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Review

Merlin, a multi-suppressor from cell membrane to the nucleus

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ABSTRACT

Recent evidence suggests that the neurofibromatosis type 2 (*NF2*) gene encoded protein merlin suppresses mitogenic signalling not only at the cell membrane but also in the nucleus. At the membrane, merlin inhibits signalling by integrins and tyrosine receptor kinases (RTKs) and the activation of downstream pathways, including the Ras/Raf/MEK/ERK, FAK/Src, PI3K/AKT, Rac/PAK/JNK, mTORC1, and Wnt/β-catenin pathways. In the nucleus, merlin suppresses the E3 ubiquitin ligase CRL4^{DCAF1} to inhibit proliferation. Gene expression analysis suggested that CRL4^{DCAF1} could also regulate the expression of integrins and RTKs. In this review, we explore the links between merlin function at the membrane and in the nucleus, and discuss the potential of targeting the master regulator CRL4 ^{DCAF1} to treat NF2 and other merlin-deficient tumours.

1. Introduction

Merlin (also known as schwannomin) is encoded by the neurofibromatosis type 2 (*NF2*) gene [1,2] and is a tumour suppressor. Mutations in the *NF2* gene, leading to loss of merlin protein, cause nervous system tumours, including schwannomas, meningiomas and ependymomas, which are part of the autosomal dominant familiar cancer syndrome NF2. Biallelic *NF2* mutations are also responsible for all spontaneous schwannomas, 50–60% of spontaneous meningiomas and 20–30% of ependymomas. In addition, loss of merlin is associated with other cancers, e.g. malignant mesotheliomas [3], glioblastomas [4,5], and has recently been linked to breast cancer [6].

Merlin/*NF2* shows high homology to the ERM (Ezrin-Radixin-Moesin) family [1,2]. Like other ERM proteins, merlin protein consists of an N-terminal FERM domain, a coil–coil segment followed by a C-terminal domain. Unlike other ERM proteins, merlin lacks the canonical actin-binding motif at its C-terminus, it interacts with the actin cytoskeleton through a actin-binding domain at its N-terminus [7]. Merlin has long been considered as a tumour suppressor regulating signalling at the membrane and cortex of cell [8]. At the membrane, merlin regulates expression and activation of integrins and tyrosine receptor kinases (RTKs) and the activation of downstream pathways, including the Ras/Raf/MEK/ERK, FAK/Src, PI3K/AKT, Rac/PAK/JNK, mTORC1 and Wnt/β-catenin pathways. Recent evidence suggests that merlin suppresses mitogenic signalling not only at the cell membrane but also in the nucleus [9]. In the nucleus, merlin suppresses the E3 ubiquitin ligase CRL4^{DCAF1} to inhibit proliferation. This review focuses on merlin suppression of the E3 ubiquitin ligase CRL4^{DCAF1} and tries to link merlin's function in the nucleus and at the membrane. We start with a short description of merlin's functions at the membrane and concentrate on those which can be linked to CRL4^{DCAF1}. We then describe merlin's function in the nucleus via CRL4^{DCAF1}. Finally we discuss the potential therapeutic strategies for NF2 and other merlin-deficient tumours.

2. At the membrane, merlin inhibits integrins and RTKs mediated mitogenic/survival signalling

2.1. Integrins and related the Rac/PAK/JNK, FAK/Src and mTORC1 pathways

The interaction between merlin and integrins was revealed in isolated and differentiated Schwann cells [10]. Loss of merlin in schwannomas leads to pathological adhesion [11,12]. Utermark et al. [12] showed that the enhanced adhesion is linked to increased expression of integrins α6, β1 and β4 at protein level and mRNA level. The upregulation of integrins α6, β1 and β4 was confirmed in an array analysis when mRNA from schwannomas was compared to Schwann cells [13]. Further activation of integrin has been linked to merlin deficiency [14,15]. Indeed, knockdown of integrin β1 with lentiviral shRNA in human schwannoma cells decreased the proliferation and adhesion upon IGFBP stimulation [14].

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Upon integrin activation and increased adhesion to the extracellular matrix, small GTPases Rac1 and Cdc42 are recruited to the membrane. This in turn activates its effectors p21 activated kinases (PAKs) and then downstream protein c-Jun N-terminal kinase (JNK). Interestingly, PAK1/2 are actually the kinases which can phosphorylate merlin at S518 [16,17]. This change of phosphorylation status of merlin switches it into a probable inactive state. Extensive research in different cell models suggests that merlin suppresses the Rac/PAK/JNK pathway [18–22]. Therefore, an activation loop between merlin and PAK was suggested [21] and merlin loss would lead to activation of the small GTPases at the membrane [11,23,24].

In addition to Rac/PAK/JNK, integrins can regulate Focal adhesion kinase (FAK) and its binding partner Src. FAK, a non-receptor tyrosine kinase localised predominantly to the site of focal adhesion, plays a central role in cell adhesion and migration [25]. Increased expression of FAK has been correlated with many types of cancers, including brain, breast, prostate and colon [26–29]. Indeed, FAK plays a critical role in cancer initiation and progression [30]. Proto-oncogene non-receptor tyrosine kinase Src and FAK, can form a dual kinase complex which phosphorylates many adaptor proteins including merlin binding partner Paxillin [31]. Due to their activation in many tumours FAK/Src are considered as promising therapeutic targets [32]. In $NF2^{-/-}$ (merlin null) tumours cells it has also been shown that FAK functions upstream of PI3K/AKT and Raf/MEK/ERK pathways to potentiate schwannoma proliferation, migration and cell survival [14,33,34]. In mouse embryonic fibroblasts (MEFs), it has been shown that FAK phosphorylation at Tyr397, which is an auto-phosphorylation site triggered by integrin, and FAK's connections with Src and PI3K are tightly regulated by merlin's expression [34]. It has been described that activated Src recruits FAK and Paxillin in $NF2^{-/-}$ mouse astrocyte cells [35]. In addition, both Src activity (Y416) and FAK phosphorylation at Y397 and Y925 (Src phosphorylation site) are significantly increased in schwannoma cells compared with Schwann cells [14,33]. Thus FAK/Src are activated downstream of integrins after merlin loss. RNA interference experiments have confirmed that integrin β1 directly functions upstream of FAK, as knockdown of integrin β1 completely abolished IGFBP1 (acting via integrin alpha 5) mediated FAK phosphorylation/activity at Y397 in human schwannoma cells [14]. Furthermore, the nuclear localisation of FAK in schwannoma cells suggests its regulation of proliferation in the cells without merlin [14].

To potentiate cell cycle progression at G1, integrins can promote activation of mTORC1 and then cyclin D1 in merlin-deficient malignant mesothelioma cells [15]. The activation of mTOR is linked to the increased survival in above mentioned merlin-deficient cells. Using the same experimental setting, López-Lago et al. also demonstrated that firstly merlin negative cells are sensitive to the mTOR inhibitor rapamycin, secondly re-introduction of merlin reduces the sensitivity to rapamycin and thirdly depletion of merlin restored the sensitivity to rapamycin in merlin positive mesothelioma cell lines [15]. Evidence of merlin regulating mTORC1 was also found in primary meningioma and schwannoma cells [36].

Thus as discussed above, integrins and downstream pathways are responsible for the pathological cell–matrix adhesion and increased proliferation in merlin-deficient tumours. However, integrins do not work on their own [37,38]. In merlin deficient tumours, in concert with integrins, receptor tyrosine kinases (RTKs) are also activated [33,39].

2.2. RTKs and related Ras/Raf/MEK/ERK, PI3K/AKT, FAK/Src and Rac/PAK/JNK

Emerging data suggest that there are at least four types of RTK involved in merlin-deficient tumours: ErbB receptors, platelet-derived growth factor receptor (PDGFR), insulin-like growth factor 1 receptor (IGF1R), and vascular endothelial growth factor receptors (VEGFRs) [40]. Accumulated evidence suggests that merlin can regulate RTK activity, possibly through their surface availability and trafficking and/or endocytosis [41]. In the schwannoma cell line HEI193, overexpression of merlin inhibits proliferation through accelerating PDGFR internalisation [42]. In primary human schwannoma cells, PDGFRβ is overexpressed and displays a postponed and impaired degradation [33]. Merlin forms a complex with PDGFR through Na+–H+ exchange regulatory cofactor NHERF (also called EBP50), which plays a role in PDGFR internalisation and recycling. Merlin could also be involved in the late stage of endocytosis by interacting with Hepatocyte Growth-factor Receptor Substrate (HRS), which regulates endosomal trafficking of the membrane receptors including EGFR [43]. Therefore merlin could play an important role in the process of PDGFR's accumulation, degradation and recycling. A similar mechanism is also discussed for other growth factor receptors, such as ErbB2 and 3, and insulin-like growth factor 1 receptor [41]. In addition, integrins, which are overexpressed in human schwannomas, could also stabilize PDGFR and delay its degradation by enhancing its autophosphorylation [44].

Ras/Raf/MEK/ERK and PI3K/AKT are two common pathways downstream of RTKs in merlin-deficient tumours. Schwannoma cells display strong activation of MEK, ERK and AKT at basal level or upon PDGF [33] and IGF stimulation (Ammoun et al., unpublished data). In addition it has been demonstrated that merlin inhibits PI3K activity by competing with PI3K for binding to PI3K enhancer long form (PIKE-L) in HEK293 cells [45].

Co-operation between integrins and RTKs is important for cells to control proliferation and survival [46]. In merlin-deficient tumours, RTK mediated signalling (mainly MAPK and PI3K/AKT) is synergised with integrin mediated signalling (Rac/PAK/JNK and FAK/Src). For example, the localisation of phospho-ERK1/2 can be altered by inhibiting PAK with small molecule inhibitor IPA3 in schwannoma cells [33] as PAK might function as a scaffold protein for MAPK cascade. AKT is also placed downstream of PAK and FAK/Src as both IPA3 and knock down of FAK downregulate the AKT activity in human schwannoma cells (Ammoun et al., unpublished data).

2.3. Contact inhibition and Wnt/β-catenin pathway

Loss of contact inhibition is part of the increased proliferation in merlin-deficient tumours. Potential mechanisms for this have been investigated by analysing the relationship between merlin and Rac/PAK, CD44, Paxillin, EGFR and other growth factor receptors and cell density dependent regulation [21,39,42,47,48]. It has been shown that merlin regulates adherens junctions (AJs) by forming a complex with E/N-cadherin and β-catenin [49]. A recent study demonstrated that Rac dependent Wnt/β-catenin signalling, which was measured by the expression of Wnt target genes and TCF activity, was found to be significantly increased in NF2 knockout mouse embryonic fibroblasts in confluent cell cultures [50]. Indeed, a study in human schwannoma cells demonstrated degradation of adherens junctions and proliferative Wnt/β-catenin signalling elevated as active β-catenin (dephosphorylated at serine 37 and threoine 41) localises to the nucleus and the Wnt targets genes *c-myc* and *cyclin D1* are upregulated in confluent human schwannoma cells [51]. Most importantly, the link between the loss of the AJ complex and the increased proliferation in human schwannoma cells is by RTK (PDGFR/Src) induced tyrosine 654 phosphorylation on β-catenin and dependent on integrin mediated Rac/PAK/JNK pathway, as depletion of PAK2 suppressed active β-catenin, c-myc, and cyclin D1. Therefore these studies suggest a model that these pathways (including Wnt/β-catenin, RTKs and

integrin mediated signalling) are coordinated and relevant for proliferation in merlin-deficient tumours.

3. In the nucleus, merlin suppresses ubiquitin E3 ligase activity

In addition to inhibiting mitogenic and survival signalling at or near the cell cortex, active merlin also enters the nucleus to suppresses the E3 ubiquitin ligase E3 CRL^{DCAF1} [52].

The strong direct interaction of merlin and the DDB1- and CUL4-associated factor 1 (DCAF1) was identified by tandem affinity purification followed by mass spectrometry. Studies have demonstrated that DCAF1 functions as a substrate receptor for the Cullin4-RING E3 ubiquitin ligase (CRL4) complex [53]. Interaction analysis suggested that merlin wildtype but not NF2 patient-derived merlin mutant (e.g. L64P), has a strong affinity to the $CRL4^{DCAF1}$ complex. Immunofluorescence studies indicate that merlin in a dephosphorylated conformation has the ability to shuttle into the nucleus and bind to $CRL4^{DCAF1}$. Further genetic experiments demonstrated that knock down of DCAF1 inhibits the hyperproliferation in merlin-deficient mesothelioma cells. Importantly, we showed that, silencing of DCAF1 in $NF2^{-/-}$ human schwannoma cells suppressed the ability of these cells to progress through G1 and to enter into S phase in response to mitogens. In contrast, knockdown of DCAF1, did not significantly affect the proliferation of control human Schwann cells, where $CRL4^{DCAF1}$ is not disinhibited. It has been demonstrated that CRL4 ligase plays an important role in genome stability, cell cycle regulation and histone methylation [54]. Microarray data showed that depletion of DCAF1 and re-expression of merlin induced similar changes in over hundreds of genes [9], thus suggesting that merlin broadly regulates gene expression by inhibiting $CRL4^{DCAF1}$ activity. Taken together, these findings indicate that $CRL4^{DCAF1}$ plays a key role in NF2-dependent hyperproliferation and tumorigenesis in human schwannomas and Merlin-deficient tumours. Merlin, which is in a non-phosphorylated status, enters into the nucleus to suppress hyperproliferation by inhibiting $CRL4^{DCAF1}$.

3.1. $CRL4^{DCAF1}$ and the Hippo pathway

During organ development, cell number is strictly regulated. The Hippo pathway controls organ size through restraining cell proliferation and promoting apoptosis in *Drosophila* and mammals [55]. The Hippo pathway also plays an important role in stem cell self-renewal and tissue regeneration [56]. Many genes in the hippo pathway are recognised as tumour suppressors as their mutations are identified in human cancers including breast and liver cancer [57,58]. The canonical Hippo pathway basically consists of a three-tier kinase cascade: Hippo activates Warts, which in turn phosphorylates Yorkie. The phosphorylation of Yorkie is critical for its cytoplasmic localisation. The involvement of merlin in the Hippo signalling pathway was initially revealed in *Drosophila*, where merlin and another FERM domain protein Expanded act upstream of the Hippo pathway to inhibit cell proliferation and apoptosis [59,60]. Studies in mammalian cells also suggested that merlin induces YAP (Yorkie Homologue) expression and localisation, which is critical for the proliferation in merlin-deficient mesothelioma and meningioma cells [61,62]. A recent study suggested that merlin might work together with a newly identified Hippo pathway component named Kibra to promote Lats1/2 (adaptor protein in the Hippo pathway) phosphorylation in HEK293 cells [63]. Re-expression of merlin or inactivation of $CRL4^{DCAF1}$ in mouse schwannoma cells induces the expression of a subset of Hippo target genes [9]. A study in progress suggested that via a still unknown mechanism merlin could regulate YAP phosphorylation, which is increased in confluent cells, through DCAF1 in the nucleus [64].

3.2. Links between $CRL4^{DCAF1}$ and membrane receptor signalling

In addition to the Hippo pathway discussed above, gene expression analysis demonstrated that depletion of DCAF1 or overexpression of merlin causes transcriptional alteration of main components of integrin and RTKs pathways, (including *itga6, itga7*, *pdgfa* and *pdgfr*b) as well as the genes jointly regulated by integrins and RTKs (e.g. *grb2* and *cav1* [9]). Taken together, the mechanism by which merlin exerts its inhibitory effects might involve a linked, two-tier system. As shown in Fig. 1, as a tumour suppressor, merlin seems to act in two linked signalling compartments: one at or close to the membrane, mainly integrin and RTKs mediated or jointly mediated signalling (including Ras/Raf/MEK/ERK, FAK/Src, PI3K/AKT, mTORC1, Rac/PAK/JNK and Wnt/β-catenin); the other in the nucleus, active merlin translocates into the nucleus to inhibit $CRL4^{DCAF1}$ ubiquitin E3 ligase activity. Importantly, the nuclear regulation of DCAF1 by merlin could enhance merlin's function at the membrane level by modifying the transcriptional level of integrins, RTKs (especially PDFGR), and their downstream effectors. The common output of these pathways is to lead to increased proliferation and survival.

4. Combination therapy and targeting ubiquitin E3 ligase $CRL4^{DCAF1}$

In addition to the disability from frequent merlin-related spontaneous cancers, the inherited tumour syndrome NF2 with multiple slow growing genetically well defined tumours of the nervous system leads to significant medical problems, i.e. substantial morbidity and reduced life span. Presently, the only treatment of NF2 is surgery and radiosurgery, chemotherapy is not effective due to the slow growth of NF2 related tumours. Surgery and radiosurgery are only locally effective and only treat a single tumour at a time; furthermore these treatments can leave patients with significant morbidity. Thus, a new pharmaceutical treatment is urgently required.

Current therapeutic strategies are focussing on targeting a single pathway with specific small molecule inhibitors or monoclonal antibodies mainly targeting RTK receptors. Indeed, EGFR/ErbB2 has been targeted with Lapatinib in sporadic and NF2-related human vestibular schwannomas [65–67]. A common RTK inhibitor, Nilotinib (targeting kinases PDGFR, BCR-ABL, and KIT), approved to treat leukaemia and other cancers, was found to be effective in a schwannoma *in vitro* model using primary tumour cells [68] and is currently being tested in a phase 0 trial with NF2-schwannoma patients. As angiogenesis has been detected in human vestibular schwannomas, another strategy is to target VEGF, which plays an important role in angiogenesis, with specific humanized antibody (e.g. Bevacizumab). Indeed, improvement of hearing and reduction of tumour growth has been found in a proportion of NF2 patients after treated with Bevacizumab [69].

However as shown in Fig. 1 and summarised in a review by Ammoun and Hanemann [40], merlin inhibits multiple pathways at membrane level, therefore an inhibitor targeting multiple targets might be needed to more effectively treat merlin-deficient tumours. Indeed a dual PDGFRβ and Raf inhibitor, Sorafenib, reduced both basal and PDGF-mediated ERK1/2 and AKT activity and decreased cell proliferation in human schwannoma cells [33]. Sorafenib is currently being tested for NF2 patients in a phase 0 trial. The idea of combination therapy was supported by other studies i.e. a dual inhibitor against mTORC1 and PI3K/AKT, such as NVP-BEZ235 [70], was suggested by James et al. [36]. In addition, targeting a common molecule, which is downstream of several pathways such as PI3K, could be a good strategy for the treatment of NF2-related tumours [14].

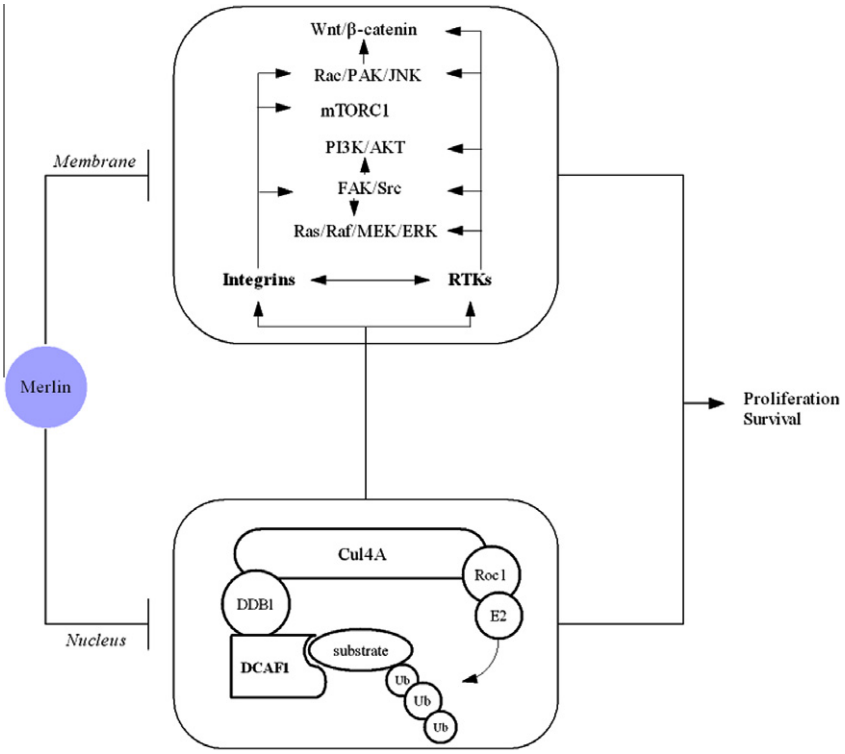

Fig. 1. Merlin inhibits multiple co-operating signalling pathways at the membrane and nucleus. At the membrane, merlin inhibits (1) integrins mediating the Rac/PAK/JNK, mTORC1, and FAK/Src pathways; (2) Receptor Tyrosine Kinases (RTKs) mediating Ras/Raf/MEK/ERK, PI3K/AKT, and (3) the Wnt/β-catenin pathways, which are also linked with integrin and RTK mediated Rac/PAK/JNK signalling. FAK/Src plays a critical role because it connects the MAPK pathways and PI3K/AKT pathway. At the nucleus, Merlin inhibits Cullin4-RING E3 ubiquitin ligase (CRL4) complex (including DDB1, Roc1) and E2 ligase through binding to the substrate receptor DCAF1. $CRL4^{DCAF1}$ could also regulate the expression of integrins and RTKs, therefore links to the membrane signalling. The common output of these pathways (upper and lower parts) is to lead to increased proliferation and survival.

However the signalling pathways in merlin-deficient tumours are complex (Fig. 1), inhibition of a single growth factor receptor or pathway may not suppress hyperproliferation of merlin-deficient tumours effectively; therefore a "master" therapeutic target would be helpful. As discussed above, DCAF1 functions upstream of RTKs/integrins/Hippo. Most importantly, depletion of DCAF1 inhibits proliferation in schwannoma cells but not normal Schwann cells. Thus, we hypothesis that $CRL4^{DCAF1}$ is such a master regulator.

Dysregulation of the ubiquitin pathway is associated with many types of diseases, including cancer and neurodegenerative diseases. The ubiquitin pathway is considered as a promising target in cancer therapy [71]. The ubiquitin proteasome complex can be targeted in multiple ways [72]. In the example of ubiquitination of the tumour suppressor p53, E1 activity can be inhibited by a cell-permeable inhibitor named PYR-41 [73], which induces apoptosis by increasing the expression levels and activity of p53. E3 and substrate interactions can also be specifically blocked by a number of peptide derivatives and small molecule inhibitors. For example, nutlins (a family of cis-imidazoline analogues), RITA (reactivation of p53 and induction of tumour cell apoptosis) and MI-63 had been developed to block the binding of p53 and E3-ligase Mdm2 [74] (murine double-minute clone 2, Hdm2 in human) specifically. Treatment of various tumour cells with these inhibitors resulted in accumulation of p53 and cell death [72,75]. Currently the approach to target E3 ligase activity closest to clinical application is to inhibit proteasomes as many proteasome inhibitors have already been approved as effective anticancer drugs [76]. Because $CRL4^{DCAF1}$ could cause downstream proteasome dependent degradation of substrates, e.g. uracil-DNA glycosylase-2 (UNG2) [77], it is interesting to test whether proteasome pathways are involved in the development of merlin-deficient tumours. The research compound MG132 is a cell-permeable tripeptide aldehyde and has been widely used as a potent inhibitor to study the functions of the ubiquitin proteasome pathways in mammalian cells. It has been reported that MG132 could induce cell cycle arrest in RT4 rat schwannoma cells (14). Our data (Zhou & Hanemann, unpublished) show that (1) human schwannoma cells treated for 24 h with 1 μM MG132 returned to a normal Schwann-cell like bipolar elongated shape, (2) the proliferation of schwannoma cells was suppressed by MG132 treatments in a dose dependent manner (3) the induction of cell-death in schwannoma cells was also observed after 48 h treatment with 10 μM MG132. Those results suggest that the merlin-related tumorigenesis is dependent, at least partly, on the ubiquitin–proteasome system. In addition, proteasome inhibitors may work on merlin-deficient tumours by restoration of phosphatase and tensin homologue (PTEN) expression, which is downregulated in human schwannoma cells [14]. PTEN functions as a negative regulator of PI3K signalling, which is one of most important survival pathways in schwannoma and other merlin-deficient cells, therefore increase of PTEN level by proteasome inhibitors will decrease PI3K signalling and lead to apoptosis.

However, there are potential shortcomings of targeting UPS with non-specific proteasome inhibitors to treat merlin-deficient tumours. They could potentially result in stabilization of unwanted proteins, e.g. an oncogene which could share a common E3 ligase with a tumour suppressor. Commonly proteasome inhibitors promote apoptosis through by stabilising IκB then preventing activation of NFκB [78]. It has been shown that IκB shares a common E3 ligase SCFβ-TrCP with β-catenin, which promotes proliferation in merlin-deficient tumours [51]. Further non-specific proteasome inhibitors, such as Bortezomib, could cause side effects e.g.

peripheral neuropathy and gastrointestinal effects, that may be due to targeting of the constitutive proteasome in these tissues [79]. Therefore it is desirable to find more specific approaches to target $CRL4^{DCAF1}$ for merlin-deficient cells.

It has been demonstrated that the ubiquitination activity of CRLs requires the covalent conjugation of an ubiquitin-like molecule, NEDD8, onto the cullin component of CRLs, in a process called neddylation. A specific CRL activity inhibitor, MLN4924, has recently been described by Soucy et al. [80]. Inhibition of NEDD8-activating enzyme (NAE) by MLN4924 leads to decreased neddylation and inhibition of CRL activity. MLN4924 forms a covalent adduct with NAE, which inhibits enzyme activity and thus prevents ubiquitination and proteasomal degradation of CRL substrate proteins [81]. MLN4924 induces apoptosis in tumour cells by deregulation of S-phase DNA synthesis (MLN-treated cells displayed initiation of DNA synthesis but not transitioning into mitosis) and suppresses human HCT116 colon cancer and H522 lung tumour xenografts in mice [80,82,83]. It has been demonstrated that DCAF1 preferentially interacts with the NEDD8-modified form of CUL4A [84], therefore there is potential to validate MLN4924 in the treatment of Merlin-deficient tumours. MLN4924 can sensitise cancer cells but not normal cells to radiation [85], however, it has been noticed that MLN4924 reduces Nedd8 activity in normal mice [80]. Therefore to evaluate the effects of MLN4924, it is important to determine whether MLN4924 has any impacts on merlin positive cells, such as Schwann cells.

5. Conclusions

Future studies that determine the substrates of $CRL4^{DCAF1}$ will help us to learn far more detail about the links between nuclear and membrane signalling in merlin deficient tumours. On the other hand, it is also interesting to investigate existing feedback from the membrane to the activation of CRL^{DCAF1} at the nuclear level in merlin-deficient cells. It is also crucial to ask whether merlin suppresses another ubiquitin E3 ligase at or near the membrane. The answers to the above questions will certainly advance our understanding the biochemical functions of merlin and the mechanism by which merlin suppresses tumorigenesis. Once we realise the complex of the signalling pathway in merlin-deficient tumours, targeting a master/key regulator such as DCAF1, which rules both the membrane and nuclear signalling, certainly will lead to a better therapeutic strategy for the NF2 and other merlin-deficient patients.

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