The FERM family proteins in cancer invasion and metastasis

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1. ABSTRACT

Metastasis is the major cause of death in patients with cancer. Metastatic cancer cells undergo dramatic molecular and cellular changes by remodeling their cell-cell and cell-matrix adhesion and their actin cytoskeleton, molecular processes that involve the activity of various signaling networks. The FERM family proteins can link transmembrane proteins to the cytoskeleton or link kinase and/or phosphatase enzymatic activity to the plasma membrane. They thus are involved not only in cell-extracellular matrix interactions and cell-cell communication but also in apoptosis, carcinogenesis and metastasis. This review will summarize the role and mechanism of FERM protein, with particular reference to the ERM and Ehm2 proteins in cancer metastasis.

2. INTRODUCTION

Metastasis, the spread of cancer from its place of origin to a secondary site, is the major cause of death in patients with cancer. Therefore, identification of the genes underlying the mechanisms of metastasis is of great interest. Tumour metastasis consists of a series of discrete biological processes known as the metastatic cascade (1). The metastatic cascade involves the detachment of cells from the primary tumour and invasion into the surrounding tissue, intravasation into the bloodstream or lymphatic system where they are transported, arrest in a capillary bed, extravasation and, finally, the survival and division of the tumour cells at the new site (2). Cell migration and invasion, critical parameters in the metastatic dissemination of cancer cells and the formation of metastasis, consist of cytoskeleton remodeling and changes in tumour cell adherence to cells and to the extracellular matrix (ECM), proteolytic degradation of surrounding tissue and motility to physically propel a tumour cell through tissue (3).

Just as the interplay of tumour suppressor and promoter genes makes a cell become cancerous, the development of metastasis requires a combination of genes whose altered expression patterns allow its progression through the metastatic cascade. Proteins involved in mediating cell invasion and metastasis have been identified and many of these proteins contain highly conserved protein interaction domains. One such family of proteins are those containing a FERM (4.1 protein, Ezrin, Radixin, Moesin) domain which functions as a protein docking surface with the cytosolic tail of transmembrane proteins such as CD44 (4).

The abbreviation FERM recognizes the first four proteins identified of the FERM protein superfamily. The first one, four-point-one protein, was identified from red blood cells and is an integral part of the membrane-associated spectrin-actin cytoskeleton and links red cell plasma membrane to the cytoskeleton, which helps maintain the shape of red blood cells and membrane stability (5-7). A second group of FERM proteins identified between 1983 and 1991 are the ERM proteins Ezrin, Radixin, and Moesin (8-10). These proteins are found in actin-rich cortical domains of epithelial cells such as microvilli and the cytocortex of other cells, and have both structural and signaling functions (11). Up to now, almost 50 FERM proteins have been found (Table 1). Many of them can be divided into Merlin/ERM (Ezrin, Radixin and Moesin) family, Protein 4.1 family, NBL4 family, Talin family, PTPH1 family, Tyrosin kinase family. All of these proteins are involved in maintaining the submembrane cytoskeleton. In some cases, they directly link transmembrane proteins to the cytoskeleton or link kinase and/or phosphatase enzymatic activity to the plasma membrane (12). Therefore, it has been suggested that these proteins are involved not only in cell-extracellular matrix interactions and cell-cell communication but also in apoptosis (13), carcinogenesis and metastasis (14).

FERM proteins in cancer

Table 1. Members of the FERM family proteins and their associated proteins.

Subgroup	Gene names	Proteins	Comments: function
Protein 4.1 family	EPB41	Protein 4.1 (4.1R, Band 4.1, EPB4.1)	A major structural element of the erythrocyte membrane skeleton.
	EPB41L1	Band 4.1-like protein 1(4.1N)	May function to confer stability and plasticity to neuronal membrane via multiple interactions, including the spectrin-actin-based cytoskeleton, integral membrane channels and membrane-associated guanylate kinases.
	EPB41L2	Band 4.1-like protein 2 (4.1G)	Interacts with FCGR1A. Interacts with TRPC4. The CTD domain interacts with FKBP2.
	EPB41L3(DAL1)	Band 4.1-like protein 3 (4.1B)	Critical growth regulator in the pathogenesis of meningiomas.
Merlin/ERM family	EZR(VIL2)	Ezrin (p81,Cytovillin,Villin-2)	In epithelial cells, required for the formation of microvilli and membrane ruffles on the apical pole. Along with PLEKHG6, required for normal macropinocytosis.
	MSN	Moesin(Membrane-organizing extension spike protein)	Probably involved in connections of major cytoskeletal structures to the plasma membrane.
	RDX	Radixin	Probably plays a crucial role in the binding of the barbed end of actin filaments to the plasma membrane.
	NF2 (SCH)	Merlin(Moesin-ezrin-radixin-like protein, Neurofibromin-2)	Nf2 is a tumour suppressor linked to neurofibromatosis and schwannomatosis.
NBL4 family	EPB41L4A (NBL4)	Band 4.1-like protein 4A (EPB41L4)	
	EPB41L4B (Ehm2)	Band 4.1-like protein 4B	Ehm2 may promote metastasis.
Talin family	TLN1(TLN)	Talin-1	Probably involved in connections of major cytoskeletal structures to the plasma membrane.
	TLN2	Talin-2	As a major component of focal adhesion plaques that links integrin to the actin cytoskeleton, may play an important role in cell adhesion.
PTPH1 family	PTPN3 (PTPH1)	Tyrosine-protein phosphatase non-receptor type 3 (PTP-H1)	May act at junctions between the membrane and the cytoskeleton. PTPN3 is a potential tumour suppressor gene.
	PTPN4	Tyrosine-protein phosphatase non-receptor type 4 (PTPase- MEG1)	May act at junctions between the membrane and the cytoskeleton.
	PTPN13 (PTPL1)	Tyrosine-protein phosphatase non-receptor type 13 (PTPE1, PTPL1, FAP-1)	Tyrosine phosphatase which regulates negatively FAS-induced apoptosis and NGFR-mediated pro-apoptotic signaling.
	PTPN14 (PEZ)	Tyrosine-protein phosphatase non-receptor type 14 (Protein-tyrosine phosphatase pez)	
	PTPN21 (PTPD1)	Tyrosine-protein phosphatase non-receptor type 21 (Protein-tyrosine phosphatase D1)	
Tyrosin kinase family	FAK1(FAK, PTK2)	Focal adhesion kinase 1(Protein-tyrosine kinase 2)	Non-receptor protein-tyrosine kinase implicated in signaling pathways involved in cell motility, proliferation and apoptosis.
	FAK2 (PYK2, PTK2B, RAFTK)	Protein-tyrosine kinase 2-beta (RAFTK, CAK-beta)	Involved in calcium induced regulation of ion channel and activation of the map kinase signaling pathway.
	JAK1	Tyrosine-protein kinase JAK1	
	JAK2	Tyrosine-protein kinase JAK2	JAK2 is linked to Budd-Chiari syndrome, polycythemia vera, essential thrombocythemia, familial myelofibrosis and acute myelogenous leukemia.
	JAK3	Tyrosine-protein kinase JAK3	JAK3 is linked to severe combined immunodeficiency.
	TYK2	Non-receptor tyrosine-protein kinase TYK2	TYK2 is linked to autosomal recessive hyper-IgE syndrome (HIES) with atypical mycobacteriosis.
others	FERMT1(KIND1, URP1)	Fermitin family homolog 1(Unc-112-related protein 1,Kindlin-1,Kindlerin,Kindlin syndrome protein)	Involved in cell adhesion. FERMT1 is linked to Kindler syndrome.
	FERMT2(KIND2, MIG2, PLEKHC1)	Fermitin family homolog 2 (Kindlin-2, MIG-2)	Participates in the connection between ECM adhesion sites and the actin cytoskeleton and also in the orchestration of actin assembly and cell shape. modulation.
	FERMT3(KIND3, MIG2B, URP2)	Fermitin family homolog 3(Unc-112-related protein 2 Kindlin-3 MIG2-like protein)	Plays a central role in cell adhesion in hematopoietic cells.
	FRMD1	FERM domain-containing protein 1	
	FRMD3 EPB41L4O	FERM domain-containing protein 3	Putative tumour suppressor gene that may be implicated in the origin and progression of lung cancer.
	FRMD4A(FRMD4)	FERM domain-containing protein 4A	
	FRMD4B	FERM domain-containing protein 4B (GRP1-binding protein GRSP1)	Member of GRP1 signaling complexes that are acutely recruited to plasma membrane ruffles in response to insulin receptor signaling. May function as a scaffolding protein.

FRMD5	FERM domain-containing protein 5	
FRMD6	FERM domain-containing protein 6	
FRMD7	FERM domain-containing protein 7	May play a specific role in the control of eye movement and gaze stability.
FRMD8 (FKSG44)	FERM domain-containing protein 8	
FARP1 (CDEP, PLEKHC2)	FERM, RhoGEF and pleckstrin domain-containing protein 1	May function as Rho-guanine nucleotide exchange factor.
FARP2 (PLEKHC3)	FERM, RhoGEF and pleckstrin domain-containing protein 2 (FIR)	Rho-guanine nucleotide exchange factor that activates RAC1. Plays a role in the response to class 3. semaphorins and remodeling of the actin cytoskeleton.
FRMPD1 (FRMD2)	FERM and PDZ domain-containing protein 1	Stabilizes membrane-bound GPSM1, and thereby promotes its interaction with GNAI1.
FRMPD2 (PDZD5C, PDZK4, PDZK5C)	FERM and PDZ domain-containing protein 2	May play a role in the regulation of tight junction formation. Binds phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P2).
FRMPD3	FERM and PDZ domain-containing protein 3	
FRMPD4 (PDZD10, PDZK10)	FERM and PDZ domain-containing protein 3	Positive regulator of dendritic spine morphogenesis and density. Required for the maintenance of excitatory synaptic transmission. Binds phosphatidylinositol-4,5-bisphosphate.
KRIT1 (CCM1)	Krev interaction trapped protein 1 (Cerebral cavernous malformations 1 protein)	Defects in KRIT1 are the cause of cerebral cavernous malformations type 1 (CCM1).
MYLIP	E3 ubiquitin-protein ligase MYLIP (MIR)	Mediates ubiquitination and subsequent proteasomal degradation of myosin regulatory light chain (MRLC).
MYO7A(USH1B)	Myosin-VIIa	Myosins are actin-based motor molecules with ATPase activity.
MYO7B	Myosin-VIIb	Myosins are actin-based motor molecules with ATPase activity.
MYO10	Myosin-X	Unconventional myosins serve in intracellular movements.
MYO15A (MYO15)	Myosin-XV	Unconventional myosins serve in intracellular movements.
PLEKHH1	Pleckstrin homology domain- containing family H member 1	
PLEKHH2	Pleckstrin homology domain- containing family H member 2	
PLEKHH3	Pleckstrin homology domain- containing family H member 3	

Data in this table were compiled according to the database: http://www.uniprot.org

The FERM domain is approximately 300 amino acid residues in length. The structure of several FERM domains has been resolved either individually or in combination with binding partners (15-18). The FERM domain consists of three subdomains that fold independently but are closely associated with one another and together form a cloverleaf with lobes of approximately 100 amino acids each. The subdomains are referred to as F1, F2, and F3 (various other designations for the three subdomains are in use including FERM-N, FERM-M, and FERM-C by the Pham protein database). The N-terminal F1 lobe is structurally related to ubiquitin, the central F2 lobe is folded like an acyl-CoA binding protein and the C-terminal F3 subdomain is similar to a pleckstrin homology (PH)/phosphotyrosine binding (PTB) domain. The three lobes of the FERM domain can interact with numerous protein-binding partners, including transmembrane ion channels (e.g. NHERF, NHE1) (19, 20) and adhesion molecules (e.g. ICAM, CD44) (21, 22) and cytoplasmic proteins such as calmodulin and membrane-associated guanylate kinases (MAGUKs) (23). In addition, FERM domains can interact with selected membrane lipids such as phosphatidylinositol-4,5-biphosphate (PIP2) (24). Thus, the FERM domain can act as an adaptor or scaffolding unit that integrates the activities of multiple membrane-associated factors (25).A special interest for the F3 domain is that it is important in target protein binding (18) and is the site of intramolecular regulation by the C-terminal domain of some FERM proteins (15).

The roles of certain members of the FERM family proteins in cancer have been extensively studied. They may be involved in the progress of cancer, such as growth, apoptosis, adhesion, invasion and metastasis. Here we will review the functions and mechanisms of some FERM proteins (Merlin/ERM proteins, protein 4.1B, NBL4 family member Ehm2, FAK) in cancer invasion and metastasis.

3. MERLIN/ERM PROTEINS IN CANCER METASTASIS

The Merlin/ERM proteins are a family of wildly distributed membrane-associated proteins that regulate the structure and function of specific domains of the cell cortex. This family consists of Ezrin, Radixin, Moesin, and Merlin. Ezrin was originally identified as a component of structures at the cell surface that contain an actin cytoskeleton, such as microvilli and membrane ruffles (8, 26), and as a protein substrate of special tyrosine kinases (27). Radixin was isolated from liver-cell adherens junctions, but seems to be primarily concentrated in the microvilli of bile canaliculi (9, 28). Radixin was identified as a protein that binds heparin, a glycosaminoglycan (29).
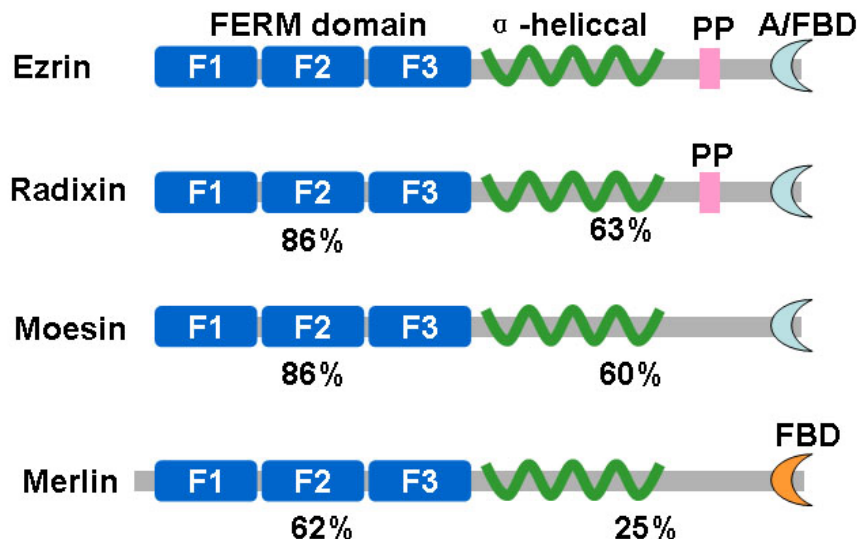
Figure 1. Domain organization and domain homology among Merlin/ERM proteins. The amino-terminal regions contain an about 300 residue FERM domain. Sequence identity to Ezrin is shown. ERM proteins show very high sequence identity, whereas Merlin is more divergent. All Merlin/ ERM proteins can form an intramolecular association through their N terminal FERM domains and C-ERMADs. ERM proteins have a carboxy-terminal filamentous (F)-actin binding domain except Merlin does not. The region between the FERM domain and C-ERMAD form an alpha-helical coiled-coil. Mammalian Ezrin and Radixin have a proline-rich region (PP). C-ERMAD: carboxy-ERM association domain; ERM: Ezrin, Radixin, Moesin; FERM: Four-point one, Ezrin, Radixin, Moesin; A/FBD: actin/FERM binding domain; FBD: FERM binding domain.

Merlin, also named schwannomin, is a product of the *Neurofibromatosis 2* (*NF2*) tumour suppressor gene (30). Merlin/ERM proteins are highly homologous and show similar domain organization (Figure 1). The conserved residues in the NH_2- and $COOH$-termini of the Merlin/ERM proteins constitute NH_2- and $COOH$-ERM association domains (N- and C-ERMADs), respectively which are responsible for mediating the observed head-to-tail association. Increasing evidence indicates that Merlin/ERM proteins can simultaneously provide regulated linkage between membrane proteins and the actin cytoskeleton, and control the surface availability of certain membrane receptors (Figure 2). They have been implicated in the determination of cell shape, membrane organization, cell polarization, migration, division and they participate in various signaling pathways. More and more evidence shows that Ezrin is highly involved in the metastasis of various tumours. The role of Moesin in tumour invasion is less documented whereas, so far, Radixin has not been implicated in this process. Merlin, a tumour suppressor, may repress the spreading of tumour cells.

3.1. Ezrin promotes cancer cell metastasis

Ezrin (cytovillin/p81/80k/Villin-2), firstly characterized as a component of microvilli in a variety of cell types, is considered the prototype member of the ERM protein family due to conserved wide spread distribution and highly homologous sequences in the FERM domain. Ezrin is localized not only at the juxta-membrane region but also in soluble cytosolic pools. The dynamic localization between these two compartments is important to determine the activity of Ezrin and is responsible for some cellular events (31, 32). Ezrin is inactive when its N-terminal FERM domain is associated with its C-ERMAD, which masks transmembrane protein binding sites in its N-terminal domain and F-actin-binding sites in the C-terminal region. Activation of Ezrin can occur by phosphorylation of threonine residues in the C-terminal domain. After stimulation by growth factor or other cellular signaling events, Ezrin is activated by binding to phosphatidyl inositol-4,5- bisphosphate (PIP2) through its N-terminal FERM domain and phosphorylation at conserved C-terminal Thr^{567} (33). Then it translocates to the membrane/cytoskeleton interface and changes its conformation to bind various membrane associated adhesion molecules and ion exchangers to the N-terminal and polymerized F-actin via its C-terminal domain, becoming fully functional (34). Ezrin plays pleiotropic functions in cellular physiological processes such as cell adhesion, motility, apoptosis and phagocytosis.

In the recent years, there has been increasing evidence that Ezrin is actively involved in the metastatic spread of various neoplasms. Overexpression and activation of Ezrin is closely related to the metastatic potential of different types of tumours, such as osteosarcoma, hepatocellular carcinoma, breast cancer, lung cancer and pancreatic carcinomas (35-41). As a linker between transmembrane proteins and cytoskeleton, Ezrin is involved in tumour metastasis by various signaling pathways and cellular processes. In some cases, Ezrin contributes to the metastatic phenotype by providing a physical connection between the plasma membrane and the actin cytoskeleton. For example, in metastatic melanoma, Ezrin mediates aberrant linkage of the cytoskeleton to various proteins, including CD44 and LAMP-1, inducing
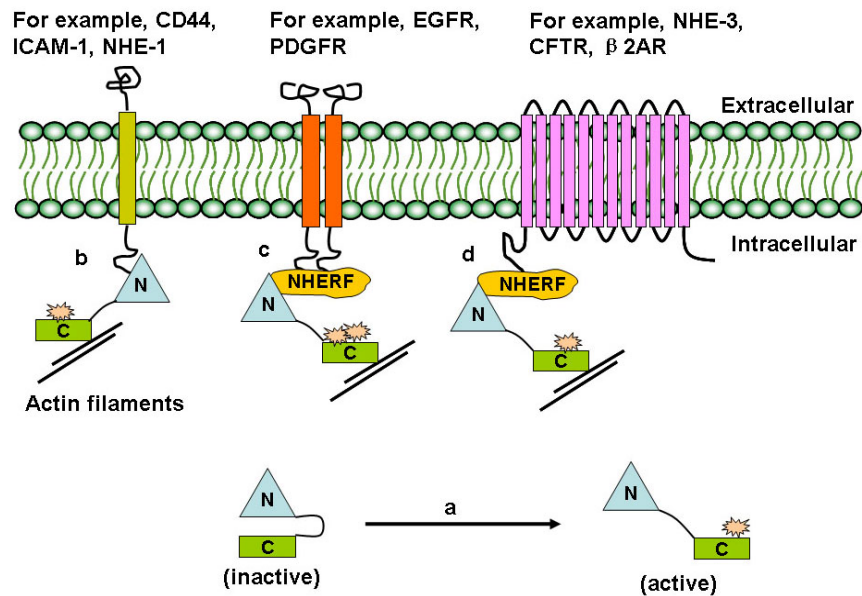
Figure 2. A model for the activation and function of Merlin/ERM proteins. Merlin/ERM proteins exist in a dormant, monomeric form in which the FERM domain is associated with the C-ERMAD. (a) Merlin/ERM proteins are activated by binding to phosphatidyl inositol-4,5- bisphosphate (PIP2) through its N-terminal FERM domain and phosphorylation at conserved C-terminal Thr by Rho-kinase or protein kinase C. (b) Activated Merlin/ERM protein can participate in membrane-cytoskeleton linkage by direct association with transmembrane proteins such as CD43, CD44, ICAM-1, ICAM-2, and so on. (c) Merlin/ERM proteins can associate with the tandem PDZ-domain-containing adapter NHERF-1, which, in turn, can directly associate with several receptors including single-pass receptors such as EGFR or PDGFR and multipass receptors such as NHE3 or CFTR (d).

marked changes in the general framework of cellular function (42). This leads also to an aberrant engagement with the extracellular microenvironment, which is directly involved in metastatic behaviour of tumour cells. In another cases, Ezrin contributes to the formation of specialized cell-surface domains assisting in both the localization of molecules in these structures and the transduction of signals from the extracellular to the intracellular microenvironment (32). For example, in CCR9-expressing acute T lymphocytic cell line MOLT4, blocking of Ezrin function induced E-cadherin to redistribute on the cell membrane, implying that down-regulation of Ezrin may inhibit metastasis of MOLT4 cells mediated by CCR9, and E-cadherin may be an effector in this process (43).

As a pleiotropic molecule, Ezrin coordinates and integrates important membrane-tyrosine-kinase-mediated signaling events and through the regulation of its tyrosine and threonine phosphorylation it mediates the signal transduction pathways orchestrated by MAPK, AKT/PKB and Rho GTPase (13, 44, 45). In prostate cancer cell, c-Myc induces cell invasion and anchorage-independent growth by regulating Ezrin protein expression in the presence of androgens. And reversely Ezrin regulates c-Myc protein levels by its phosphorylation, which results in the regulation of Akt and GSK-3beta activity, resulting in increased c-Myc protein synthesis and inhibition of its degradation (45). Ezrin might play functional roles in modulating morphology, growth, motility and invasion of pancreatic cancer cells, and that the Erk1/2 pathway may be involved in these roles (41).

Ezrin also promotes tumour metastasis by other mechanisms. For example, Ezrin is important for the interaction between hepatocyte growth factor (HGF) and its receptor c-Met, and Ezrin overexpression has been related to the HGF/c-Met associated tumour metastasis (37). Overexpression of Ezrin inactivates NF2 tumour suppressor in glioblastoma and delocalizes NF2 from the cortical compartment releasing its inhibition on Rac1, leading to decreased cell proliferation and motility (46).

Thus, Ezrin exerts multiple effects on metastastic cascade, including (i) heterotypic adhesion, (ii) tumour-cell extravasation (e.g., into the lung parenchyma), (iii) invasiveness, (iv) adhesion between cancer cells and the surrounding tissue, and (v) reduced susceptibility to apoptotic stimuli (21, 36, 47).

3.2. Moesin promotes cancer cell metastasis
Moesin, a member of the ERM family, is an actin-binding protein that plays an imperial role in cell motility by linking the actin cytoskeleton to a variety of membrane-anchoring proteins (10, 48). Like Ezrin, the activity of Moesin is negatively regulated by intramolecular association between its N-terminal FERM and C-terminal domains thus preventing its binding to F-actin and membrane proteins. Conformational changes triggered by the binding of the FERM domain to PIP2 followed by the phosphorylation of the conserved C-terminus Thr^{558} regulate its association with both the membrane and the actin cytoskeleton. When activated through phosphorylation of Thr^{558}, Moesin induces actin

depolymerization and reassembly toward the cell membrane edge (49). Actin remodeling is involved in cancer transformation and metastasis (50). It is the primary step, not only for cancer cell metastasis (51), but also for endothelial and neuron cell migration (52, 53). Moesin is therefore also involved in tumour metastasis (54, 55). For example, Moesin gene expression is shown to be strongly associated with metastatic phenotypes of cervical cancer (56). Moesin is strongly upregulated in metastatic breast cancer cells and is misregulated in cancers with a poor prognosis (57-60).

As a crosslinker between membrane proteins and actin cytoskeleton, Moesin can coordinate and integrate some important signaling pathways induced by growth factors and cytokines. VEGF-C upregulates and activates Moesin protein through RhoA/ROCK-2 pathway, then leads to enhanced cell motility and promotes cervical cancer metastasis (54).

In some cases, Moesin plays important role in invasion of cancer cells. Using short interfering RNA (siRNA), Estecha *et al.* showed that Moesin was crucial for invasion by melanoma cells in 3D matrices and in early lung colonization. Using live imaging, they showed that following initial adhesion to the endothelium or 3D matrices, Moesin was redistributed away from the region of adhesion, thereby generating a polarized cortex: a stable cortical actin dome enriched in Moesin and an invasive membrane domain full of blebs. Polarized Moesin played a role in orienting Rho activation, Myosin II contractility, and cortical actin stability, which is crucial for driving directional vertical migration instead of superficial spreading on the fluid-to- solid tissue interface. This mechanism of cortical polarization could sustain extravasation of fluid-borne tumour cells during the process of metastasis (55).

Although Moesin has overlapping functions with Ezrin due to high sequence similarity, they also display some contrasting role, depending on the cell types and cellular locations. Estecha *et al.* explored the role of Ezrin and Moesin in melanoma cell invasion of 3D collagen matrices and in transmigration through endothelial cells. They showed that following early attachment of the cells to the collagen matrix or to the endothelium, Moesin distribution was clearly polarized, accumulating to the dorsal surface of the cell, and being excluded from the attachment point. In contrast, Ezrin distribution was found in blebs at the sites of cell invasion. Moreover, whereas Moesin-depleted cells were less invasive in 3D collagen matrix, Ezrin-depleted cells acquired an elongated shape and were more prone to 3D matrix invasion (55).

Although a few studies clearly provide substantial progress in the understanding of Moesin function during cell invasion *in vitro* and *in vivo*, the roles of Moesin in tumours are inconsistent. In a study by Ichikawa *et al.*, Moesin level was found to be elevated in benign melanocytic naevi and malignant melanomas but was significantly reduced in invasive melanomas and metastasis (48). Moesin was not expressed in normal pancreatic ducts, exocrine acinar cells and islets. In pancreatic cancer, however, Moesin proteins were localized in the membrane and cytoplasm of tumour cells, and 66.7% of cancer tissues were positive with Moesin immunoreactivity. Moesin expression levels of cancer cells in lymph node metastasis (LNM)-positive tumours were significantly higher than those in LNM-negative tumours (61). While another study showed that Moesin knock-down increased migration, invasion, and metastasis and influenced extracellular matrix organization of pancreatic cancer cell (62). In the normal skin and the melanocytic tumours, the expression pattern of Moesin was largely similar to that of CD44 standard. In about two-thirds of the patients with metastatic lymph node, homogeneous cytoplasmic expression was detected in the metastatic lymph nodes. In addition, the squamous cell carcinoma cell line SQUU-B with high metastatic potential showed more reduced levels of membrane-bound Moesin than SQUU-A with low metastatic potential (60). Taken together, the functions of Moesin in tumours are diverse depending on the type of tumours and need to be studied further.

3.3. Tumour suppressor Merlin inhibits tumour metastasis

Tumour suppressor gene neurofibromatosis-2 (NF2) was identified in neurofibromatosis type 2, a familial cancer syndrome that features the development of multiple nervous system tumours including schwannomas and meningiomas (30, 63). The *NF2* gene is located on human chromosome 22q12 (64) and alterations of the gene have been detected in the germline of NF2 patients and in sporadic NF2-associated tumours (65). It has been documented that mutations and deletions of the *NF2* gene lead to development of NF2-associated tumours and that loss of heterozygosity (LOH) of the gene is associated with sporadic schwannomas, ependymomas, and meningiomas (66-68). The *NF2* gene mutations have also been found in thyroid cancer, mesotheliomas, and melanoma albeit less frequently (69). The *NF2* gene encodes a protein called Merlin or schwannomin that displays significant homology to ERM family proteins (70). Merlin and ERM proteins share a similar molecular structure comprised of 3 functional domains: an amino (N)-terminal FERM domain, an alpha-helical region, and a carboxyl (C)-terminal domain (12). Merlin lacks the C-terminal F-actin binding region that is present in the other ERM proteins (71) but contains a unique N-terminal 17 residue sequence that confers localization to cell-cell boundaries (72). 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 (16, 73). Head-to-tail self-association of Merlin results in the "closed" conformation, which is required for its tumour suppressor activity (74). Phosphorylation of Merlin at its COOH terminus especially at Ser[518] abolishes the head-to-tail self-association and leads to an "open" conformation and loss of tumour suppressor activity (75, 76). Several kinases including p21-activated kinase 1 and 2 (PAK1/2) and cAMP dependent protein kinase A (PKA) phosphorylate Merlin at Ser[518], which leads to the open inactive conformation (75, 77-79). Phosphorylation of Merlin at serine 10 of its N terminus by PKA site can also

regulate its activity. Mutation of serine 10 (S10A) affects not only cellular morphology, but also actin cytoskeleton organization and dynamics *in vivo*, as Merlin S10A reduces the amount of cellular F-actin and Merlin S10D stabilizes F-actin filaments. Dephosphorylation of serine 10 leads to defects in migration, possibly through altered ability of the cells to form lamellipodia (78).

Like the ERM proteins, Merlin has been implicated in the regulation of membrane organization and cytoskeleton-based cellular processes such as adhesion, migration, cell-cell contact, spreading, proliferation, and signal transduction (80-82). Unlike ERM proteins, Merlin functions as a negative growth regulator or tumour suppressor. Merlin is a multifunctional protein and involved in integrating and regulating the extracellular cues and intracellular signaling pathways. Accumulating evidence indicates that Merlin plays an essential role in regulating cell morphology and motility (83) and that loss of Merlin results in dramatic changes in actin cytoskeleton organization and cell adhesion (12). Tumours developed in the heterozygous NF2 knockout mice (NF2+/-) are highly motile and display metastatic proclivity (84). Almost all schwannoma cells display disorganized stress fibers, altered spreading, and increased membrane ruffling (85, 86), which can be reversed by re-expression of Merlin (86). Studies have shown that Merlin inhibits actin assembly induced by Arp2/3 and Rac (87, 88). Even though a recent study suggested that the tumour-suppression function of Merlin is independent of its role as an organizer of the actin cytoskeleton in Schwann cells (81), other studies have clearly demonstrated an essential role of Merlin in inhibiting tumour cell motility and invasion (76, 89). Loss of NF2 function is associated with the development of multiple cancers in humans and mice (76, 84, 90, 91). In humans, NF2 mutations are associated with familial and sporadic tumours of the nervous system and with other sporadic cancers such as mesothelioma, whereas heterozygous *Nf2* mutant mice develop bone, liver, and other tumours that are highly metastatic (92-94).

Although a few studies have indicated that Merlin is important in regulating cell motility and metastasis, the mechanism by which this occurs is still unclear. Overexpression of Merlin in mouse melanoma B16F10 cells changes/redistributes the cell surface heparan sulfate glycosaminoglycans (HSGAGs) and these specific changes of glycosaminoglycan organization reduce the metastatic activity of B16F10 cells (95). Re-expression of Merlin in malignant mesothelioma (MM) markedly inhibits cell motility, spreading and invasiveness of MM cells by attenuating FAK phosphorylation at the critical phosphorylation site Tyr^{397} and disrupts the interaction of FAK with its binding partners Src and p85, the regulatory subunit of phosphatidylinositol-3-kinase (89). Merlin is known to bind paxillin, beta1-integrin and focal adhesion kinase, members of focal contacts, multi-protein complexes that mediate cell adhesion to the extracellular matrix. In Merlin-deficient Schwannomas, showing pathological adhesion to the extracellular matrix, RhoA signaling via the Rho kinase ROCK and Rac1 plays significant roles in the pathological adhesion of Schwannoma cells (96).

4. PROTEIN 4.1B INHIBITS CANCER METASTASIS

Protein 4.1B belongs to the protein 4.1 subgroup, which includes four well-defined members: erythroid protein 4.1 (4.1R), the best-known and characterized member, 4.1G (general), 4.1N (neuronal), and 4.1 B (brain). Each protein 4.1 family member possesses three highly conserved domains: N-terminal FERM domain, spectrin-actin-binding domain (SABD), and C-terminal domain (CTD) (2) (97). In addition to these domains, 4.1 proteins possess several unique domains: U1, U2 and U3. Although the functions of these domains are not known, their sequences are distinct from each other and thus might specify unique protein interactions that underlie the functional differences between Protein 4.1 family members (98, 99). Protein 4.1B or type II brain 4.1 is a product of gene *Epb41l3*, originally isolated as the cDNA DAL-1 from a screen of lung adenocarcinomas (99). It is subcellularly localized at the plasma membrane in regions of cell-cell contact (98). Like other members of the 4.1 family, 4.1B is thought to be involved in tethering the F-actin cytoskeleton to membrane proteins (100).

4.1B, or a truncated form of this protein (DAL-1, known as Deleted in Adenocarcinoma of the Lung-1), is frequently lost in brain, lung, and breast cancers, suggesting a tumour suppressor role of the protein (99, 101-103). Loss of heterozygosity has been found in the chromosome 18p11.3 region where DAL-1 maps in 38% of lung, brain and breast tumours (99). Microarray analysis of rat sarcomas indicated that 4.1B is significantly downregulated in metastasising sarcoma cells. Further study showed that 4.1B may function as a metastasis suppressor by supporting orderly arrangements of actin stress fibres and suppressing the enhanced cell motility and chemotaxis associated with increased metastatic potential (2). In a screen for genes involved with prostate cancer metastasis, 4.1B was down-regulated in highly metastatic tumour cells. Loss of 4.1B promotes metastasis in an orthotopic xenotransplant model of prostate cancer. In the transgenic adenocarcinoma of the mouse prostate (TRAMP) tumour model, 4.1B deficient mice developed aggressive, spontaneous carcinomas at a significantly higher frequency than did 4.1B-heterozygous mice and that these tumours often metastasized to local lymph nodes (97).

The mechanisms by which 4.1B inhibits metastasis remain unclear. One study has reported that overexpression of 4.1B induces Rac1-dependent JNK signaling (104). Another work has also shown that 4.1B can interact with a potential tumour suppressor, {alpha}v{beta}8 integrin (105), and this interaction has been confirmed in PC-3 cells (97).

5. EHM2 PROMOTES CANCER METASTASIS

Ehm2 (for expressed in high-metastatic cells), belonging to the NBL4 subfamily, was first discovered in 1996 and its gene was cloned in 2000 (106, 107). Human Ehm2 was characterized in a human fibrosarcoma cell line model of steroid-regulated cytoskeletal reorganization and

shown to be androgen-regulated (108, 109). Sanjay Chauhan *et al.* performed tissue expression analysis of the human Ehm2 gene and found that there are two Ehm2 protein isoforms, isoform1 and 2. Isoform 1 is brain-specific, containing 913 amino acids, while isoform 2 exists in testes, prostate and breast. Compared with isoform1, isoform 2 misses the carboxy terminal region including an additional 409 amino acids of unknown function (108). Amino acid sequence comparisons of the Ehm2 subfamily proteins showed that Ehm2 proteins encode an extended F3 subdomain, which together with the F1 and F2 subdomains, constitutes an about 325 amino acid FERM domain spanning residues 70-407, lack of recognizable protein motifs in the about 100 amino acid C-terminus of Ehm2. This maybe suggest that the Ehm2 protein function as a constitutively active FERM signaling protein that is controlled by transcriptional regulation, rather than autoregulation involving intramolecular folding of subdomains.

Ehm2 was expressed in high- but not in low-metastatic K-1735 and B16 murine melanoma cells. It was proposed that alterations of the expression levels of Ehm2 are likely to be linked to one or more steps of cancer metastasis through regulation of interaction between cell surface transmembrane proteins and cytoskeletal proteins (106). The biological functions of Ehm2 and its roles in many cancers are not well-known. Wang *et al.* have reported that expression of the FGFR-4 Arg[388] variant results in increased expression of Ehm2 in prostate epithelial cells. They also found that Ehm2 expression is upregulated in prostate cancer cell lines and prostate cancer tissues. Increased expression of Ehm2 leads to decreased adhesion to collagen IV, which has been associated with metastasis in cancers. Analysis of tissue microarrays revealed increased Ehm2 expression that is associated with biochemical recurrence following radical prostatectomy, which is indicative of more aggressive disease (110). The *Drospholia* ortholog of Ehm2, called Yurt, has been shown to be required for epithelial cell migration during embryogenesis and loss of function Yurt mutations are lethal, suggesting that its mammalian homolog may play a role in cancer metastasis by altering cell migratory properties (111). Our recent study showed that increased Ehm2 expression correlated with poor prognosis and metastasis in breast cancers. Further study showed that Ehm2 promoted the invasion ability of breast cancer cells via regulation of MMP9 (112,113).

6. THE ROLE OF FAK IN TUMOUR METASTASIS

Focal adhesion kinase (FAK) is an approximately 120 kD nonreceptor tyrosine kinase. It is composed of an N-terminal FERM domain, a central kinase domain, and a C-terminal domain that includes the focal adhesion targeting (FAT) sequence responsible for FAK's localization to focal adhesions. The FERM domain of FAK has been described to be involved in interactions with other proteins, including the cytoplasmic region of integrins (114), the FERM domain of Ezrin (115), the pleckstrin homology domain of the Tec family kinase Etk (116), and the receptors for HGF (117), platelet-derived growth factor

(PDGF) and epidermal growth factor (EGF) (118). It also plays a critical role in the regulation of FAK activation through an intramolecular interaction with the kinase domain of FAK (119). Direct contact between the FERM domain and kinase domain of FAK masks its catalytic activity. Growth factors or the clustering of integrins facilitate the rapid autophosphorylation of Y^{397} and exposure of the docking site for Src family kinases, this in turn recruits Src-family protein tyrosine kinases, resulting in the phosphorylation of Tyr^{576} and Tyr^{577} in the FAK activation loop and full catalytic FAK (120,121). The mutually activated FAK/Src complex then initiates a cascade of phosphorylation events and new protein-protein interactions to trigger several signaling pathways. These FAK signaling pathways have been shown to regulate a variety of cellular functions both in normal and cancer cells.

FAK is an important protein tyrosine kinase that acts downstream of various growth factors and extracellular matrix components. It is involved in cytoskeleton remodeling, formation and disassembly of cell adhesion structures, and in the regulation of Rho-family GTPases. Therefore, FAK is widely accepted as an important promoter of directional cell movement. FAK plays a prominent role in tumour progression and metastasis through its regulation of both cancer cells and their microenvironments including cancer cell migration, invasion, epithelial to mesenchymal transition, and angiogenesis. A role for FAK in cell migration was first suggested by the observation of increased activation of FAK in the migrating keratinocytes in epidermal wound healing (122). Overexpression of FAK has been reported in a number of cancers, including metastatic prostate carcinoma, ovary cancer, hepatocellular carcinoma, breast cancer and colon cancer (123-127). In analyses of FAK expression in benign and malignant human breast and colon tissues, FAK expression is correlation with preinvasive and invasive phenotypes (125). Both FAK and phosphorylated FAK Tyr^{397} were overexpressed in hepatocellular carcinoma (HCC) samples, which were correlated with tumour stage, vascular invasion and intrahepatic metastasis in HCC. Furthermore, HCC cell adhesion, migration and invasion were substantially impaired by siRNA-mediated knockdown of FAK expression, whereas cell growth, apoptosis and cell cycle distribution were not affected (127).

The overexpression and increased activities of FAK are associated with motility and invasion of cancer cells, however the exact mechanism is still unknown. A number of downstream signaling pathways have been identified to mediate FAK-stimulated cancer cell migration. Autophosphorylation of FAK at Y^{397} and its association with Src at the site is essential for FAK's ability to promote cell migration. The migratory phenotype of colon cancer cells is controlled by the combined activities of Src and FAK, and the recruitment of FAK to adhesive sites results in its phosphorylation by Src and other peripheral tyrosine kinases (128). Another mechanism of FAK promotion of cell migration involves its interactions with phosphatidylinositol 3'-kinase (PI3K). A FAK mutant that

selectively disrupted its binding to PI3K, but retained binding to Src and induction of Cas phosphorylation, failed to promote cell migration (129). Inhibition of PI3K by wortmannin or LY294002 prevented FAK-stimulated cell migration (129). In human lung cancer cell line A549, increased phosphorylation of FAK by fibronectin led to the activation of its downstream targets, Src, ERK1/2, PI3K, and Akt, which caused the activation of MMP9/calpain-2 and MMP9/RhoA and induced lung cancer metastasis (130). Other signaling pathways also participate in FAK-stimulated cancer cell migration. In B16F10 melanoma cells, the Y925F-mutation of FAK suppressed metastasis by downregulation of the phosphorylation of Erk, the expression of VEGF, and the association of FAK with paxillin (131). FAK knockdown downregulated MMP-2 and MMP-9 expression levels and activities in HCC cells (127).

7. SUMMARY AND PERSPECTIVES

FERM family proteins play important roles in many cellular events, including tumour progress and metastasis, by acting as an adaptor or scaffolding unit that integrates the activities of multiple membrane-associated factors through their FERM domain. Here we only reviewed six kinds of FERM proteins, most of them are overexpressed in many cancers and promote cancer metastasis. Besides the proteins above mentioned, other FERM proteins also participate in cancer metastasis. For example, Talin1 overexpression enhanced prostate cancer cell adhesion, migration and invasion by activating AKT signals and anoikis resistance (132). The expression of nonreceptor tyrosine kinase Pyk2 was upregulated in invasive glioma cells and silencing Pyk2 expression inhibited glioma cell migration *in vitro* (133, 134). Mutations within the FERM domain, particularly those within the F3 module, significantly inhibited the activity of FERM proteins and this inhibition correlated with loss of stimulation of migration (131, 135). So FERM proteins have emerged as promising molecular targets for cancer therapeutic strategies. It would be interesting to determine the cooperativity and redundancy of FERM proteins in tumour progress and metastasis, which might be important for developing clinical drugs based on FERM domain.

8. ACKNOWLEDGEMENTS

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Abbreviations: FERM: Four-point-one protein, ezrin, radixin, moesin; ECM: extracellular matrix; ERM: ezrin, radixin, moesin; PH: pleckstrin homology; PTB: phosphotyrosine binding; MAGUK: membrane-associated guanylate kinase; C-ERMAD: carboxy-ERM association domain; A/FBD: actin/FERM binding domain; FBD: FERM binding domain; PIP2: phosphatidyl inositol-4,5-bisphosphate; HGF: hepatocyte growth factor; LOH: loss of heterozygosity; SABD: spectrin-actin-binding domain; CTD: C-terminal domain; LNM: lymph node metastasiss; FAK: focal adhesion kinase; FAT: focal adhesion targeting; PDGF: platelet-derived growth factor; EGF: epidermal growth factor; HCC: hepatocellular carcinoma.

Key Words: FERM protein, Invasion, Metastasis, Ezrin, Radixin, Moesin, Merlin, 4.1B, Ehm2, FAK, Review

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