FEBS Letters xxx (2014) xxx–xxx

journal homepage: www.FEBSLetters.org

Review

Molecular insights into *NF2*/Merlin tumor suppressor function

Jonathan Cooper *, Filippo G. Giancotti *

Cell Biology Program, Sloan Kettering Institute for Cancer Research, Memorial Sloan Kettering Cancer Center, New York, NY, United States

ARTICLE INFO

Article history:
Received 26 February 2014
Revised 1 April 2014
Accepted 2 April 2014
Available online xxxx

Edited by Shairaz Baksh, Giovanni Blandino and Wilhelm Just

Keywords:
Merlin
Neurof**i**bromatosis Type **2**
Hippo signaling pathway
Contact inhibition
DDB1 and **C**ul4-**A**ssociated **F**actor **1**
CRL4 E3 ubiquitin ligase

ABSTRACT

The FERM domain protein Merlin, encoded by the *NF2* tumor suppressor gene, regulates cell proliferation in response to adhesive signaling. The growth inhibitory function of Merlin is induced by intercellular adhesion and inactivated by joint integrin/receptor tyrosine kinase signaling. Merlin contributes to the formation of cell junctions in polarized tissues, activates anti-mitogenic signaling at tight-junctions, and inhibits oncogenic gene expression. Thus, inactivation of Merlin causes uncontrolled mitogenic signaling and tumorigenesis. Merlin's predominant tumor suppressive functions are attributable to its control of oncogenic gene expression through regulation of Hippo signaling. Notably, Merlin translocates to the nucleus where it directly inhibits the $CRL4^{DCAF1}$ E3 ubiquitin ligase, thereby suppressing inhibition of the Lats kinases. A dichotomy in *NF2* function has emerged whereby Merlin acts at the cell cortex to organize cell junctions and propagate anti-mitogenic signaling, whereas it inhibits oncogenic gene expression through the inhibition of $CRL4^{DCAF1}$ and activation of Hippo signaling. The biochemical events underlying Merlin's normal function and tumor suppressive activity will be discussed in this Review, with emphasis on recent discoveries that have greatly influenced our understanding of Merlin biology.

1. Introduction

The ability of normal cells to survive and proliferate is dictated by environmental cues, including intercellular and matrix adhesions as well as the presence of growth factors. Normal cells undergo growth arrest when detached from the extracellular matrix or when they come into contact with adjacent cells and form intercellular junctions [1]. Neoplastic cells evade contact inhibition of proliferation, which leads to the disruption of tissue organization – a distinguishing event in cancer [2]. The *NF2* (**N**eurof**i**bromatosis Type **2**) tumor suppressor gene encodes the FERM (**4**.1 protein/**E**zrin/**R**adixin/**M**oesin) domain protein Merlin, which is coordinately regulated by intercellular adhesion and attachment to the extracellular matrix [3–7]. Cadherin engagement inactivates PAK, causing an accumulation of the active, dephosphorylated form of Merlin. Notably, since integrin attachment to the extracellular matrix activates PAK, Merlin can therefore be regulated independently of contact-mediated signaling events [7].

Neurofibromatosis type 2 patients carry a single mutated *NF2* allele and develop a highly specific subset of central and peripheral nervous system tumors. NF2-associated tumors include schwannomas, meningiomas, and ependymomas, which arise from the Schwann cells comprising the myelin sheath surrounding sensory and motor neurons, arachnoid cap cells within the arachnoid villi in the meninges, and ependymal cells lining the CSF-filled ventricles of the brain and central spinal canal, respectively. In addition to the autosomal dominant NF2 disorder, non-germline Merlin deficiency is a driving force in sporadic occurrences of nearly all vestibular schwannomas, a majority of meningiomas, and a notable fraction of ependymomas. Moreover, Merlin was found to be inactivated in a large proportion of malignant mesotheliomas [8,9] and to a lesser extent in other solid tumors [10–13].

Due in large part to Merlin's high sequence homology to the ERM (**E**zrin/**R**adixin/**M**oesin) family of cytoskeletal linker proteins, it was widely assumed that Merlin suppresses mitogenic signaling at the cell cortex to mediate contact inhibition and tumor suppression [14]. Active Merlin suppresses Rac-PAK signaling [7,15–17], restrains activation of mTORC1 independently of Akt [18,19], inhibits PI3K-Akt and FAK-Src signaling [20,21], and negatively regulates the EGFR-Ras-ERK pathway [22,23]. However, the mechanisms by which Merlin inhibits these pathways remain unclear, and furthermore, the relative contribution of these pro-mitogenic signals to various Merlin-deficient malignancies is unknown. Interestingly, Merlin activates the Hippo tumor suppressor pathway to suppress the transcriptional coactivators YAP/TAZ in mammals or the *Drosophila* ortholog Yorkie, revealing a conserved role for Merlin in regulation of organ size, stem cell behavior, and cell proliferation [24–26]. Notably, few germline or somatic mutations of

* Fax: +1 212 639 3917 (F.G. Giancotti).
E-mail addresses: cooperj1@mskcc.org (J. Cooper), F-Giancotti@ski.mskcc.org (F.G. Giancotti).

http://dx.doi.org/10.1016/j.febslet.2014.04.001
0014-5793/

Hippo pathway components have been discovered in human tumors – the exception being Merlin, which remains as one of the only bona fide tumor suppressors in the Hippo pathway.

Merlin's upstream regulation has been extensively characterized, and recently reported models of regulation and post-translational modification will be highlighted in this Review. Post-translational modification of Merlin is vitally important to its conversion between dormant and growth-inhibitory states, where the dephosphorylated and more open state is now considered to be active in contact inhibition and tumor suppression [27]. However, without crystallographic analyses of full-length Merlin in both its active and inactive conformations, structural inferences drawn from biochemical experiments must be approached with caution. Apropos of the myriad upstream signals and modifications affecting Merlin, the downstream biochemical functions of Merlin have been the subject of intensive research for two decades, yet a consensus mechanism for Merlin's function in normal tissues has not been reached. It is becoming apparent that Merlin functions primarily in two branches – contact inhibition of proliferation and tumor suppression. Although these branches are naturally intertwined, the distinct locations of Merlin function and respective interactors in those subcellular compartments lend credence to a concept of a bimodal function.

Recent studies among the ever-increasing breadth of NF2 literature have revealed important high-affinity interactors governing Merlin's biochemical function. Notably, Merlin regulates tight junction-associated Angiomotin to inhibit Rac signaling [28], and nuclear-localized Merlin inhibits the $CRL4^{DCAF1}$ E3 ubiquitin ligase to suppress oncogenic gene expression [29]. Moreover, Merlin's regulation of YAP/TAZ is a burgeoning and highly provocative field due to the multifarious roles of Hippo signaling in organ growth, stem cell maintenance, and cancer. Recent studies have also shed light on Merlin's conformational changes that regulate its intramolecular associations and downstream signaling, providing fundamental insight into Merlin's regulation and biochemical function. In this Review, we will explore Merlin's rich biochemical background and examine new insights that are shaping our understanding of Merlin's role in normal biology and how its loss leads to the deregulation of a multitude of signaling pathways leading to tumorigenesis.

2. The NF2 gene

NF2 maps to the long arm of chromosome 22 and encodes two Merlin isoforms. The longer, dominant Merlin isoform 1 (Merlin-1 or Merlin), has an extended carboxy-terminal tail that is encoded by exon 17 (Fig. 1A). Merlin isoform 2 (Merlin-2), on the other hand, contains the alternatively spliced exon 16 which ends in a stop codon, encoding 11 unique residues following amino acid 579 as compared to Merlin-1 [10]. Notably, Merlin-2 lacks carboxy-terminal residues required for intramolecular binding between the amino-terminal FERM domain and the carboxy-terminal tail, possibly leading to a constitutively open conformation [30,31]. Recent studies have found that Merlin-2 inhibits cell proliferation and attenuates downstream mitogenic signaling to the same extent as Merlin-1 [27,32].

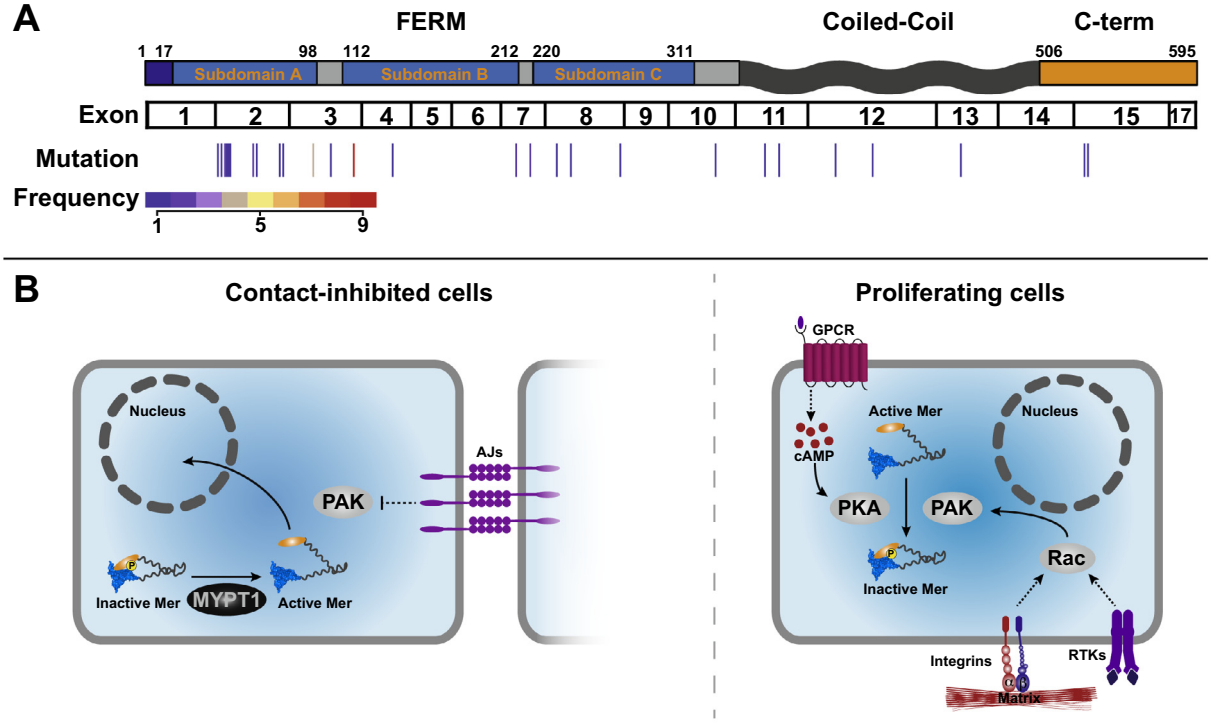

Fig. 1. (A) Merlin structure and overview of pathogenic mutations. Merlin is a 595-residue protein divided into three structurally distinct regions – an amino-terminal FERM domain, an α-helical coiled-coil domain, and a carboxy-terminal hydrophilic tail. Merlin's FERM domain is further subdivided into three globular subdomains. *Nf2* encodes 16 exons, terminating with exon 17 in the canonical Merlin isoform. The positions of patient-derived missense mutations or single residue deletions are indicated below the exon schematic, with mutation frequency indicated by a heat map. Either these mutations underlie NF2 tracked within a family, were found in two or more unrelated patients, or experimental evidence was obtained to confirm their pathogenicity. Mutational data were obtained from [48] and [29]. (B) Adhesion-mediated regulation of the Merlin activation cycle. (Left) In contact-inhibited cells, dephosphorylated Merlin accumulates as a result of intercellular adhesions which lead to PAK inhibition. Merlin may also be activated via MYPT1-mediated dephosphorylation. (Right) Conversely, in proliferating cells, integrin-mediated anchorage to the cell matrix and stimulation of receptor tyrosine kinases (RTKs) activate Rac, in turn activating PAK and leading to phosphorylation of Merlin at Serine 518. In response to high cyclic AMP levels, PKA also phosphorylates Merlin at serine 518. Serine 518 phosphorylation increases the interdomain binding between Merlin's carboxy-terminus and FERM domain, maintaining Merlin in a more closed, inactive form.

Please cite this article in press as: Cooper, J. and Giancotti, F.G. Molecular insights into *NF2*/Merlin tumor suppressor function. FEBS Lett. (2014), http://dx.doi.org/10.1016/j.febslet.2014.04.001