Title: Merlin, a "magic" linker between extracellular cues and intracellular signaling pathways that regulate cell motility, proliferation, and survival.

Authors: Stamenkovic I, Yu Q

Journal: Current protein & peptide science

Year: 2010 Sep

Volume: 11

Issue: 6

Pages: 471-84

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serveur académique lausannois

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Faculté de biologie
et de médecine

Merlin, a "Magic" Linker Between the Extracellular Cues and Intracellular Signaling Pathways that Regulate Cell Motility, Proliferation, and Survival

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Abstract

Genetic alterations of neurofibromatosis type 2 (NF2) gene lead to the development of schwannomas, meningiomas, and ependymomas. Mutations of *NF2* gene were also found in thyroid cancer, mesothelioma, and melanoma, suggesting that it functions as a tumor suppressor in a wide spectrum of cells. The product of *NF2* gene is merlin (moesinezrin-radixin-like protein), a member of the Band 4.1 superfamily proteins. Merlin shares significant sequence homology with the ERM (Ezrin-Radixin-Moesin) family proteins and serves as a linker between transmembrane proteins and the actin-cytoskeleton. Merlin is a multifunctional protein and involved in integrating and regulating the extracellular cues and intracellular signaling pathways that control cell fate, shape, proliferation, survival, and motility. Recent studies showed that merlin regulates the cell-cell and cell-matrix adhesions and functions of the cell surface adhesion/extracellular matrix receptors including CD44 and that merlin and CD44 antagonize each other's function and work upstream of the mammalian Hippo signaling pathway. Furthermore, merlin plays important roles in stabilizing the contact inhibition of proliferation and in regulating activities of several receptor tyrosine kinases. Accumulating data also suggested an emerging role of merlin as a negative regulator of growth and progression of several non-NF2 associated cancer types. Together, these recent advances have improved our basic understanding about merlin function, its regulation, and the major signaling pathways regulated by merlin and provided the foundation for future translation of these findings into the clinic for patients bearing the cancers in which merlin function and/or its downstream signaling pathways are impaired or altered.

Keywords

CD44; cancer stem cell; hippo signaling pathway; merlin; neurofibromatosis type 2; receptor tyrosine kinase

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INTRODUCTION

Merlin is the product of neurofibromatosis type 2 (*NF2*) gene and a member of the Band 4.1 superfamily proteins. It serves as a linker between transmembrane proteins and the actin-

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cytoskeleton. Mutations and deletions of merlin cause NF2, which is characterized by the development of schwannomas, meningiomas, and ependymomas. Mutations of the *NF2* gene have also been found in other cancers, suggesting that merlin regulates a variety of cancer types. Increasing amount of evidence indicated that merlin regulates the functions and activities of cell surface receptor tyrosine kinases (RTKs) and adhesion/extracellular matrix (ECM) receptors and serves as a key regulator of several important signaling pathways that regulate cell motility, proliferation, and survival. These important discoveries and recent advances are summarized in the following sections.

MERLIN ACTS AS A TUMOR SUPPRESSOR IN THE NF2 ASSOCIATED TUMORS AND MERLIN MUTANTS PROMOTE TUMORIGENESIS

Neurofibromatosis type 2 (NF2) familial cancer syndrome is a dominantly inherited autosomal disease characterized by the development of NF2-associated tumors including schwannomas, meningiomas and ependymomas in the central and peripheral nervous system [1-9]. The *NF2* gene is located on human chromosome 22q12 [10] and alterations of the gene have been detected in the germline of NF2 patients and in sporadic NF2-associated tumors [11]. It has been well established that mutations and deletions of the *NF2* gene lead to development of NF2-associated tumors and that loss of heterozygosity (LOH) of the gene is associated with sporadic schwannomas, ependymomas, and meningiomas [12-14]. The *NF2* gene mutations have also been found in thyroid cancer, mesotheliomas, and melanoma albeit less frequently [15]. The *NF2* gene product is merlin (Moesin-Ezrin-Radixin Like Protein), also known as schwannomin, which belongs to the Band 4.1 protein family [13,14] and shares significant sequence homology with the ERM proteins, namely ezrin [16], radixin [17] and moesin [18] Fig. (**1**). Merlin has a conserved tri-lobe NH2-terminal Four point one, Ezrin, Radixin, Moesin (FERM) domain, a central alpha-helical region, and a COOH-terminal tail [19,20] Fig. (**1**).

Genetic analysis of NF2 patient samples demonstrated that deletions in the NH2-terminal FERM domain of merlin occur frequently and are associated with early tumor onset and poor prognosis [13,21,22]. Overexpression of several merlin mutants causes excessive proliferation of *Drosophila* wing epithelial cells through interfering with activity of endogenous wild type merlin [23]. In addition, loss of merlin is embryonic lethal both in mouse and fly, which implies broad roles of merlin during key stages of embryonic development [24,25]. Furthermore, the heterozygous merlin knockout mice (NF2+/-) develop metastatic osteosarcomas, fibrosarcomas, and hepatocellular carcinomas. Nearly all of these tumors have lost their wild type NF2 allele [26], suggesting that merlin may serve as a tumor suppressor in a wider spectrum of cells and that loss of merlin function may play an important role in tumor growth and progression.

MERLIN HAS CONSERVED STRUCTURE/DOMAIN ORGANIZATION AND ITS FUNCTION IS REGULATED BY POSTTRANSLATIONAL MODIFICATION AND PROTEOLYTIC CLEAVAGE

The *NF2* gene consists of 17 exons [15]. There are at least 10 known isoforms of human merlin and the two most common ones are isoform I and II, which differ at their COOH-terminal ends with the segments, encoded either by exon 16 or 17, respectively [27-32] Fig. (**1**). Merlin isoform I contains 595 amino acids whereas isoform II has 590 amino acids with estimated molecular weights of approximately 65-70 kDa [20]. Similar to the ERM proteins, merlin has conserved structure and domain organization [33], which consists of a tri-lobe globular NH2-terminal FERM domain, a central alpha-helical region, and a charged hydrophilic COOH-terminal tail [9,20,34]. Unlike the ERM proteins, however, merlin lacks the conventional COOH-terminal actin-binding site but contains an unconventional actin-binding site in its

NH2-terminus [35]. In addition, merlin can be linked to the actin-cytoskeleton through other actin-binding proteins such as βII-spectrin or by forming heterodimers with the ERM proteins.

The highest sequence homology between merlin and the ERM proteins is in the conserved tri-lobe FERM domain Fig. (**1**). Merlin and the ERM proteins interact with numerous membrane-associated proteins through their NH2-terminal domains. Merlin can form homodimers with each other and heterodimers with the ERM family proteins through head-to-tail intra- and inter-molecular association, which regulates its function [20,34,36]. The conserved residues in the NH2- and COOH-termini of merlin and the ERM proteins constitute NH2- and COOH-ERM association domains (N- and C-ERMADs), respectively Fig. (**1**) which are responsible for mediating the observed head-to-tail association. Functions of merlin and the ERM proteins are, respectively, positively and negatively regulated by these intra- and inter-molecular associations [37,38].

Head-to-tail self-association of merlin results in the "closed" conformation, which is required for its tumor-suppressor activity [39]. Unlike isoform 1, merlin isoform 2 is believed to be incapable of forming the head-to-tail association and therefore lacks the tumor suppressor activity [39,40]. Phosphorylation of merlin at its COOH terminus especially at Ser518 abolishes the head-to-tail self-association and leads to an "open" conformation and loss of tumor-suppressor activity [20,39,41] Fig. (**1**). Several kinases including p21-activated kinase 1 and 2 (PAK1/2) and cAMP-dependent protein kinase A (PKA) phosphorylate merlin at Ser518 [41-46], which leads to the open inactive conformation. PAK1-4 are a group of serine/threonine kinases that function immediately downstream of Rac [41,42]. Merlin inhibits PAK1 activity through a feedback loop by binding to its PBD domain (Rac/Cdc42 binding domain of PAK), which results in inhibition of PAK1 recruitment to the focal adhesions [41]. Loss of merlin results in increased PAK1 activity. In addition, a recent study indicated that erbin (ERBB2 interacting protein) and merlin complexes bind and inactivate the GTPase-bound PAK2 in epithelia [47]. Erbin is a PDZ protein that acts as an adaptor for the receptor tyrosine kinase ERBB2/HER2 [48]. In addition to PAK1, AKT phosphorylates merlin at Thr230 and Ser315, which promotes merlin degradation by proteasome [49]. Conversely, myosin phosphatase MYPT1–PP1 dephosphorylates merlin at Ser518, which results in merlin activation [50,51]. Merlin can also be cleaved by calpain, a calcium-dependent cysteine protease, in schwannomas and meningomas [52], implying that merlin can be inactivated and down-regulated by calpain-mediated proteolytic cleavage.

MERLIN INTERACTS WITH NUMEROUS TRANSMEMBRANE AND INTRACELLULAR PROTEINS

Studies have shown that merlin [36,53] binds to numerous transmembrane and intracellular proteins (for a thorough review see [9]) including the hyaluronic acid (HA) receptor, CD44 [35,54,55], integrin β1 [56], layilin [57], DCC [58], CD43 [59], FAT—a large protocadherin [60], NHE-RF/EBP50 [61-65], Caspr/paranodin [66], paxillin [67,68], actin [69-71], N-WASP [72], βII-spectrin [73], microtubules [74,75], EG1/magicin [76], SCHIP1 [77], MYPT-1-PP1δ [50], RIβ PKA (RIβ subunit of protein kinase A) [78,79], PAK-1/2 [41], calpain [80], HRS (hepatocyte growth factor-regulated tyrosine kinase substrate) [81], syntenin [82], PIKE-L [83], Grb2 [84], NGB [85], RalGDS (Ral guanine nucleotide dissociation stimulator) [86], RhoGDI [87], TRBP (transactivation-responsive RNA-binding protein) [88], eIF3c (eukaryotic initiation factor subunit c) [89], and CRM1/β exportin [90] (Table **1**). These merlin-binding partners are likely play important roles in exerting the effects of merlin on the signaling pathways mediated by RTKs, adhesion and extracellular matrix (ECM) receptors, PI3K/AKT/mTOR, and small GTPases (Table **1**).

MERLIN REGULATES CELL MOTILITY AND INVASION

Like the ERM proteins, merlin serves as a linker between the plasma membrane and the actin cytoskeleton and regulates cytoskeleton remodeling, cell motility, and cell proliferation in response to the extracellular signals [91-96]. Accumulating evidence indicates that merlin plays an essential role in regulating cell morphology and motility [97] and that loss of mer lin results in dramatic ch anges in actincytoskeleton organization and cell adhesion [19]. Merlin and the ERM proteins display a similar subcellular localization and they are localized predominantly in the areas of dynamic cytoskeleton remodeling such as microspikes, membrane ruffles [19, 55,98,99], and the cellular structures that are critical for cell motility and invasion [37,100]. Tumors developed in the heterozygous NF2 knockout mice (NF2+/-) are highly motile and display metastatic proclivity. Almost all schwannoma cells display disorganized stress fibers, altered spreading, and increased membrane ruffling [101], which can be reversed by re-expression of merlin [69]. Studies have shown that merlin inhibits actin assembly induced by Arp2/3 and Rac [72,102]. Even though a recent study suggested that the tumor-suppression function of merlin is independent of its role as an organizer of the actin cytoskeleton in Schwann cells [103], other studies have clearly demonstrated an essential role of merlin in inhibiting tumor cell motility and invasion [104,105].

As a linker between transmembrane proteins and the actin-cytoskeleton, merlin is uniquely positioned to regulate cell proliferation in response to the cues/signals derived from their microenvironment. Contact inhibition of cell proliferation is essential for maintaining tissue homeostasis. Its loss is a hallmark of transformation and leads to increased tumor cell proliferation, motility, and invasion [106]. Studies have demonstrated that merlin is dephosphorylated and activated in confluent cells and that merlin accumulates and stabilizes at the adherens junctions in keratinocytes and fibroblasts [101,107,108]. Furthermore, merlin mediates contact inhibition of cell growth through its interaction with CD44 [108,109] or by blocking recruitment of Rac to the plasma membrane [110]. Accordingly, loss of merlin destabilizes the cadherin-containing cell-cell junctions, leading to loss of the contact inhibition [101] and increased Rac activity, lamellipodia formation, and increased cell motility [42]. We have shown recently that inhibition of the CD44-hyaluronan (HA) interaction by merlin contributes to the tumor suppressor activity of merlin [54] and that CD44 functions upstream of merlin [111].

MERLIN FUNCTIONS UPSTREAM OF THE HIPPO SIGNALING PATHWAY THAT CONTROLS CELL PROLIFERATION AND SURVIVAL AND EXERTS MERLIN'S INHIBITORY EFFECT ON CANCER GROWTH AND PROGRESSION

The Hippo signaling pathway plays an essential role in regulating cell proliferation and survival [112] and recent results placed merlin upstream of *Drosophila* Hippo signaling pathway [113-116]. There are two FERM domain-containing proteins in *Drosophila*, merlin (*mer*) and expanded (*ex*), that negatively regulate cell growth [113,114,117]. Inactivation mutations of *mer* or *ex* result in reduced apoptosis and increased cell proliferation [113,114]. Analyses of *mer* and *ex* double mutants indicate that they function in at least a partially redundant manner upstream of the Hippo (Hpo)/Salvador (Sav)/Warts (Wts)/Mats signaling pathway [113]. In *Drosophila*, expanded was recruited by a protocadherin, Fat, to the plasma membrane [60, 118]. Hpo and Wts are Thr/Ser kinases of Ste20 and NDR (nuclear Dbf2-related), respectively, whereas Sav and Mats (Mob1 as a tumor suppressor) are scaffold proteins. In *Drosophila*, Hpo phosphorylates and activates Wts, which in turn phosphorylates and inhibits Yorkie (*Yki*), a transcriptional co-activator [119]. Inactivation of Hpo/Wts signaling results in up-regulation and activation of *Yki*, which in turn up-regulates *cyclin E* and *Drosophila* Inhibitor of Apoptosis Protein 1 (DIAP1), resulting in increased proliferation and survival [117,119]. Mammalian

homologs of *Drosophila* mer, Hpo, Wts, Yki, and DIAP are mammalian merlin [117], Mammalian Sterile Twenty-like (MST) kinase1 and 2 (MST1/2)[112,120,121], Large tumor suppressor 1 and 2 (LATS1/2) [122-124], YAP (Yes-Associated Protein) [125,126], and cIAP1/2 (cellular inhibitor of apoptosis 1 and 2) [127]. In general, merlin, MST1/2, and LATS1/2 function as tumor suppressors and regulate the activity of downstream protooncogenes, YAP and TAZ (Transcriptional co-activator with PDZ-binding motif), through phosphorylation of their conserved HXRXXS motif. There are two isoforms of YAP, YAP1 and YAP2, which contain one or two WW protein interaction domains, respectively [128].

LATS kinases interact with their co-activator hMOB1 (human Mps-one binder one), which is required for their efficient autophosphorylation [129], whereas full activation of LATS1/2 is mediated through their phosphorylation by MST1/2 [130,131]. MST1/2-activated LATS1/2 in turn phosphorylates YAP, which leads to cytoplasmic retention and inactivation of YAP [132-134]. MST1/2 form complexes with a scaffold protein, human Salvador (hSAV or hWW45 for human WW domain-containing protein with a predicted molecular mass of approximately 45 kDa), which is the mammalian ortholog of *Drosophila* Salvador [135]. hSAV is required for activation of MST1 and heterozygous hSAV knockout mice are prone to tumor development, suggesting that it functions as a tumor suppressor [135,136].

Like MST1/2, LATS1/2 genes encode serine/threonine kinases that display anti-tumor activity [137,138]. MST1/2 kinases are down regulated in human colorectal cancer and sarcomas [139,140] and LATS1/2 are down regulated in human astrocytomas, sarcomas, acute lymphoblastic leukemia, and breast cancer as a result of promoter hypermethylation [112, 141]. Loss of LATS1 causes predisposition to soft-tissue sarcomas and ovarian tumors in mice [142]. Mouse embryonic fibroblasts (MEFs) derived from the LATS2-null mice, acquire a growth advantage and display a profound defect in the contact inhibition of growth [143]. By contrast, overexpression of LATS1 induces G2/M arrest and subsequent apoptosis [137] whereas LATS2 inhibits G1/S transition and suppresses RasV12-induced transformation of NIH 3T3 cells [138]. Furthermore, overexpression of LATS1 suppresses YAP-mediated cellular transformation, epithelialmesenchymal transition (EMT), and tumorigenesis [137, 144,145]. Together, these results suggest that MST1/2 and LATS1/2 serve as tumor suppressors in a variety of human cancers. MST1/2 kinases also play important roles in regulating the cellular stress response [146].

Similar to *Drosophila* Yki, YAP rescues pupal lethality caused by overexpression of *hpo* or *wts* in *Drosophila* [119]. The human orthologs of *hpo* and *wts* also rescue their corresponding *Drosophila* mutants [147-149]. YAP, a mammalian ortholog of Yki, and TAZ [150], a homolog of YAP, are overexpressed in human malignancies, including liver, prostate, lung, ovarian, colon, pancreatic, and nervous system cancers [112] and display oncogenic activity [125, 126,132,134,151-153]. Overexpression of YAP or TAZ leads to loss of contact inhibition and anchorage-independent growth, and to EMT [125,134,144,145,154], whereas knockdown of either molecule inhibits tumorigenicity [126,132,152]. As a transcriptional co-activator, YAP promotes proliferation and inhibits apoptosis by up-regulating expression of cyclin E and cIAP1/2, respectively. In contrast to a vast majority of results that demonstrate the pro-tumor activity of YAP and the tight negative regulation of YAP localization/activity by upstream mammalian Hippo signaling components, one report provided evidence suggesting loss or reduction of YAP expression in human breast tumors and that MST2/LATS1 positively regulates YAP activity [155]. This apparent discrepancy needs to be resolved in the future.

Until recently, it was unknown whether the *Drosophila* Hpo—Wts—Yki signaling pathway is conserved as a tumor-suppressor pathway in mammalian cells. Recent studies have suggested that the Hippo signaling pathway is conserved in mammalian cells and plays important roles

in regulating the progression of human glioma and response of glioma to a variety of stresses including the one resulted from a chemotherapeutic agent [104,111,134,152,153]. We showed that merlin is absent or down regulated in high-grade gliomas and that increased expression of merlin inhibits glioma cell motility and invasiveness and sensitizes their response to chemotherapy and irradiation. Re-expression of merlin dramatically inhibits subcutaneous and intracranial growth of human gliomas whereas merlin depletion promotes glioma growth *in vivo*. Furthermore, we established that re-expression of merlin in human glioma cells up regulates expression and activity of Lats2, inhibits activity of YAP, and reduces cIAP1/2 expression suggesting that merlin inhibits glioma growth and progression by regulating the activity of mammalian Hippo signaling pathway [104]. Very recently, we showed that CD44 functions upstream of mammalian Hippo signaling pathway and attenuates the stress- induced activation of the hippo signaling pathway [111] Fig. (**2**). Together, these results indicate that mammalian Hippo signaling pathway plays important roles in regulating cancer progression and the responses of cancer cells to therapeutic treatments and suggest that components and regulators of this emerging signaling pathway can be used as therapeutic targets for a variety of cancer types.

MERLIN AND CD44 IN CANCER STEM CELLS

Both merlin and the ERM proteins interact with the cytoplasmic tail of CD44 [59,92]. CD44 is a major cell surface receptor for hyaluronan (HA), an abundant ECM component, and composed of an extracellular domain that contains an HA-binding domain and a membrane-proximal region, a transmembrane domain, and a COOH-terminal cytoplasmic tail. Functionally, CD44 mediates the cell-cell and cell-matrix adhesion, cell migration, and signaling [156-158]. CD44 is often up regulated in malignant tumors, serves as a predictor of poor prognosis in several cancer types, and promotes tumor progression and metastasis [156-159]. A study showed that treatment of confluent tumor cells with HA leads to dephosphorylation/activation of merlin, which mediates contact inhibition of cell growth through its interaction with CD44 [108]. In contrast, we have shown that increased expression of wild type merlin in schwannoma cells inhibits CD44-HA binding and subcutaneous schwannoma growth *in vivo* whereas a merlin deletion mutant that lacks CD44-binding domain but not other NH2-terminal deletion mutants of merlin is incapable of inhibiting the tumor growth. Together, these results demonstrated that merlin exerts its tumor suppressor function, at least in part, by negatively regulating CD44 function [54].

Increasing evidence suggests the existence of a small population of specialized cancer cells that display stem cell properties, commonly referred as cancer stem cells (CSCs). CSCs are characterized by their ability to self-renew, differentiate into various lineages, and reconstitute the cellular hierarchy of the original tumors upon serial xenotransplantations [160-162]. CSCs are highly resistant to chemo- and radio-therapy and are believed to be responsible for tumor recurrence following therapeutic intervention [160-162]. CD44 has been identified as one of the consistent markers of CSCs in a variety of malignancies, including leukemia, breast, colon, ovarian, prostate, pancreatic, and head and neck cancers [156,160-161,163-172]. Studies have shown that CD44 plays an essential role in engraftment of leukemia stem/initiating cells in the bone marrow, which is required for leukemia development [163,164], and that CD44 plays the functional role in colorectal cancer stem cells [172]. These results point toward potentially important roles of CD44 in the formation, maintenance, and/or function of cancer stem cells, even though additional studies are required to establish these potential roles of CD44 in CSCs and to reveal the underlying mechanisms. Recent studies have also demonstrated important contributions of the components of mammalian Hippo signaling pathway in regulating the stem cell niche, stem cell self-renewal, maintenance, and differentiation [173-176]. Merlin plays a critical role in maintaining normal structure and function of the hematopoietic stem cell (HSC) niche and the NF2-deficient animals display increased stem cell pool size and increased

mobilization of HSC to the circulation [176]. TAZ, a homolog of YAP, was found to play important role in regulating mesenchymal stem cell differentiation [173] and controlling human embryonic stem-cell self-renewal [175] whereas YAP is enriched in embryonic and neural stem cells [174]. It is conceivable that, as an upstream regulator of mammalian Hippo signaling pathway [111], CD44 may exert some degree of control over CSC behavior through regulating the Hippo signaling pathway.

MERLIN REGULATES THE ACTIVITIES OF SEVERAL RECEPTOR TYROSINE KINASES

Merlin is often enriched in lipid rafts [177], which is required for receptor internalization from the plasma membrane and regulation of their downstream signaling [178,179]. Recent studies have demonstrated the important contribution of merlin in regulating the distribution, aggregation, and availability of several cell surface receptors, especially receptor tyrosine kinases (RTKs), in the plasma membrane [100,180,181]. In *Drosophila*, merlin (*mer*) and expanded (*ex*) cooperatively modulate receptor endocytosis and signaling. Loss of *mer* and *ex* up-regulates several growth-factor receptors including Notch, Patched, and epidermal growth factor receptor (EGFR) due to impaired receptor endocytosis and degradation [182]. This leads to accumulation of these receptors on the cell surface and corresponding activation of downstream signaling such as Ras-ERK, JNK, Rac, Pak, and FAK that leads to increased cell proliferation [41,51,105,182,183]. Similar results have been obtained in mammalian cells with respect to EGFR [184]. However, merlin seems to block the internalization of ligand-bound EGFR, an event that is necessary for the EGF-EGFR-mediated signaling, and sequester EGFR into a non-signaling membrane compartment [100,185], which results in reduced cell proliferation. Merlin might regulate the endocytosis of RTKs through its interaction with hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) or syntenin [82,184,186]. HRS is an endosomal protein that is required for RTK trafficking from early endosomes to lysosomes where they are degraded whereas syntenin is a PDZ domain-containing adaptor that is co-localizes with the PIP2 in early endosomes [187]. Studies have shown that HRS inhibits RTK signaling and schwannoma cell proliferation and that merlin lost its inhibitory effect on cell proliferation in HRS-null mouse fibroblasts [81], supporting the possibility that merlin regulates RTK endocytosis through HRS. This hypothesis needs to be tested in the future experiments.

Recent studies also demonstrated that merlin regulates glial cell growth in ErbB2- and Src-dependent manner [188] and that merlin inhibits schwannoma cell proliferation by promoting platelet-derived growth factor receptor (PDGFR) degradation [189]. Merlin also reduces cell surface levels of ErbB2 and ErbB3, which leads to inhibition of their downstream mitogenic signaling pathways, whereas loss of merlin elevates levels of the ErbB receptors in primary Schwann cells and levels of insulin-like growth factor 1 receptor and PDGFR in the peripheral nerves of NF2-mutant mice and in human schwannomas [103]. Furthermore, merlin also negatively regulates the downstream signaling pathways of PDGFR [183]. In contrast, CD44 is known to serve as a co-receptor of c-Met and ErbB2 and enhances their signaling [111, 190,191]. Together, these results suggest an important role of merlin in regulating the activity and availability of several RTKs that play essential roles in tumor initiation and progression. Additional works are required to determine how this type of regulation is achieved.

MERLIN IS INVOLVED IN SEVERAL ADDITIONAL IMPORTANT SIGNALING TRANSDUCTION PATHWAYS

Tight control of cell proliferation, survival, and motility is critical for maintaining normal tissue homeostasis. Studies have shown that increased expression of merlin not only inhibits cell

proliferation and promotes apoptosis, but also impairs cell-cell and cell-matrix adhesion, as well as cell spreading and motility [21,37,40,101,110], suggesting that merlin is involved in the signaling pathways that regulate these cellular functions. Merlin has been shown to reverse Ras-induced transformation [192,193]. The Rho family of small GTPases, Rho, CDC42, and Rac, are essential downstream effectors of the Ras signaling [194]. Overexpression of merlin inhibits Rac-mediated anchorage independent growth and cell transformation [42]. One mechanism whereby merlin mediates contact inhibition of growth is by blocking recruitment of Rac to the plasma membrane [110]. Another is by inhibiting Ras and Rac activation [183, 195]. Consistent with this notion, loss of merlin function enhances Rac activity [196]. Conversely, Rac1 activation results in merlin phosphorylation and an "open" conformation, leading to inactivation of its tumor suppressor activity [42]. Merlin and Rac therefore appear to engage in mutual inhibition. Merlin mutations were also found to increase STAT3 and STAT5 signaling [197] whereas merlin negatively regulates rapamycin complex 1 (mTORC1) activity. Loss/knockdown of merlin leads to mTORC1 activation in malignant mesothelioma, meningioma, and schwannoma and increased meningioma and schwannoma growth [198, 199].

CONCLUSIONS AND PERSPECTIVES

Merlin is a multifunctional protein that is involved in integrating and regulating extracellular cues and intracellular signaling pathways that control cell fate, shape, motility, proliferation, and survival. Merlin can achieve these tasks by coordinating with the cell-cell and cell-matrix adhesion receptors, which help merlin to sense the cell-cell contact and cell-matrix adhesion, and by regulating the activities of cell surface RTKs. This review summarized the significant progresses in the following areas: establishment of the contributions of genetic alterations of *NF2* gene to phenotypes of the NF2 associated tumors; determination of the domain and structure organization of merlin; identification of many merlin binding partners and the signaling pathways regulated by merlin; establishment of the cellular functions of merlin and the roles of merlin and merlin mutants in the initiation and progression of several cancer types; and elucidation of the mechanisms underlying the merlin functions. Furthermore, recent advances also allowed us to envision several areas of interests for future studies. These studies will not only improve our basic understanding about merlin function, its regulation, and the signaling pathways involving merlin, but also help translate the most relevant recent findings into the clinic for treatment and management of patients bearing the cancers in which merlin function and its downstream signaling pathways are impaired or altered. These areas include:

1. Identification of Potential Targets for NF2 Cancer Therapy and Beyond

Merlin has many interacting partners, which can be classified into several major signaling pathways: Hippo, PI3K/AKT/mTOR, RTKs, and small GTPases [5,9,200,201] (Table **1**). Pharmacological inhibitors of some of these signaling pathways are currently available, and it will be interesting to investigate their efficacy in the mouse models and patients bearing tumors with dysregulated merlin signaling pathways (Fig. (**2**)).

2. Integration of Extracellular and Intracellular Signals by Merlin

Results obtained thus far have clearly indicated that merlin serves as an essential linker that integrates signals from extracellular microenvironment into intracellular signaling pathways that regulate cell fate, motility, proliferation, and survival. Although the underlying mechanisms remain to be elucidated, the interactions between merlin and cell surface RTKs and between merlin and adhesion receptors such as CD44, E-cadherin, and FAT may offer some clues. Additional works are required to establish clear connections between these molecules and their corresponding downstream signaling events.

3. Interplay between Merlin and ERM Proteins During Cancer Development

Merlin is closely related to the ERM proteins and there is evidence to suggest that the ERM proteins and merlin serve as positive and negative regulators, respectively, in organizing cortical actin and in regulating cell growth [38,196]. However, compared to merlin, our understanding of the contribution of the ERM proteins to tumorigenesis and tumor progression is limited. The major signaling pathways downstream of the ERM proteins and the interplay between merlin and the ERM proteins that may exist during tumor initiation and progression are largely unknown. Since merlin and ERM proteins are co-expressed in most cell types with overlapping sub-cellular localizations, form heterodimers with each other, and share common binding partners, functional interplays between merlin and the ERM proteins within at least some of the signaling pathways regulated by merlin are very likely. The functional relationship and contribution of these interactions to the tumor initiation and progression will be important to elucidate.

4. The Potential Roles of CD44, Merlin, and the Hippo Signaling Pathway in Cancer Stem Cells

Recent studies have suggested contributions of the components of mammalian Hippo signaling pathway in regulating the stem cell niche, as well as in stem cell maintenance and differentiation [173-176]. It will be of great interests to determine whether and how CD44, merlin, and the mammalian Hippo signaling pathway contribute to these aspects of CSCs.

5. Merlin in Endocytosis: How Merlin Regulates Function of Cell Surface Receptors

Merlin is localized to endocytic vesicles [197,202] and may play an important role in endocytosis and vesicle trafficking [9,96]. Merlin can silence RTK activity by regulating their endocytosis [176,185] and merlin is known to regulate the activities of small GTPases, which play essential roles in endocytosis and exocytosis. However, little is known about how these events are interrelated and how RTK endocytosis regulated by merlin contributes to tumor initiation and progression, which should be interesting to explore.

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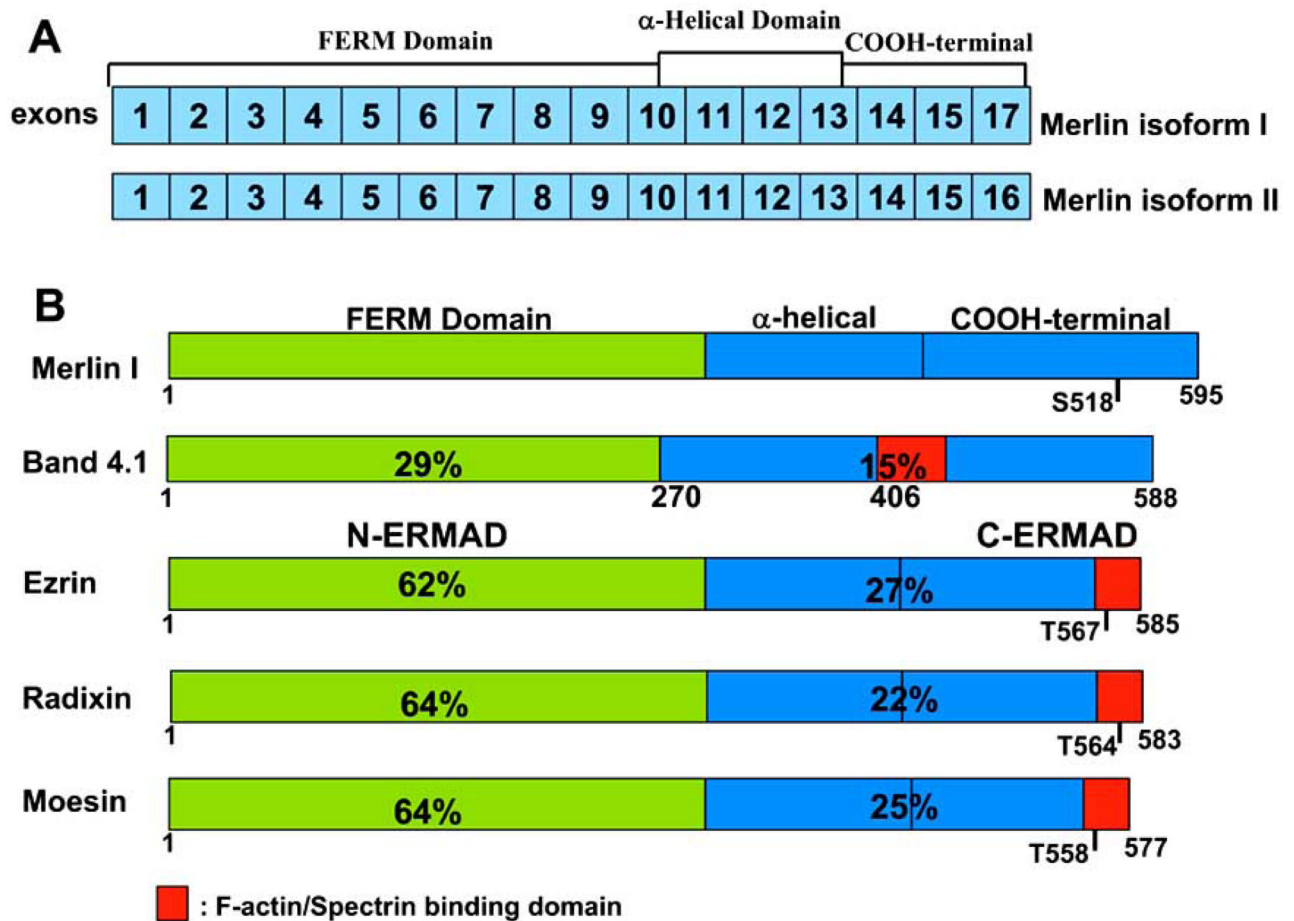
Fig. (1).
Exon organization and domain structure of merlin isoforms in relation to ezrin–radixin–moesin (ERM) proteins. **A**, The *NF2* gene consists of 17 exons. Two most common merlin isoforms, isoform I and II, differ at their COOH-terminal ends with the segments, encoded by either exon 16 or 17, respectively. Merlin isoform I contains 595 amino acids whereas isoform II has 590 amino acids with estimated molecular weights of approximately 65-70 kDa. **B**, Domain organization and domain homology between merlin, band 4.1 protein, ezrin, radixin, and moesin are outlined.
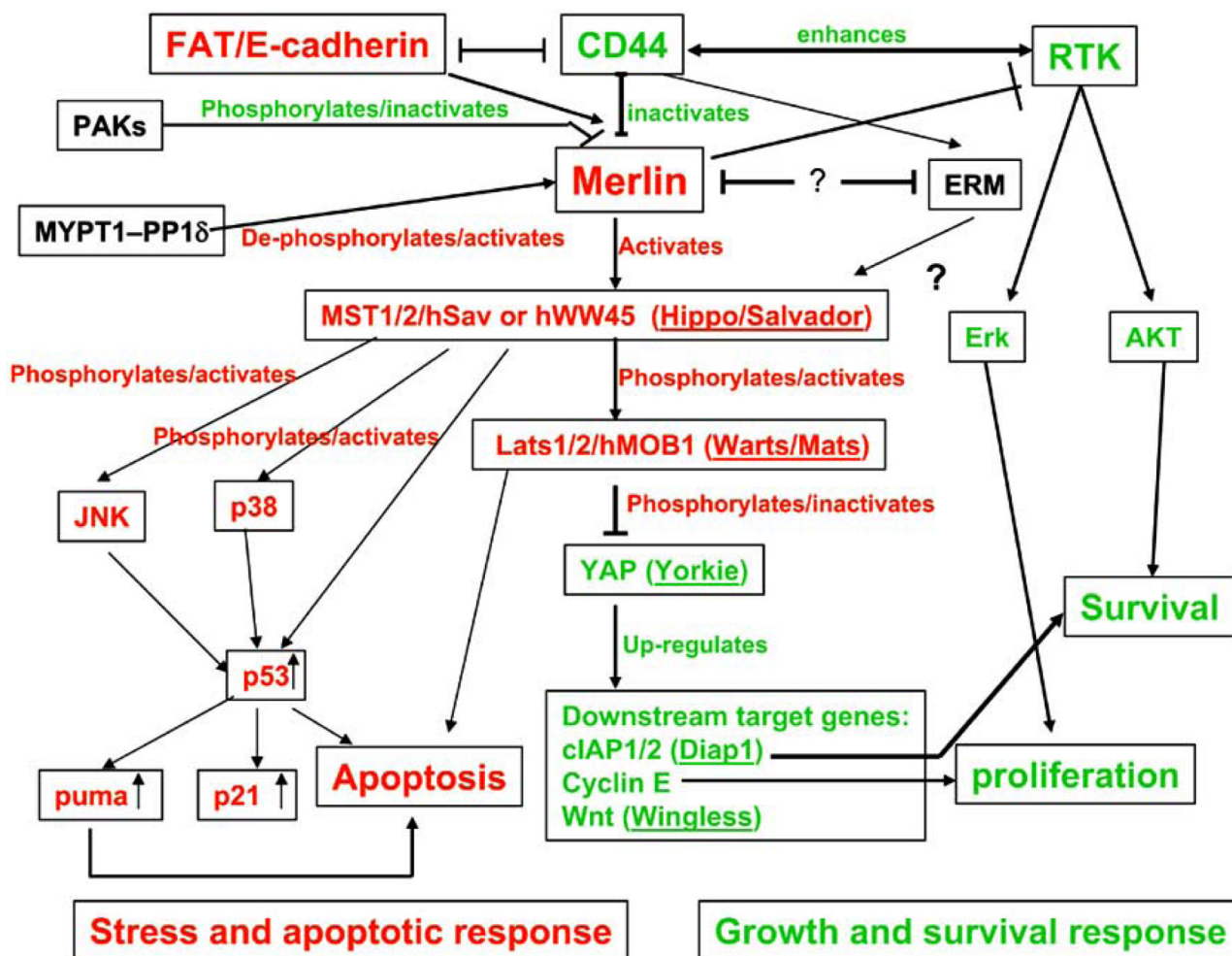
Fig. (2).
A working model of merlin-mediated signaling events and their potential cross-talks (the components of *Drosophila* Hippo signaling pathway are underlined): merlin functions upstream of the mammalian Hippo (merlin-MST1/2-LATS1/2-YAP) and JNK/p38 signaling pathways and plays an essential role in regulating the cell response to the stresses and stress-induced apoptosis as well as to proliferation/survival signals. Merlin antagonizes CD44 function and inhibits activities of RTKs and the RTK-derived growth and survival signals. CD44 function upstream of mammalian Hippo signaling pathway attenuates the stress induced activation of the hippo signaling pathway, however, enhances activities of RTKs.

Table 1

The Proteins that Interact with Merlin

Protein Family/ Signaling Pathway	Protein that Interacts with Merlin	Effect on the Function of the Merlin-Binding Protein or on Merlin	Biochemistry	Biologic Function Affected or Potentially Affected	Tumor Biology Relevance
Adhesion/ ECM receptors	CD44	Negatively	CD44-HA interaction	Contact inhibition and the ECM adhesion	Inhibits pro-tumor activity of CD44
	DCC	Positively	ND	Cell adhesion and neurite outgrowth	Enhances inhibition of tumor growth
	Layilin	Negatively	ND	Focal adhesions and hyaluronan mediated signaling	ND
	β1-integrin	Negatively	Indirect interaction through paxillin	Integrin signaling and crosstalk between integrin and ErbB-2	ND
PDZ domain proteins	NHERF	Negatively	Inhibits MAP kinase signaling through erbin	MAPK signaling and AJ formation	ND
	Syntenin	Negatively	ND	Endosomal localization and cell growth	Inhibits tumor progression
Cytoskeleton/Structural proteins	Actin	Positively	Polymerization of F-actin	Cytoskeletal organization	ND
	βII-spectrin	Positively	Organization of actin	Organizing Cytoskeleton and focal adhesions	ND
	Microtubules	Positively	Microtubules polymerization	Organizing Cytoskeleton	ND
	N-WASP	Negatively	Inhibitor of actin polymerization	Actin polymerization	ND
	paxillin	Positively	Actin binding protein Inhibit ErbB-2 signaling	Focal adhesions	ND
Band 4.1 family	ERM proteins	Reciprocally negative	Formation of heterodimers	Organizing Cytoskeleton, cell shape and motility	ND
Endocytosis	HRS	Positively	Reduction of abundance of EGFR and activity of Stat3	Endocytic trafficking; cell proliferation, spreading, and motility	Growth inhibition
Receptor tyrosine kinases	EGFR	Negatively	Preventing EGFR internalization	Inhibits EGFR signaling	Growth inhibition
	PDGF receptor	Negatively	ND	Promotes PDGFR degradation	Growth inhibition
	AKT	Reciprocally negative	ND	Inhibits survival signaling	ND
PI3 kinase/AKT/mTOR signaling pathway	PIKE	Negatively	Inhibits PI3K activity	Inhibits PI3K signaling	ND
	mTOR	Negatively	Inhibits mTOR activity	Inhibits mTOR signaling	Inhibits tumor growth
	eIF3c (eukaryotic initiation factor subunit c)	Negatively	Protein translation	Cell proliferation	Inhibits tumorigenesis

Protein Family/ Signaling Pathway	Protein that Interacts with Merlin	Effect on the Function of the Merlin-Binding Protein or on Merlin	Biochemistry	Biologic Function Affected or Potentially Affected	Tumor Biology Relevance
	TRBP (transactivation-responsive RNA-binding protein)	Negatively	Promotes ubiquitination and degradation	Protein translation and cell proliferation	Inhibits the oncogenic activity of TRBP
MAPK activity	Grb2	Negatively	Inhibits Raf-ERK pathway	Inhibits MAPK signaling	ND
	Magicin	Negatively	Inhibits Grb2	Inhibits MAPK signaling	ND
Hippo signaling pathway	MST1/2	Activated	Activates Hippo signaling pathway	Cell proliferation and apoptosis	Growth inhibition, anti-tumor progression
	Ras	Negative	ND	Inhibits Ras signaling	Inhibits Ras-induced transformation
	Rac	Reciprocally negative	ND	Blocking recruitment of Rac to the plasma membrane	Inhibits anchorage independent growth and cell transformation
Small GTPases	MAP (merlin associated protein)	Positively	Inhibits Rab GTPase	ND	ND
	RalGDS	Negatively	Inhibits Ral GTPase	Cell proliferation and survival, cell motility	Inhibits tumor incidence and growth
	RhoGDI	Positively	Inhibit Small GTPase signaling	Cell proliferation and motility	Growth inhibition
	MYPT-1-PP1δ	Positively	De-phosphorylation	Activates merlin signaling	Inhibits tumorigenesis
Modulating merlin activity	PAKs	Reciprocally negative	PAK1 phosphorylation merlin at S518; merlin binds to the Cdc42/Rac-binding domain of PAK1	Cell proliferation and survival, cell motility	Promotes tumorigenesis
	PKA	Negatively	Phosphorylation at S518	Promotes proliferation	ND
Merlin proteolytic cleavage	Calpain	Negatively	Cleavage of merlin	Loss of merlin function	Increases tumorigenicity

ND = Not Determined.