

## Theme Issue Article

# Roles of the multifunctional glycoprotein, emmprin (basigin; CD147), in tumour progression

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### Summary

Emmprin (basigin; CD147) is a widely distributed cell surface glycoprotein that belongs to the Ig superfamily and is highly enriched on the surface of malignant tumour cells. Emmprin is involved in numerous physiological and pathological systems and exhibits several molecular and cellular characteristics, but a major function of emmprin is stimulation of synthesis of several matrix metalloproteinases. In tumours, emmprin most likely stimulates matrix metalloproteinase production in stromal fibroblasts and endothelial cells as well as in tumour cells themselves by a mechanism involving homophilic interactions be-

tween emmprin molecules on apposing cells or on neighbouring cells after membrane vesicle shedding. Membrane-associated cofactors, including caveolin-1 and annexin II, regulate emmprin activity. Emmprin induces angiogenesis via stimulation of VEGF production, invasiveness via stimulation of matrix metalloproteinase production and multidrug resistance via hyaluronan-mediated up-regulation of ErbB2 signaling and cell survival pathway activities. Although the detailed mechanisms whereby it regulates these numerous phenomena are not yet known, it is clear that emmprin is a major mediator of malignant cell behavior.

### Keywords

Matrix metalloproteinases, angiogenesis, invasiveness, chemoresistance, tumour-stroma interaction

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## Introduction

The progression of cells to malignancy is characterized by emergence of several properties, including self-sufficiency with respect to growth-promoting signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, invasiveness, and metastatic potential (1). A central creed in cancer research over the past few decades has been that acquisition of these properties is triggered by genetic mutations of oncogenes and tumour suppressor genes within developing tumour cells. However, both old and new research has also implicated tumour-stroma interactions in each of the critical steps in cancer progression. For example, sustained angiogenesis, which arises from tumour-host interactions, has been added to the 'canonical' list of properties characteristic of developing tumours (1). The stromal compartment of a tumour contains a variety of host cells, including endothelial cells, fibroblasts, and inflammatory cells and it is becoming increasingly appreciated that these host-derived cells infiltrate into tumour tissue, interact with tumour cells, and are subsequently conscripted by tumour

cells to produce an array of soluble and insoluble factors that stimulate tumour angiogenesis, growth, and metastasis. Accumulating evidence suggests a prominent role for emmprin in mediating interactions both between tumour cells themselves and between tumour cells and "hijacked" host stromal cells to promote a number of events during cancer progression. One of the important and most studied functions of emmprin is its role in induction of matrix metalloproteinase (MMP) production via cell interactions – thus the derivation of its name: extracellular matrix metalloproteinase inducer (2).

## Structure and functional activity of emmprin

Emmprin was identified independently in numerous systems as an antigen or cDNA of unknown function or as a factor of known function but unknown identity. Consequently numerous names (basigin, neurothelin, OX-47, gp42, CE9, 5A11, M6, HT7) were used to designate the same protein in different species and tissues of origin. Emmprin was designated CD147 at the Sixth In-

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ternational workshop and Conference on Human Leukocyte Differentiation Antigens (3). Several studies have shown that emmprin and its homologs belong to the Ig superfamily with homology to both the Ig variable (V) domain and MHC class II b-chains (4–7). A family of molecules related but not identical to emmprin has been described (8–10).

### Emmprin as an inducer of matrix metalloproteinases

In the 1980s, the laboratory of Chitra Biswas was attempting to identify a factor shed from the surface of tumour cells which was responsible for stimulation of interstitial collagenase (matrix metalloproteinase-1; MMP-1) production by fibroblasts (11–13). Biswas's studies became of great interest to the oncology community with the discovery that stromal fibroblasts within human tumours were producing most MMPs, rather than the tumour cells themselves (14–16). Subsequent experiments showed that the source of most of the MMP-1-stimulating factor produced by B16 murine melanoma cells and LX-1 human lung carcinoma cells (originally called tumour collagenase stimulating factor or TCSF) was plasma membrane-derived and could act via direct cell-cell interaction or via shedding of the factor from the cell surface (13, 17). It was also found to be capable of stimulating production of several matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9, MMP-14, MMP-15) by fibroblasts (18–23) and endothelial cells (24). Likewise, it was found that emmprin was not only produced by malignant tumour cells but also by non-malignant cells, albeit in much lower concentrations (25, 26). Thus when TCSF was fully characterized as a 58 kDa transmembrane glycoprotein of the Ig superfamily by Biswas et al. (2), it was renamed emmprin. On cloning of emmprin cDNA, it became apparent that emmprin is identical to human basigin and the M6 antigen present on membranes of leukocytes from patients with rheumatoid arthritis. Mouse emmprin (basigin) was demonstrated to have the same MMP stimulatory effect as its human counterpart (27).

### Emmprin structure and functional interactions

Emmprin is a glycoprotein containing two C2-like immunoglobulin extracellular domains, a transmembrane domain, and a cytoplasmic domain (4–7). The presence of a highly conserved acidic residue (glutamic acid) and leucine zipper-like sequences within the hydrophobic sequence of the transmembrane domain suggests that intramembrane associations are likely to occur with other membrane proteins. The overall amino acid sequence identity between mouse and rat emmprin is 94%, between human and mouse emmprin is 58% and between mouse and chicken is 45% (7, 28). Most of the differences between species lie within the extracellular domains. The transmembrane domain is almost totally conserved across species whereas the cytoplasmic domain is only moderately conserved. For example, the transmembrane domain is completely conserved between human, mouse, and chick, and ~80% between these vertebrates and *Drosophila* (29). However, the human cytoplasmic domain has only 68% homology with chick and the external domains only 34% homology. Current evidence indicates that the N-terminal Ig domain is required for stimulation of fibroblast MMP production by human emmprin (2, 30, 31). Murine emmprin (basigin) has been shown to stimulate MMP production (27) but it remains to be

seen whether emmprin homolog preparations from more distant species, which have very different ectodomain sequences, also stimulate MMP production. Emmprin has three conserved N-glycosylation sites that are variably glycosylated. Glycosylation is also known to be important for emmprin function (19, 30) and differences in glycosylation, and therefore activity, are regulated by interaction with caveolin-1 (32).

Similar to other members of the Ig superfamily, emmprin forms homo-oligomers in a *cis*-dependent manner in the plasma membrane; the N-terminal Ig-like domain is necessary and sufficient for oligomerization, probably through hydrophobic interactions (33). The MMP-inducing function of emmprin in part involves the molecule acting as a counter-receptor for itself (30). This homophilic counter-receptor binding activity of emmprin requires the N-terminal Ig domain, but in this case interaction is in a *trans* manner. Inhibition of this homophilic interaction interferes with MMP production and MMP-dependent invasion in tumour cells (30). Emmprin also interacts with integrins  $\alpha 3\beta 1$  and  $\alpha 6\beta 1$  (34), probably via the N-terminal Ig domain (35). A subset of emmprin molecules, separate from that which associates with integrin, associates with caveolin-1 in lipid rafts. The second Ig domain is required for lipid raft association. Over-expression of caveolin-1 causes a decrease in clustering of emmprin on the cell surface and decreased induction of MMP-1, thereby contributing to the onco-suppressive effects of caveolin-1 (35). Association with caveolin-1 also prevents formation of highly glycosylated forms of emmprin and consequently blocks emmprin aggregation and activity (32). Annexin II also interacts with emmprin and is required for its activity in stimulating MMP production (H. Guo et al, submitted for publication). This is of interest in view of the finding that at least two other cell surface-associated proteases, plasminogen activator (36) and cathepsin B (37), interact with annexin II suggesting a coordinating function for annexin II in assembling proteases at the cell surface. In addition to stimulating production of MMPs, emmprin also binds MMP-1 and retains it at the cell surface, an arrangement that may promote turnover of pericellular collagen (38).

### The emmprin gene

The emmprin gene consists of seven exons and six introns spanning 7.5 kb (39, 40). The 5' upstream sequence of the emmprin gene contains no TATA or CAAT box but has a CpG-rich island. A 470-bp fragment upstream of the coding region of emmprin has been shown to promote its transcription. A 30-bp element of this sequence (-142 to -112 bp) which contains a binding site for Sp1, was also demonstrated to be important for emmprin transcription (41). The nuclear protein, pinin, may negatively regulate emmprin expression (42); this may be of importance since pinin is down-regulated in several types of cancer cell (43).

### Other functions of emmprin

A knockout mouse has been produced in which emmprin (basigin) is lacking (44, 45). Although experimental challenges in the null mouse have been limited, much has been learned about the functions of emmprin. The null mutant is small, and usually unable to undergo implantation, possibly due to alterations in MMPs required in the reproductive process. Those embryos that implant survive past birth, but the mature offspring are sterile

and have deficiencies in spermatogenesis, fertilization, sensory and memory functions, and mixed lymphocyte responses (44, 45). It is clear from numerous studies of various tissue and organ systems that emmprin is multifunctional and that stimulation of MMP production is not common to all of these systems. For example defects in retinal development in the basigin null mouse are most likely due to failure of monocarboxylate transporter-1 (MCT-1), a lactose transporter, to integrate into the plasma membranes of Muller cells and retinal pigmented epithelium (46, 47). Recent studies have demonstrated clearly that emmprin is an essential chaperone for assembly of MCT-1 and MCT-4 into membranes (48, 49). Emmprin may also be involved in immune cell interactions and activation (3, 50–54), viral entry into cells (55), cell-cell interactions in the developing nervous system (56), blood-brain barrier development and maintenance (57, 58), cyclophilin function (54, 55), and calcium mobilization (59). A unifying mechanism for the role of emmprin in these phenomena has not been elucidated. Although many of these functions of emmprin are unlikely to depend on its ability to up-regulate MMP production, stimulation of MMPs may be the basis of emmprin function in disease processes such as heart failure (60, 61), atherosclerosis (41), arthritis (62–64) and lung injury (65, 66). In each of these cases, there is a close correlation between increased emmprin and MMP expression.

## Role of emmprin in cancer

### Regulation of tumour growth, invasion and angiogenesis

The pathological consequences of elevated emmprin expression in tumour growth and invasion were directly demonstrated using emmprin-overexpressing cancer cells. MDA-MB-436 human breast cancer cells are normally slow-growing cells when they are implanted into nude mice. However, when these cells are transfected with emmprin, they adopt a more aggressive phenotype, exhibiting both accelerated growth and increased invasiveness (67). MMP-2 and MMP-9 expression was increased in the emmprin-enhanced tumours. A new observation arising out of this and other studies (30) was the realization that emmprin stimulates MMP production in tumour cells themselves in addition to stromal cells. This most likely occurs via homophilic interactions between emmprin molecules on adjacent cells (30), as described in the previous section. However, it is likely that MMP production in homotypic cancer cell interactions also requires the cytoplasmic domain of emmprin (68). Using MDA-MB-231 human breast cancer cells engineered to express different levels of emmprin, the potential role of increased expression of emmprin in tumours was further elucidated (69). In both *in vitro* and *in vivo* studies using these cells, emmprin was found to increase production of VEGF in the tumour cells. *In vivo*, increased emmprin expression accelerated tumour growth, accompanied by enhanced tumour angiogenesis partially due to a significant up-regulation of VEGF and MMPs in both tumour and stromal compartments. Co-localization of mouse (i.e. host) emmprin, VEGF and MMP to angiogenic blood vessels suggested direct involvement of these molecules in tumour angiogenesis, supporting a new paradigm in which tumour cell surface emmprin plays a key role in regulating tumour angiogenesis and growth (69).

### Host-tumour cell interactions

It is now well established that many of the MMPs found in tumours are produced mainly by peritumoral stromal cells rather than by tumour cells themselves. For example, examination of MMP expression patterns in clinical tumour specimens has shown that these fibroblasts produce tumour-associated interstitial collagenase (MMP-1), stromelysin-1 (MMP-3), stromelysin-3 (MMP-11), and gelatinase A (MMP-2) in breast, colon, lung, skin and head and neck cancers (70–73). On the other hand, examination of emmprin expression in clinical samples by a variety of means has revealed that in most cases emmprin is primarily expressed by tumour cells themselves, e.g. in cancers of the lung (73), breast (73), bladder (74, 75), ovary (76), brain (77), and in lymphomas (31), although in some tumours emmprin expression has been noted in stromal fibroblasts and endothelial cells (76, 78).

The receptor on fibroblast cells that is responsible for emmprin-mediated stimulation of MMP or VEGF production has remained elusive (18, 19). It has been shown that emmprin serves as its own counter-receptor in homotypic cancer cell interactions, thus stimulating MMPs via homophilic emmprin interaction (30). One could speculate the same mechanism may also be applicable for cancer cell-fibroblast interaction. However, this hypothesis has appeared unlikely since only very low levels of emmprin expression can usually be detected in resting fibroblast cells. A more careful study of the dynamics of emmprin expression led to the discovery of a novel positive feedback regulatory mechanism of emmprin expression that provides an explanation for the potential role of emmprin as its own counter-receptor in cancer cell-fibroblast interactions (23). When fibroblasts are exposed to an emmprin stimulus, emmprin expression is up-regulated in these cells at both RNA and protein levels. Newly synthesized emmprin is then presented on the cell surface and serves as the counter-receptor for emmprin-dependent signaling between tumour cells and fibroblasts (23). In a nude mouse xenograft model, over-expression of emmprin in human tumour cells results in a profound increase in mouse emmprin expression in host cells, both in the periphery of the tumour and in stromal cells infiltrated into the tumour tissue (69). This novel regulatory mechanism is supported by recent findings of emmprin mRNA expression in peritumoral fibroblasts in ovarian carcinoma (76) and in some breast cancer cases (78). However, this homophilic interaction may only account for emmprin-mediated tumour-host cell interactions in some tumours since emmprin expression has not been detected in the stroma of some other tumours, including melanoma (21), lung and breast cancer (73). In many cases, tumour cells and stromal cells are separated by distances that would not permit direct cell interactions, thus raising the question whether, and if so, how emmprin is released from tumour cells and translocated from the tumour cell to the stromal cell surface. The possibility that emmprin is released from the cell surface by shedding, as a result of either proteolytic scission or by shedding of membrane vesicles, was raised in early studies (11, 79). The former mechanism has been questioned (80) but a recent study indicates that full length emmprin is released from the surface of cells via constitutive microvesicle shedding (81), which is an active process in many tumour cells (82). Shedding can be amplified on exposure of cells to phorbol

esters through a signaling cascade dependent on protein kinase C, calcium mobilization, and mitogen-activated protein kinase kinase (MEK 1/2) acting upstream of Erk 1/2. However, these vesicles are unstable and rapidly breakdown to release full-length, bioactive emmprin (81).

### Emmprin and signal transduction

The abnormally high levels of emmprin in cancer cells have recently been attributed to dysregulation of EGFR signaling. Amphiregulin, acting through interaction with EGFR, promotes tumour progression through emmprin-induced increases in production of MMPs by fibroblasts and endothelial cells (83). The signaling events downstream of emmprin interactions that result in stimulation of MMP production are not yet established but MAPK p38 has been implicated in induction of MMP-1 production (84) and activation of 5-lipoxygenase and phospholipase A2 in MMP-2 production (80). Investigation of the mechanism whereby emmprin promotes tumour growth *in vivo* (67) led to *in vitro* experiments demonstrating that emmprin induces anchorage-independent growth (85), a phenomenon that is characteristic of malignant cancer cells and that reflects resistance to anoikis, i.e. apoptosis caused by cell detachment from extracellular matrix. Further investigation showed that emmprin stimulates cell survival pathway signaling, including phosphorylation of Akt, Erk and FAK. These effects of emmprin were shown to depend on stimulation of production of hyaluronan, a pericellular polysaccharide (85, 86). The increase in anti-apoptotic signaling in turn leads to increased multidrug resistance and is dependent on hyaluronan-induced ErbB2 and cell survival signaling pathways (86, 87). Interestingly, it has been shown that multidrug resistant cancer cells express increased amounts of emmprin, and

as a consequence produce higher levels of MMP-1, MMP-2, and MMP-9 (22). Although it has not yet been fully established, the stimulatory effects of emmprin on MMP production and invasiveness may also be mediated through hyaluronan-induced signaling (88). In addition, a disruptive role for emmprin in calcium mobilization through G-protein sensitive pathways has been demonstrated in tumour cells, and it was suggested that this effect of emmprin enhances the metastatic potential of hepatoma cells (59). In the light of these findings, Metuximab, a murine HAB18 F(ab')<sub>2</sub> fragment specific for emmprin (59) (also known as LICARTIN), has been developed in the iodine<sup>131</sup>-labeled form and is currently being tested for safety and clinical efficacy in hepatocellular carcinoma.

### Conclusions

Emmprin expression in primary breast and ovarian cancer tissue correlates with tumour size and staging, and is predictive of poor prognosis (76, 89). Correlations between emmprin expression and malignancy have also been demonstrated in other cancers, e.g. bladder carcinomas (74), lung carcinomas (73), gliomas (77), melanomas (21), and lymphomas (31). Of particular interest is the finding that emmprin is frequently expressed in micrometastases (89, 90). In addition to these findings in human patients, experimental studies have demonstrated that emmprin induces several malignant properties associated with cancer, including invasiveness, angiogenesis, anchorage-independent growth and chemoresistance. Consequently, the development of effective therapeutic interventions targeted to emmprin would provide a novel and potentially powerful alternative to current treatments.

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