

FERM family proteins and their importance in cellular movements and wound healing (Review)

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Received February 2, 2014; Accepted March 10, 2014

DOI: 10.3892/ijmm.2014.1775

Abstract. Motility is a requirement for a number of biological processes, including embryonic development, neuronal development, immune responses, cancer progression and wound healing. Specific to wound healing is the migration of endothelial cells, fibroblasts and other key cellular players into the wound space. Aberrations in wound healing can result in either chronic wounds or abnormally healed wounds. The protein 4.1R, ezrin, radixin, moesin (FERM) superfamily consists of over 40 proteins all containing a three lobed N-terminal FERM domain which binds a variety of cell-membrane associated proteins and lipids. The C-terminal ends of these proteins typically contain an actin-binding domain (ABD). These proteins therefore mediate the linkage between the cell membrane and the actin cytoskeleton, and are involved in cellular movements and migration. Certain FERM proteins have been shown to promote cancer metastasis via this very mechanism. Herein we review the effects of a number of FERM proteins on wound healing and cancer. We show how these proteins typically aid wound healing through their effects on increasing cellular migration and movements, but also typically promote metastasis in cancer. We conclude that FERM proteins play important roles in cellular migration, with markedly different outcomes in the context of cancer and wound healing.

Contents

1. Cancer and wound healing - similarities in cellular physiology and pathology
2. Cellular migration and wound healing
3. The FERM protein superfamily
4. The FERM domain
5. ERM proteins - background
6. ERM proteins and cancer

7. ERM proteins and wound healing
8. Protein 4.1R - background
9. Protein 4.1R in human disease and wound healing
10. Ehm2 - background
11. Ehm2 in human disease and wound healing
12. Conclusion

1. Cancer and wound healing - similarities in cellular physiology and pathology

Cellular migration is fundamental to both wound healing in health and cancer metastasis in disease. What separates these two diametrically opposed processes is the control and termination of this movement. When cellular migration is absent, wounded edges are stationary, resulting in chronic wounds; when migration is overactive, abnormal scarring results. In cancer, excessive cellular motility predisposes to metastasis, which is the cause of 90% of human cancer deaths (1,2). Epithelial-mesenchymal transition (EMT) refers to the morphological changes observed during embryogenesis and foetal development, cutaneous wound repair and neoplastic invasion and metastases (3,4). In response to wounding, keratinocytes at the wound edge are transformed from a sedentary to a migratory phenotype. They lose their cell-cell and cell-extracellular matrix (ECM) attachments, rearrange their actin cytoskeleton and express a variety of mesenchymal markers (such as vimentin) (5). However, in contrast to cancer progression, the changes observed in these wound edge keratinocytes are short lived and reversible (4). Maintaining this 'Goldilocks' type requirement of migration (not too much, not too little) is essential for health, and understanding which factors regulate such a process is of interest both to cancer biologists and wound healing scientists.

Historically, it was in the context of excessive inflammation, rather than excessive cellular migration, that the cross-over between cancer and wound healing was first recognised. As early as 1863, Virchow observed that excessive inflammation predisposed to cancer formation (6). Over 100 years later, Dolberg *et al* used an ingenious animal model to demonstrate this link (7). Chickens injected with the Rous sarcoma virus were observed to develop cancer not only at the site of injection, but also at distant body sites which were wounded. Furthermore, the application of an anti-inflammatory therapy inhibited cancer generation in these distant wounds (8). In his

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Key words: cancer, cell migration, FERM domain, FERM proteins, wound healing

Table I. Inflammatory conditions known to predispose to malignant transformation.

Pre-malignant inflammatory condition	Cancer-associated outcome	(Refs.)
Barrett's oesophagus	Oesophageal cancer	(101)
Hepatic cirrhosis	Hepatic cancer	(102)
Gastric ulceration	Gastric cancer	(103)
Inflammatory bowel disease (ulcerative colitis)	Colorectal cancer	(104)
Chronic pancreatitis	Pancreatic cancer	(105)
Burn wound	Squamous cell carcinoma (SCC, Marjolin's ulcer)	(106)

landmark study, Dvorak succinctly suggested that tumours represent 'wounds that do not heal' (9).

Currently, the link between chronic inflammatory states predisposing to cancer formation is well established (Table I). Other links between wound healing and cancer include cell growth, angiogenesis and the formation of fibrous tissue/ECM (10). These processes are appropriately regulated during physiological wound healing, whilst being uncontrolled in cancer. Of interest to our group is the regulation of cellular movements, in the context of both cancer metastasis and wound healing. During our search for key molecular markers involved in wound healing, we noted, as have other authors (11), marked similarities between molecular effectors of cancer and wound healing. Whilst this correlation is not absolute, there is a sufficient overlap to warrant further attention to this association (12). For example, Pedersen *et al* performed microarray analysis on keratinocytes during wound healing and compared this to keratinocytes from squamous cell carcinomas (SCCs) and normal tissue (13). They noted that keratinocytes from the wound edge transiently mimic their SCC transformed counterparts, with the most notable exception between the groups in gene expression profile relating to control of growth. Thus, keratinocytes captured whilst undergoing healing would share a number of similarities with their neoplastic counterparts. However, this overlap is not just confined to keratinocytes; fibroblasts also show remarkable parallels with their response to both wound healing and cancer progression. Chang *et al* cultured fibroblasts in serum, recreating a condition found only in the context of wound healing (14). They analysed the resultant gene expression profile, and showed a similar profile alteration in tumour-associated fibroblasts or tumour cells. Furthermore, the continued expression of this 'wound healing' genotype in breast, lung and gastric carcinomas predicts an increase in metastasis and death.

2. Cellular migration and wound healing

Common to all tissue repair processes is the migration of cells required for healing into the wound space, including endothelial cells (15), fibroblasts (16) and epithelial cells (17). Cells migrate through a process of 'crawling' motility, consisting of four well conserved steps: protrusion of the leading edge, adhesion to the substratum, de-adhesion and retraction of the rear (18). For directional movement, cells acquire a polarised morphology (19), responding to chemokines (chemotactic cytokines) or other extracellular signals. At the cell front (the 'leading edge'), actin assembles both lamellipodia and

filopodia. It is these lamellipodia that adhere to the substratum and provide an anchor from which a force can be generated (20). Key to both lamellipodia and filopodia production is the assembly and protrusion of actin (20). Once a leading edge has been created, actin becomes polarised with a forward 'barbed' end and a backward 'pointed' end (both named due to myosin decoration of actin forming an arrow-head type pattern), and ATP fuels the disassembly of posterior actin filaments, and the assembly of anterior ones (21). Thus actin moves forward relative to the cell.

In order for these actin movements to be converted into cellular locomotion, there needs to exist a link between the cytoskeleton and the cellular membrane. The protein 4.1R, ezrin, radixin, moesin (FERM) family of proteins, so named as an acronym of its founding four members (protein 4.1R, ezrin, radixin and moesin), is a well described family involved in membrane-cytoskeletal interactions. We have already noted the important role FERM family proteins play in cancer progression (22). Herein we update and summarise these pertinent findings, and compare the effects of this protein family on wound healing and tissue repair.

3. The FERM protein superfamily

Members of the FERM superfamily are characterised by the presence of a conserved FERM domain at the N-terminus of the molecule, and often a spectrin/actin binding domain (SABD) at the C-terminus (23). Protein 4.1R, originally referred to as erythrocyte band protein 4.1, was identified as an abundant protein in human erythrocyte ghosts, localising to the cytoskeleton and stabilising its shape, and the first of the FERM proteins to be identified (24-26). By 1991 three more FERM proteins were discovered; ezrin (27), radixin (28) and moesin (29) (also known as ERM proteins). Prior to the now commonly accepted title, the FERM domain has been known by a variety of names, including the 30 kDa domain, 4.1^{N30}, the membrane-cytoskeletal-linking domain, the ERM like domain, the ezrin like domain of the band 4.1 superfamily, the conserved N-terminal region, the membrane attachment domain (23) and the talin, ezrin, radixin, moesin (TERM) domain (30).

The FERM family has grown considerably since this initial discovery of protein 4.1R, with more than 40 members identified to date (31) (Fig. 1). On the basis of protein sequence similarity, this superfamily was divided in 1994 into five subgroups: band 4.1 proteins, ERM-related proteins, talin-related molecules, protein tyrosine phosphatase (PTP) proteins and novel band 4.1-like 4 (NBL4) proteins (32).

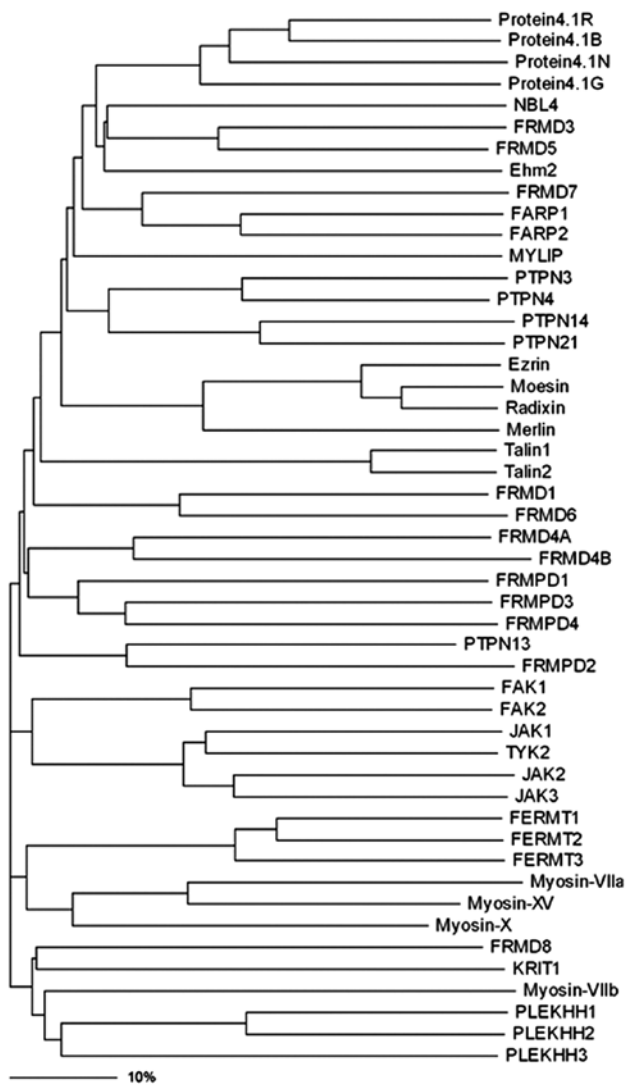


Figure 1. Phylogenetic tree of the protein 4.1R, ezrin, radixin, moesin (FERM) family proteins. Phylogenetic analyses was performed using the ClustalW (<http://www.ebi.ac.uk/clustalw/>), and the dendrogram tree was drawn by using the Treeview (version 1.6.6, <http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>). The bar scale shows the identity shared by the proteins in their amino acid sequences. NBL4, novel band 4.1-like protein 4; FRMD, FERM domain-containing protein; Ehm2, expressed in high metastatic cells 2; FARP, FERM RhoGEF and pleckstrin domain-containing protein; MYLIP, myosin regulatory light chain interacting protein; PTPN, protein-tyrosine phosphatase non-receptor-type; FRMPD, FERM and PDZ domain-containing protein; FAK, focal adhesion kinase; JAK, janus kinase protein; TYK, non-receptor tyrosine-protein kinase; FERMT, fermitin family homolog; KRIT, Krev interaction trapped protein; PLEKHH, pleckstrin homology domain-containing family H member.

4. The FERM domain

The FERM domain is a 30 kDa, N-terminal, cysteine rich, basically charged, globular molecule, initially noted as it was resistant to mild chymotrypsin treatment (24). It is hydrophobic and contains numerous β -sheet structures, suggesting a tight, compact structure which may account for its resistance to proteolytic degradation (33). The FERM domain has been shown to consist of three lobes, the F1, F2 and F3 domains (also known as FERM-N, FERM-M and FERM-C subdomains), in a number of FERM proteins (34,35). Pearson *et al*

suggested that, although the FERM domain is composed of three smaller domains, these three function together as a single unit and are structurally rigid upon activation (36). The F1 and F2 domains appear to be the most highly conserved amongst the FERM domain proteins, whilst F3 encodes for more specific protein binding sites (34,36). 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 (36). The FERM domain can bind a variety of proteins, lipids and molecules (Table II). Inactive FERM proteins are retained in the cytoplasm in a closed conformation, which masks transmembrane protein binding sites in both the FERM domain and the C-terminal region (36). Activation of the protein results in weakening of this interaction and subsequent exposure of the FERM domain and active C-terminal region (Fig. 2). For example, when phosphatidylinositol-4,5-bisphosphate (PIP₂) binds to the FERM domain of ezrin, its conserved C-terminal at Thr⁵⁶⁷ becomes phosphorylated, thus opening and activating the protein (37). Ezrin can then translocate to the membrane/cytoskeleton interface, change its conformation, and undertake binding with various partners.

Certain FERM proteins have been well characterised with regards to human disease, cancer progression and wound healing. The following sections review the effects of certain key FERM proteins, ezrin, radixin, moesin, protein 4.1R and the novel protein expressed in highly metastatic cells 2 (Ehm2) in human disease and wound healing.

5. ERM proteins - background

Ezrin (cytovillin/p81/80k/villin-2), initially discovered in 1983 (38) as an 81 kDa component of intestinal microvilli, is the most well characterised of these proteins and considered by some to be the prototype member of the ERM protein family due to its conserved wide spread distribution and highly homologous sequences of the FERM domain. Although ezrin (along with radixin and moesin) is expressed in most cultured cells, it has been shown to be highly concentrated in the gastrointestinal tract, lungs and kidneys, and is expressed in epithelial and mesothelial cells (39-41). Ezrin is localised to the juxta-membrane region and to soluble cytosolic pools. The dynamic localization between these two compartments is important in determining ezrin activity and is responsible for its cellular action (42,43). Ezrin is involved in a myriad of cellular functions, such as cell adhesion, motility, apoptosis and phagocytosis.

Moesin (membrane-organising extension spike protein) was initially isolated from bovine uterus and characterised as a possible heparin receptor (29,44). Moesin is concentrated in specialised microdomains, namely in the intracellular core of microextensions, such as filopodia, microvilli, microspikes and retraction fibers (45). Similar to ezrin, moesin is important in cellular motility and migration. Moesin is responsible for the formation of cortical actin complexes when activated (following phosphorylation of Thr⁵⁵⁸), and inducing actin depolymerisation and reassembly toward the cell membrane edge (46). In the context of cervical cancer, moesin-associated cellular motility has been shown to be upregulated by vascular

Table II. The various binding partners of the N-terminal FERM domain.

Class of FERM domain binding partner	Specific binding partner	(Refs.)
Membrane proteins	Band 3 protein	(107)
	Glycophorin C and D	(108,109)
Phosphoproteins	p55	(107,109)
	ERM-binding phosphoprotein 50 (EBP50)	(110)
Adhesion molecules	CD44	(111)
	Inter-cellular adhesion molecule 1 (ICAM1)	(112)
	Inter-cellular adhesion molecule 2 (ICAM2)	(112)
	Na ⁺ /H ⁺ exchanger (NHE1)	(113)
Membrane lipids	PIP2	(114)
Cytoplasmic proteins	Calmodulin	(115)
Other proteins	Na ⁺ /H ⁺ exchanger regulatory factor (NHERF)	(116)

FERM, protein 4.1R, ezrin, radixin, moesin; PIP2, phosphatidyl inositol-4,5- biphosphate.

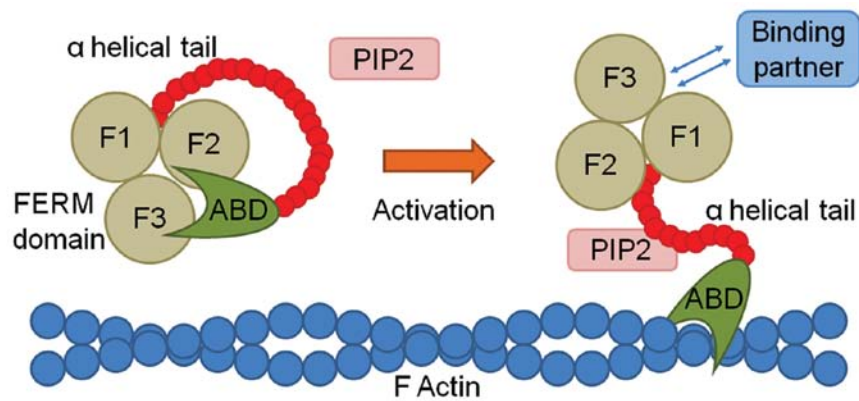


Figure 2. Schematic diagram demonstrating the activation and binding of protein 4.1R, ezrin, radixin, moesin (FERM) family proteins. The coiled FERM protein (left) is composed of three lobes (F1, F2 and F3), an α helical tail, and an actin binding domain (ABD), which is maintained in a closed conformation, and thus does not allow binding. On activation, in this instance by phosphatidyl inositol-4,5-bisphosphate (PIP2), the FERM protein opens, allowing the ABD to bind to F actin, and the FERM domain to bind to one of its various binding partners.

endothelial growth factor C (VEGF-C) and mediated by the RhoA/ROCK-2 signalling pathway (47).

In 1989, radixin, an 82-kD protein, was purified from a rat liver and shown to be a barbed end-capping protein associated with the undercoat of the cell-to-cell adherens junction (28). Subsequently, it was shown to be predominantly located to the hepatocyte microvilli (48). It shows high (>84%) levels of sequence homology with ezrin and moesin (49). Radixin has been shown to interact with the GABA_AR α 5 subunit, a component of an inhibitory neurotransmitter receptor in neurons, at extra-synaptic sites (50). Thus, radixin may be indirectly important in modulating neuronal network activity.

6. ERM proteins and cancer

Over the years, more data has become available outlining the role ERM proteins play in cancer biology. Ezrin is the most studied protein in this context, whilst moderate work has been performed on moesin and very little on radixin. As FERM

family members, and consistent with their role as membrane cytoskeletal linkers, ERM proteins typically promote cancer progression and metastasis (22). For example, the overexpression of ezrin results in the greater metastatic potential of osteosarcoma, hepatocellular carcinoma, breast cancer, lung cancer and pancreatic carcinoma (51-56). In the context of metastatic melanoma, ezrin deletion reduces important interactions with CD44, merlin and Lamp-1, and thus their recruitment (57). The resultant aberrant engagement with the surrounding microenvironment reduces cellular movements, thereby reducing the metastatic potential of these cells. When ezrin was knocked down in the CCR9-expressing acute T lymphocytic cell line, MOLT4, cell polarisation disappeared. Furthermore, E-cadherin translocation from the cytoplasm to the cell membrane was reduced, as was the invasive ability of the cells (58).

Ezrin also interacts with other proteins through which it has an effect on cancer. For example, the cytoskeletal-related protein, neurofibromatosis type 2 (NF2), acts as a cancer

suppressor in the context of glioblastomas. However, ezrin has been shown to reduce NF2 expression via aberrant intracellular recruitment and thus intermolecular association. The resulting reduced levels of NF2 are observed in approximately one-third of glioblastoma cell lines and tumours (59).

Moesin has also been shown to promote metastasis in certain types of cancer. It has already been mentioned that moesin promotes cervical cancer metastasis through the RhoA/ROCK pathway (47). Greater levels of moesin (and also radixin) were found in pancreatic tumours which had metastasised to regional lymph nodes, compared to non-metastasising cancers (60). The knockdown of moesin using siRNA reduces the invasion of melanoma cells into 3D matrices, in part due to a loss of cellular polarisation (61).

7. ERM proteins and wound healing

Active ERM proteins have been examined using a phospho-ERM antibody, with affinity for ezrin, radixin and moesin, by a number of authors. For example, Jensen and Larsson observed that both CD44 and ERM proteins were co-localised to luminal F-actin domains in endothelial cells *in vitro* and *in vivo* (62). After wounding of the confluent monolayer of cells *in vitro*, focal F-actin branching points appeared, with CD44 and ERM proteins again co-localised to this area, and associated with phosphorylated protein kinase (PK)C. The inhibition of PKC activity resulted in inhibited wound healing with reduced ERM-F-actin interaction and associated F-actin branching points. The authors concluded that ERM proteins, through the stimulation of PKC, play an important role in endothelial migration and thus, healing. These data complement the work by Ng *et al* who showed that ERM protein phosphorylation by PKC improved *in vitro* wound healing in MCF7 breast cancer cells (63).

It is possible to study neuronal regeneration following injury *in vitro* using a neuron transection model. Haas *et al* initially showed that phosphorylated ERM proteins were localised to neuronal processes that formed several hours after neural transection, but were notably absent from relatively mature and stable cultured cells (64). They subsequently showed that the activation of ERM proteins was dependant on Rho kinase, with Rho kinase inhibition reducing phosphorylated ERM levels and subsequent response to wounding (65).

Tsuda *et al* showed that the signalling adaptor protein Crk associates with ERM proteins and enhances cell motility in 293T and 3Y1 (fibroblast) cells towards hyaluronic acid (66). Crk results in the translocation of ERM proteins to microvilli in the 3Y1 cell line, although this function was, as with the neuron transection model above, suppressed by Rho kinase. The authors concluded that Crk promotes cellular migration and motility in fibroblasts through its effects on ERM proteins.

Crepaldi *et al* utilised a kidney-derived epithelial cell line, LLC-PK1, to analyse the effect of ezrin overexpression on hepatocyte growth factor (HGF)-induced renal wound healing and epithelial migration (67). Cells overexpressing ezrin demonstrated enhanced migration and tubulogenesis in response to HGF compared to both a wild-type (WT) control, and a control overexpressing a truncated variant of ezrin, and thus concluded that ezrin promotes renal wound healing. The effects of moesin and lung injury were investigated using

moesin knockout mice (68). These mice were compared to WT mice, and both had intra-tracheal bleomycin to simulate widespread lung injury. Moesin knockout mice developed more pronounced lung injury and fibrosis, following an abnormal cytokine and chemokine response, and had lower survival rates when compared to WT mice.

Moesin knockout mice were also used for investigating the role of moesin in hepatic injury (69). Hepatic stellate cells (HSCs) undergo activation in response to injury, and are important in hepatic wound healing and fibrosis. Response to hepatic wounding, caused by both common bile duct ligation and focal thermal denaturation, were compared in moesin knockout mice and WT controls. In moesin knockout mice inflammatory infiltrate, fibrosis and collagen deposition at the injury margin was markedly reduced compared to the WT mice. *In vitro* migration assay of moesin knockout HSCs demonstrated a significant reduction in migration compared to WT controls.

8. Protein 4.1R - background

Protein 4.1R is a 80 kDa protein, initially found localised to the cytoskeleton on human erythrocytes, which binds to a variety of other proteins (adducin, tropomyosin, tropomodulin, dematin and protein p55 (70) and together stabilises the erythrocyte characteristic biconcave disc shape (71). Protein 4.1R consists of four structural domains (72); an N-terminal FERM domain, a 16 kDa domain of unknown function, SABD and a 22 kDa C-terminal domain which has been shown to interact with some proteins that form tight junctions, such as occludin, zonula occludens (ZO)-1 and ZO-2 (73). It is the most studied member of the protein 4.1 family, which consists of protein 4.1R (red blood cells), 4.1G (general), 4.1N (neuronal) and 4.1B (brain) (74).

A decrease in protein 4.1R expression due to chromosomal deletion results in hereditary elliptocytosis, which is characterised by abnormally shaped erythrocytes, haemolysis and splenomegaly (75). Abnormal erythrocyte architecture has been documented in protein 4.1R null mice (76). A similar model suggested that protein 4.1R deficiency results not only in the diminution of actin, but also in a reduction of various transmembrane proteins, including glycophorin C, XK, Duffy and Rh, and results in the conformational change of band 3 and Kell epitopes (77). Protein 4.1R can also be deactivated by different methods. For example, in the presence of calcium, calmodulin decreases the affinity of protein 4.1R for the spectrin-actin complex, thus reducing the mechanical stability of the cellular membrane (78). Furthermore, protein 4.1R can be phosphorylated by a number of agents, including casein kinase, tyrosine kinase, PKA and PKC (79,80). This phosphorylation reduces its ability to associate with spectrin approximately five-fold, with an increase in the relaxation of the cytoskeleton and associated cellular flexibility (81).

9. Protein 4.1R in human disease and wound healing

Although initially found to localise to human erythrocytes, it subsequently became clear that protein 4.1R is expressed in a variety of nucleated cells, is located in several different subcellular compartments, and is required for other diverse functions (82-85). Protein 4.1R has been shown to link with

β -catenin in gastric epithelial cells, and such cells lacking protein 4.1R show impaired cell-cell contacts and disordered gastric glands (86).

The role of protein 4.1R in wound healing was recently investigated by Chen *et al* using protein 4.1R^{-/-} mice (and their cultured keratinocytes) (87). Protein 4.1R deficient mice displayed delayed dermal wound healing. *In vitro*, protein 4.1R was shown to be present in the cytoplasm and the leading edge of moving cells. The absence of protein 4.1R resulted in reduced adhesion, spreading, migration and motility of keratinocytes. Focal adhesion complexes failed to localise in the absence of protein 4.1R. Furthermore, β 1 integrin levels were reduced, and shown to directly link to this protein. Of note, protein 4.1R deficient keratinocytes showed greater levels of 4.1G and 4.1N, without any change in 4.1B levels, suggesting that these former two proteins compensate in part for the absence of protein 4.1R (87). Protein 4.1R has also shown to be important in the migration of endothelial cells. Ruiz-Sáenz *et al* showed that protein 4.1R interacts with Ras GTPase-activating-like protein 1 (IQGAP1), which acts as a scaffolding protein near the cell cortex at the leading edge of migrating cells, and both binds and cross-links actin filaments (88). The authors demonstrated that protein 4.1R was recruited to the leading edge of migrating cells, where it recruits and binds IQGAP1 through its FERM domain. The lack of protein 4.1R significantly slowed cellular migration.

10. Ehm2 - background

In 1996, Hashimoto *et al* identified four novel proteins in both high and low metastatic murine melanoma cells. Ehm2 was the second novel protein to be found in the highly metastatic (and notably absent in the low metastatic) K-1735 murine melanoma cell line, with an mRNA size of 4.0kb (89). By the year 2000, the same team had cloned Ehm2 (90). The authors demonstrated that the Ehm2 gene encodes a protein with 527 amino acids, with a 41% amino acid identity with the FERM domain. Further analysis revealed that Ehm2 showed the greatest homology with NBL4, and was thus classified in the NBL4 subfamily. A human homologue, with significant homology (83% identity, mapped to chromosome 9) was identified, along with seven rat clones and one pig clone, again with significant homology (83-92%), suggesting that Ehm2 is a highly conserved gene (90).

In 2003, Chauhan *et al* characterised Ehm2 in a human fibrosarcoma cell line model (HT-AR1 cells) of steroid-induced cytoskeletal reorganisation, and demonstrated that Ehm2 was androgen (dihydrotestosterone)-, but not dexamethasone-regulated (91). They analysed Ehm2 mRNA levels in a variety of human tissues, and found the expression of a 3.8 kb transcript (isoform 1), which was expressed in greater levels in the testis, with a lower expression in prostate and breast tissue. An additional 2.3 kb Ehm2 transcript was also found in these three tissues, with their transcripts only differing in the length of the 3' untranslated region (3'UTR). In addition, the human brain contained high levels of a 5.6 kb Ehm2 transcript, encoding a protein of 913 amino acids (isoform 2), with an additional 409 amino acids of unknown function. Further analysis of the Ehm2 protein revealed that it appeared to encode only an extended FERM domain located 70 amino acids from

the N-terminus. Unlike other FERM domain proteins, the C terminus did not appear to encode any additional protein function. The authors subsequently hypothesised that Ehm2 either encodes a novel activity, or possibly, functions as a dominant negative modulator of other FERM proteins (92). Phylogenetic tree analysis revealed that Ehm2 belongs to a subfamily consisting of at least nine members from seven different species, including the *Drosophila* Yurt gene (93).

11. Ehm2 in human disease and wound healing

Wang *et al* investigated the role of Ehm2 in prostate cancer. Ehm2 mRNA and protein levels were higher in immortalised prostate cancer cell lines (LNCaP, PC-3 and LAPC4) compared with the immortalised prostate epithelial cell line (PNT1a) (94). Similarly, Ehm2 mRNA levels, as analysed by qRT-PCR, were significantly greater in prostate cancer tissues compared to benign controls. Immunohistochemical (IHC) analysis confirmed a significantly greater expression of Ehm2 protein in prostate cancer tissues compared with benign controls, with cancers associated with PSA recurrence following radical prostatectomy having a significantly greater staining compared to those without recurrence. Using transient transfection and siRNA knockdown in PNT1 and LAPC4 cells respectively, the authors demonstrated that increased Ehm2 levels were associated with decreased adhesion to collagen IV. Schulz *et al* subsequently confirmed that Ehm2 mRNA levels were significantly increased in cancerous compared to non-cancerous prostate samples, and also showed that higher levels of Ehm2 expression (mRNA) significantly increased the likelihood of biochemical recurrence (95). Other authors have used microarray technology to show that Ehm2 transcript levels are greater in neoplastic prostate samples (96-98).

Yu *et al* recently demonstrated that elevated levels of Ehm2 mRNA and protein were found in breast cancer tissues compared to controls, analysed with qRT-PCR and IHC (99). Higher levels were observed in node-positive disease (vs. node-negative), more advanced disease [TNM (tumour, node, metastasis)-4 disease vs. TNM1-3], higher levels of the Nottingham prognostic index (a marker of prognosis) and evidence of metastasis. There was a significant reduction in disease-free survival in patients with high levels of Ehm2. *In vivo* data using MCF7 Ehm2 knockdown cells showed Ehm2 enhanced growth and invasion; however, there was no effect on motility or adhesion. Ehm2 knockdown was associated with the reduced mRNA and protein expression of MMP9.

We recently demonstrated that Ehm2 is upregulated in samples obtained from the periphery of acute healing wounds compared to chronic non-healing wounds (100). Furthermore, chronic wounds which subsequently healed displayed a greater cellular staining for Ehm2 than those which failed to heal, which was noted particularly at the leading keratinocyte edge of the healing wounds (Fig. 3A). In order to investigate the mechanisms behind this observation of improved wound healing, we created a stable Ehm2 knockdown in the HaCaT keratinocyte cell line. *In vitro* experiments revealed that Ehm2 knockdown slowed cellular adhesion, migration and motility (Fig. 3B-D), without affecting cellular growth, apoptosis or cell cycle rates. These data suggest that Ehm2 acts as a pro-migratory protein, in common with other FERM family proteins, and promotes wound healing through the process of re-epithelialisation.

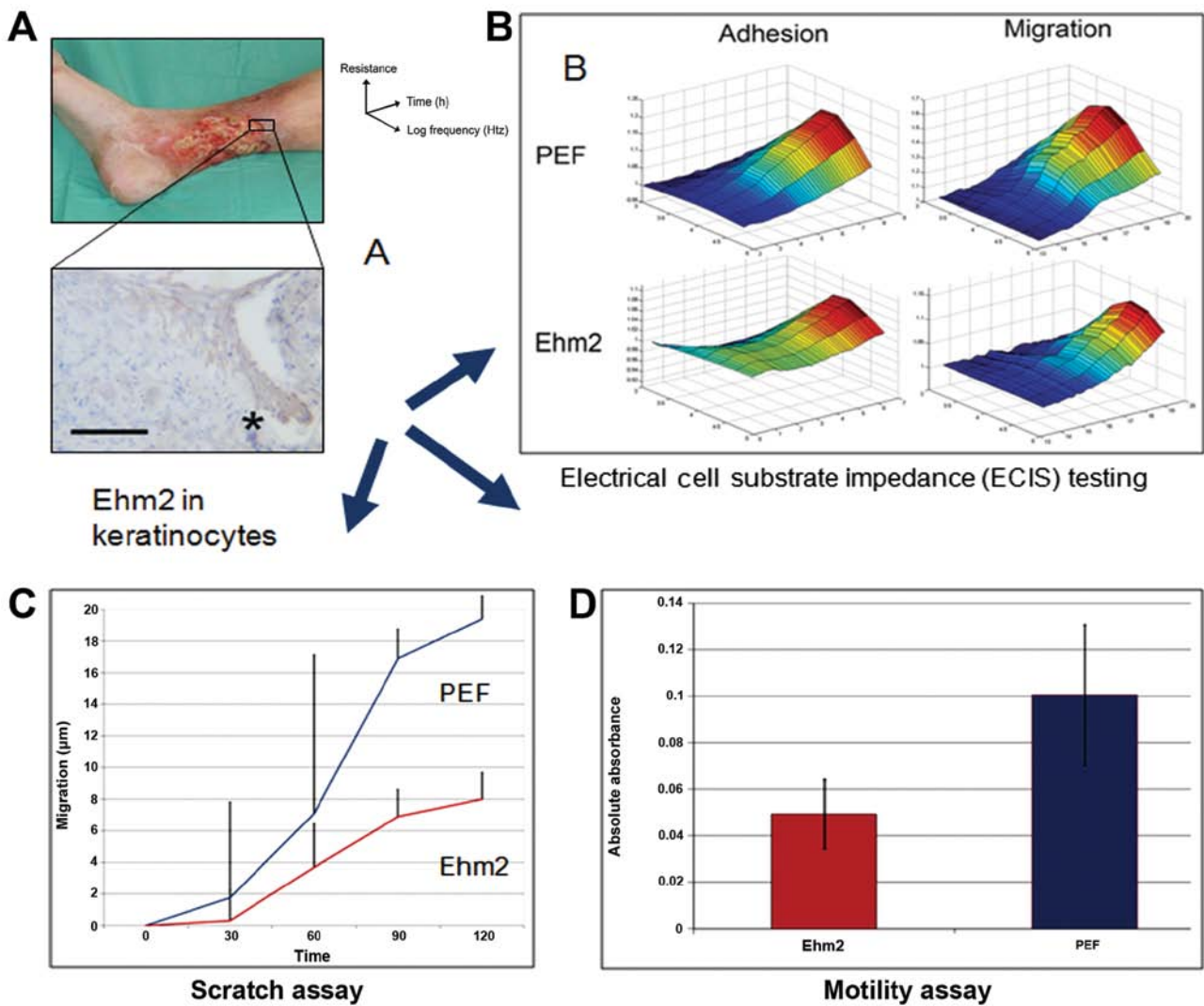


Figure 3. Example of data relating to the protein 4.1R, ezrin, radixin, moesin (FERM) protein expressed in high metastatic cells 2 (Ehm2) in wound healing. (A) Biopsies [immunohistochemical (IHC) staining, $\times 100$ magnification; line represents $0.1 \mu\text{m}$] taken from the periphery of healing chronic wounds showed greater staining of Ehm2 in keratinocytes than those that were non-healing, particularly noted in the leading edge of keratinocytes as they migrated over the wound bed (*). (B-D) Subsequent *in vitro* experiments revealed that Ehm2 knockdown in a HaCaT keratinocyte model significantly slowed cellular motility in a variety of models compared to plasmid controls. (B) 3D data created by the electric cell-substrate impedance sensing (ECIS™) software used to analyse cellular adhesion and migration. Shown is a typical individual ‘run’ in both the Ehm2 knockdown (labelled ‘Ehm2’) and plasmid cell line showing that Ehm2 knockdown slows both adhesion and migration compared to plasmid control. (C) Results from 3 repeats (means \pm SEM) of a standard scratch/wounding assay comparing the wound healing rates of Ehm2 knockdown (red) and plasmid control (blue) cell lines at 30 min intervals. Migrated distances were significantly lower as measured at 90 and 120 min ($p < 0.01$, Student's t-test). (D) Analysis of beads motility assay of Ehm2 knockdown (labelled Ehm2) and plasmid control cell lines. Results shown are the means (\pm SEM) of 3 repeats. Ehm2 knockdown results in a marked reduction in motility (with absorbance used as a proxy measurement of cellular number, measured using a spectrophotometer) when compared to the plasmid control ($p = 0.15$, 2-tailed Student's t-test). Images derived from Bosanquet *et al* (100), used with permission from the Journal of Dermatological Science.

12. Conclusion

The migration of cells is essential not only for wound healing, but also for embryonic development, neuronal development and immune responses, and is also crucial for cancer metastasis. This brief review highlights the role that certain FERM proteins, the ERM proteins, protein 4.1R and Ehm2, play in wound healing and other diseases. Although there is some heterogeneity amongst this family, the FERM proteins generally promote the migration of cells, such as keratinocytes and endothelial cells, through their role as mediators between the cytoskeleton and the cell membrane, linking to a large number

of different receptors. It is suggested that it is through these various mechanisms (i.e., re-epithelialisation and angiogenesis) that wound healing occurs.

This positive wound healing effect has been noted across a variety of models and in differing cell types. However, whilst *in vivo* results can be suggestive of an important role within wound healing, evidence corroborated with *in vivo* tissue data, or animal models, are often lacking. Whilst this does not necessarily detract from the conclusions, it highlights the need for further studies to fully assess the significance of FERM protein alterations in human tissue repair and regeneration.

Acknowledgements

The authors wish to thank the Welsh Government (A4B scheme) for supporting this study.

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