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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