional network and elevated expression of FOXM1 are associated with high-grade meningiomas [42,47]. Interestingly, while *RBBP4*, one of the MuvB core complex members, is located on chr1p, its expression was not significantly changed in type C tumors. How chr1p loss might lead to the switch from the repressor form of the DREAM complex to the activator form remains to be discovered.

Taken together with previous work, these results suggest that chr22q/*NF2* loss is a requisite for the development of aggressive meningiomas which also harbor chr1p losses. *NF2* mutation and chromosomal losses may be two distinct processes that work in parallel, but they are early events in tumorigenesis. This also explains the findings highlighted by Dewan *et al.* in which two tumors within a single *NF2* patient can have dramatically different clinical courses [51].

## 4. Merlin Signaling

### 4.1. Molecular Conformation

Early insights into merlin function were gleaned from its sequence homology to the FERM family of proteins. Canonical ERM proteins are comprised of a N-terminal FERM domain, followed by an alpha-helix domain and a C-terminal domain. Typically, ERM proteins are maintained in a dormant state by an intramolecular association between N-terminal FERM and C-terminal domains.

Upon phosphorylation of a conserved threonine residue at the C-terminal domain by Rho kinase, ERM proteins undergo a conformation change, unmasking sites at the C-terminal domain for the binding of F-actin and other membrane proteins, thereby rendering it active [52,53].

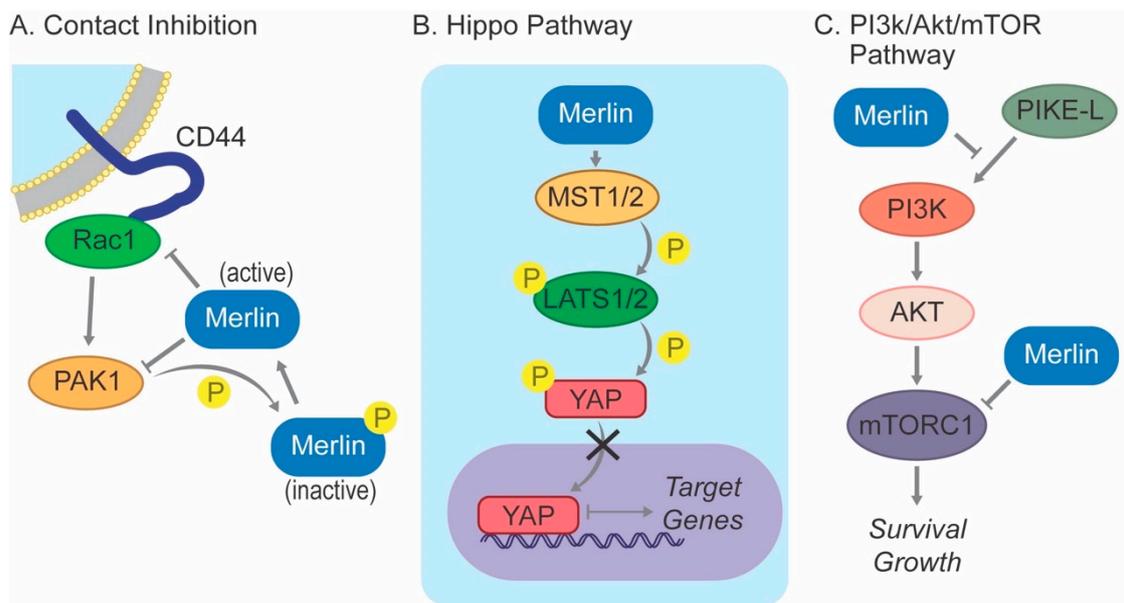
While bearing some similarity to other ERM proteins, the process by which merlin undergoes conformational change and the relative importance of its open and closed state to its scaffolding and tumor suppressor function remain a source of controversy. Traditionally, phosphorylation of merlin at Serine 518 by p21 activated kinase 1 (PAK1) or protein kinase A (PKA) versus dephosphorylation mediated by MYPT1–PP1 was believed to mediate the transition between its open and closed conformations [54–56]. Initial studies demonstrated that the tumor suppressor activity of merlin was dependent upon the non-phosphorylated, closed conformation of merlin. However, detailed structural analyses of merlin using fluorescence energy transfer analysis showed that the hyperphosphorylation of Serine 518 or the expression of phosphomimetic and non-phosphorylatable S518D and S518A mutations had only subtle effects on the conformation of merlin [57]. Moreover, studies based on small-angle neutron scattering and immunoprecipitation showed that the phosphorylation of Serine 518 stabilized the closed form rather than promoting an open conformation as initially thought, and that the phosphorylated form of merlin was nonetheless able to interact with its target proteins [58]. Two recent studies reported the interaction between merlin and phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), which promotes its open conformation and anti-proliferative activity, whose effects may be distinct from those mediated by phosphorylation at Serine 518 [57,59]. Clearly, the mechanism of the conformational change of merlin is different from that of typical FERM proteins. Merlin's activity might depend on other factors and, hence, remains an interesting and active area of investigation.

#### 4.2. Contact Inhibition

Merlin functions as a tumor suppressor in a wide range of cancers. However, relatively few studies have investigated its molecular mechanisms specific to meningioma pathogenesis. Consequently, the understanding of merlin signaling in meningiomas needs to be supplemented by insights from studies in other cancers and cell types.

Early studies suggested that merlin's tumor suppressor activity is related to its contact inhibition of proliferation. In various cell types, merlin is upregulated and hypophosphorylated with an increasing degree of cell confluency [54]. Dephosphorylated, active merlin preferentially interacts with and inhibits CD44, a cell-to-cell adhesion molecule and receptor for hyaluronan, an abundant extracellular matrix (ECM) component [60]. Treatment of sub-confluent schwannoma cells with hyaluronan rapidly induced dephosphorylation of merlin and inhibited cell growth, which was abolished by mutation in the hyaluronan binding domain of CD44. CD44 also constitutively associates with various RTKs that mediate growth factor signaling. Consistent with this notion, siRNA-mediated knockdown of merlin in schwannoma cells increased the levels of ErbB2/ErbB3 RTK, suggesting that merlin normally functions to reduce the availability of RTKs at the plasma membrane [61].

In addition, merlin-mediated contact inhibition is critically regulated by a reciprocal interaction with Ras-related C3 botulinum toxin substrate 1 (RAC1) and its downstream kinase PAK1 (Figure 2). In confluent cells, activation of PAK1 by RAC1 is sufficient to release the cells from contact inhibition [55]. However, active (dephosphorylated) merlin suppresses the recruitment of RAC1 to the plasma membrane, preventing the activation of RAC1 and PAK1. On the other hand, PAK1 phosphorylates merlin at Serine 518, and its subsequent inactivation prevents the translocation to the plasma membrane, mitigating the inhibitory effect of merlin on RAC1. Merlin-deficient meningioma cell lines demonstrate increased expression of PAK1 compared to normal arachnoid cap cells, and knocking down PAK1 expression using doxycycline-inducible shRNA or treatment with PAK1 inhibitors inhibited cell proliferation in vitro and tumor growth in xenograft models [62].



**Figure 2.** Merlin signaling. (A) Active, de-phosphorylated Merkin inhibits Rac1 and PAK1, key mediators of contact inhibition; (B) Merkin activates the Hippo pathway, leading to YAP phosphorylation and its sequestration from the nucleus; (C) Merkin is a negative regulator of PIKE-L and mTORC1.

#### 4.3. Hippo Pathway

The regulation of the Hippo pathway by merlin is better characterized in meningiomas. This evolutionarily conserved signaling pathway, first identified in *Drosophila melanogaster*, inhibits cell proliferation and promotes apoptosis to limit organ size during normal development and suppress tumorigenesis [63]. It relies upon a kinase cascade including macrophage-stimulating 1/2 (MST1/2), salvador family WW domain-containing protein 1 (SAV1, also called WW45), and large tumor suppressor 1/2 (LATS1/2) to phosphorylate yes-associated protein (YAP), leading to the sequestration of this key transcriptional coactivator from the nucleus, thereby inhibiting the transcription of target genes associated with proliferation and survival (Figure 2).

The suppression of merlin using NF2 siRNA in established meningioma cell lines inhibited contact-dependent inhibition of growth and promoted cell cycle progression in association with increased levels and elevated nuclear co-localization of YAP [64]. In addition, nuclear YAP immunoreactivity was revealed in 92% of merlin-negative tumors, further suggesting that merlin is a negative regulator of the Hippo pathway in meningiomas. A separate study also confirmed a complementary pattern of merlin expression and nuclear YAP expression, although, unexpectedly, nuclear YAP expression was found even in merlin-positive tumors [65]. Furthermore, nuclear YAP expression was increased when meningioma cell lines were plated at sparse cell density and in less rigid extracellular matrix, suggesting that inhibition of YAP-mediated Hippo signaling pathway by merlin is dependent on cell-to-cell contact and upstream adhesion molecules.

#### 4.4. PI3K/AKT/mTOR Pathway

Phosphoinositide 3-kinase (PI3K)/AKT signaling is involved in the regulation of cell growth and proliferation [66]. Growth factor stimulation triggers the production of phosphatidylinositol (3,4,5) triphosphate (PIP3) by PI3K, leading to the phosphorylation and activation of downstream AKT at the plasma membrane and subsequent activation of mammalian target of rapamycin complex (mTORC), resulting in the translation of target proteins. It has been shown that merlin inhibits the activation of PI3K by binding phosphatidylinositol 3-kinase enhancer-L (PIKE-L) [67]. The finding that a subset of meningiomas have activating E17K mutations in AKT supports a crucial role of

this pathway in meningioma biology [36,37,39,43]. High-grade meningiomas showed higher levels of phosphorylated AKT compared to benign tumors, further supporting a role for this pathway in merlin-driven meningioma pathogenesis [68]. Conversely, inhibition of AKT phosphorylation decreased meningioma growth in several in vitro studies [69,70].

Merlin has also been identified as a negative regulator of mTORC1 (Figure 2). This association was initially suspected from the observation that cultured primary human merlin-deficient meningioma cells exhibited a strikingly enlarged morphology compared to non-neoplastic arachnoid cap cells from the same patient [71], bearing similarity to tuberous sclerosis, wherein mutations in TSC1 and TSC2 lead to aberrant activation of mTORC1 [72]. Additional studies demonstrated that merlin-deficient primary meningioma cell lines and tumors exhibit constitutive activation of mTORC1, and conversely, exogenous expression of wild-type, but not mutant, merlin inhibited mTORC1 signaling [73]. Interestingly, merlin-deficient mTORC1 activation was independent of upstream PI3K/Akt and ERK signaling, which traditionally activate this signaling in response to various mitogenic stimuli. Therefore, the non-canonical mechanism by which the loss of merlin induces mTORC1 signaling is unknown.

mTORC1 inhibition is a validated therapeutic strategy in various types of cancers, and several orally bioavailable mTORC1 inhibitors are currently FDA-approved, including temsirolimus and everolimus. Despite the incomplete understanding of the interaction between merlin and mTORC1, mTORC1 inhibitors have been tested in various in vitro and in vivo meningioma models and patients. Temsirolimus and everolimus treatment significantly decreased viability and proliferation of a meningioma cell line in a concentration-dependent manner, and temsirolimus significantly reduced tumor burden in xenograft models [74]. Interestingly, shRNA-mediated downregulation of merlin rendered the meningioma cells more resistant to mTORC1 inhibition, presumably due to merlin deficiency-mediated constitutive upregulation of mTORC1 activity. In a small prospective phase 2 trial of 17 patients with progressive or refractory symptomatic meningiomas, concurrent treatment with bevacizumab and everolimus demonstrated overall median progression-free survival of 22 months [75]. An additional trial with the mTORC1/2 inhibitor AZD2014 is currently ongoing for patients with neurofibromatosis type 2-associated meningiomas (NCT 02831257) and recurrent high-grade meningiomas (NCT 03071874).

## 5. Animal Models

### 5.1. Xenograft Models

Xenograft models rely upon the implantation of human meningioma cells into immunocompromised mice. Their usefulness as a tool to investigate the role of merlin in meningioma pathogenesis is limited by several factors. Primary cells isolated from surgical samples, especially from benign tumors, do not reliably generate tumors, and the methods for their intracranial delivery have not been standardized [76]. In our experience, intracranial implantation of high-grade meningiomas can lead to xenograft formation; however, these do not serially transplant. Thus, large amounts of the original tumor are necessary to continue to develop numerous xenografts from a single tumor.

Most investigators have therefore relied upon established cell lines for xenograft experiments, such as the well-characterized BenMen1 line derived from a WHO grade I meningothelial meningioma which recapitulates key histologic and genetic features of the parent tumor, including *NF2* mutation [60,61]. However, this cell line has been retrovirally transduced with the human telomerase reverse transcriptase (*hTERT*) gene, in order to bypass senescence, with unclear alterations in underlying tumor biology [77]. The less well characterized HBL-52 cell line was derived from a transitional grade I optic canal meningioma, but this cell line harbors the *TRAF7* driver mutation [78–80]. Other cell lines are derived from high-grade meningiomas such as the IOMM-Lee and CH-157MN cell lines, and these cell lines demonstrate the genomic instability seen in more aggressive parent tumors [61,64,65]. However, the IOMM-Lee cell line has intact *NF2*, rendering it unsuitable for studies of merlin function [79]. Overall, these cell lines have no comparable controls, and their predetermined or laboratory-altered

genetics are unlikely to account for the full complexity of their real-life counterparts. Finally, due to the immunocompromised nature of the host, potentially important immune interactions cannot be analyzed.

### 5.2. Genetically Engineered Mouse Models (GEMM)

Homozygous deletion of *Nf2* in mice is embryonically lethal, and heterozygous *Nf2* knockout mice develop osteosarcomas but not meningiomas [81]. Therefore, initial efforts to create a *Nf2* deletion-based GEMM of meningiomas relied upon a conditional knockout approach. Given the lack of known arachnoid-specific promoters at the time, an adenovirus encoding recombinant Cre was injected into the subdural space of mice harboring two copies of floxed *Nf2* allele (*Nf2*<sup>lox/lox</sup>), driving arachnoid-specific deletion of *Nf2*. Remarkably, this was sufficient to induce a range of benign meningioma encompassing the transitional, meningothelial, and fibroblastic subtypes, although only a minority of injected mice ultimately developed meningiomas [82]. Additional studies demonstrated that the arachnoid-specific deletion of cyclin-dependent kinase inhibitor (*Cdkn2ab*), frequently deleted in high-grade meningiomas, increased meningioma frequency and the development of grade II and III meningiomas in mice [83]. Shortly after these initial studies, prostaglandin D2 synthase (PTGDS) was identified as a specific marker for meningioma precursor cells. By crossing transgenic mice expressing Cre under the *PTGDS* promoter to *Nf2*<sup>lox/lox</sup> mice, biallelic *Nf2* inactivation in meningioma precursor cells was achieved, without the need for exogenous Cre delivery [84]. This resulted in the generation of meningothelial and fibroblastic meningiomas in the majority of animals. Taken together, these results provide proof for a fundamental role of merlin in meningioma induction and provide ideal models for further investigation into merlin signaling, especially incorporating insights from recent genetic studies based on next-generation sequencing.

## 6. Conclusions

Merlin/*NF2* loss is a key driver in the development of both syndromic and most sporadic meningiomas. Next-generation sequencing studies have provided a framework for an increasingly sophisticated categorization of meningiomas into two groups: non-*NF2* mutants and *NF2* mutants. It is intriguing, however, that the two types of *NF2*-inactivated meningiomas (type B and C) seem to have different underlying molecular mechanisms and dramatically different clinical outcomes. How the loss of merlin function leads to two very different biological dysregulations is yet to be investigated. These insights need to be further investigated through large-scale NGS studies and, more importantly, biochemical and molecular studies to reveal therapeutically relevant targets.

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## References

1. Asthagiri, A.R.; Parry, D.M.; Butman, J.A.; Kim, H.J.; Tsilou, E.T.; Zhuang, Z.; Lonsler, R.R. Neurofibromatosis type 2. *Lancet Lond. Engl.* **2009**, *373*, 1974–1986. [[CrossRef](#)]
2. Stamenkovic, I.; Yu, Q. Merlin, a “magic” linker between extracellular cues and intracellular signaling pathways that regulate cell motility, proliferation, and survival. *Curr. Protein Pept. Sci.* **2010**, *11*, 471–484. [[CrossRef](#)] [[PubMed](#)]
3. Petrilli, A.M.; Fernández-Valle, C. Role of Merlin/*NF2* inactivation in tumor biology. *Oncogene* **2016**, *35*, 537–548. [[CrossRef](#)] [[PubMed](#)]

4. Trofatter, J.A.; MacCollin, M.M.; Rutter, J.L.; Murrell, J.R.; Duyao, M.P.; Parry, D.M.; Eldridge, R.; Kley, N.; Menon, A.G.; Pulaski, K. A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. *Cell* **1993**, *72*, 791–800. [[CrossRef](#)]
5. Rouleau, G.A.; Merel, P.; Lutchman, M.; Sanson, M.; Zucman, J.; Marineau, C.; Hoang-Xuan, K.; Demczuk, S.; Desmaze, C.; Plougastel, B. Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. *Nature* **1993**, *363*, 515–521. [[CrossRef](#)]
6. Evans, D.G.; Howard, E.; Giblin, C.; Clancy, T.; Spencer, H.; Huson, S.M.; Laloo, F. Birth incidence and prevalence of tumor-prone syndromes: Estimates from a UK family genetic register service. *Am. J. Med. Genet. A* **2010**, *152A*, 327–332. [[CrossRef](#)]
7. Baser, M.E.; Friedman, J.M.; Wallace, A.J.; Ramsden, R.T.; Joe, H.; Evans, D.G.R. Evaluation of clinical diagnostic criteria for neurofibromatosis 2. *Neurology* **2002**, *59*, 1759–1765. [[CrossRef](#)]
8. Evans, D.G.; Huson, S.M.; Donnai, D.; Neary, W.; Blair, V.; Newton, V.; Harris, R. A clinical study of type 2 neurofibromatosis. *Q. J. Med.* **1992**, *84*, 603–618.
9. Ahronowitz, I.; Xin, W.; Kiely, R.; Sims, K.; MacCollin, M.; Nunes, F.P. Mutational spectrum of the *NF2* gene: A meta-analysis of 12 years of research and diagnostic laboratory findings. *Hum. Mutat.* **2007**, *28*, 1–12. [[CrossRef](#)]
10. Parry, D.M.; Eldridge, R.; Kaiser-Kupfer, M.I.; Bouzas, E.A.; Pikus, A.; Patronas, N. Neurofibromatosis 2 (NF2): Clinical characteristics of 63 affected individuals and clinical evidence for heterogeneity. *Am. J. Med. Genet.* **1994**, *52*, 450–461. [[CrossRef](#)]
11. Kluwe, L.; Mautner, V.F. A missense mutation in the *NF2* gene results in moderate and mild clinical phenotypes of neurofibromatosis type 2. *Hum. Genet.* **1996**, *97*, 224–227. [[CrossRef](#)] [[PubMed](#)]
12. Ruttledge, M.H.; Andermann, A.A.; Phelan, C.M.; Claudio, J.O.; Han, F.Y.; Chretien, N.; Rangaratnam, S.; MacCollin, M.; Short, P.; Parry, D.; et al. Type of mutation in the neurofibromatosis type 2 gene (*NF2*) frequently determines severity of disease. *Am. J. Hum. Genet.* **1996**, *59*, 331–342. [[PubMed](#)]
13. Kluwe, L.; MacCollin, M.; Tagatigiba, M.; Thomas, S.; Hazim, W.; Haase, W.; Mautner, V.F. Phenotypic variability associated with 14 splice-site mutations in the *NF2* gene. *Am. J. Med. Genet.* **1998**, *77*, 228–233. [[CrossRef](#)]
14. Baser, M.E.; Kuramoto, L.; Joe, H.; Friedman, J.M.; Wallace, A.J.; Gillespie, J.E.; Ramsden, R.T.; Evans, D.G.R. Genotype-phenotype correlations for nervous system tumors in neurofibromatosis 2: A population-based study. *Am. J. Hum. Genet.* **2004**, *75*, 231–239. [[CrossRef](#)]
15. Evans, D.G.; Wallace, A.J.; Wu, C.L.; Trueman, L.; Ramsden, R.T.; Strachan, T. Somatic mosaicism: A common cause of classic disease in tumor-prone syndromes? Lessons from type 2 neurofibromatosis. *Am. J. Hum. Genet.* **1998**, *63*, 727–736.
16. Kluwe, L.; Mautner, V.; Heinrich, B.; Dezube, R.; Jacoby, L.B.; Friedrich, R.E.; MacCollin, M. Molecular study of frequency of mosaicism in neurofibromatosis 2 patients with bilateral vestibular schwannomas. *J. Med. Genet.* **2003**, *40*, 109–114. [[CrossRef](#)]
17. Moyhuddin, A.; Baser, M.E.; Watson, C.; Purcell, S.; Ramsden, R.T.; Heiberg, A.; Wallace, A.J.; Evans, D.G.R. Somatic mosaicism in neurofibromatosis 2: Prevalence and risk of disease transmission to offspring. *J. Med. Genet.* **2003**, *40*, 459–463. [[CrossRef](#)]
18. Mautner, V.F.; Lindenau, M.; Baser, M.E.; Hazim, W.; Tagatigiba, M.; Haase, W.; Samii, M.; Wais, R.; Pulst, S.M. The neuroimaging and clinical spectrum of neurofibromatosis 2. *Neurosurgery* **1996**, *38*, 880–885; discussion 885–886. [[CrossRef](#)]
19. Otsuka, G.; Saito, K.; Nagatani, T.; Yoshida, J. Age at symptom onset and long-term survival in patients with neurofibromatosis Type 2. *J. Neurosurg.* **2003**, *99*, 480–483. [[CrossRef](#)]
20. Mautner, V.F.; Tagatigiba, M.; Lindenau, M.; Fünsterer, C.; Pulst, S.M.; Baser, M.E.; Kluwe, L.; Zanella, F.E. Spinal tumors in patients with neurofibromatosis type 2: MR imaging study of frequency, multiplicity, and variety. *AJR Am. J. Roentgenol.* **1995**, *165*, 951–955. [[CrossRef](#)]
21. Dirks, M.S.; Butman, J.A.; Kim, H.J.; Wu, T.; Morgan, K.; Tran, A.P.; Lonser, R.R.; Asthagiri, A.R. Long-term natural history of neurofibromatosis Type 2-associated intracranial tumors. *J. Neurosurg.* **2012**, *117*, 109–117. [[CrossRef](#)] [[PubMed](#)]
22. Goutagny, S.; Bah, A.B.; Henin, D.; Parfait, B.; Grayeli, A.B.; Sterkers, O.; Kalamarides, M. Long-term follow-up of 287 meningiomas in neurofibromatosis type 2 patients: Clinical, radiological, and molecular features. *Neuro-oncology* **2012**, *14*, 1090–1096. [[CrossRef](#)] [[PubMed](#)]

23. Rutledge, M.H.; Sarrazin, J.; Rangaratnam, S.; Phelan, C.M.; Twist, E.; Merel, P.; Delattre, O.; Thomas, G.; Nordenskjöld, M.; Collins, V.P. Evidence for the complete inactivation of the NF2 gene in the majority of sporadic meningiomas. *Nat. Genet.* **1994**, *6*, 180–184. [[CrossRef](#)] [[PubMed](#)]
24. Gutmann, D.H.; Giordano, M.J.; Fishback, A.S.; Guha, A. Loss of merlin expression in sporadic meningiomas, ependymomas and schwannomas. *Neurology* **1997**, *49*, 267–270. [[CrossRef](#)] [[PubMed](#)]
25. Lomas, J.; Bello, M.J.; Arjona, D.; Alonso, M.E.; Martinez-Glez, V.; Lopez-Marin, I.; Amiñoso, C.; de Campos, J.M.; Isla, A.; Vaquero, J.; et al. Genetic and epigenetic alteration of the NF2 gene in sporadic meningiomas: NF2 in Meningiomas. *Genes. Chromosomes Cancer* **2005**, *42*, 314–319. [[CrossRef](#)]
26. Riemenschneider, M.J.; Perry, A.; Reifenberger, G. Histological classification and molecular genetics of meningiomas. *Lancet Neurol.* **2006**, *5*, 1045–1054. [[CrossRef](#)]
27. MacCollin, M.; Chiocca, E.A.; Evans, D.G.; Friedman, J.M.; Horvitz, R.; Jaramillo, D.; Lev, M.; Mautner, V.F.; Niimura, M.; Plotkin, S.R.; et al. Diagnostic criteria for schwannomatosis. *Neurology* **2005**, *64*, 1838–1845. [[CrossRef](#)]
28. Koontz, N.A.; Wiens, A.L.; Agarwal, A.; Hingtgen, C.M.; Emerson, R.E.; Mosier, K.M. Schwannomatosis: The overlooked neurofibromatosis? *AJR Am. J. Roentgenol.* **2013**, *200*, W646–W653. [[CrossRef](#)]
29. Hulsebos, T.J.M.; Plomp, A.S.; Wolterman, R.A.; Robanus-Maandag, E.C.; Baas, F.; Wesseling, P. Germline mutation of INI1/SMARCB1 in familial schwannomatosis. *Am. J. Hum. Genet.* **2007**, *80*, 805–810. [[CrossRef](#)]
30. Piotrowski, A.; Xie, J.; Liu, Y.F.; Poplawski, A.B.; Gomes, A.R.; Madanecki, P.; Fu, C.; Crowley, M.R.; Crossman, D.K.; Armstrong, L.; et al. Germline loss-of-function mutations in LZTR1 predispose to an inherited disorder of multiple schwannomas. *Nat. Genet.* **2014**, *46*, 182–187. [[CrossRef](#)]
31. Kehrer-Sawatzki, H.; Farschtschi, S.; Mautner, V.-F.; Cooper, D.N. The molecular pathogenesis of schwannomatosis, a paradigm for the co-involvement of multiple tumour suppressor genes in tumorigenesis. *Hum. Genet.* **2017**, *136*, 129–148. [[CrossRef](#)] [[PubMed](#)]
32. Merker, V.L.; Esparza, S.; Smith, M.J.; Stemmer-Rachamimov, A.; Plotkin, S.R. Clinical features of schwannomatosis: A retrospective analysis of 87 patients. *Oncologist* **2012**, *17*, 1317–1322. [[CrossRef](#)] [[PubMed](#)]
33. Bacci, C.; Sestini, R.; Provenzano, A.; Paganini, I.; Mancini, I.; Porfirio, B.; Vivarelli, R.; Genuardi, M.; Papi, L. Schwannomatosis associated with multiple meningiomas due to a familial SMARCB1 mutation. *Neurogenetics* **2010**, *11*, 73–80. [[CrossRef](#)]
34. Melean, G.; Velasco, A.; Hernández-Imaz, E.; Rodríguez-Álvarez, F.J.; Martín, Y.; Valero, A.; Hernández-Chico, C. RNA-based analysis of two SMARCB1 mutations associated with familial schwannomatosis with meningiomas. *Neurogenetics* **2012**, *13*, 267–274. [[CrossRef](#)] [[PubMed](#)]
35. van den Munckhof, P.; Christiaans, I.; Kenter, S.B.; Baas, F.; Hulsebos, T.J.M. Germline SMARCB1 mutation predisposes to multiple meningiomas and schwannomas with preferential location of cranial meningiomas at the falx cerebri. *Neurogenetics* **2012**, *13*, 1–7. [[CrossRef](#)] [[PubMed](#)]
36. Clark, V.E.; Erson-Omay, E.Z.; Serin, A.; Yin, J.; Cotney, J.; Ozduman, K.; Avşar, T.; Li, J.; Murray, P.B.; Henegariu, O.; et al. Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. *Science* **2013**, *339*, 1077–1080. [[CrossRef](#)] [[PubMed](#)]
37. Brastianos, P.K.; Horowitz, P.M.; Santagata, S.; Jones, R.T.; McKenna, A.; Getz, G.; Ligon, K.L.; Palescandolo, E.; Van Hummelen, P.; Ducar, M.D.; et al. Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. *Nat. Genet.* **2013**, *45*, 285–289. [[CrossRef](#)]
38. Reuss, D.E.; Piro, R.M.; Jones, D.T.W.; Simon, M.; Ketter, R.; Kool, M.; Becker, A.; Sahm, F.; Pusch, S.; Meyer, J.; et al. Secretory meningiomas are defined by combined KLF4 K409Q and TRAF7 mutations. *Acta Neuropathol.* **2013**, *125*, 351–358. [[CrossRef](#)]
39. Clark, V.E.; Harmanci, A.S.; Bai, H.; Youngblood, M.W.; Lee, T.I.; Baranoski, J.F.; Ercan-Sencicek, A.G.; Abraham, B.J.; Weintraub, A.S.; Hnisz, D.; et al. Recurrent somatic mutations in POLR2A define a distinct subset of meningiomas. *Nat. Genet.* **2016**, *48*, 1253–1259. [[CrossRef](#)]
40. Agnihotri, S.; Suppiah, S.; Tonge, P.D.; Jalali, S.; Danesh, A.; Bruce, J.P.; Mamatjan, Y.; Klironomos, G.; Gonen, L.; Au, K.; et al. Therapeutic radiation for childhood cancer drives structural aberrations of NF2 in meningiomas. *Nat. Commun.* **2017**, *8*, 186. [[CrossRef](#)]
41. Bi, W.L.; Greenwald, N.F.; Abedalthagafi, M.; Wala, J.; Gibson, W.J.; Agarwalla, P.K.; Horowitz, P.; Schumacher, S.E.; Esaulova, E.; Mei, Y.; et al. Genomic landscape of high-grade meningiomas. *NPJ Genom. Med.* **2017**, *2*, 15. [[CrossRef](#)] [[PubMed](#)]

42. Harmanci, A.S.; Youngblood, M.W.; Clark, V.E.; Coşkun, S.; Henegariu, O.; Duran, D.; Erson-Omay, E.Z.; Kaulen, L.D.; Lee, T.I.; Abraham, B.J.; et al. Integrated genomic analyses of de novo pathways underlying atypical meningiomas. *Nat. Commun.* **2017**, *8*, 14433. [[CrossRef](#)] [[PubMed](#)]
43. Patel, A.J.; Wan, Y.-W.; Al-Ouran, R.; Revelli, J.-P.; Cardenas, M.F.; Oneissi, M.; Xi, L.; Jalali, A.; Magnotti, J.F.; Muzny, D.M.; et al. Molecular profiling predicts meningioma recurrence and reveals loss of DREAM complex repression in aggressive tumors. *Proc. Natl. Acad. Sci. USA* **2019**. [[CrossRef](#)] [[PubMed](#)]
44. Modha, A.; Gutin, P.H. Diagnosis and treatment of atypical and anaplastic meningiomas: A review. *Neurosurgery* **2005**, *57*, 538–550; discussion 538–550. [[CrossRef](#)] [[PubMed](#)]
45. Pearson, B.E.; Markert, J.M.; Fisher, W.S.; Guthrie, B.L.; Fiveash, J.B.; Palmer, C.A.; Riley, K. Hitting a moving target: Evolution of a treatment paradigm for atypical meningiomas amid changing diagnostic criteria. *Neurosurg. Focus* **2008**, *24*, E3. [[CrossRef](#)] [[PubMed](#)]
46. Aghi, M.K.; Carter, B.S.; Cosgrove, G.R.; Ojemann, R.G.; Amin-Hanjani, S.; Martuza, R.L.; Curry, W.T.; Barker, F.G. Long-term recurrence rates of atypical meningiomas after gross total resection with or without postoperative adjuvant radiation. *Neurosurgery* **2009**, *64*, 56–60; discussion 60. [[CrossRef](#)]
47. Vasudevan, H.N.; Braunstein, S.E.; Phillips, J.J.; Pekmezci, M.; Tomlin, B.A.; Wu, A.; Reis, G.F.; Magill, S.T.; Zhang, J.; Feng, F.Y.; et al. Comprehensive Molecular Profiling Identifies FOXM1 as a Key Transcription Factor for Meningioma Proliferation. *Cell Rep.* **2018**, *22*, 3672–3683. [[CrossRef](#)]
48. Kia, S.K.; Gorski, M.M.; Giannakopoulos, S.; Verrijzer, C.P. SWI/SNF mediates polycomb eviction and epigenetic reprogramming of the INK4b-ARF-INK4a locus. *Mol. Cell. Biol.* **2008**, *28*, 3457–3464. [[CrossRef](#)]
49. Kadoch, C.; Copeland, R.A.; Keilhack, H. PRC2 and SWI/SNF Chromatin Remodeling Complexes in Health and Disease. *Biochemistry* **2016**, *55*, 1600–1614. [[CrossRef](#)]
50. Sadasivam, S.; DeCaprio, J.A. The DREAM complex: Master coordinator of cell cycle-dependent gene expression. *Nat. Rev. Cancer* **2013**, *13*, 585–595. [[CrossRef](#)]
51. Dewan, R.; Pemov, A.; Dutra, A.S.; Pak, E.D.; Edwards, N.A.; Ray-Chaudhury, A.; Hansen, N.F.; Chandrasekharappa, S.C.; Mullikin, J.C.; Asthagiri, A.R.; et al. First insight into the somatic mutation burden of neurofibromatosis type 2-associated grade I and grade II meningiomas: A case report comprehensive genomic study of two cranial meningiomas with vastly different clinical presentation. *BMC Cancer* **2017**, *17*, 127. [[CrossRef](#)] [[PubMed](#)]
52. Gary, R.; Bretscher, A. Ezrin self-association involves binding of an N-terminal domain to a normally masked C-terminal domain that includes the F-actin binding site. *Mol. Biol. Cell* **1995**, *6*, 1061–1075. [[CrossRef](#)] [[PubMed](#)]
53. Pearson, M.A.; Reczek, D.; Bretscher, A.; Karplus, P.A. Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. *Cell* **2000**, *101*, 259–270. [[CrossRef](#)]
54. Shaw, R.J.; Paez, J.G.; Curto, M.; Yaktine, A.; Pruitt, W.M.; Saotome, I.; O'Bryan, J.P.; Gupta, V.; Ratner, N.; Der, C.J.; et al. The Nf2 Tumor Suppressor, Merlin, Functions in Rac-Dependent Signaling. *Dev. Cell* **2001**, *1*, 63–72. [[CrossRef](#)]
55. Okada, T.; Lopez-Lago, M.; Giancotti, F.G. Merlin/NF-2 mediates contact inhibition of growth by suppressing recruitment of Rac to the plasma membrane. *J. Cell Biol.* **2005**, *171*, 361–371. [[CrossRef](#)]
56. Jin, H.; Sperka, T.; Herrlich, P.; Morrison, H. Tumorigenic transformation by CPI-17 through inhibition of a merlin phosphatase. *Nature* **2006**, *442*, 576–579. [[CrossRef](#)]
57. Hennigan, R.F.; Foster, L.A.; Chaiken, M.F.; Mani, T.; Gomes, M.M.; Herr, A.B.; Ip, W. Fluorescence Resonance Energy Transfer Analysis of Merlin Conformational Changes. *Mol. Cell. Biol.* **2010**, *30*, 54–67. [[CrossRef](#)]
58. Ali Khajeh, J.; Ju, J.H.; Atchiba, M.; Allaire, M.; Stanley, C.; Heller, W.T.; Callaway, D.J.E.; Bu, Z. Molecular Conformation of the Full-Length Tumor Suppressor NF2/Merlin—A Small-Angle Neutron Scattering Study. *J. Mol. Biol.* **2014**, *426*, 2755–2768. [[CrossRef](#)]
59. Chinthalapudi, K.; Mandati, V.; Zheng, J.; Sharff, A.J.; Bricogne, G.; Griffin, P.R.; Kissil, J.; Izard, T. Lipid binding promotes the open conformation and tumor-suppressive activity of neurofibromin 2. *Nat. Commun.* **2018**, *9*, 1388. [[CrossRef](#)]
60. Morrison, H. The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. *Genes Dev.* **2001**, *15*, 968–980. [[CrossRef](#)]
61. Ahmad, Z.; Brown, C.M.; Patel, A.K.; Ryan, A.F.; Ongkeko, R.; Doherty, J.K. Merlin knockdown in human Schwann cells: Clues to vestibular schwannoma tumorigenesis. *Otol. Neurotol. Off. Publ. Am. Otol. Soc. Am. Neurotol. Soc. Eur. Acad. Otol. Neurotol.* **2010**, *31*, 460–466. [[CrossRef](#)] [[PubMed](#)]

62. Chow, H.-Y.; Dong, B.; Duron, S.G.; Campbell, D.A.; Ong, C.C.; Hoeflich, K.P.; Chang, L.-S.; Welling, D.B.; Yang, Z.; Chernoff, J. Group I Paks as therapeutic targets in NF2-deficient meningioma. *Oncotarget* **2015**, *6*, 1981. [[CrossRef](#)] [[PubMed](#)]
63. Watt, K.I.; Harvey, K.F.; Gregorevic, P. Regulation of Tissue Growth by the Mammalian Hippo Signaling Pathway. *Front. Physiol.* **2017**, *8*, 942. [[CrossRef](#)] [[PubMed](#)]
64. Striedinger, K.; VandenBerg, S.R.; Baia, G.S.; McDermott, M.W.; Gutmann, D.H.; Lal, A. The Neurofibromatosis 2 Tumor Suppressor Gene Product, Merlin, Regulates Human Meningioma Cell Growth by Signaling through YAP. *Neoplasia* **2008**, *10*, 1204–1212. [[CrossRef](#)] [[PubMed](#)]
65. Tanahashi, K.; Natsume, A.; Ohka, F.; Motomura, K.; Alim, A.; Tanaka, I.; Senga, T.; Harada, I.; Fukuyama, R.; Sumiyoshi, N.; et al. Activation of Yes-Associated Protein in Low-Grade Meningiomas Is Regulated by Merlin, Cell Density, and Extracellular Matrix Stiffness. *J. Neuropathol. Exp. Neurol.* **2015**, *74*, 704–709. [[CrossRef](#)] [[PubMed](#)]
66. Noorolyai, S.; Shajari, N.; Baghbani, E.; Sadreddini, S.; Baradaran, B. The relation between PI3K/AKT signalling pathway and cancer. *Gene* **2019**, *698*, 120–128. [[CrossRef](#)]
67. Rong, R.; Tang, X.; Gutmann, D.H.; Ye, K. Neurofibromatosis 2 (NF2) tumor suppressor merlin inhibits phosphatidylinositol 3-kinase through binding to PIKE-L. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 18200–18205. [[CrossRef](#)]
68. Mawrin, C. Different Activation of Mitogen-Activated Protein Kinase and Akt Signaling Is Associated with Aggressive Phenotype of Human Meningiomas. *Clin. Cancer Res.* **2005**, *11*, 4074–4082. [[CrossRef](#)]
69. Bush, M.L.; Oblinger, J.; Brendel, V.; Santarelli, G.; Huang, J.; Akhmametyeva, E.M.; Burns, S.S.; Wheeler, J.; Davis, J.; Yates, C.W.; et al. AR42, a novel histone deacetylase inhibitor, as a potential therapy for vestibular schwannomas and meningiomas. *Neuro-oncology* **2011**, *13*, 983–999. [[CrossRef](#)]
70. Weller, M.; Roth, P.; Sahm, F.; Burghardt, I.; Schuknecht, B.; Rushing, E.J.; Regli, L.; Lindemann, J.P.; von Deimling, A. Durable Control of Metastatic AKT1-Mutant WHO Grade 1 Meningothelial Meningioma by the AKT Inhibitor, AZD5363. *J. Natl. Cancer Inst.* **2017**, *109*, 1–4. [[CrossRef](#)]
71. James, M.F.; Lelke, J.M.; Maccollin, M.; Plotkin, S.R.; Stemmer-Rachamimov, A.O.; Ramesh, V.; Gusella, J.F. Modeling NF2 with human arachnoidal and meningioma cell culture systems: NF2 silencing reflects the benign character of tumor growth. *Neurobiol. Dis.* **2008**, *29*, 278–292. [[CrossRef](#)] [[PubMed](#)]
72. Kwiatkowski, D.J. Tuberous sclerosis: From tubers to mTOR. *Ann. Hum. Genet.* **2003**, *67*, 87–96. [[CrossRef](#)] [[PubMed](#)]
73. James, M.F.; Han, S.; Polizzano, C.; Plotkin, S.R.; Manning, B.D.; Stemmer-Rachamimov, A.O.; Gusella, J.F.; Ramesh, V. NF2/Merlin Is a Novel Negative Regulator of mTOR Complex 1, and Activation of mTORC1 Is Associated with Meningioma and Schwannoma Growth. *Mol. Cell. Biol.* **2009**, *29*, 4250–4261. [[CrossRef](#)] [[PubMed](#)]
74. Pachow, D.; Andrae, N.; Kliese, N.; Angenstein, F.; Stork, O.; Wilisch-Neumann, A.; Kirches, E.; Mawrin, C. mTORC1 Inhibitors Suppress Meningioma Growth in Mouse Models. *Clin. Cancer Res.* **2013**, *19*, 1180–1189. [[CrossRef](#)]
75. Shih, K.C.; Chowdhary, S.; Rosenblatt, P.; Weir, A.B.; Shepard, G.C.; Williams, J.T.; Shastri, M.; Burris, H.A.; Hainsworth, J.D. A phase II trial of bevacizumab and everolimus as treatment for patients with refractory, progressive intracranial meningioma. *J. Neurooncol.* **2016**, *129*, 281–288. [[CrossRef](#)]
76. McCutcheon, I.E.; Friend, K.E.; Gerdes, T.M.; Zhang, B.-M.; Wildrick, D.M.; Fuller, G.N. Intracranial injection of human meningioma cells in athymic mice: An orthotopic model for meningioma growth. *J. Neurosurg.* **2000**, *92*, 306–314. [[CrossRef](#)]
77. Püttmann, S.; Senner, V.; Braune, S.; Hillmann, B.; Exeler, R.; Rickert, C.H.; Paulus, W. Establishment of a benign meningioma cell line by hTERT-mediated immortalization. *Lab. Investig. J. Tech. Methods Pathol.* **2005**, *85*, 1163–1171. [[CrossRef](#)]
78. Akat, K.; Mennel, H.-D.; Kremer, P.; Gassler, N.; Bleck, C.K.E.; Kartenbeck, J. Molecular characterization of desmosomes in meningiomas and arachnoidal tissue. *Acta Neuropathol. (Berl.)* **2003**, *106*, 337–347. [[CrossRef](#)]
79. Mei, Y.; Bi, W.L.; Greenwald, N.F.; Agar, N.Y.; Beroukhim, R.; Dunn, G.P.; Dunn, I.F. Genomic profile of human meningioma cell lines. *PLoS ONE* **2017**, *12*, e0178322. [[CrossRef](#)]
80. Akat, K.; Bleck, C.K.E.; Lee, Y.-M.A.; Haselmann-Weiss, U.; Kartenbeck, J. Characterization of a novel type of adherens junction in meningiomas and the derived cell line HBL-52. *Cell Tissue Res.* **2008**, *331*, 401–412. [[CrossRef](#)]

81. McClatchey, A.I.; Saotome, I.; Mercer, K.; Crowley, D.; Gusella, J.F.; Bronson, R.T.; Jacks, T. Mice heterozygous for a mutation at the Nf2 tumor suppressor locus develop a range of highly metastatic tumors. *Genes Dev.* **1998**, *12*, 1121–1133. [[CrossRef](#)] [[PubMed](#)]
82. Kalamarides, M. Nf2 gene inactivation in arachnoidal cells is rate-limiting for meningioma development in the mouse. *Genes Dev.* **2002**, *16*, 1060–1065. [[CrossRef](#)] [[PubMed](#)]
83. Peyre, M.; Stemmer-Rachamimov, A.; Clermont-Taranchon, E.; Quentin, S.; El-Taraya, N.; Walczak, C.; Volk, A.; Niwa-Kawakita, M.; Karboul, N.; Giovannini, M.; et al. Meningioma progression in mice triggered by Nf2 and Cdkn2ab inactivation. *Oncogene* **2013**, *32*, 4264–4272. [[CrossRef](#)]
84. Kalamarides, M.; Stemmer-Rachamimov, A.O.; Niwa-Kawakita, M.; Chareyre, F.; Taranchon, E.; Han, Z.-Y.; Martinelli, C.; Lusi, E.A.; Hegedus, B.; Gutmann, D.H.; et al. Identification of a progenitor cell of origin capable of generating diverse meningioma histological subtypes. *Oncogene* **2011**, *30*, 2333–2344. [[CrossRef](#)]



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