Chapter 18
Unravelling the Roles of Proteinases in Cell Migration In Vitro and In Vivo

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Abstract Metalloproteinases have been implicated in cell migration in many in vitro model systems which involving tumour cell or leukocyte migration. Here the similarities between the migration mechanisms of amoebae, leukocytes and tumour cells are discussed with a particular focus on recent studies of metalloproteinase dependence in tumour cell migration in three dimensional matrices. Some novel in vivo model systems where metalloproteinases or serine proteinases have been explored are discussed. Finally certain matricryptic sites exposed following metalloproteinase remodelling of the extracellular matrix are also considered.

Introduction

Metalloproteinase involvement in cell migration has been an area of research interest for several decades. Cellular interactions with the extracellular matrix have been investigated with molecular approaches and sophisticated microscopy allowing visualisation of a range of cell types in two and three dimensions (2D and 3D) in vitro as well as more recently in vivo. Many reviews have covered the mechanisms underlying cell migration, including the roles of the cytoskeleton in driving the cell forward as well as signalling networks regulating this process. Here, approaches to the study of cell migration will be reviewed, with an emphasis on recent studies regarding the roles of metalloproteinases and some serine proteinases in cell migration in 2D and 3D as well as the roles of some matricryptic sites in extracellular matrix-driven migration.

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Overview of Cell Migration

Leukocyte Cell Migration and Some Comparisons with Amoeba

The amoeba *Dictyostelium discoideum* has provided much knowledge regarding chemokinetic migration and cytoskeletal organisation. Over several decades the similarities between lymphoid cell migration with that of *Dictyostelium* have been identified (reviewed in Bagorda et al. 2006). Chemotactic migration is essential to *Dictyostelium's* life cycle: free-living amoebae detect bacteria (the food source) through chemotactic migration towards folic acid, a bacterial metabolism product. In periods of stress such as low food availability, amoebae become responsive to cAMP (cyclic adenosine monophosphate), which is secreted by neighbouring amoebae and to which the cells respond chemotactically (Bagorda et al. 2006). Cell migration is a critical part of the inflammatory response to injury when leukocytes are recruited to the site of injury. During an inflammatory response the endothelium becomes 'activated' where endothelial cells produce various pro-inflammatory molecules that include cytokines, chemokines and cell adhesion molecules (CAMs), all of which play a role in the recruitment of leukocytes and/or platelets to the damaged tissue (reviewed in Libby et al. 2002). In normal physiology the damaged area of, for example, wounded skin is cleared of dead cells and tissue debris by phagocytes and repair begins by cell proliferation and the laying down of new matrix. Under normal conditions inflammation subsides but, for reasons not yet clear, in diseases such as cancer and atherosclerosis the tissue becomes chronically inflamed.

Leukocyte—Endothelial Cell Interactions

The recruitment of monocytes to the endothelium and subsequent transendothelial migration involves multiple steps and requires tight regulation. Initial tethering of monocytes is followed by rolling along the endothelial surface and finally firm, sustained adhesion (reviewed in Worthylake and Burridge 2001). Monocyte binding to the endothelium elicits signals that facilitate the migration of monocytes through the endothelial monolayer. Initial tethering and rolling are primarily mediated by members of the selectin family, whereas firm adhesion involves members of the integrin family and their ligands. However, it is becoming increasingly apparent that there are a series of overlapping roles between different classes of CAMs with evidence of integrins also participating in initial tethering events (reviewed in Steeber et al. 2005). Integrins and their ligands play an essential role in the firm adhesion and subsequent migration of leukocytes.

Selectins are a family of transmembrane glycoproteins. Leukocyte (L-) selectin is constitutively expressed, whereas endothelial (E-) selectin is induced by inflam-

matory cytokines (reviewed in Blankenberg et al. 2003). Platelet (P-) selectin is primarily expressed in platelets but is also present in endothelial cells. Initial tethering of monocytes to the endothelium can be mediated by selectins and their carbohydrate-based ligands. Whilst selectins have not been described in *Dictyostelium*, this organism does express cell surface cadherin-like molecule DdCAD-1 (reviewed in Bowers-Morrow et al. 2004), which may be important in amoeba cell–cell interactions. Aggregation in *Dictyostelium* is mediated by the lectins discoidins I and II, which interestingly both contain an RGD site (Gabius et al. 1985).

Inflammation is an important feature of tumour progression and features described initially for cellular transmigration in, for example, atherosclerosis are also relevant in cancer. Thus, tumour cell extravasation is influenced by shear flow (Dong et al. 2005) just as the development of atherosclerotic lesions occurs primarily in regions where blood flow is disturbed (reviewed in Libby et al. 2002). Under conditions of laminar shear stress, endothelial cells are ellipsoid and orientated in the direction of flow whereas in regions of disturbed flow endothelial cells lose their uniform orientation and become more polygonal in shape (reviewed in Lusis 2000). In addition, disturbed flow can augment the expression of leukocyte adhesion molecules (reviewed in Libby et al. 2002).

In the context of cancer, cytokines and chemokines are secreted by tumour cells which attract leukocytes including neutrophils and macrophages (reviewed in Coussens and Werb 2002), which in turn produce a wide range of mediators. Cytokine production by tumour cells and associated macrophages also impacts on the development of angiogenesis and thus on tumour invasion. A number of cytokines produced by tumour cells, including anti-inflammatory IL10 (reviewed in Tedgui and Mallat 2006), reduce the T cell response to tumours (Coussens and Werb 2002). Cytokines have a very wide range of activity related to inflammation, including the induction of other cytokines, chemokines, CAMs and proteases, and therefore make a huge contribution to the chronic inflammatory state.

Infection has been associated with tumour development in a number of cancers (reviewed in Coussens and Werb 2002). In general, at sites of infection neutrophils respond to fMLP (formyl-Met-Leu-Phe) released by bacteria which signals through a G Protein-coupled receptor (GPCR). Chemokines (chemotactic cytokines) such as monocyte chemoattractant protein (MCP)-1 and fractalkine are small proteins that direct the migration of circulating leukocytes to sites of inflammation or injury, again signalling through GPCR (reviewed in Charo and Taubman 2004). In addition to chemotactic roles, chemokines can regulate the adhesion of leukocytes to the endothelium by modulating integrin-mediated adhesion (reviewed in Worthylake and Burridge 2001). *Dictyostelium* chemotaxis towards both folic acid and cAMP is mediated through GPCRs (Bagorda et al. 2006), indicating some of the close parallels between neutrophil and *Dictyostelium* migration.

Integrins in Leukocyte Transendothelial Cell Migration

In order for tumour cells or leukocytes to migrate across the endothelial monolayer during extravasation, regulation of CAMs is required. In inflammation, following initial tethering of leukocytes to the endothelium via selectins, which also mediate rolling of cells over the endothelial surface, sustained adhesion to the endothelium is induced through the binding of integrins α4β1 to VCAM-1 and $\alpha_{L/M}\beta2$ to ICAM-1 (reviewed in Worthylake and Burridge 2001). Cell migration requires cycles of adhesion and detachment in order for a cell to move forward, which suggests crosstalk between different classes of integrins. Tumour cells may also adhere to the endothelium through selectins but do not seem to roll along the endothelial surface (reviewed in Miles et al. 2007). Very similar mechanisms are used by tumour cells to attach firmly to the endothelium, particularly α4β1 integrin–VCAM-1 interactions as well as α6β1 binding to as yet to be defined ligands, but presumably including laminin(s), depending on the integrin profile of particular tumour cells (reviewed in Miles et al. 2007).

The integrin αvβ3 is up-regulated on the lumen of the activated endothelium (Hoshiga et al. 1995). Expressed in monocytes and certain tumour cells, this integrin pair can bind to several ECM proteins including vitronectin and fibronectin (Wayner et al. 1991). In addition, integrin αvβ3 binds platelet endothelial (PE) CAM-1, a member of the immunoglobulin family that is expressed in monocytes and at the intercellular junctions of endothelial cells (Piali et al. 1995) and these molecules have been implicated in monocyte transendothelial migration. In monocytic cell lines engagement of αvβ3 integrin decreases β2 integrin binding to ICAM-1 and promotes transendothelial cell migration (Weerasinghe et al. 1998), indicating that αvβ3 integrin may play a key role in integrin crosstalk. In tumour cell extravasation, this hierarchy of integrin ligation has not been explored although crosstalk between different integrin partners has been reported during cell migration over ECM components (Galvez et al. 2002).

Until very recently, *Dictyostelium* were thought to lack integrins but Cornillon et al. (2006) have now identified an adhesion molecule sibA (similar to integrin beta) which has certain β-integrin features. Mutants in sibA demonstrate a smaller contact area with the underlying substrate than wild-type cells (which could be thought of as a defect in cell spreading) and numbers of cells adhering to the substrate were significantly reduced under a flow of medium. At the molecular level, sibA has a von Willebrand A (VWA) domain highly homologous to β integrin VWA domain, a transmembrane domain and conserved NPxY motifs in the cytoplasmic domain. SibA is expressed at the cell surface and can interact with talin, a cytoskeletal protein important in cell adhesion (Cornillon et al. 2006). A disintegrin containing protein AmpA, possibly related to ADAM or ADAMTS proteinases but lacking a metalloprotease domain, is critical to cell migration in *Dictyostelium* and it has been postulated that this protein may bind to DdCad-1 (Varney et al. 2002). It is also interesting to speculate whether AmpA could be a ligand for the integrin-like sibA.

Roles of Proteinases in Migration of Leukocytes and Tumour Cells Through Endothelial Cell Barriers

Interaction of leukocytes with the endothelium and sub-endothelial ECM results in the up-regulation of metalloproteinases, including MMP2 (Romanic and Madri 1994) and more recently MT1-MMP (Matias-Roman et al. 2005). In fact in the latter study, MT1-MMP was shown to be required during human monocyte migration through filters coated with fibronectin or the extracellular portion of ICAM-1 or VCAM-1 (made as Fc fusions) in a chemotactic assay. In the same study, monocyte transmigration, induced by the chemoattractant MCP-1 through TNFα-activated endothelial cells in vitro, was also dependent on MT1-MMP, demonstrated by antibody inhibition studies (Matias-Roman et al. 2005). However, transmigration of monocytes through resting endothelial cells (i.e. not treated with TNFα) was independent of MT1-MMP. Of relevance here is the observation that TIMP1 inhibits monocyte migration across a resting endothelial layer also implying a role for MMPs but independent of MT-MMPs (Bar-Or et al. 2003). Matias-Roman et al. (2005) also further demonstrated that MT1-MMP co-localised with profilin, a marker of the leading edge of migrating cells, in monocytes cultured on TNFα-activated endothelial cells. Arroya's group also showed that monocytes migrating over fibronectin, VCAM-1 or ICAM-1 displayed clustered MT1-MMP, whereas cells on BSA had diffuse MT1-MMP staining.

More recently, MT1-MMP has been shown to shed ICAM-1 and to play a key role in transendothelial cell migration (Sithu et al. 2007). In this study, endothelial cells over-expressing ICAM-1 promoted transendothelial migration in a TIMP2- and TIMP3-dependent manner. ICAM-1 was shown to bind to MT1-MMP, co-localising in endothelial cell surface ruffles. This distribution of MT1-MMP was disrupted when ICAM-1 cytoplasmic tail mutants were expressed and since trans-endothelial cell migration was markedly reduced in these mutants but partially rescued by MT1-MMP, a complex role for MT1-MMP is suggested in this process. These results are in keeping with those of Matias-Roman et al. (2005) since MT1-MMP was shown to cluster when the cells were migrating over an ICAM-1 substrate. The study of leukocyte extravasation in MT1-MMP-null mice would be of interest, if these mice could tolerate such investigation. Leukocytes derived from MT1-MMP-null mice implanted into wild-type mice would answer the question whether MT1-MMP is required on the leukocyte side of the equation. In models of shear stress, ICAM-1 is shed by MMP9 (Sultan et al. 2004) and by ADAM17 in response to PMA stimulation (Tsakadze et al. 2006), indicating that both cellular and stimulus context are critical to specific proteinase involvement.

Just as leukocytes exit the vasculature at sites of inflammation or injury, tumour cell extravasation involves interaction with the endothelium at metastatic locations as well as sub-endothelial matrix. Tumour cells use many of the signals described above for leukocytes including selectins and chemokines to orchestrate invasion and metastasis (reviewed in Coussens and Werb 2002). In the next section, recent studies exploring roles of metalloproteinases in both tumour cells and leukocytes in

in vitro and in vivo models will be reviewed, highlighting recent studies where different models have yielded intriguing data.

Leukocyte and Tumour Cell Migration in 2D and 3D

Many studies have been performed which have shed light on metalloprotease involvement in cell migration including MMPs and ADAMs in cancer (VanSaun and Matrisian 2006, Arribas et al. 2006) and MMPs in vascular remodelling (Newby 2005). Studies in 2D, where visualisation of cell migration has been possible using either time-lapse videomicroscopy for assessing random cell migration or the Dunn chemotaxis chamber, were exploited to great effect by Anne Ridley and colleagues in the study of Rho GTPases in cell migration (Ridley et al. 2003 and references therein). Over the last 10 years, increasingly 3D studies and, more recently, in vivo studies have been performed, revealing many important insights into cell migration including potential roles for metalloproteinases. Several recent reviews by Ken Yamada and colleagues raised important issues regarding the study of cell migration in 2D including the as yet unresolved question of whether cells migrating in 3D have a lamellipodium which is seen extensively in 2D (reviewed in Evan-Ram and Yamada 2005). Other studies suggest that cells migrating in a 3D matrix do have invadapodia (*see* Chapter 10 by Mueller et al. this volume). In addition, it has been observed that microglia (brain macrophages) exhibit highly motile filapodia sampling their environment (Nimmerjahn et al. 2005) although Even-Ram and Yamada (2005) have speculated that such activity may relate to the unavoidable damage incurred when preparing such specimens. In vivo models clearly will provide vital information regarding the migration of cells within their social context but these studies are still complemented by those in vitro where dissection of mechanisms is more tractable.

A halfway house between 2D systems and in vivo migration are the various 3D matrices which have been developed starting from predominantly type-I collagen gels through tumour extracts (e.g. Matrigel where laminin-1 predominates) and fibrin gels. Each of these systems have yielded interesting data and enabled hypotheses to be tested. Most relevant to this chapter are the data which have emerged from the groups of Steve Weiss and Peter Friedl.

Early studies on leukocyte cell migration in 3D collagen gels suggested that collagen degradation was not required in this context (Schor et al. 1983). This area was taken up by Friedl and colleagues using sophisticated microscopic techniques to explore tumour-cell and T-cell migration in detail (Wolf et al. 2003a, b). These authors have used quenched-fluorescent collagen to track degradation of collagen gels during cell migration. Friedl and colleagues have suggested that when proteinases are inhibited, T cells and tumour cells adopt an amoeboid-like migration and can still penetrate collagen gels. Similarly, Sahai and Marshall (2003) demonstrated that tumour cells migrating in a 3D collagen gel in the presence of a cocktail of proteinase inhibitors adopt a rounded morphology and require Rho signalling.

More recently, Friedl and colleagues have explored mechanisms of migration of tumour cells in greater detail using collagen gels where most of the collagen is unlabelled with a small component of quenched fluorescent collagen (Wolf et al. 2007). The authors suggest that MT1-MMP is involved in 'collective cell migration' of tumour cells. HT1080 cells over-expressing MT1-MMP expressed this MMP at the leading edge but an area of proteolysis is detected, with a collagen-cleavage site antibody, just behind the leading edge of the cell (Wolf et al. 2007). In this chapter, the authors show significant inhibition of cell migration in a 3D matrix with the MP inhibitor BB2516 (marimastat). The authors then go on to observe that when cultured in a cocktail of proteinase inhibitors (inhibiting all classes of proteinases) HT1080 cells can continue to migrate, deforming the collagen matrix and migrating as single cells. They comment that twofold enhanced migration speed is observed in proteinase competent cells. Overall the Friedl group argues that 'collective-cell migration' requires collagenolysis but single-cell migration is not impaired in the absence of MT1-MMP activity (*see* Fig. 18.1a and b for summary). These authors have the view that it is not necessary for epithelial cells to undergo EMT in order to become invasive, but this remains controversial and probably tumour dependent. The single-cell migration observed is reminiscent of earlier studies in 3D-collagen gels from a number of groups, including that of Jean Paul Thiery where in the absence of proteinase inhibitors (though in the presence of serum and thus in the presence of some inhibitors) cell migration in chains of cells was observed following EMT (e.g. Tucker et al. 1990).

The question arises as to how do the Friedl group's recent studies fit in with studies by those groups who have reported critical roles for MMPs in, for example, tumour cell migration (reviewed in Sounni and Noel 2005)? Notable amongst these studies are those of the Weiss group. In a series of papers, Weiss and co-workers have elegantly demonstrated that there is a requirement of three members of the MT-MMP family in the migration of tumour cells through collagen gels, chick chorioallantoic membrane (CAM) or intact peritoneal basement membrane barriers (Hotary et al. 2000, Sabeh et al. 2004, Hotary et al. 2006). Hotary et al. (2000) investigated collagen gel invasion by MDCK cells in response to HGF and showed that transfection of these cells with MT1-, MT2- or MT3-MMPs accelerated invasion into collagen gels (with variations observed between these enzymes). It is relevant to note that invasion by MDCK cells in response to HGF is observed following 12 days of culture and the accelerated invasion is observed after 3 days of culture. Studies with cells isolated from MT1-MMP-/- mice showed that these cells display no collagen invasion over a 6-day period, and crucially this phenotype is rescued by transfection with MT1-MMP (Sabeh et al. 2004, Fig. 18.1C and D). In addition, these authors report that MT1-MMP-/- fibroblasts become trapped in the collagen-rich dermis of 4-week-old mice (Sabeh et al. 2004). The recent work (Hotary et al. 2006) indicates that proteolytic degradation of basement membrane ECM components is essential for the invasion over 8 days seen with several different tumour cell lines of carcinoma origin (summarised in Fig. 18.2B). The Friedl group study migration within a type-I collagen gel is observed over a 24 h period, and it is possible that the differences reported may reflect the

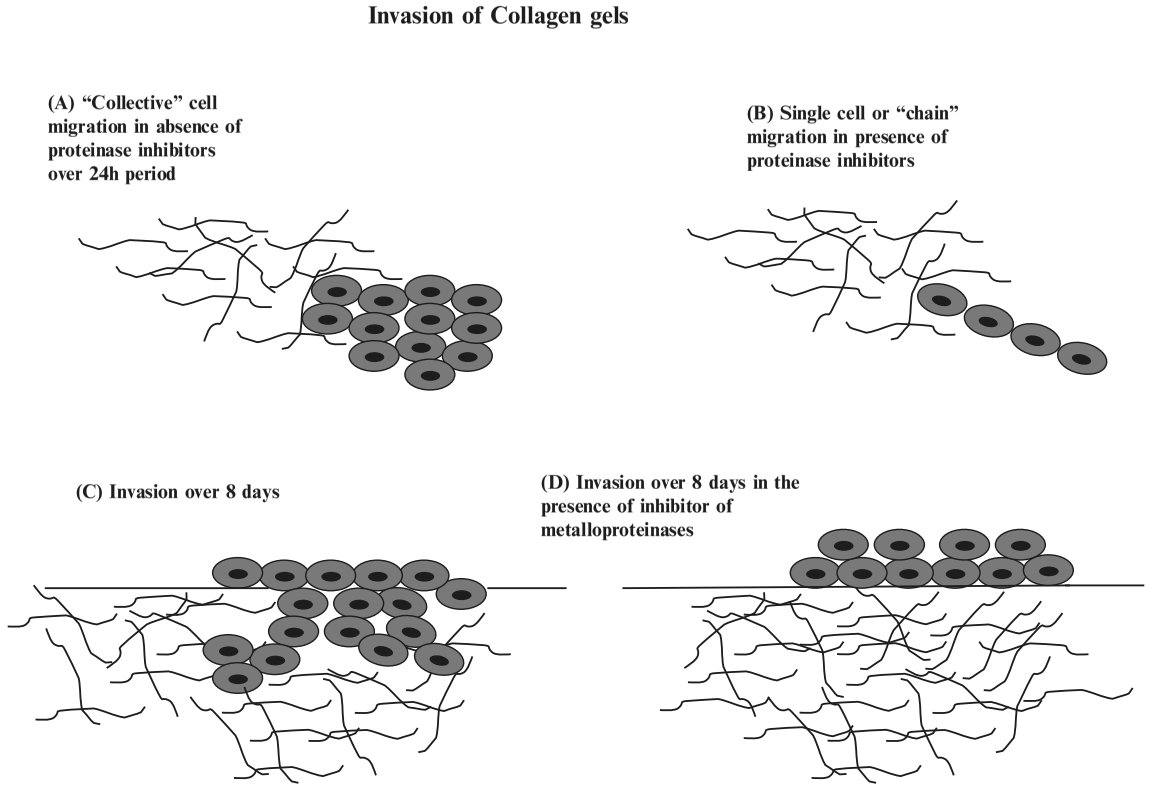

Fig. 18.1 Short- and long-term invasion of collagen gels by tumour cells. **a** Tumour cells invading through collagen gels up to 24 h seem to move as a group. **b** When proteinases are inhibited in these short-term assays, cells migrate as single cells. **c** Tumour cells invading collagen gels over 6 days in the absence of proteinase inhibitors. **d** Tumour cell invasion is completely blocked by metalloproteinase inhibitors

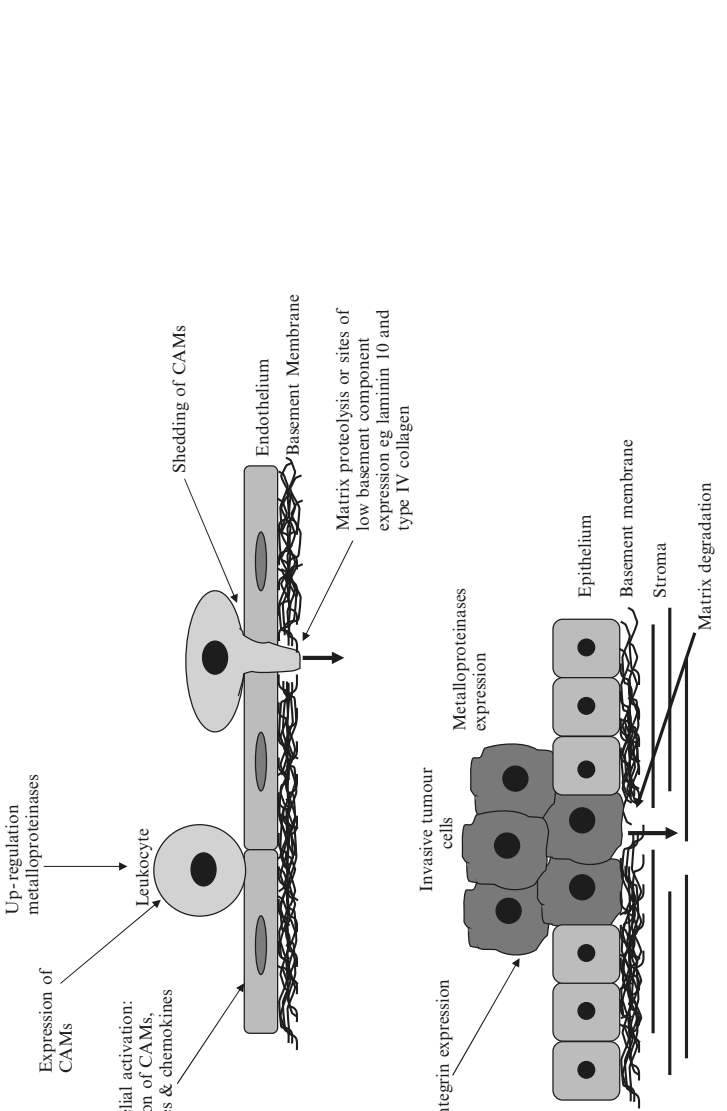

Fig. 18.2 Models of cell migration during normal or pathological events. **a** Activated endothelium attracts leukocytes to regions of infection or injury. Leukocytes attach to endothelial cells and migrate through the monolayer. Matrix proteolysis by the up-regulation of metalloproteinase expression or low expression of matrix proteins allows leukocytes to migrate through the basement membrane barrier. **b** Tumour invasion across intact basement membrane barriers over long time periods (12 days) in vitro. Expression of metalloproteinases enables invasive cells to migrate through an intact basement membrane and in vivo would allow migration to regions distant from the site of origin (*CAM*, cell adhesion molecule)

time-points studied. The data from Hotary et al. (2006) would suggest that the single-cell migration observed by Friedl and colleagues in cells treated with proteinase inhibitors and migrating in reconstructed 3D type-I collagen matrices is not sufficient to allow migration through an intact basement membrane. Friedl's group has largely used the highly motile HT1080 cell line of fibrosarcoma origin in their studies, although in their most recent study they have performed experiments with MDA-MB-231 cells, again revealing proteolytic degradation behind the leading edge, although the localisation of MT1-MMP was not determined in these cells (Wolf et al. 2007). As Mueller et al. (Chap. 21 this volume) comment, the temporal generation of the ¾ collagen neoepitope is unknown and it is not inconceivable that this may take some time to be revealed as the cell moves forward.

The recent data reviewed above suggest that short-term invasion assays in 3D collagen matrices are not dependent on MMP function but invasion of intact basement membranes and longer-term invasion of collagen gels requires certain MT-MMPs.

Cell Migration Studies In Vivo

Several studies in genetically manipulated mice have suggested roles of proteinases in cell migration. For example, TIMP1-null mice exposed to bleomycin develop an enhanced neutrophilia in the lung (Kim et al. 2005), which is suggestive of increased cell migration due to proteolytic action of a TIMP1-sensitive proteinase (ADAM10 or MMPs excluding some MT-MMP family members). Possible sites of action include MMP7 degradation of syndecan-1 (Li et al. 2002) or E-cadherin (McGuire et al. 2003) or cleavage of basement membrane components. The role of MMP7 is particularly supported by the fact that MMP7-null mice are protected from bleomycin lung injury with few cells penetrating the alveolar epithelial cell layer to enter the lung space (Li et al. 2002). In this case, in wild-type mice generation of a gradient of the chemokine KC is set up by MMP7 cleavage of syndecan-1. A number of recent studies in MMP-deficient mice have demonstrated crucial roles for several MMPs in cheomokine (and cytokine) processing which impact on neutrophil cell migration (reviewed in Van Lint and Libert 2007).

Intravital miscroscopy has begun to revolutionise our knowledge of cell migration in vivo, particularly with respect to leukocytes and tumour cells. Early intravital microscopy tumour studies by Ann Chambers and co-workers showed that the MP inhibitor batimastat suppressed B16 melanoma liver metastases-associated angiogenesis although tumour extravasation was not altered (Wylie et al. 1999). The pioneering work of Sussan Nourshargh and colleagues has allowed the mechanisms underlying leukocyte migration in vivo to be elucidated, for example during migration through the basement membrane encountered by leukocyte and tumour cells alike, once the endothelial barrier has been crossed. Recently, this group has established that neutrophils transmigrate through venules and then through the pericyte basement membrane by locating sites of lower expression of

key components of the basement membrane (Wang et al. 2006). Immunolocalization studies reveal that whilst expression of the proteoglycan perlecan was uniform, identifiable 'exit points' had severely decreased immunostaining for type-IV collagen and laminin-10 (summarised in Fig. 18.2A). Of relevance to these data, Van Agtmael et al. (2005) have shown that in the normal retina the basement membrane has a non-uniform thickness, indicating that there may be sites of differential expression of ECM components in this tissue as well. Nourshargh's group has also shown that neutrophil elastase and other serine proteinases play an important role in migration through the venule BM (Young et al. 2007). Whilst NE-null mice do not show a defect in cytokine-induced transmigration, the serine proteinase inhibitor aprotinin blocks transmigration, indicating that additional serine proteinases are involved. Any role for metalloproteinases in generation of sites of lower basement membrane component expression has yet to be explored.

Thus far few studies have addressed the potential roles of MPs in transendothelial cell migration in vivo. Ann Ager and colleagues demonstrated that MPs have some roles in transendothelial migration of lymphocytes by pre-incubating lymphocytes with a hydroxamate MP inhibitor and thus inhibiting ADAMs and MMPs (Faveeuw et al. 2001). These authors demonstrated that lymphocytes pre-treated with the hydroxamate inhibitor had higher levels of surface L-selectin and accumulated within the endothelial lining of high endothelial venules. Venturi et al. (2003) have uncovered a role for L-selectin shedding in neutrophil entry into the peritoneum in a mouse model of inflammation. L-selectin shedding, prevented by replacement of the membrane proximal cleavage site with the equivalent sequence from the E-selectin molecule, resulted in a significantly increased leukocyte migration into the peritoneum, although rolling was not altered, as visualised by intravital microscopy (Venturi et al. 2003). In studies of EAE, the transmembrane receptor dystroglycan, expressed in the brain parenchymal basement membrane, is cleaved by MMPs 2 and 9 (but not MMPs 1, 3, 7 and 8) and as a result macrophages penetrate the blood–brain barrier in this inflammatory condition (Agrawal et al. 2006). Mice null for MMPs 2 or 9 are resistant to EAE and macrophages fail to infiltrate.

Zebrafish is a very attractive model system for in vivo analysis of cell migration due to its transparency, the availability of genetic mutants and the accessibility of pharmacological inhibitors. Very recently, Philippe Herbomel's group has observed neutrophil and macrophage migration in vivo in zebrafish and uncovered the remarkable fact that whilst primitive neutrophils are rapidly attracted to sites of injury or infection they barely phagocytose bacteria, whereas macrophages attracted simultaneously phagocytose bacteria in great quantities (Le Guyader et al. 2007). Zebrafish have also been used to study tumour cell migration, and a recent study with intraperitoneal injection of several human tumour cell lines and murine B16 melanoma shows that HT1080 cells adopt an amoeboid-like migration and are highly invasive whereas B16 cells show a mesenchymal type of migration (Stoletov et al. 2007). MDA-435 cells showed mixed amoeboid and mesenchymal morphology and were poorly invasive but MDA-435 carcinoma cells over-expressing RhoC (associated with human metastasis) became highly invasive and

exhibited amoeboid-like cell migration in the zebrafish (Stoletov et al. 2007). Co-injection experiments with parent MDA-435 cells (which do not invade in the zebrafish) revealed that the Rho-C expressing MDA cells did not modify the migration rate of control MDA-435 cells. The authors conclude that this suggests that the alteration in invasion of Rho-C is intrinsic to the cell line and that proteolytic mechanisms are not implicated in the enhanced migration of RhoC-expressing cells. Whilst the data seem compatible with the idea that path clearing is not occurring (which could enhance cell migration of control cells) perhaps a further experiment with co-injection of proteinase inhibitors is warranted. Another recent study indicates that chronic inflammation, with hallmarks of human diseases such as psoriasis, is induced in a zebrafish with a mutation in HAI-1 (hepatocyte growth factor activator inhibitor-1), an inhibitor of the serine proteinase matriptase (Mathias et al. 2007). Intravital microscopy of this mutant crossed with zebrafish with GFP-tagged neutrophils reveals that these cells display periods of random migration with a loss of polarity and adoption of a rounded morphology whilst pausing as well as periods of persistent migration. The authors observed that a COX-2 inhibitor blocked neutrophil migration and induced a similar rounded morphology, suggesting that inflammatory mediators whose production involves COX-2 are active in vivo. Zebrafish is thus emerging as an excellent model for the study of cell migration in vivo. Embryonic development in *Xenopus laevis* is another very interesting model for in vivo analysis of metalloproteinases in cell migration. MMP7 has been localised to migrating macrophages in *Xenopus* (Harrison et al. 2004), and recent studies indicate that in vivo macrophage migration inhibited morpholino knockdown of XMMP7, 9 and 18 (Matt Tomlinson and Grant Wheeler, personal communication).

Matricryptic Sites in Cell Migration

Matricryptic sites in matrix components (termed matricryptins by Davis et al. 2000) are sites which become exposed largely through proteolytic cleavage and which have novel biological activities. Recent reviews cover some of the major matricryptic sites (Bellon et al. 2004, Tran et al. 2005), and so here brief consideration will be given to those which relate most closely to the cell migration studies described above.

Type-IV Collagen

A number of cryptic anti-angiogenic factors have been described in type-IV collagen generated, for example, by MMP cleavage of tumour basement membrane sources (reviewed in Mundel and Kalluri 2007). In some cases, these fragments have been reported to block tumour cell migration as well through effects on

MT1-MMP (reviewed in Pasco et al. 2005), although other studies reveal pro-migratory cryptic sites within type-IV collagen which bind to αvβ3 integrins (Xu et al. 2001). It would be of interest to determine the integrins involved in migration through intact basement membrane in the type of experiment described in Hotary et al. (2006) as well as the nature of any type-IV collagen fragments generated in this model system.

Laminin 5

Degradation of the γ2 chain of rat laminin 5 has been reported to expose a cryptic site resulting in enhanced migration of tumour cells (Koshikawa et al. 2000). However, other studies have shown that human γ2 and α3 chains of laminin 5 are degraded by BMP-1 and not by MT1-MMP or MMP2 (Amano et al. 2000). Recent studies indicate that MMP7 cleaves the β3 chain of laminin 5 generating a 90 kDa fragment (Remy et al. 2006). This group demonstrates that MMP-7-degraded laminin 5 promotes migration of the colon carcinoma cell line HT29 and that MMP7 and laminin 5 co-localise in cells on the outer borders of cellular colonies. Signalling events generated by this cleavage event remain to be determined but this model opens new avenues for investigation.

Proteoglycans

Proteoglycans are inhibitory to axon regeneration in both the peripheral nervous system and the central nervous system (CNS; reviewed in Busch and Silver 2007). Work from several laboratories has demonstrated that treatment of CNS with chondroitinase ABC (ChABC), which cleaves GAG chains, results in enhanced neural repair (Barritt et al. 2006). Recent studies indicate that perineuronal nets in rat brain contain several proteoglycans including aggrecan, brevican, neurocan and phosphocan (Deepa et al. 2006). Whilst the mechanisms by which ChABC exerts its effects remain unclear (reviewed in Crespo et al. 2007), exposure of pro-migratory sites in proteoglycans or other ECM components remains a possibility. Previous work indicated that MMPs could also promote regeneration in the PNS again by cleaving proteoglycans, though in this case one would presume through cleavage of the protein core (Krekoski et al. 2002). Larsen et al. (2003) demonstrated that MMP9 enhances remyelination by degradation of another inhibitory proteoglycan NG2. Nerves and blood vessels grow into normally avascular intervertebral discs, and ChABC treatment of intervertebral discs results in endothelial cell migration and proliferation (Johnson et al. 2005). It is still unclear whether proteoglycan degradation exposes cryptic sites or simply removes a barrier to migration. It is of relevance to note that exencephaly occurs in the brains of perlecan-null mice, attributed to the removal of an intact basement membrane barrier (Costell et al. 1999).

Type-I Collagen

In these recent investigations of cell migration in 3D (described above), the potential roles of matricryptic sites in the ECM have yet to be explored. Studies of cell migration in 2D have revealed that denaturation of type-I collagen (Davis 1992) or degradation of type-I collagen by MMP-13 into classical ¾ and ¼ fragments (Messent et al. 1998) leads to the exposure of an RGD site which can then become available for binding through αvβ3 integrins. Exposure of vascular smooth cells to purified ¾ collagen fragments results in markedly enhanced migration in response to PDGF-BB, visualised by time-lapse videomicroscopy (Stringa et al. 2000). This is of interest since αvβ3 integrin has some role in pathological vascular smooth muscle cell migration (reviewed in Newby 2005).

Conclusion

Much remains to be uncovered regarding proteolytic mechanisms involved in cell migration in 3D as well as in vivo. The use of complex in vitro model systems should result in the generation of exciting hypotheses to be tested in vivo, where organisms such as zebrafish should allow both genetic and microscopic analysis. The new era of intravital imaging using quantum dots with their advantages of photostability, tunability to narrow emission spectra and potential as drug delivery vehicles (Stroh et al. 2005) as well as second harmonic imaging of collagen will undoubtedly extend dramatically our understanding of cell migration in vivo. In combination with genetic approaches, these advanced microscopic techniques will allow the interplay of cells with neighbouring cells and with their surrounding matrix to be determined at a level which may allow more astute in vivo discrimination between, for example, proteinase inhibitors with subtly different biochemical specificities.

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