Review

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Extracellular matrix determinants of proteolytic and non-proteolytic cell migration

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Cell invasion into the 3D extracellular matrix (ECM) is a multistep biophysical process involved in inflammation, tissue repair, and metastatic cancer invasion. Migrating cells navigate through tissue structures of complex and often varying physicochemical properties, including molecular composition, porosity, alignment and stiffness, by adopting strategies that involve deformation of the cell and engagement of matrix-degrading proteases. We review how the ECM determines whether or not pericellular proteolysis is required for cell migration, ranging from protease-driven invasion and secondary tissue destruction, to non-proteolytic, non-destructive movement that solely depends on cell deformability and available tissue space. These concepts call for therapeutic targeting of proteases to prevent invasion-associated tissue destruction rather than the migration process *per se*.

Cell migration and tissue remodeling

Cells migrating through interstitial tissue or a basement membrane are confronted with 3D tissue structures that provide a substrate for adhesion and traction, and also impose biomechanical resistance against the moving cell body. In cancer invasion, neoplastic cells activate migration and tissue-penetration programs and escape from their primary location by crossing basement membranes and infiltrating interstitial tissues. In chronic inflammation and tissue repair, migrating leukocytes and fibroblasts migrate through the ECM and, simultaneously, contribute to tissue reorganization. Accordingly, a frequent hallmark of cell invasion and tissue remodeling is the upregulation of proteolytic enzymes generated by both migrating cells and reactive stromal cells [1–3]. Despite their common association during disease processes *in vivo*, however, the mechanistic link between cell invasion and often pathologic degradation of tissue structures is controversial [4–7]. We review here when and how proteolytic ECM degradation in cancer invasion and physiological and disease-associated tissue remodeling is mandatory, when it is optional, and when it is dispensable for cell migration through connective tissue. We aim to provide a physicochemical model of the protease requirements in invasive cell migration and discuss the implications for therapeutically targeting proteases, with a focus on tissue invasion by cancer cells and other types of tissue remodeling, such as in inflammation, wound healing and bone remodeling.

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Cell determinants in migration

Proteolytic cell invasion into tissues is a cyclic five-step process with secondary adaptation reactions dependent on the molecular properties of the cell and the ECM encountered [8]. After cell polarization and initial protrusion (step 1), the newly formed leading edge attaches to ECM fibers (step 2) followed by cell surface-localized proteolysis degrading the underlying or surrounding ECM to generate space for the advancing cell body (step 3) [9–12]. Concomitantly, actomyosin contraction leads to local traction and force generation, deforming both the cell body and ECM (step 4) which, with turnover of adhesion bonds near the cell rear, is followed by retraction of the trailing edge (step 5) [9].

Each migration phase may undergo adaptation dependent on molecular and/or physical determinants of both cell and substrate. Cellular determinants comprise (i) the volume and deformability of the cytoplasm depending upon its spatiotemporally adaptive cytoskeletal structure, contractility and cortical rigidity, and (ii) the volume and deformability of the nucleus controlled by lamin and chromatin content and structure [13,14]; each parameter is variable and responsive to the environment encountered.

Accordingly, cell protrusion may lead to very different leading edge morphologies with distinct force-generation capabilities, including lamellipodia, pseudopodia or spherical blebs [15–18]; actomyosin contraction may generate high, low or no traction force [15,19,20]; and cell–matrix interactions may generate strong, weak or no proteolytic ECM remodeling [5,8]. Thus, at molecular and physical scales, cell migration is a plastic and adaptive process that enables the cell to accommodate different environments and 'fine-tune' each migration step.

Space determinants of ECM

Both normal and deregulated cell migration can take place along or within virtually all extracellular tissues, such as

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basement membrane, collagen-rich interstitial tissue, fibrin, cartilage and even bone (Figure 1). Chemical and physical determinants of tissue composition and structure include (i) protein and other molecular content, and (ii) chemical assembly and crosslink state [21–23], which together determine (iii) the physical ECM scaffold geometry, that is alignment, density and the resulting pore size [11,24,25], as well as (iv) tissue deformability or stiffness [21,26]. All of these may vary considerably between different tissues as well as locally within the same tissue [19,24,27,28]. Generally, tissues are either loose and soft, such as loose connective tissues, embryonal stroma or provisional wound matrix, or dense and stiff, such as mature connective tissues, tumor stroma (i.e. around neoplastic breast ducts) or bone [19,27–31].

The ECM scaffold structures through which cells migrate *in vivo* can be classified according to the geometry of protein polymer and space available for the moving cell body, and most of these basic geometries can be recapitulated *in vitro* using ECM-based or synthetic migration models (Figure 1). 2D protein layers, such as basement membranes, are 0.1–1 μm thick and relatively dense layers that consist of laminins, crosslinked collagen type IV, link proteins and proteoglycans, and contain small regions of reduced protein density with pore-like configuration measuring 3–20 μm^2 in area (i.e. 2–5 μm in diameter) designated 'low-expression regions' [32]. The geometry of such a porous 2D surface is recapitulated by polycarbonate filter membranes containing pore-like vertical channels 1–8 μm in diameter, with coatings of basement membrane components that introduce a structural ECM barrier inside the pore (Figure 1a). Loose fibrillar collagen meshworks as present in most connective tissues consist of networks of crosslinked collagen type I or III fibers or bundles and interfibrillar gaps and pores that are variable in shape, diameter and spatial arrangement (i.e. not forming a tube or an elongated cleft). Loosely structured connective tissue collagen is most closely mimicked *in vitro* by reconstituted 3D collagen matrices or synthetic polymer scaffolds generating networks of tunable pore size (Figure 1b). In addition to random fibrillar networks forming a discontinuously ECM-structured scaffold, aligned ECM bundles, as well as cell surfaces, impose smooth 2D-like interfaces surrounded by 3D tissue, morphologically forming elongated gaps and tunnels [9,24]. These structures include packed collagen bundles consisting of multiple aligned fibers, myofibers or blood vessels decorated with basement membrane, and nerve strands suited for providing contact guidance along the preformed interface [33]. Such interface-dependent tracks are mimicked by microchannel models with circular or rectangular cross-sections, with diameters of several micrometers, which are used for probing cell deformability, elongation and migration capacity (Figure 1c). Particularly narrow clefts with submicrometer heights are present in slit-like migration chambers used for probing cell protrusions and for high-resolution imaging (Figure 1d; P. Peters, unpublished data). Last, mineralized bone or teeth provide a rigid 2D ECM landscape that lacks interstitial spaces, thus supporting horizontal but prohibiting vertical cell migration. Such tight and rigid structures with a relatively smooth 2D surface are mimicked by continuous ECM or mineral layers on glass or other hard synthetic homogeneous materials (Figure 1e). For mechanistic studies, the chemical and physical parameters intrinsic to each *in vivo* tissue and corresponding *in vitro* model substantially impact upon the morphological and molecular mechanisms by which cells engage with polymeric substrate and available space.

Principles of proteolytic migration and ECM processing

Moving cells, in response to varying substrate properties and often co-engaged cytokine and growth factor signals, upregulate and/or activate different classes of enzymes in a time- and space-controlled manner [1,2,34]. The enzyme classes involved are cathepsins, serine proteases, and/or matrix metalloproteinases (MMPs), which together contribute to degradation of all ECM molecules via three spatially distinct mechanisms, including (i) diffuse proteolysis executed by diffusing proteases released from the cell, (ii) cell surface-associated proteolysis generated by membrane-inserted or -tethered proteases, and (iii) intracellular degradation by proteases confined in lysosomes [11,35,36].

Diffuse proteolysis of structural ECM components is executed by soluble enzymes outside the cell pericellularly or further away from the cell surface (Figure 2a). Enzymes originate either from the cell, for example exocytosed MMPs or cathepsins, or are delivered systemically with body fluids, including interstitial or vascular fluids, for example plasminogen. Enzymes diffuse into the extracellular space in either active form or as proenzymes to become activated at the cell surface [36–38]. Active enzymes then distribute and temporarily engage with ECM substrates to execute cleavage, either locally or as gradual lysis in widely distributed areas [27,39,40]. As a consequence, diffuse proteolysis leads to non- or poorly focalized cleavage of multimeric ECM proteins and gradual loss of substance at arbitrary sites [39–42].

In a specialized form of diffuse juxtacrine proteolysis, osteoclasts form a ring-like adhesive sealing zone to bone and release cathepsins in a spatially confined manner for diffuse substrate degradation (Figure 2a, right) [27,38,43]. Both secreted wide-ranging and juxtacrine proteolysis are generally not strictly associated with cell migration, but are instead associated with relatively random substrate disintegration required for connective tissue turnover or the lysis of provisional tissue, such as a fibrin clot; they further contribute to pathologic 'overshoot' tissue destruction, such as bone degradation during osteoarthritis or malignant plasmocytomas [27,36,44,45].

Contact-dependent proteolysis is the topographically controlled degradation of ECM polymers, including collagens, fibrin, fibronectin or laminins, which surround and touch the cell passively or adhere to the cell surface actively via binding to specific receptors. Proteases executing cell contact-dependent proteolysis are either tethered at the cell surface via receptors – for example, MMP-2 is bound to αvβ3 integrins or to MMP-14 – or are inserted in the plasma membrane by a transmembrane domain; examples include seprase and membrane-type matrix metalloproteinases (MT-MMPs) (Figure 2b) [46,47]. Cell-surface proteases, when co-engaged with ECM-binding adhesion

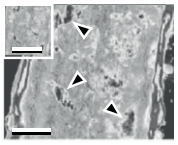
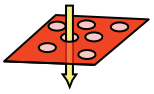
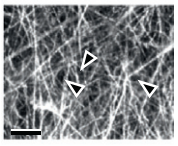
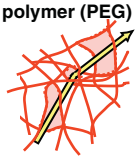
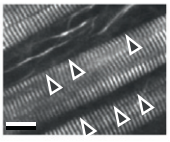
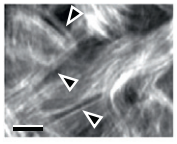
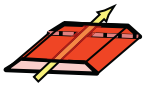
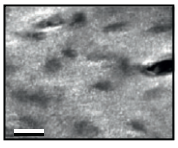
	***In vivo* structure**			***In vitro* model**		
	Morphology	Tissue type	Ref.	Type of scaffold	Physical migration structure	Ref.
(a)	Basement membrane 	Epithelial and endothelial basement membranes	[32,68,73]	**Porous synthetic surface** 	Flat pore through a synthetic surface (Boyden chamber)	[73,88,89]
(b)	Collagen-rich dermis 	Randomly organized fibrillar ECM; provisorial matrix after wounding; loose primordial connective tissue	[24,28,30, 31,63, 76,90]	**Collagen matrix, synthetic fibrillar polymer (PEG)** 	Fibril meshwork with discontinuous pores of variable cross-section	[11,91]
(c)	Perimuscular stroma 	Wide gaps and trails with parallel interfaces; perimuscular or perineural tracks; perivascular loose stroma	[33,76]	**Synthetic microchannels** 	Continuous microchannels of tubular shape (Capillary-like)	[75,92]
(d)	Dermis 	Narrow clefts in collagen-rich mature connective tissue	[24,28,93]	**Synthetic flat microchannels** 	Continuous slit-like channel with opposing 2D surfaces	[94] P. Peters, (unpublished)
(e)	Bone 	Tight calcified collagen in bones and teeth	[27,95]	**Synthetic surface** 	Thin homogeneously glass-coated dentin, apatite, or collagen	[27]

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Figure 1. **Structural *in vivo* ECM determinants and related *in vitro* migration models**. Examples of interstitial tissue types *in vivo* monitored by *in situ* fluorescence **(a)** or second harmonic generation microscopy **(b–e)**, displaying the geometries of their pro-migratory structures (left) and the closest experimental counterpart models *in vitro* (right). **(a)** Basement membrane from a murine cremaster venule (anti-laminin-α5 immunostaining of an *ex vivo* sample). **(b,c)** Collagen fiber scaffold of upper and deep mouse dermis (3D reconstruction of an *in vivo* sample). **(d)** Human dermis (3D reconstruction of an *ex vivo* sample). **(e)** Lamellar bone surface (3D *in vivo* reconstruction of lamellar bone of the mouse tibia). Black areas (arrowheads) correspond to regions of low laminin content in **(a)**, pores **(b)**, channel-like guidance structures **(c)**, or slit-like clefts in **(d)**, that may serve as guidance structures to migrating cells. Black areas in the bone indicate vessel tracks (asterisk) and lacunae of osteocyte positions (not marked). The cartoons for *in vitro* models indicate the space-confining protein or polymer (red), bordered space (light red), and the direction of migration (arrow). Bars, 10 μm (a); 20 μm (a, inset; b–f). Reprinted images in **(a)** are with permission from Elsevier [32], **(b)** with permission from John Wiley & Son [76], and **(d)** with permission from Elsevier [24].

molecules or directly bound to ECM substrate, show a tendency to multimerize and form larger protein complexes [5,48]. As a consequence, contact-dependent proteolysis leads to localized matrix breakdown of only the ECM in direct vicinity to the cell surface and, in conjunction with cell migration, to a precisely re-aligned matrix defect while the structural integrity of ECM components nearby remains preserved (Figures 2b,d) [11,49,86]. Contact-dependent proteolysis underlies invasive processes, such as vascular sprouting, cartilage and bone remodeling during development, and cancer cell invasion [50–52]. Consequently, contact-dependent cleavage is relevant for cell migration-dependent ECM patterning leading to coordinated formation and restructuring of multicellular tissues.

Last, intracellular proteolysis leads to the processing or degradation of presolubilized ECM particles inside cells (Figure 2c), a process that also takes place following

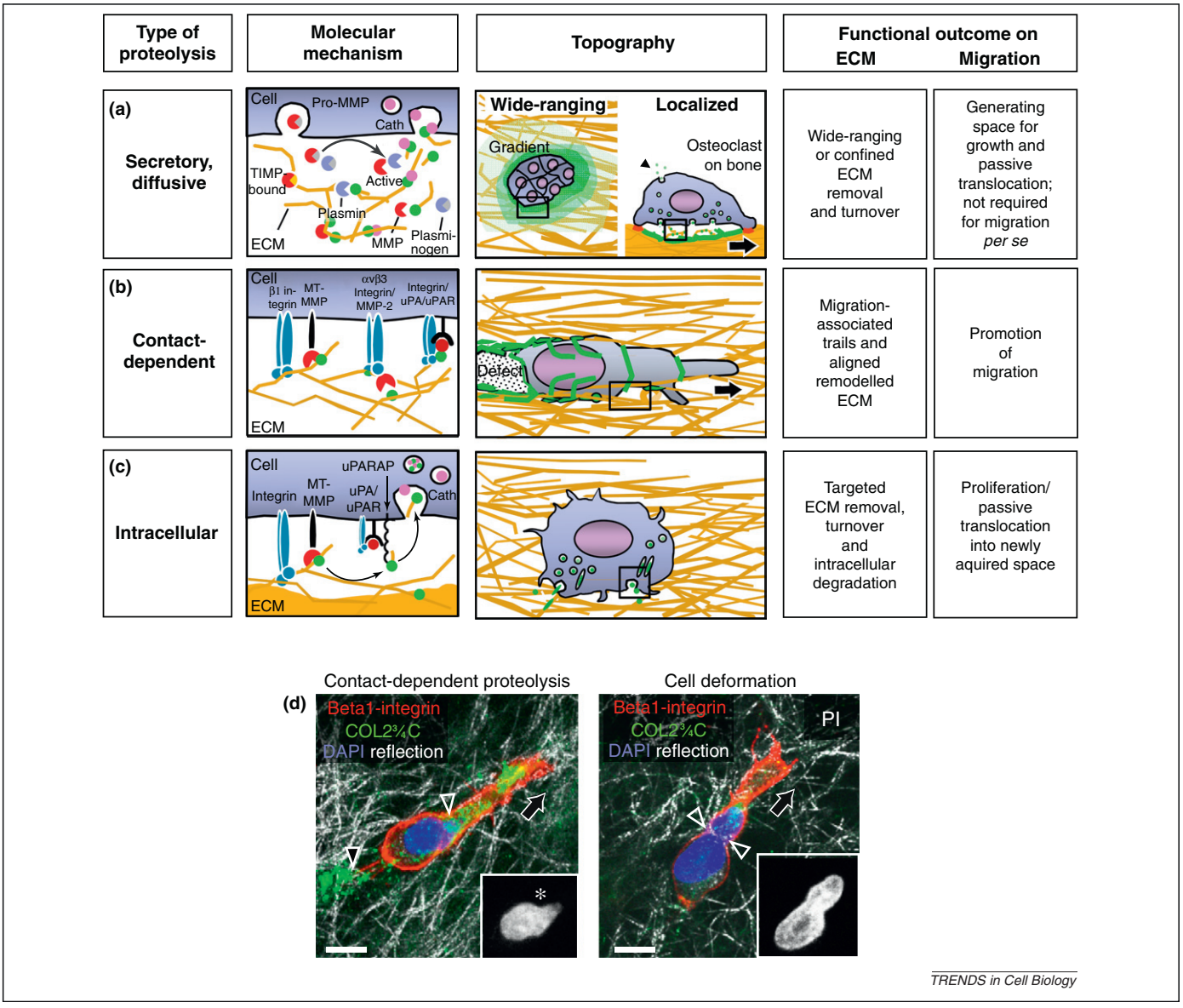

Figure 2. Modes of ECM proteolysis in 3D tissue. (a) Diffuse ECM degradation. Cells placed on or within ECM degrade tissue structures diffusely or nearby via diffuse proteolysis by soluble proteases. Protease precursors, such as MMPs, are released and activated at the cell surface by a cascade of proteolytic events to diffuse away from the cell [37,82–84]. As an example of wide-ranging ECM degradation, inactive MMP-2 (red/grey circle) is secreted and activated (red open circle) by membrane-bound uPA/uPAR or MMP-14, and then released into the extracellular space to cleave ECM (green circles). MMP-2 activity is blocked by soluble TIMPs released into the extracellular space and is thereby tightly regulated (red/yellow circle). Extracellular plasminogen (purple/gray circle) becomes activated by membrane-bound uPA/uPAR to plasmin to cleave fibrin during clot lysis [40]. Likewise, cathepsins (cath) located in caveolae and/or released into the extracellular space cleave ECM pericellularly [36,39]. For localized bone matrix-digestion, osteoclasts release lytic enzymes towards the underneath location confined by an actin-generated sealing zone, where collagen is pre-degraded (green), followed by phagocytosis, transcytosis and exocytosis (black arrowhead; adapted from [38]). **(b)** Cell surface-associated contact-dependent ECM degradation. ECM structures contacted by integrins are cleaved by adjacent cell surface-bound proteases, such as MT1-MMP/TIMP-2/MMP-2 complexes, MMP-2 complexed with α3β3 integrins, or uPA/uPAR, degrading native collagen fibers, predigested collagen or fibrillar fibronectin, respectively. Processed ECM polymers are either realigned (green lines) or endocytosed. **(c)** Intracellular ECM degradation. Partially degraded, preprocessed ECM fragments or monomers are internalized by the mannose receptor or the homologous uPARAP/Endo180 receptor complexed with uPA/uPAR and β1 integrins [35,36]. Intracellular degradation is abundant in macrophages and fibroblasts during ECM remodeling [85]. **(d)** Protease-dependent and non-proteolytic cancer cell migration. Contact-dependent proteolysis of migrating HT1080/MT1-MMP cell within a 3D collagen lattice (left), compared with a cell in the presence of a broad-spectrum protease inhibitor cocktail (PI). With contact-dependent proteolysis intact (empty arrowhead, left image), the nucleus retains an ellipsoid, poorly deformed shape (*, inset). Proteolytic path (black arrowhead). With proteases inhibited, the inability to degrade the ECM leads to deformation of the nucleus (empty arrowheads). Thick black arrows (a,b,d) indicate the direction of movement. Green ECM denotes proteolytic degradation zones. Black encircled rectangles in (a–c) indicate topography for molecular mechanism depicted in the left column. Not discussed here are the specific proteolytic cell structures that mediate contact-dependent ECM degradation, including lamellipodia, pseudopodia and lateral spikes, podosomes and invadopodia, and regions of cell compression [10,86,87].

extracellular proteolysis. ECM uptake and digestion removes ECM from the interstitium for internal degradation or recycling not only during connective tissue, cartilage or bone turnover in development, hemostasis and repair [35,53], but also during pathologic collagen deposition [54], and supports malignant cell growth by removal of tumor-surrounding ECM [55]. Solid ECM, such as collagen, is cleaved by secreted or cell-surface proteases into soluble fragments, followed by receptor-mediated endocytosis [56]. In fibroblasts, chondroblasts or osteoclasts, the urokinase plasminogen activator receptor-associated protein (uPARAP/Endo180) receptor binds to collagen fragments and mediates their internalization, followed by lysosome acidification and intracellular degradation by cathepsins [36,53,56]. Activated macrophages endocytose collagen via mannose receptors, followed by digestion in lysosomes [54].

These mechanistically and topographically distinct modes of ECM degradation do not function in isolation, but instead form concurrent and complementary systems for ECM scaffold remodeling and degradation involved in tissue organization and growth, and, when deregulated, in tissue destruction or neoplastic invasion [35,36,44,57].

Principles of protease-independent cell migration

In the absence of proteolytic ECM breakdown – either by intrinsic lack of protease availability, as in T lymphocytes, or the presence of endogenous or therapeutically applied protease inhibitors – migrating cells accommodate the pre-existing ECM structure by protease-independent mechanisms [5,58,59]. The ECM structure remains undegraded and the migrating cell must therefore change its own shape and, depending on ECM compliance, temporarily deform the ECM and widen the available space by pushing [5,13,60]. Consequently, depending on local ECM elasticity and the effective space available, cells employing protease-independent mechanisms are dependent on physical strategies to secure migration; these include (i) deformation of the cell body and nucleus, which thereby fit themselves into pre-existing space (Figure 1d, right) [11,61]; (ii) the transient deformation of tissue structures by actomyosin-mediated outward pushing during migration [60,62]; or, if an ECM region is impenetrably narrow, (iii) retraction of the leading edge, reorientation of the migration direction and bypassing of the obstacle by movement along available alternative interphases [58]. Thus, cells possess a portfolio of distinct proteolytic and protease-independent mechanisms to *de novo* generate or negotiate tissue space for migration.

Decision-making in proteolytic and non-proteolytic migration

The reciprocity of physical parameters and protease function between ECM and the cell determines to what extent and how cell migration occurs in the presence or absence of substrate cleavage (Figure 3, Box 1). First, the advancing cell body and in particular its most rigid compartment, the

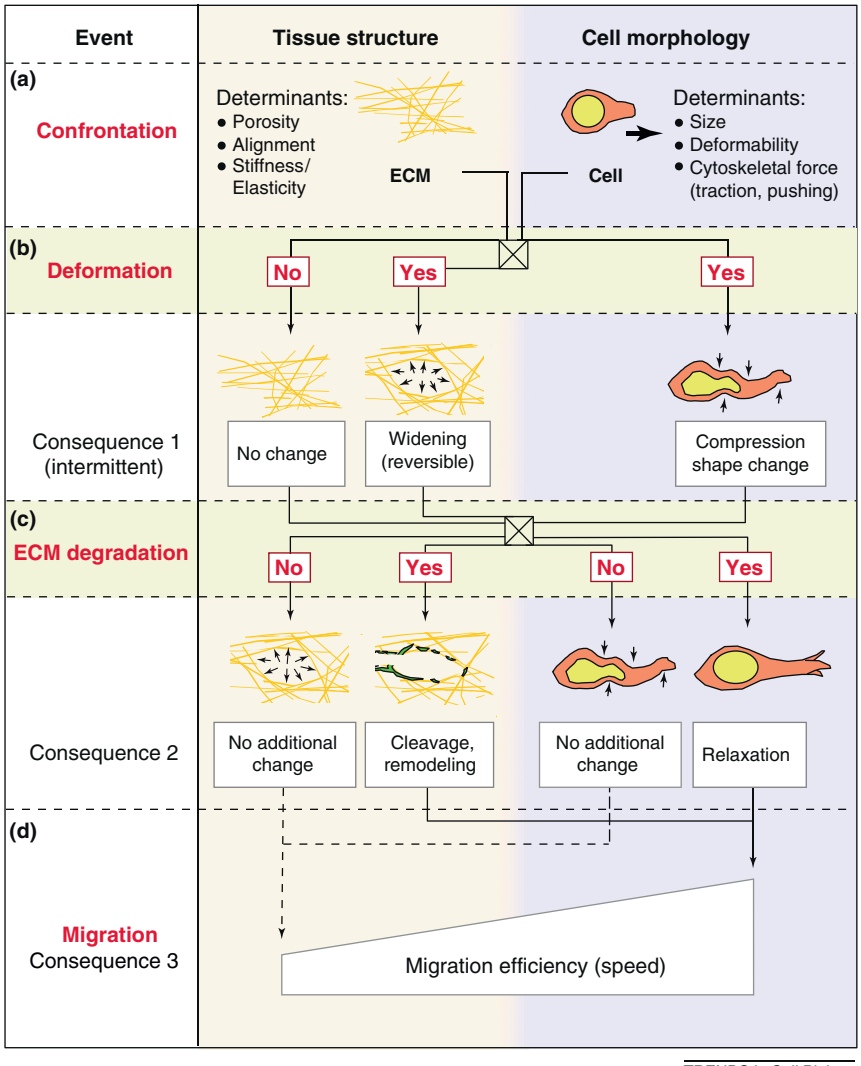

Figure 3. Decision-making in cell invasion through 3D tissue. Stepwise physicochemical events and 'decisions' (the diagonally crossed square) during dynamic cell confrontation with 3D ECM depicting the perspectives of both the tissue structure and cell morphology, leading to either a proteolytic or non-proteolytic migration mode. The decision model and resulting migration efficiency assume a matching scenario between pore diameter smaller than the cell diameter, but large enough to allow protease-independent migration supported by combined ECM deformation, or by cell shape change alone. The rate-limiting spatial conditions leading to complete migration arrest in non-proteolytically moving cells remain to be determined.

Box 1. Proteolytic and non-proteolytic cell migration - interdependence with tissue structure

Dependent on the tissue structure encountered (compare Figure 1), cells engage distinct mechanisms to bypass an obstacle.

Basement membranes. For transmigration through a vessel basement membrane, leukocytes preferentially pass through pore-like regions of reduced laminin and collagen IV content [68–70]. During inflammation *in vivo*, monocytes and neutrophils vigorously deform the cell body and nucleus 1 to 3 μm in cross-section [69]. Simultaneously, neutrophils employ proteases, particularly MMP-8 and serine proteases, and monocytes employ MMP-9 [71] and thereby may both enlarge pre-existing or forming *de novo* pores [72]. The mechanics of *trans*-pore migration is mimicked by porous filter assays, however, coating with reconstituted basement membrane or isolated ECM components probably fails to mimic the structural density and crosslink status of basement membranes. Tumor cells, presumably because of their larger and possibly less deformable cell bodies, are largely dependent on pericellular proteolysis to migrate through basement membrane *ex vivo*, generating pore-like matrix defects several micrometer in diameter [73], however, it is unclear when, *in vivo,* tumor cell penetration through basement membranes fully depends upon proteolytic pore widening or when cell deformation suffices.

Synthetic non-degradable materials. These include rigid polymers or glass used as templates for pores, channels or narrow 2D surface slits, and are, in contrast to most *in vivo* substrates, non-deformable and non-degradable by cells, therefore the size and deformability of a cell alone determines successful cell translocation or migration arrest.

3D loose fibrillar collagen. In connective tissues collagen provides linear fiber tracks along which the moving cell aligns and glides, and pores through which the cell body passes [5,58,74]. *In vitro*, 3D collagen networks are slightly deformable and, with sufficient porosity (i.e. pore cross-section exceeding 5 to 20 μm^2; K.W., unpublished data), migrating cells deform both their cell body and, to a minor extent, matrix fibers, thereby permitting cell translocation [5,58]. The spatial limits of pore geometry and cell deformability for proteolytic and non-proteolytic cell migration through collagen-rich tissue, and the rate-limiting parameters controlling cell deformation, remain to be defined [6].

2D interfaces of complex geometry formed by protein polymers or cell surfaces. These deliver continuous scaffolds for guidance next to channels and clefts for cell passage [9]. Whereas moving cells *in vitro* accommodate narrow tubes or clefts in the submicrometer range by shape change alone [75] (P. Peters, personal communication), the protease requirements for cell passage through heterogeneously organized tissue gaps and clefts *in vivo* remain unknown.

nucleus, are physically confronted with spatial ECM constraints, such as fibers bordering a pore, gap or trail (Figure 3a). As a consequence, the moving cell body imposes pressure and subsequently, as elasticity permits, displaces fibers in both forward and outward directions, thereby deforming the cell body and ECM structures. Thus, in the first event, here operationally termed a 'decision', both the cell and ECM deform to a variable degree (Figure 3b). Confrontation and intermittent reciprocal deformation take place in both proteolytic and non-proteolytic migration, as well as in dense and loose tissues, albeit to different extents, given that tissue regions of high rigidity, such as compact collagen bundles, bone or *in vitro* plastic polymers, are refractory to cell-driven deformation [27,29]. The ability or inability of the migrating cell to proteolytically degrade physically challenging ECM barriers therefore determines the extent of local ECM widening and cell deformation (Figure 3c). With the ECM locally cleaved, the newly generated space releases ECM-imposed confinement and allows the cell and its nucleus to adopt a less compressed, 'relaxed' morphology [11,13]. Conversely, in the absence of degradation, reciprocal compression remains undiminished until the cell has deformed sufficiently and passed through the resistance zone, until both cell shape and ECM scaffold revert to their original geometry and remain structurally unchanged [5,58]. Thus, each physicochemical step impacts the steady-state migration efficiency. At ECM porosity below the cross-section of the cell body, contact-dependent ECM degradation supports cell migration by creating small matrix openings that match the cell diameter, and this both limits the need for cell and ECM deformation and speeds up migration rates (Figure 3d, right) ([6,11]; K.W. and P.F., unpublished data). In the absence of proteolysis, migration continues on the condition that both matrix porosity and cell deformation provide sufficient adaptation for transmigration, albeit at the expense of speed as a function of increased ECM density [11] (Figure 3d, left). This spatiotemporal physicochemical model based on cell and ECM deformation, and on proteolytic substrate degradation, may be useful in predicting outcomes in both protease-competent and -ablated cells migrating in ECM of different density, structure and stiffness (Box 1).

Concluding remarks

Structural tissue discontinuities, including pores, gaps, channels and slits, provide constitutive space and guidance for migrating cells, whereas additional space is gained transiently by cell and ECM deformation and permanently by cell-mediated proteolytic ECM remodeling. Thus, space, deformability of both ECM and the cell, and protease activity together determine the outcome for cell migration *in vitro* and, probably, *in vivo* [5,44,49,52]. Furthermore, additional crosslink-mediated stiffening contributes to increased matrix alignment [21,26,29,63], and thereby may affect the conditions for both proteolytic and non-proteolytic cell migration. As an example, breast cancers are frequently associated with increased interstitial collagen deposition and LOX-mediated matrix rigidity [26]; this leads to collagen fiber realignment, bundling and stiffening, with dense collagen bundles bordering longitudinal gap-like channels along which invading tumor cells may invade the host tissue by proteolysis-independent mechanisms [25,64]. In a breast cancer model, trails of least resistance along thick collagen fibers were shown to guide amoeboid-moving single cells through wide collagen-free gaps and spaces, without signs of migration-associated collagen fiber degradation or reorganization [65]. Thus, with interstitial gaps and spaces available, neoplastic or other cells can employ contact guidance and shape change to find permissive tissue routes for enhanced migration, and therefore probably remain uncompromised by therapeutic protease inhibition [5,11,62]. Therapeutic targeting of pericellular proteolysis, although considered to be an approach for reducing neoplastic invasion and metastasis, may thus only poorly inhibit cancer cell migration, given migration-permissive ECM discontinuities in interstitial tissues and basement membranes (Figure 1, Box 2). As a consequence of both cell invasion- and chronic inflammation-mediated diffuse, contact-dependent and intracellular ECM degradation, however, the resulting net loss of ECM structure could compromise tissue integrity and function

Box 2. Monitoring proteolytic and protease-independent cell migration *in vitro* and *in vivo*.

The ECM conditions and the extent and type of proteolysis required for tissue invasion remain controversial [6,9]. We summarize here the technical and conceptual considerations that may help to clarify the rate-limiting conditions of proteolytic and protease-independent cell migration in 3D tissue environments.

(i) Cell migration into 3D tissues is a multiparametric process. *In vitro* analyses of cell invasion should thus include the coregistration of migration speed or end-point translocation, cell morphology changes during migration, and tissue porosity.

(ii) Structural imaging techniques – including confocal backscatter, second and third harmonic generation microscopy, together with fluorescent contrast imaging – allow visualization of multiple connective tissue components with guidance and/or barrier function during cell migration *in vitro* and *in vivo*, including fibrillar collagen, elastin fibers, muscle strands, myelinated nerves, perfused blood vessels, lymph vessels and fat cells [11,33,76–78]. Accordingly, time-resolved coregistration of tissue structures and moving cells will show whether these tissue scaffolds are unaltered, temporally displaced, structurally reorganized, or even degraded during cell invasion.

(iii) To monitor conclusively the effect of protease inhibitors on cell migration *in vitro* and *in vivo*, the following parameters warrant coregistration: the migration efficacy, ECM structure before, during and after migration, molecular *in situ* evidence for ECM cleavage, and (uncompromised) cell viability. In particular, targeting of proteases *in vivo* will depend upon coregistering effective local protease inhibitor delivery and efficacy (e.g. by using fluorescent activity reporters) [79–81].

[3,11,66]. Thus, chronic proteolytic tissue remodeling eventually leads to tissue destruction and loss of function, such as tumor-induced tissue ulceration or bone destruction by osteoarthritis or tumor metastases. Therefore, instead of targeting interstitial cell migration and dissemination, therapeutic protease inhibition could effectively inhibit the local degradative tissue remodeling by MMPs, serine protease or cathepsins, and, thus, prevent or delay secondary potentially life-threatening consequences. Future research is required to test whether therapeutic inhibition of protease systems involved in connective tissue remodeling and destruction is suited to preserve the integrity and function of important tissue structures for prolonged time periods, similar to the bone protection provided by bisphosphonates that delay bone demineralization by bone metastases [67]. Rather than preventing invasion and metastatic dissemination, protease inhibitor-based therapy could therefore reduce tissue consumption and disruption by cancer cells and, hence, increase both the quality and expectancy of life in patients.

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