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The biology of cell locomotion within three-dimensional extracellular matrix

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Abstract. Cell migration in three-dimensional (3-D) extracellular matrix (ECM) is not a uniform event but rather comprises a modular spectrum of interdependent biophysical and biochemical cell functions. Haptokinetic cell migration across two-dimensional (2-D) surfaces consists of at least three processes: (i) the protrusion of the leading edge for adhesive cell-substratum interactions is followed by (ii) contraction of the cell body and (iii) detachment of the trailing edge. In cells of flattened morphology migrating slowly across 2-D substrate, contact-dependent clustering of adhesion receptors including integrins results in focal contact and stress fiber formation. While haptokinetic migration is predominantly a function of adhesion and deadhesion events lacking spatial barriers towards the advancing cell body, the biophysics of the tissues require a set of cellular strategies to overcome matrix resistance. Matrix barriers force the cells to adapt their morphology and change shape and/or enzymatically degrade ECM components, either by contact-dependent proteolysis or by protease secretion. In 3-D ECM, in contrast to 2-D substrate, the cell shape is mostly bipolar and the cytoskeletal organization is less stringent, frequently lacking discrete focal contacts and stress fibers. Morphologically large spindle-shaped cells (i.e., fibroblasts, endothelial cells, and many tumor cells) of high integrin expression and strong cytoskeletal contractility utilize integrin-dependent migration strategies that are coupled to the capacity to reorganize ECM. In contrast, a more dynamic ameboid migration type employed by smaller cells expressing low levels of integrins (i.e., T lymphocytes, dendritic cells, some tumor cells) is characterized by largely integrin-independent interaction strategies and flexible morphological adaptation to preformed fiber strands, without structurally changing matrix architecture. In tumor invasion and angiogenesis, migration mechanisms further comprise the migration of entire cell clusters or strands maintaining stringent cell-cell adhesion and communication while migrating. Lastly, cellular interactions, enzyme and cytokine secretion, and tissue remodeling provided by reactive stroma cells (i.e. fibroblasts and macrophages) contribute to cell migration. In conclusion, depending on the cellular composition and tissue context of migration, diverse cellular and molecular migration strategies can be developed by different cell types.

Key words. Collagen matrix; cell migration; integrins; CD44; matrix metalloproteinases; attachment; detachment; tissue remodeling; tumor invasion; antibody blocking; haptokinetic.

Introduction

Active cell motility is essential in physiological tissue development and homeostasis [1–5], including embryological morphogenesis, wound healing, immune surveillance, and inflammation, as well as neoplastic tumor cell dissemination and metastasis. Cell migration in the body mainly occurs within the connective tissue consisting of three-dimensional (3-D) multimerized extracellular matrix ligands of nonrandom texture. Cellular interactions with the extracellular matrix (ECM) are primarily provided by cell adhesion receptors mediating a range of different cell functions such as cell attachment and polarity, growth and differentiation, as well as adhesion and migration [3–10]. The 3-D architecture of the ECM appears to provide complex information not present if cells merely attach to a two-dimensional

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(2-D) ligand-coated surface [10]. In 3-D collagen lattices, but not however during attachment to a collagen-coated surface, integrin $\alpha 2\beta 1$ is upregulated in fibroblasts and melanoma cells, suggesting that, besides receptor-mediated biochemical signaling, the biomechanical architecture of the tissue has a major impact on cell function [11]. To a degree previously not anticipated, cell-matrix interactions can control cellular phenotype, cell growth, and behavior not only in normal cells but also in tumor cells. Sometimes, matrix-derived signaling can even override a given genotype, e.g., by restoring growth arrest and a differentiated phenotype in cells bearing a neoplastic genotype [7].

For migration through 3-D tissues, cellular mechanisms to overcome biophysical matrix resistance comprise adhesion and deadhesion events mediated by adhesion molecules coupled to shape change as well as proteolysis of ECM components provided by serin proteases and metalloproteinases [reviewed in refs 12, 13; see also Ivaska and Heino, and Johansson et al. in this issue]. In this review, cellular and molecular parameters of cell migration are emphasized with reference to tumor cell invasion and motility in 3-D tissues as opposed to migration on planar substrates.

Biology of cell migration—the three-step concept

The current concept of cell migration and adhesion receptor function is based on the haptokinetic migration across surfaces of diverse cell types, including fibroblasts and keratinocytes [reviewed in refs 4, 5, 8]. The haptokinetic migration of fibroblasts involves at least three interdependent functional elements, namely attachment of the leading edge, cell contraction, and detachment of the rear end.

Receptor-mediated attachment

At the leading edge of migrating fibroblasts, ruffling of filopodia is followed by the protrusion of the leading pseudopod and the formation of new attachments to the substrate [14, 15]. The pseudopod protrusion and ruffling of filopodia require actin polymerization and are initiated and maintained by contact of adhesion receptors binding to the underlying substrate. Cell-matrix attachments can be mediated by multiple receptor families, and most knowledge is available on the promigratory function of the integrin receptor family [reviewed in ref. 4; see also Ivaska and Heino in this issue]. Other potentially promigratory adhesion receptors include cell surface proteoglycans, such as syndecans, phosphacan, and CD44, as well as other receptors, including CD36, CD47/integrin-associated protein, and the dystroglycan complex [reviewed in ref. 16]. In the case of integrin-mediated migration, upon or immediately preceding to firm attachment, integrins cluster in the cell membrane to ligand-binding sites [17, 18]. Subsequently, integrin cytoplasmic domains recruit intracellular signaling and adapter proteins, resulting in the assembly of 3-D multimolecular cytoskeleton-binding complexes that have been designated as focal adhesions [19–21]. Focal adhesions are spear tip-like structures of up to 10 μm in length and 0.5 μm in width connecting the ventral plasma membrane to the ECM [22]. They are composed of densely packed transmembrane receptors attaching to extracellular substrate and intracellular proteins linking the cytoskeleton to outside structures, as detected in fibroblasts, smooth muscle cells, endothelial and epithelial cells, and platelets [19]. The cytoplasmic phase of focal adhesions contains structural proteins (e.g., α-actinin, talin, vinculin, zyxin, F-actin), adapter proteins (e.g., Grb2, Sos), and signaling molecules (focal adhesion kinase (FAK), C-terminal Src kinase (Csk), PI-3-kinase, phospholipase Cγ (PLCγ), Rho, Ras, Raf, mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (MAPK/ERK = MEK), Jun N-terminal kinase) [reviewed in refs 6, 20]. The cytoplasmic domain of $\beta 1$ integrins directly interacts with cytoskeletal proteins such as talin and α-actinin, but also with signaling molecules such as FAK and integrin-linked kinase [6]. The assembly of focal adhesions is thought to be initiated by ligand-induced integrin dimerization and clustered leading to protein tyrosine phosphorylation and cytoskeletal rearrangement [18, 23, 24]. Among the signaling pathways triggered by clustered $\beta 1$ integrins are the MAP kinase cascade, protein kinase C, phosphatidyl inositol hydrolysis, and low molecular-weight G proteins, such as Rho [6, 23]. Intracellular signals, on the other hand, can change the affinity of integrins for their extracellular ligands further contributing to attachment [reviewed in ref. 25]. Besides direct attachment to ECM, additional adhesion and signaling are provided by growth factors [e.g. epidermal growth factor (EGF), nerve growth factor, platelet-derived growth factor-β, insulin-like growth-factor] and chemokines, either in soluble or substrate-bound form [10, 26]. Ultimately, focal adhesions provide attachment-dependent outside-in signaling [3] leading to the recruitment and polymerization of actin monomers and the formation of actin cables or bundles (stress fibers) [8, 21].

Cell contraction

Following substrate binding at the leading edge, a gradient of binding and traction forces is established between the front and the rear of the cell [reviewed in refs 27, 28] resulting in forward movement of the cell in

relation to the underlying substrate. This cell contraction is most likely provided by myosin motors inserting between actin bundles, leading to the rearward traction of focal contacts relative to the cell body [4, 29, 30].

Cell contraction not only leads to cell displacement, but also has an impact on the strength of focal contacts. While the assembly of focal contacts follows ligand-induced outside-in signaling, cluster strengthening, maturation, and maintenance appear to be dependent on force-related, signaling-induced integrin accumulation (inside-out signaling) that are in direct relation to the traction forces applied to these bonds [8, 20]. Based on experiments using integrin ligands coupled to beads, the stiffness of the substrate was predicted to favor the formation of focal adhesions and strengthen integrin-cytoskeleton linkages [8]. Reduced integrin clustering and the disassembly of focal adhesions is seen after inhibition of the low molecular-weight protein Rho as well as impairment of cell contractility by blocking myosin light chain kinase activity or myosin-actin interactions [20, 29]. This is consistent with the concept that biomechanical traction, hence substrate rigidity as well as the force provided by cell contraction, support the strengthening of integrin-cytoskeleton interactions [8, 31]. Force-dependent strengthening of focal contacts, on the other hand, implies that cells migrating at low adhesive and contractile forces may utilize less developed or no focal contacts for interaction with the substrate (see below) [32].

Detachment

Following cell contraction, focal contacts are resolved at the trailing edge via at least two mechanisms. Integrin-ligand interactions are thought to be weakened by affinity modulation leading to a low-affinity binding state [33], favoring integrin release from the substrate [4, 34]. Receptor detachment from the ECM is followed by endocytosis, anterograde trafficking of integrin-containing vesicles and fusion of these vesicles with the cell membrane for receptor recycling to the leading edge [35]. Alternatively, substantial amounts of substrate-bound integrins are released and deposited on the substrate by tyrosine phosphorylation-dependent events [4, 36].

Migration as a cycling process

For attachment and detachment, varying degrees of integrin-ligand binding strength can be achieved by either altering the affinity of individual integrin receptors without changing receptor density (affinity regulation) [25, 32, 37] or by regulating the number of available receptors in a given contact region (avidity regulation) [38]. The concept of affinity modulation in the absence of changes in surface expression was established for platelets, leukocytes, and neurons [25, 39]. If integrins expressed at the cell surface and ligand density of the substrate remain constant, alterations in avidity can be induced by outside-in signaling ranging from an increase to complete loss of integrin function [25]. Neurons lose their capacity to attach to and migrate on laminin at an intermediate stage of their differentiation, although laminin-binding integrins remain continuously expressed at high levels on these cells throughout development [39]. Apart from affinity modulation, recent findings suggest that fibronectin ligand binding can occur by avidity regulation via $\beta 1$ integrin multimerization in the absence of increases in receptor affinities [38]. These findings imply that changes in the redistribution state, i.e., receptor clustering, may favor ligand capture [39] even in the absence of increased receptor affinity.

The subtle balance of adhesion and sufficiently rapid detachment allows maximal migration rates at intermediate attachment strength [40] resulting in the paradigm of intermediate adhesion [5, 28, 41] and/or transient high-affinity binding [5] of cell migration.

This three-step model of cell haptokinetic migration implies that graded attachment, contraction, and detachment form a cycle of events without obvious starting and end point. In this process, focal adhesions are considered as specific structures needed for cell attachment, spreading and locomotor activity, as well as forming 'hot spots' for cell signaling [20, 23]. Implicitly, focal adhesions are not static but highly dynamic structures that are constantly remodeled in size, shape, and molecular assembly [21]. It is thought that the formation and resolution of cell-matrix interactions lead to varying degrees of focal adhesion assembly and cytoskeletal organization into stress fibers. Cell-substratum adhesion is a time-dependent process typically requiring 15 min or more before focal adhesions can mature into stable interactions [19, 44]. Consequently, focal adhesions and stress fibers are most prominently found in relatively static processes such as stable cell attachment and cell spreading in the absence of significant cell displacement [42, 43] as well as contraction of ECM, presumably resulting from slow turn-over of established focal adhesions [20].

Disassembly and/or dynamic turnover of focal adhesions are seen in cell motility and in many transformed cells. In locomotive cells, focal adhesions, then termed focal contacts, are frequently of smaller size, incompletely assembled, less stable [46, 47] and may also contain molecules not present in focal adhesions, including myristoylated alanine-rich C-kinase substrate (MARCKS) and gelsolin [21]. Fibroblasts and neural crest cells can lack or develop only transient focal adhesions while migrating [45, 46]. The weakening of

focal adhesions is an early step in the onset of migration. In the presence of interleukin-8, previously resident fibroblasts acquire motile, chemokinetic behavior and lose focal adhesions both in number and size [48]. On the other hand, delayed or reduced disassembly of focal contacts, for example in FAK-deficient cells or, conversely, after constitutive activation of Rho are accompanied by more stable focal adhesions and reduced cell motility [49]. In rapidly moving cells such as leukocytes, focal contacts are usually completely lacking [32]. Vigorously migrating microglia cells of ameboid morphology generally lack stress fibers; however, the lipopolysaccharide-induced transition to a sessile state is accompanied by cell flattening and spreading and the formation of stress fibers inserting into the cores of attaching microspikes [50]. These findings suggest that increased migration dynamics are associated with (or even result from) decreased focal contact assembly, and vice versa [32, 43, 51].

3-D cell migration models

Investigations on haptokinetic migration across 2-D surfaces have helped to define the dynamics in receptor-mediated cell-substrate interactions that lead to signal transduction, cytoskeletal organization, and cell motility. Because the limited 2-D nature of planar substrates does not reflect the 3-D architecture of the ECM, cell-matrix interactions have been further investigated in a 3-D tissue context. Some in vitro models mimicking a 3-D tissue environment are suitable for direct visual access to migrating cells. In vivo, cell motility and positioning in interstitial tissues is monitored by intravital microscopy. Other 3-D tissue-based migration models, most notably explant confrontation assays and stationary organ cultures, ECM and cartilage invasion assays, and sponge matrix culture, have been reviewed elsewhere [52]. Although the latter assays permit analysis of net cell migration on the basis of endpoint determination, they lack the possibility to visualize migratory action, e.g., by time-lapse videomicroscopy.

3-D collagen matrix models

Cell motility including tumor invasion usually occurs in connective tissues and, therefore, may be particularly dependent on interactions with ubiquitously expressed collagen. Collagen as substrate is present even if laminin (in basement membranes) and fibronectin (in embryonic basement membranes and wound-healing pathways) are absent. 3-D hydrated collagen lattices have been widely used for in vivo-like cell culture [53]. In vitro, collagen solutions at concentrations ranging from 0.6 to 3.5 mg/ml are polymerized to form interconnected smooth fibers of 50–300 nm in diameter and multimerized fibrillar bundles ranging from 100 to 1000 nm in diameter [53–55]. The process of collagen self-assembly and in vitro fibrillogenesis depends on the type of collagen preparation, concentration, and temperature, resulting in filament morphology and texture resembling in vivo collagen fiber characteristics [53, 55, 56].

Cell suspensions may be mixed with the collagen solution prior to polymerziation thus trapping the cells between fibers and bypassing initial adhesion steps [57, 58]. The density of collagen fibers then has a major effect on cell migration in collagen lattices: optimal migration rates of neoplastic and nonneoplastic cells are dependent on intermediate collagen concentrations (1.5–2 mg/ml) and reduced migrating cell ratios and velocities are seen at increased or reduced fiber density [59, 60]. Alternatively, cells are placed on top of the lattice for subsequent attachment and penetration of the preformed lattice [61, 62].

For the generation of oriented collagen lattices, parallel fiber alignment is achieved by fluid drainage, application of mechanical force (M. Gunzer, P. Friedl, B. Niggemann, E. B. Bröcker, E. Kämpgen and K. S. Zänker, submitted), or as a function of an electric or magnetic field and temperature applied during polymerization [63, 64]. Matrix architecture and fibril orientation can be visualized at low detail and contrast by phase contrast microscopy [63]. For higher contrast and resolution, scanning electron microscopy for fixed [65] or confocal reflection contrast [55] for fixed and unfixed lattices are used.

Overall, 3-D collagen lattices are considered as a defined and appropriate substrate to study diverse aspects of cell behavior in vitro, including positioning and migration [66], cell growth and differentiation [7], and matrix contraction [11]. The fiber distribution and biophysical architecture of collagen lattices, i.e., the sparsity and random order of the fibers, most closely resemble interstitial soft tissues, dermis, and the network-like stroma of the lymph node cortex [53, 67]. In collagen lattices, fibroblasts retain their tissue phenotype including polarized morphology [53, 68] and in vivo-like positioning such as the formation of the supracellular perpendicular network-like orientation of dermal fibroblasts or parallel alignment of tendon fibroblasts [66]. However, the extent to which the actual substrate conditions in vitro (i.e., elasticity and rigidity) correspond to the biophysics of tissues in vivo remains to be established.

Multicomponent matrices

In tissues, collagen is associated with various other components like fibronectin and glycosaminoglycans/proteoglycans. To further approximate the structural

complexity of in vivo tissues, collagen lattices are supplemented with other ECM components including fibronectin [69], hyaluronan (HA), and other glycosaminoglycans [54, 70], and link proteins [54]. The orthotopic self assembly of such multicomponent matrices is a critical issue. The presence of noncollagenous molecules has important regulatory and frequently inhibitory effects on the collagen self-assembly reaction [56, 71]. In some cases, arbitrary matrix assembly can be replicated in vitro. The retention and ultrastructural resemblance of multicomponent lattices to some in vivo tissues such as embryonic tissues have been described [54]. In other cases, ECM assembly requires cells and occurs at the cell surface. Fibronectin, for example, is polymerized to a pericellular network by cells expressing activated integrin $\alpha 5\beta 1$, αv integrins, or nonintegrin receptors at the cell surface [72]. On the basis of spontaneous dimeric interactions, plasma fibronectin binds to native collagen fibers, but does not self-assemble into a fibronectin lattice [69]. Similar to fibronectin, HA and chondroitin sulfate decorate the core of collagen fibers and are enriched at irregularly polymerized intersections [54, 69]. In addition, HA forms interconnected lattices filling gaps and pores between collagen fibers [54, 73]. In vivo, HA is further multimerized by other molecules including chondroitin sulfate proteoglycan, forming complex lattices of varying degrees of viscosity [74]. It is not known how the tissue-specific amount and density of immobilized, fibril-bound HA relative to the soluble HA can be biophysically translated to in vitro lattices. For cell migration studies using 3-D collagen lattices supplemented with HA, inconclusive modulation of migratory responses have been reported. HA-induced effects vary from activating to inhibitory, and sometimes no effect is obtained at all [70, 75–77]. These discrepancies may be partially explained by the fact that the stability of HA-HA assembly and the amount of polymerized HA versus the remaining nonattached fraction are a function of the molecular mass and the purity of the utilized HA preparation as well as the presence or absence of link proteins that have not yet been sufficiently standardized for such models [74, 78].

Basement membrane-like matrices

In an attempt to mimic the complex basement membrane structure, the supernatant of a Schwannoma cell line (Matrigel) predominantly containing laminin, collagen IV, and nidogen was established [79]. Matrigel is polymerized to a thin matrix layer covering the filter of a modified transmigration chamber, resulting in a selective barrier imposed by the matrix [79]. This assay is thought to mimic cellular penetration of a basement membrane layer and is widely used for cell adhesion and invasion studies [7, 80]. Cell invasion through Matrigel-coated filters requires a combination of haptokinetic migration across the Matrigel surface and the penetration of the matrix layer giving access to the underlying filter pores. Migration is usually quantified by endpoint determination; however, a combination with time-lapse videomicroscopy may give more detailed information about the different phases of migratory action (P. Friedl, unpublished observations). Because of differences in molecular composition and biophysical properties of the lattice, the cellular and molecular mechanisms governing migration of different cell types in Matrigel compared to collagen lattices frequently differ with respect to integrin usage, intracellular signaling, and matrix metalloproteinase (MMP) function [80, 81].

Chorioallantoic membrane model

The chorioallantoic membrane of the chick embryo provides a highly vascularized substrate of sparse ECM components that is used for xenotransplantation of tumor cell suspensions and solid tumor explants for studies on cell invasion, angiogenesis, and metastasis [82]. Because the ECM is sparse and because tumor growth and local invasion are monitored using histological morphometry, direct invasiveness as a consequence of cell motility cannot be clearly distinguished from invasive growth (i.e., resulting from local growth-related tissue destruction) [83]. Furthermore, the effect of interfering cell-cell interactions between tumor cells and the upper ectodermal layer cannot be ruled out [52, 83]. Hence, although cell migration within the 3-D mesodermal layer of the membrane will eventually occur and contribute to metastasis, controlled assessment of the relative contribution of cell motility to overall invasion and metastatic spread requires additional direct visual assessment of cell migration, i.e., by intravital microscopy [84].

Intravital microscopy and cell migration

For direct in vivo monitoring of cell behavior in the tissues, intravital microscopy allows quantitative detection of native or fluorescently labeled cells upon transendothelial migration or migration in the tissue. Cells are either injected intravenously or locally implanted, and the action of activatory or inhibitory substances can be monitored directly [85]. Several organs have been used in the past for migration studies, i.e., the rabbit ear [86; reviewed in ref. 52], the rat mesentery [85], the lymph node [reviewed in ref. 87], or the chorioallantoic membrane [84]. For monitoring cell migration in the tissue, the target organ must be of high transparency or, alternatively, the cells must be fluorescently labeled [88] in order to assure sufficient bright-

ness and contrast of visual assessment, allowing time-lapse videomicroscopy and subsequent quantitative analysis of cell trafficking [87].

Data assessment and quantification of cell migration

Cell migration in 3-D ECM models can be monitored by indirect endpoint determination or by direct visual analysis, i.e., time-lapse videomicroscopy and computer-assisted cell tracking. The invasive capacity of cells layered on top of a preformed 3-D tissue is quantified after fixation and cell staining using the number of cells invaded after a given time interval, their penetration depth, and/or the extent of matrix contraction, all of which are likely locomotion-associated parameters [11, 59, 61, 62, 89]. Such endpoint determination approaches allow the distinction of functional subpopulations, i.e., nonadherent, adherent but nonmobile, and the invading and mobile fraction [90, 91], the analysis of adhesion receptor function as well as of migration-enhancing factors including cytokines [90] and additional ECM components [75]. As a disadvantage of invasion-based 3-D matrix models, i.e., after layering the cells on top of a preformed matrix, penetration into the matrix requires an initial adhesion step which is dependent on functional adhesion receptors [92]. Furthermore, the extent to which the true path of a cell is represented by the final invasion depth is unresolved [93], suggesting that the readout may sometimes be hampered by a limited sensitivity and precision of endpoint determination [93, 94].

To directly and quantitatively assess cell migration in 3-D tissues in vitro and in vivo, computer-assisted cell tracking was introduced for the reconstruction of individual cell paths (trajectories) [57, 64, 85, 93, 94]. The quantitative data analysis is based on a random selection of cells prior to cell tracking and data analysis [93] allowing the extraction of diverse aspects of cell motility (fig. 1). Continuous-time detection of locomotor population dynamics represents the number of cells actually migrating within the minimal step interval (mobile fraction), their average step length (velocity), and the mean population displacement including stopping cells (speed) [58, 94]. Single-cell analysis (fig. 1B) and the detection of locomotor subpopulations are required for a correct representation of stop-go patterns and varying degrees of motility within heterogeneous source populations [58]. Derived from the path structure (fig. 1C) are the migratory persistence and angular distribution of the cell path over time (i.e., angular turn to the left or to the right); these parameters represent important characteristics in cell polarity and cytoskeletal organization [95]. The migratory persistence (fig. 1C) and directionality (fig. 1D) of the cells towards a source of cytokines ('positive chemotaxis') [76] or in the opposite direction ('negative chemotaxis') [96] are detected on a single-cell basis or for populations, using stochastic models [97]. The continuous-time quantification of randomly selected cell paths is a powerful strategy to determine cellular and molecular principles of cell migration in 3-D tissues [9].

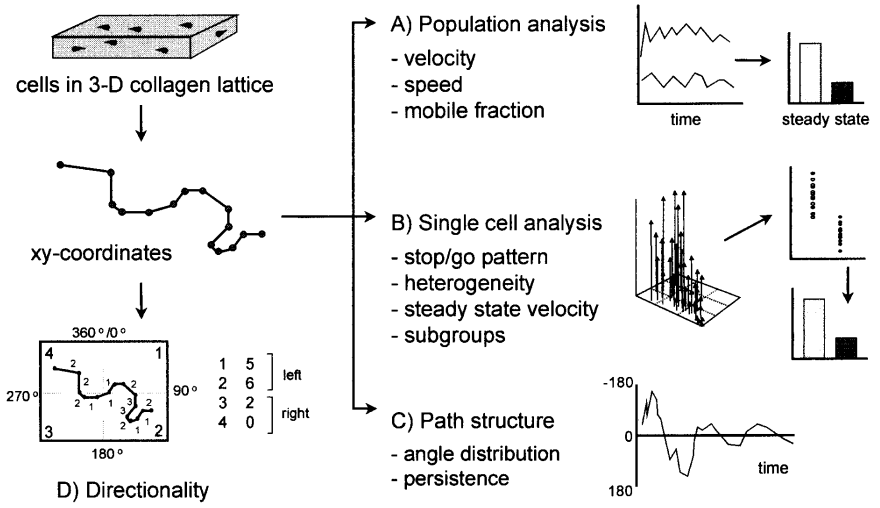

Figure 1. Computer-assisted cell tracking and quantitative data analysis. Cells are incorporated into a 3-D collagen matrix, recorded by time-lapse videomicroscopy (not shown) and analyzed by computer-assisted cell tracking of randomly selected cells (30–60 cells/experiment; migrating and nonmigrating included), as described by Friedl et al. [93]. Different quantitative parameters either at the individual cell level or for population analysis are obtained from the x- and y-coordinates.

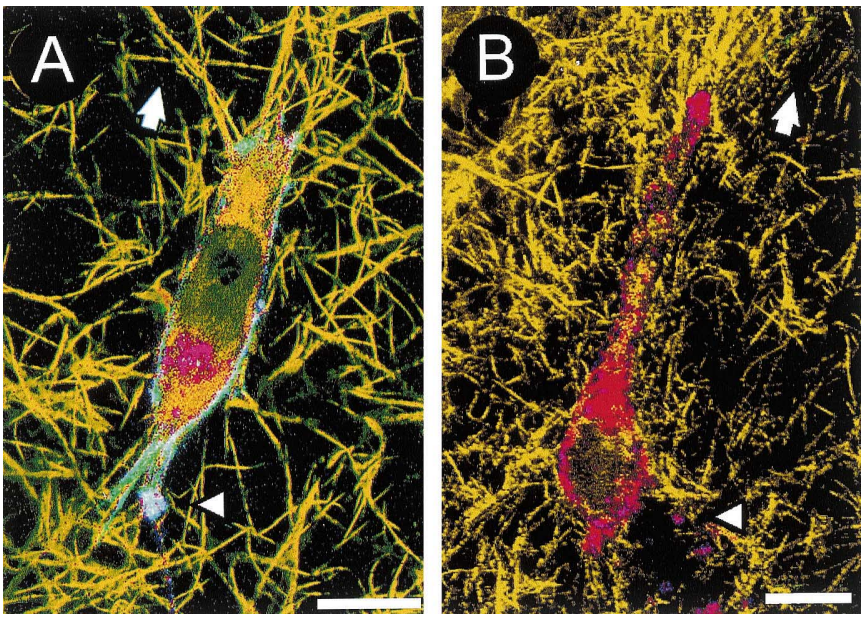

Figure 2. Cell-matrix interactions and remodeling by highly invasive MV 3 melanoma cells migrating through a 3-D collagen lattice. False-color images of confocal reflection of collagen fibers (brown) and immunofluorescence of $\beta 1$ (*A*) or $\alpha 2$ (*B*) integrins, as represented by central sections of the cells. Bar = 12 μm. (Note the difference in resolution detail with × 63 objective (*A*) as compared to × 40 (*B*). (*A*) Double-labeling of membrane-bound integrins (light blue) clustering at interaction to collagen fibers in comparison to the fraction of $\beta 1$ integrins internalized upon migration (red). Cells were initially labeled with anti-$\beta 1$ antibody (red) and incorporated into the collagen lattice. After 20 h, the lattice was fixed and stained for denovo integrin expression on the surface (light blue). At the detachment zone, $\beta 1$ integrins are released into the lattice (white arrowhead). (*B*) Formation of a matrix defect upon cell detachment and deposition of $\alpha 2$ integrins (red) into the area of remodeled collagen (arrowhead). Arrows indicate the direction of migration, as assessed by time-lapse videomicroscopy.

Principles governing cell migration in 3-D tissues

The three-step concept of cell migration

In 3-D tissues, the basic elements of cell migration including cell polarization, flowing of the leading cell process for polarized attachment, contraction of the cell body, and detachment at the trailing edge appear to be maintained in fibroblasts, leukocytes, and tumor cells [51, 55, 68, 98]. In 3-D collagen lattices, the migration of MV3 melanoma cells (fig. 2) and fibroblasts involves adhesion of the leading pseudopod to the substrate and $\beta 1$ integrin clustering at interactions to collagen fibers at the leading edge (fig. 2A) [99]. Upon subsequent cell movement, $\beta 1$ integrins are further clustered at fiber insertions towards lateral cell portions and the trailing edge (fig. 2A) [55]. For cell detachment, integrins are either released into the collagen lattice or internalized (fig. 2A,B) [55, 70]. In 3-D collagen lattices, similar to migration on collagen- or fibronectin-coated surfaces, migration of tumor cells and fibroblasts is consistently inhibited by anti-$\beta 1$ or anti-$\alpha 2$ antibody [70, 100; K. Maaser and P. Friedl, unpublished data), suggesting that for these cells adhesion is required for migration in a 3-D matrix environment [reviewed in ref. 9]. Although more detailed analysis of attachment and detachment forces and the 3-D cytoskeletal organization remain to be established, the principal elements of the three-step haptokinetic migration concept appear to be retained in 3-D ECM. However, in 3-D tissues, cell migration may contain additional specialized features not present in haptokinetic migration, namely differences in cytoskeletal organization and cellular strategies to overcome biophysical matrix resistance relative to the size of the cell body.

Cell shape and pseudopod kinetics

A surface does not impose mechanical resistance towards the cell body. Consequently, adaptation of cell shape in response to preexisting matrix structures is lacking in migration across surfaces [98, 101]. In fibroblasts migrating on 2-D substrate, adhesion-driven adaptation of cell shape and cytoskeletal organization result in an extended, flat morphology [68]. At the leading edge, one or more broad and flat pseudopods with ruffling spikes at the tip ('ruffling membrane

form') result from integrin engagement towards the lower side [4, 53], whereas the site of detachment is more narrow [68]. Ruffling filopodia develop new contacts with the underlying substrate and the lamellipod grows outwards to form a sheet between progressively attaching filopodia [65]. Because new interactions are formed continuously, a continuous 'persistent gliding' mode of migratory action is observed in many cell types [102].

In in vivo tissues and in 3-D collagen lattices, fibroblasts lack this well-spread morphology of their counterparts moving on 2-D substrate; likewise, the persistent gliding mode of action and the geometry of interaction points differ [65]. In 3-D ECM, a predominantly bi- to tripolar or stellate spindle-shaped morphology results from traction forces provided by contractile cell-matrix interactions at both the leading and trailing edge [9, 53, 55, 68]. Unlike flat ruffling membranes in 2-D models, matrix-binding pseudopodia are cylindrical and covered three-dimensionally with microspikes and small lamellipodia or ruffles at or close to fiber insertions in 3-D ECM [53, 55, 65, 66; K. Maaser and P. Friedl, unpublished data]. The cylindrical form could represent a strategy to minimize cell resistance towards the matrix network. Although cylindrical pseudopods as compared to flattened lamellipods represent environmental variants of the same cell compartment [65, 98], marked differences are present in focal contact formation and the structure of the actin cytoskeleton.

Focal contacts, stress fibers, and force generation

The focal contact is the locus of the shortest distance between the cell and substratum and is frequently the site of stress fiber termination in 2-D adhesion and migration models [68]. On 2-D substrate, the size of focal contacts is positively correlated with attachment strength and inversely correlated with the migratory rate [39] although a complete loss of focal contacts is rarely seen in 2-D migration models [39, 103]. Stress fibers represent solid actin bundles involved in static, tension-generating cell-matrix interaction, hence potentially counteracting rather than favoring migration [43]. Consequently, the formation of stress fibers is most prominent in static cells attaching to 2-D substrate [103]. Although focal contacts and stress fibers provide a well-established model for studying the interactions of cells with 2-D ECM, cells surrounded by 3-D tissue usually lack such cytoskeletal specializations [6, 20, 29]. In free-floating hence collapsing collagen lattices in vitro, and in granulation tissues in vivo, fibroblast migration occurs in the absence of actin bundling and stress fiber formation [104, 105] exhibiting a diffuse cortical F-actin distribution over the entire cell body [32, 66, 68]. In rapidly migrating leukocytes exposed within a 3-D extracellular matrix, stress fibers are completely absent, and diffuse cortical actin polymerization is developed [32, 50, 106, 107]. This is reminiscent of the striking lack of stress fibers in *Dictyostelium* [108, 109]. Hence, focal contact formation and stress fibers appear not to be essential for efficient movement within 3-D tissues, whereas upon wound healing in vivo and in anchored collagen lattices, stress fibers are seen in static cells in the process of matrix contraction [104, 105]. Taken together, these findings emphasize that the model of focalized integrin clustering, focal contact assembly and, ultimately, formation of force-generating actin cables requires further refinement for cell migration in 3-D environments.

Adhesion and cell migration

Haptokinetic cell migration across surfaces occurs in the presence of insignificant biophysical resistance of the substrate towards the advancing cell body. Migration across a 2-D surface does require certain tensile binding strength to the substrate implying that, in theory, an adhesion molecule will support migration as soon as substrate binding is dynamically linked to the cytoskeleton. Besides integrins, other surface molecules including CD44 and the receptor for HA-associated motility (RHAMM), immunoglobulin family members, and cadherins also mediate cell guidance and migration on suitable 2-D substrate [110].

Because cell migration is secondary to attachment in most 2-D assay systems [111], perturbation of adhesion usually impairs migration, suggesting a promigratory function of the receptor in question [4, 100, 103]. Complete loss of $\beta 1$ integrin expression by genetic disruption of the $\beta 1$ gene in embryonic stem cells or in F9 embryonal carcinoma cells is accompanied by reduced adhesion and an impaired ability to migrate on ECM proteins such as fibronectin [112, 113]. However, it is obvious that there is not a direct correlation between adhesivity and the cellular ability to migrate [34, 39, 41]. Reducing adhesion forces may favor migration by facilitating detachment in models of high adhesivity [34, 39, 41]. For example, laminin and mesosin act as antiadhesion molecules on olfactory cells attaching to otherwise highly adhesive substrata; this reduction in attachment, in turn, results in the disassembly of focal contacts and the onset of migration [39].

Differences in biochemical and biomechanical substrate conditions imply that certain adhesion receptors supporting migration in haptokinetic models will not function in 3-D models and that other receptors providing for example anchorage on surfaces will provide migration in 3-D tissue. In 3-D tissues, ligand availability and biophysical matrix resistance may be orders of magnitude higher than those present in 2-D haptokinetic migration. Consequently, some cells may be trapped by

3-D matrix fibers, while for others, e.g., deformable cells, a low interaction strength may be sufficient to support migration. Thus, in contrast to migration across surfaces, 3-D tissues in vitro and in vivo favor integrin-dependent as well as integrin-independent cell-substrate interaction strategies to mediate cell migration, depending on the cell type and the tissue of investigation.

Integrin-dependent migration. Large and slowly migrating cells such as fibroblasts and tumor cells migrating in 3-D collagen matrices and/or contracting them are predominantly dependent on $\alpha 2\beta 1$ integrin, as shown by antibody blocking studies [11, 70, 100, 114; reviewed in ref. 9]. Adhesion-related clustering of $\beta 1$ integrins at points of attachment and traction of collagen fibers at the leading edge of migrating fibroblasts and melanoma cells appear to confirm the concept of ligand-induced receptor clustering [18] at stable interactions to individual collagen fibers (fig. 2A,B) [55, 70, 99]. After blocking of integrin-ligand interaction, the cells retain or acquire a spherical shape despite continuous cytoskeletal oscillations and shape change (running on the spot), and redirect integrins to an even distribution [70]. These data suggest that specific integrin-dependent adhesion is the mode of cell-fiber interaction in these cells. Such integrin-mediated interactions are not only required for the development, elongation, and anchoring of leading pseudopods, but also for the traction and radial alignment of fibers towards the cell body (fig. 2) ranging from 20 up to 100 μm in forward direction [99]. Hence, the forces involved are considerable. In vivo, inhibition experiments with antibodies, antagonistic peptides, or antisense oligonucleotides have suggested that $\beta 1$ integrins are important for the migration of neural crest cells, myoblasts, and neuroblasts [reviewed in ref. 115].

Integrin-independent migration. Adhesion-independent migration is a controversial issue. Leukocyte migration in attachment-dependent migration models is reduced by adhesion-perturbing antibodies against $\beta 1$ integrins in vitro [92, 100, 116, 117] and in vivo [85], although never fully abolished. In 3-D collagen lattices, T lymphocytes, monocytes, and dendritic cells continue to migrate after blocking of $\beta 1$ integrins as assessed by cell-tracking techniques [9, 51]. In T cells, no promigratory compensation by $\beta 2$, $\beta 3$, and αV integrins or CD44 is observed in collagen lattices after blocking of $\beta 1$ integrins [32]. In vivo, $\beta 1$ integrin-deficient null embroys die shortly after implantation, yet $\beta 1$-deficient cells populate most tissues in chimeric embryos, including those whose formation was previously considered $\beta 1$ dependent [113, 118]. Despite their major deficits in vitro (see above), $\beta 1$-null embryonal stem cells readily migrate into the inner cell mass of blastocysts and position themselves correctly for ectodermal and mesodermal differentiation [113]. $\beta 1$-Deficient hematopoietic stem cells proliferating and differentiating in the yolk sac readily emigrate into the blood system, but fail to transmigrate into other organs such as liver and thymus [119]; despite the absence of $\beta 1$ integrins, a principal capacity to migrate through the ECM of the yolk sac appears to be retained [119]. Similarly, in ESB lymphoma cells, the targeted disruption of the $\beta 1$ integrin gene leads to greatly reduced metastatic capacity to liver and spleen; however, in skeletal muscle, septal interstitial tissue is strongly invaded [120]. These findings suggest that in some tissues predominantly consisting of sparse fibrillar collagen, residual migration is developed independent of $\beta 1$ integrins. In the rat mesentery in vivo, the migration velocity of neutrophils is reduced by adhesion-perturbing antibody against $\beta 1$ integrins [85]; however, considerable residual migratory action is retained in the presence of antibody. The inability to fully block neutrophil migration by anti-$\beta 1$ antibodies is reminiscent of persistent migration of locomotor subpopulations of T cells detected in 3-D collagen lattices in the presence of blocking anti-integrin antibodies [32, 76].

Taken together, these investigations indicate that, in collagen lattices and in vivo, significant cell migration occurs independent of $\beta 1$ integrins and other integrins under conditions that were not predicted by haptokinetic migration assays. One explanation for such discrepant results could be differences in the assay systems. In vivo, the migratory action and cell-cell interactions involved are not assessed directly. In chimeric mice, $\beta 1$-deficient cells might be able to translocate passively via interaction with wild-type neighboring cells that utilize $\beta 1$-dependent migration [113, 121]. Another explanation might be that the loss of integrins in totipotent cells might allow the development of compensatory pathways which are otherwise silent, e.g., by integrins other than $\beta 1$ [72], whereas, the possibility of counter-regulation might not be as dominant in short-term antibody or peptide inhibition experiments. Furthermore, growth factors that are present in vivo but absent in vitro can gain important functions when integrins are absent [26, 115]. Lastly, it cannot be ruled out that biophysical mechanisms such as shape change (see below) and nonspecific low-affinity interactions with the tissue environment provided by multiple surface glycoproteins via charged or uncharged residues are able to mediate migration. It will be important to know whether the residual migration seen in many cell types after blocking or gene disruption experiments in vitro [76, 117] or in vivo [85] results from adhesive multiplicity by overlapping adhesion specificities or rather from cellular capacity to translocate via shape change.

One possible explanation comes from studies on motility mechanisms in ameboid cells. In ameba, defined molecular structures such as clustered integrins, focal

contacts, and stress fibers stabilizing polarized cell shape, substrate adhesion, and force generation are absent [106, 108]. Such 'ameboid' cells comprise *Dictyostelium*, T and B lymphocytes, some tumor cells and, to some extent, neutrophils and monocytes, all of which move while interacting with only a small part of their surface to the substrate [9, 122]. Common features in ameboid migration are small cell size, high migration velocities up to 30 μm/min provided by a cyclic process of pseudopod extension and retraction of relatively low adhesivity to the substrate [32, 107, 122, 123]. The protrusion of pseudopods and pseudopod kinetics occur independently of physical contact to the substrate, and are hence substrate independent, which sets ameboid cells apart from integrin-guided pseudopod protrusion and contact formation in fibroblasts and neurons [109, 124]. Highly dynamic, ameboid migration strategies exemplify a case of cell motility of low adhesiveness [117] that sometimes might be largely uncoupled from integrin-dependent traction forces [32] and, therefore, do not fully conform with the paradigm of haptokinetic multistep migration of graded attachment and cytoskeletal traction forces [9, 27, 51, 109]. This type of migration may be particularly effective in 3-D tissues, allowing multimeric low-affinity interactions to the substrate instead of unilateral adhesive attachment.

Contact guidance

Contact guidance is defined as the migration of cells along physical structures of defined shape, e.g., the border of a glass coverslip or along 3-D aligned ECM lacking a gradient of chemotactic or haptotactic factors [63, 125]. Contact guidance is considered to be important in embryonic morphogenesis, axon formation, and wound healing. Tissue fiber orientation is either a constitutive feature of the tissue architecture, e.g., alignment of collagen fibers in the dermis or lymphatic organs [67], or temporarily induced by traction, such as the orientation of fibrin fibers in provisional wounds induced by platelet and fibroblast contraction.

The cellular and molecular mechanisms underlying contact guidance involve multiple events. (i) Contact-dependent formation of focal contacts and actin polymerization at interactions to ECM [8, 18] results in contact-mediated protrusion of leading pseudopod(s) along these strands. (ii) Cell protrusion is favored by gaps and pathways of least resistance, e.g., between aligned fiber strands [55, 99]. (iii) Migration may occur along paths of maximum substrate rigidity favoring integrin-cytoskeleton linkages [31]. (iv) Finally, contact guidance is provided by matrix strands created and maintained by traction from the migrating cell itself. Initial traction and reorientation of the matrix leads to radial orientation of fibers towards the emerging leading edge (fig. 2A,B), as detected by confocal reflection microscopy of migration dynamics in fibroblasts and MV3 melanoma cells [9]. After cell polarization has been completed, migration tends to continue further along these fiber strands resulting in high directional persistance within matrices of initially random fiber order [55, 63]. Similarly, the striking migratory persistence found in locomoting multicellular complexes or clusters (see below) follows a radial field of polarized and directional fibers oriented towards a limited number of dominant, highly motile cells at the leading edge ('pathfinder cells') [95, 126]. In all of these cases, the length axis of the cell orients parallel to matrix strands, and migration, occurs along these structures.

Cellular strategies to overcome extracellular matrix resistance

Three principally different mechanisms by which cells might overcome biophysical matrix barriers have been described: (i) shape change, (ii) contact-dependent matrix remodeling, and (iii) cell-independent proteolysis by secreted proteases. The first mechanism leads to reversible alterations of the ECM structure, i.e., traction and bending of fibers, whereas the two latter processes cause irreversible changes of both matrix protein structure and overall matrix architecture.

Biophysical elements in cell migration. Upon migration, cells adapt morphologically to the ECM environment, finding and utilizing a path of least resistance suitable for migration. In the chorioallantois model using migrating neutrophils, graded lateral protrusions towards fiber strands ('footholds') stabilize the cell and push the cell body forward [101]. Constriction rings at locations of narrow texture function as anchors and support propulsion of the cytoplasm by cytoskeletal contraction ('squeezing'), leading to cell translocation [51, 101]. Hence these cells adapt their shape according to the biophysical properties of the matrix environment [101]. In 3-D collagen matrices, T-cell and dendritic-cell migration occur along preexisting fibrillar strands while areas of dense network are rather circummigrated than penetrated [32, 127]. In these cells, fiber bending is minimal (up to 5 μm) and changes in matrix architecture are not detected [32, 51, 127], supporting the concept that migration can occur independent of structural matrix remodeling. These observations are in line with findings from in vitro wound-healing models using relatively soft and sparse 3-D fibrin gels as substrate. In fibrin gels, nonneoplastic fibroblasts continue to migrate in the presence of physiological and pharmacological broad-spectrum inhibitors of MMPs [81] or the broad-spectrum serin proteinase inhibitor aprotinin [128], indicating migration strategies independent of proteolysis. Hence, if pores and matrix gaps preformed

in the tissue offer a sparse or elastic enough substrate in relation to the size and flexibility of a given cell body, shape change may be a sufficient strategy to overcome matrix barriers and mediate migration, suggesting a nonproteolytic mechanical process in these models [32, 81, 101].

Contact-dependent proteolysis and matrix remodeling. Membrane-bound proteinases play an important role in the proteolytic remodeling of the ECM [81, 129, 130]. Cell surface localization of several MMPs including MMP-2, MMP-9, and membrane-type (MT)-MMPs have been shown for normal fibroblasts and endothelial cells, but also for malignant cells like melanoma cells, breast cancer cells, and neoplastic keratinocytes [130–132]. From a mechanistic perspective, migrating cells might be forced to focally lower the level of structural and molecular organization of the surrounding matrix, if the cell body does not permit sufficient biophysical adaptation to preexisting matrix gaps. Consequently, membrane-bound proteinases are thought to allow highly localized and tightly controlled proteolysis that cleaves matrix fibrils only to an extent required for the cell body to translocate, as seen in melanoma cells forming tube-like matrix defects while migrating (fig. 2B, arrowhead).

Surface location of proteases occurs via two different mechanisms, either expression of endogenous transmembrane proteins or by binding of extracellular proteases to integral membrane receptors. In both cases, the cell surface must physically bind to the ECM substrate to perform proteolysis.

An important class of membrane-anchored MMPs comprises the family of MT-MMPs [129]. Four members of the MT-MMP family (MT1–4-MMP) have been identified, functioning as individual enzymes and degrading multiple cross-linked ECM components, including collagen and fibrin [81, 129, 133, 134]. In addition, MT-MMPs can be integrated in ternary complexes in the plasma membrane. The prototype of these ternary complexes contains pro-MMP-2, tissue inhibitor of MMPs (TIMP)-2, and MT1-MMP. Activated MT1-MMP acts as receptor of TIMP-2, and this complex then binds and activates soluble pro-MMP-2 or pro-MMP-13 [129, 135]. In haptokinetic migration, fibroblasts overexpressing MT1-MMP on the surface cause focal degradation of fluorescently labeled gelatin films [131]. Upon detachment, MT1-MMP-containing vesicles are deposited on the substrate [131]. Hence, pericellular lysis accompanying single-cell movement is likely to contribute to the migratory action. Alternatively, MT-MMP function could be a coincident parameter that results from genetically forced overexpression or overall cell activation rather than from the specific signals required for matrix cleavage favoring migration.

A further family of transmembrane receptors, designated ADAMs (derived from 'a disintegrin and metalloproteinase domain'), contain both a disintegrin and a metalloproteinase domain potentially providing both attachment and proteolysis in close topical proximity [reviewed in ref. 136]. ADAMs are important candidate molecules for the integration of cell adhesion and migration, and related matrix remodeling.

Secreted proenzymes present in the tissue are bound and activated by cell surface proteins [129]. Pro-MMP-2 secreted by the migratory cells themselves or by resident stroma cells can be bound and activated by MT-MMPs [137]. Alternatively, latent MMP-2 is captured by the cell surface via $\alpha V\beta 3$ integrin to become activated for matrix degradation [130]. Similarly, the serine protease urokinase-type plasminogen activator (uPA) binds to its receptor (uPAR), which further colocalizes with integrin $\alpha V\beta 5$ on migratory keratinocytes and carcinoma cells [138] or with $\beta 2$ integrins on monocytes [139].

Besides directly interacting with cell surface receptors, pro-MMPs can bind to ECM proteins and/or degradation products located at the cell surface. Cell surface association of pro-MMP-9 is mediated by the $\alpha 2$ chain of type IV laminin [132]. Likewise, latent MMP-2 interacts with pericellular type I collagen that itself is anchored to $\beta 1$ integrins on the cell surface [140]. In both cases, integrins are targeted by pro-MMPs that require further activation. It was therefore proposed that a latent pool of inactive MMPs is recruited into focal contacts to be released by competing ECM ligands, thereby increasing the local proteolytic potential close to relevant ECM interactions [132, 141].

By focalizing proteolytic events at or near the cell surface, matrix-degrading enzymes might be effective even in the presence of high inhibitor concentrations. Besides widening preexisting matrix gaps, cell surface proteases may also enhance migration by favoring cell detachment either by cleavage of adhesion molecules or by degrading surface-bound ECM components.

Direct evidence for physiological surface expression of MMPs and its function in cell motility comes from endothelial cells migrating in 3-D matrices. Cultivation of primary rat capillary endothelial cells in 3-D collagen lattices but not, however, attachment to collagen surfaces, leads to the expression of MT1-MMP and MMP-2 accompanied by sprouting and the formation of endothelial cell networks [80]. Collagen degradation, formation of cell-cell interactions, and cellular organization are reduced by MMP inhibitor BB-2516 (marimastat) suggesting that MMP function is important in multicellular endothelial sprouting and, presumably, vessel formation. Similarly, MT1-MMP activity independent of MMP-2 favors endothelial sprout formation in 3-D fibrin gels but not, however, the migration of endothelial cells across 2-D fibrin-coated surfaces [81].

The ability of MT1-MMP to direct this invasion was critically dependent on its localization on the cell surface, underlining the importance of cell-mediated focalized proteolysis in some migratory processes [81].

Secretion of matrix-degrading enzymes. The secretion of matrix-degrading enzymes is present in processes of enhanced cell migration and tissue remodeling during morphogenesis, inflammation, wound healing, and tumor invasion [142]. In many of these events, degradation of ECM by secreted enzymes is assumed to be a prerequisite for the cells to migrate into native or provisional tissue matrix [143, 144; see also Johansson et al. in this issue]. An important candidate enzyme for freely diffusible protein degradation is the protease plasmin which is activated by uPA after binding to its receptor (uPAR) on invading tumor cells and/or reactive stromal cells [reviewed in ref. 145]. Several variables may determine the tissue distribution of proteases, such as production rate, diffusion efficiency, and the amounts of protease inhibitors present in the tissues. Secretion of active enzyme in the presence of high inhibitor concentrations will lead to a very limited diffusion distance of enzymatic activity, hence approximating short-range or focalized proteolysis. At lower inhibitor concentrations, more diffuse proteolysis could reduce biophysical matrix resistance at rather distant matrix sites beyond the direct reach of the cell body. Addition of soluble neutrophil elastase or porcine pancreatic elastase to 3-D collagen lattices results in the directional migration of airway fibroblasts [146]. It is possible that digestion of the matrix by soluble elastase facilitates fibroblast migration by generating ECM fragments with enhanced chemotactic properties [147]. Alternatively, secreted proteases could selectively and/or partially degrade certain components of the ECM only, resulting in a general 'softening' of tissue or subtle widening of preexisting matrix gaps and pores, and reduce physical barriers.

Morphologically visible proteolysis is present in collagen lattices upon long-term culture of primary squamous cell carcinoma or melanoma explants (M. Tusch, Y. Hegerfeldt and P. Friedl, unpublished data). However, uncontrolled ECM degradation could impede the invasive process, since cells require sufficient substrate with which to interact for migration and survival. Repeated attempts to detect diffuse proteolysis from collagen lattices upon tumor cell invasion have not confirmed the release of measurable amounts of collagen degradation products [148, 149] or cell-independent general widening of the matrix architecture [55, 95].

The relative contribution of soluble versus cell-bound MMP function was recently investigated for U251.3 glioma and HT-1080 fibrosarcoma cells contracting 3-D collagen matrices in vitro. Collagen contraction requires endogeneously expressed MMP-2 in association with MT1-MMP as well as $\beta 1$ integrin function [150]. In the absence of endogenous MMP-2, matrix contraction was not restored by high amounts of exogenously added soluble active MMP-2 [150]. These data suggest a relatively minor function for soluble MMP-2 in the remodeling of 3-D collagen lattices in comparison to the capture of cell-derived enzyme.

In addition to the secretion of soluble proteases, MMPs can be deposited with membrane vesicles that also contain $\beta 1$ integrins, CD44, and other cell surface molecules [131, 141]. Such membrane deposits are shed by migrating tumor cells into areas of remodeled matrix along tracks of previous migration (fig. 2A,B, white arrowheads) [55, 131]. The functional significance of matrix-bound membrane vesicles and smears is unclear; however, a putative function may reside in the maintenance of facilitated migration for following cells along such successfully established paths [55].

To date, genetic approaches have yielded several matrix proteinase-deficient mice, including knockout mice for uPA, MMP-2, MMP-3, MMP-7, MMP-9, MMP-11, and MMP-12 [reviewed in ref. 13]. Although some of these mutant mice develop limited morphologic abnormalities, all mutant mice lack a lethal embryonic phenotype and deliver healthy offspring. More detailed analysis has shown alterations in some migration-related processes such as pathologic inflammatory reactions, reduced angiogenesis, or delayed tumor progression in injection or implantation models, supporting a function of individual MMPs in cell motility [13, 151]. The overall minimal phenotype of MMP deletion mutants is thought to result from redundancy and functional compensation by other related enzymes [13]. Hence, multiply MMP-deficient mice will be required to further elaborate the combined contribution of MMPs to cell motility and tissue remodeling.

Tumor cell invasion and migration

For cancer spread and metastasis, cell migration through the connective tissue is a prerequisite for lymphatic or hematogeneous dissemination. As established for nonneoplastic cells, tumor cell motility through the tissue depends on the cooperation of adhesive and proteolytic mechanisms. Migrating tumor cells must cross multiple barriers such as basement membranes, interstitial tissue stroma, and cell-cell junctions, covering distances up to several hundreds of micrometers. A number of reports have demonstrated that integrins and matrix-degrading proteases influence local tumor growth, survival, and metastasis [reviewed in ref. 152]. However, there is no simple concept as to how adhesion receptors and proteases might be involved in tumor cell invasion and motility, and how this function

in cell migration might be set apart from effects on tumor cell proliferation and survival. For example, disrupting cellular interactions with the surrounding matrix by antibody perturbation or genetic ablation experiments might lead to apoptosis and, consequently, reduced invasiveness [153]. In this case, impaired migration would be secondary to apoptosis, concealing changes in motility mechanisms per se. Furthermore, many of the observed effects appear to be cell type specific, depending on cell-specific parameters such as size and morphology, cell-cell interactions, and the relative expression level and activity state of different integrins and MMPs. Finally, the tumor stroma varies in ECM structure and composition, and bystander cells such as fibroblasts and leukocytes may add novel binding and signaling structures regulating tumor cell invasion and survival.

Cellular pattern of tumor cell migration

Based on histological observations and in vitro studies, at least two different tumor invasion mechanisms have been described [95, 154]: the migration of individual cells and the invasion and migration of cell aggregates or clusters (fig. 3).

Single cells. The invasion of individually migrating cells corresponds to the locomotion of single cells after losing homophilic adhesion to neighboring cells [2, 155]. Mutation or downregulation of receptors mediating homophilic cell-cell interactions such as cadherins and catenins and/or an impaired ability to assemble adherens junctions favors changes in signaling, cytostructure, and cell-cell communication, leading to the release of cells from the primary tumor [7, 156]. In normal breast epithelial cells, direct activation of the low molecular-weight G protein Rac1 disrupts homophilic cell-cell interactions that are normally mediated by E-cadherin and, hence, induces migration in collagen matrices [156, 157]. Similarly, engagement of integrin-linked kinase (ILK) leads to a downregulation of E-cadherin promoting the locomotion of individual cells [158], offering a molecular explanation for tissue infiltration by scattered individual tumor cells in epithelial tumors [159].

After detachment, disseminated individual cells invade adjacent tissue stroma maintaining cell-matrix rather than cell-cell interactions while migrating. In some tumor cell lines, there is little inhibition of locomotion if individual tumor cells encounter other neoplastic cells or resident stroma cells. This phenomenon was described as a loss of contact inhibition of movement in malignant cells [160] which may set tumor cells apart from more tightly controlled migratory and cell-cell interaction signals in normal cells [160]. Taken together, this migration type has established the 'single-cell paradigm' of cell migration which is generally represented in studies on the molecular mechanism of tumor cell motility in vitro and in vivo.

Individually migrating cells may follow either a slow and adhesive 'stromal,' fibroblast-like type of migration or rather more rapid and ameboid crawling (fig. 3). In morphologically large cells of high integrin expression (such as fibrosarcoma and many other tumor cells), a slow migratory action is accompanied by integrin clustering to patches and/or streaks at interactions to matrix fibrils, accompanied by migration-associated remodeling of the ECM [9, 32, 46]. Using intravital microscopy, such a slow type of migration was observed for *ras*-transformed fibroblasts migrating within the mesenchymal layer of the chorioallantoic membrane after extravasation [84].

Other tumor cell types may utilize more rapid ameboid migration strategies [155]. Ameboid locomotion in the interstitial tissue was first detected in V2 carcinoma cells, using intravital microscopy of the rabbit ear chamber and time-lapse cinematography [86]. After intravenous injection, V2 cells adhere to vessel walls, transmigrate within minutes and then show continuous ameboid locomotion through the tissue [86]. In V2 cells and mammary carcinoma cells, in vivo migration velocities range from 3 to 10 μm/min [86, 88, 161], reminiscent of speeds observed in neutrophils in the rat mesentery (7–20 μm/min) [85] or lymphocytes in 3-D collagen lattices (4–18 μm/min) [58]. Ameboid morphology and high speed set certain cancer cell types [2, 88, 155, 162]], including V2 carcinoma cells, bronchial and mammary carcinoma cells, lymphoma and leukemia cells apart from larger, morphologically complex and slower cells, such as melanoma cells [55] and HT-1080 fibrosarcoma cells [51; H. Lü and P. Friedl, unpublished data].

Migration of cell clusters. The dissemination of coherent masses of tumor satellites is histologically observed in tumors of epithelial origin (e.g., invasive oral carcinoma and adenocarcinoma) or melanoma [154]. In vivo, such coherent cell groups retain desmosomes and other cell-cell junctions [163], protrude into the tissue preferentially along paths of least resistance such as clefts, natural cleavage planes or tissue spaces (e.g., fiber bundles, nerves, blood vessels) [83] and can be detected in lymphatic vessels [164] and in peripheral blood [165, 166]. Using time-lapse videomicroscopy, the coordinated protrusion, detachment, and migration of neoplastic cell clusters from primary tumor explants is seen upon culture in 3-D collagen lattices [99, 126]. The leading edge of such clusters is composed of highly motile cells ('pathfinder cells') creating driving force and a highly persistent and directional migration type, whereas the trailing edge is passive [95, 99]. This coordinated cluster migration is similar to that of cells from *Xiphophorus* (platyfish) melanoma developing cluster

movement on a coverglass [167]. The polarized ruffling of dominant pathfinder cells appears to be maintained by cell-cell interactions and contact inhibition rather than a structurally determined difference [99; P. Friedl, unpublished data]. Within the cluster, $\beta 1$ integrins are heterogeneously expressed and redistribute to cell-matrix as well as cell-cell interactions, supporting not only migration and migratory persistence but also cell-cell interactions and cluster polarity [121]. In collagen lattices, neoplastic cell clusters are present in about one-third of primary explants from different human tumors, including invasive oral squamous cell carcinomas, breast cancer, cutaneous melanoma, and rhabdomyosarcoma. In contrast, no cluster development is obtained in liquid culture or using single-cell suspensions [95], underlining the importance of a 3-D ECM substrate. How in vitro invasion and migration of cell clusters correlate with the aggressiveness of a given tumor remains to be determined. However, cluster migration would explain the spread of heterogeneous sets

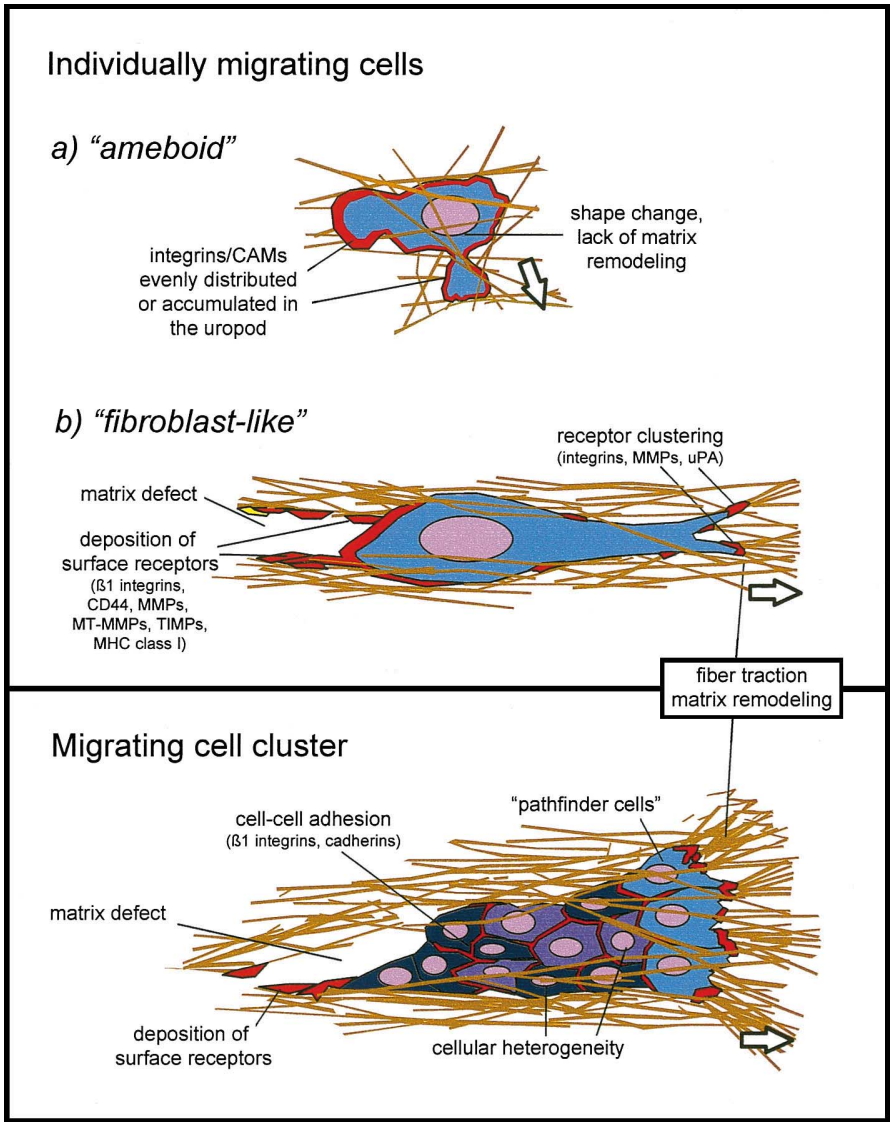

Figure 3. Cellular pattern of migrating tumor cells. Schematic diagrams represent cell morphology, receptor distribution, and interactions with ECM fibers, as detected by confocal fluorescence and reflection microscopy [9, 98]. Abbreviations: CAMs, cell adhesion molecules, MMPs, matrix metalloproteinases; uPA, urokinase-type plasminogen activator, TIMPs, tissue inhibitor of matrix metalloproteinases; MHC, major histocompatibility complex.

of tumor cells, minimizing cell loss, favoring high local MMP expression [168], and protecting cancer cells from immunological assault.

Molecular mechanisms of tumor cell migration

A multitude of cell surface receptors has been implicated in tumor invasion and migration, among them integrins $\alpha 2\beta 1$ and $\alpha v\beta 3$, CD44, and CD44 splice variants [11, 43, 89, 169, 170]. In 3-D tissues, the cellular environment constitutively surrounds and interacts with the cell surface; thus adhesion receptor-mediated signals might be expected to operate constitutively. However, there are no overall generalities concerning adhesion receptor function in tumor cell invasion. Additionally, whether migration of tumor cells strictly follows the three-step concept of cell migration or whether more diverse, potentially less regulated migration mechanisms are involved is not completely resolved.

Integrins in cancer invasion

While integrin signaling in neoplastic and nonneoplastic cells has been extensively studied in the past few years [reviewed in ref. 171] it has been difficult to differentiate their precise function in cancer cell motility and invasion from other integrin-related functions such as cell cycle control and prevention of apoptosis.

The overall picture now predicts that integrin function in the migration of several neoplastic cells follows similarly tightly regulated principles, as previously described for fibroblasts [70, 100, 172]. $\beta 1$ integrin levels in neoplastic cells in vivo range from increased levels to profound losses, depending on the tumor type examined [100, 171]. The $\alpha 2$ integrin subunit was initially described as a melanoma progression marker [89]. High $\alpha 2\beta 1$ expression is correlated with a more invasive and metastatic phenotype than low $\alpha 2\beta 1$ expression in melanoma [11, 170] and rhabdomyosarcoma [169] cells. After blocking of $\alpha 2\beta 1$ integrins, migration and other migration-associated cell functions, such as cell polarization, shedding of cell surface receptors, and the remodeling of ECM are greatly impaired [70], suggesting invasion-promoting effects of $\beta 1$ integrins. Furthermore, in fibroblasts and osteosarcoma cells, interaction with a 3-D fibrillar collagen matrix leads to $\alpha 2\beta 1$-mediated upregulation of MMP-1 and MMP-2 at the mRNA and protein level [173, 174]. Because both MMP-1 and MMP-2 were shown to cleave type I collagen, integrin-collagen interaction appears able to increase the cellular capacity for substrate degradation and invasion [173].

In marked contrast, in breast carcinoma, reduced $\alpha 2\beta 1$ expression confers a less differentiated and more invasive phenotype, whereas $\alpha 2\beta 1$ reexpression restitutes cell differentiation, restores contact inhibition of migration and, consequently, abrogates tumorigenesis [7, 175]. Hence, depending on the cell type and the context of cell matrix interactions, $\alpha 2\beta 1$ integrin can function as migration-promoting receptor in one cell type, whereas in another cell type, invasion is ablated and differentiation restored.

Increased expression of $\alpha v\beta 3$ in melanoma cells appears to be correlated with increased metastatic potential of melanoma cells. The adhesive function of $\alpha v\beta 3$ to its ligands vitronectin, fibrinogen, laminin, or denatured collagen supports haptokinetic migration in different tumor cells [176, 177]. $\alpha v\beta 3$ also binds and activates matrix-degrading MMP-2 which in turn contributes to tumor cell dissemination [130]. In addition, $\alpha v\beta 3$ prevents apoptosis in tumor cells and, consequently, favors tumorigenesis [178]. The overall picture, however, now suggests that the most important $\alpha v\beta 3$-related function resides in the prevention of apoptosis in tumor-related angiogenesis, thereby supporting endothelial cell motility and sprouting [179–181]. Hence, the contribution of $\alpha v\beta 3$ integrins to tumor progression and motility appears to be secondary to sustained survival of both tumor cells and blood vessels.

A similarly complex picture now emerges for the fibronectin receptor $\alpha 5\beta 1$. Integrin $\alpha 5\beta 1$ promotes the migration of fibroblasts and tumor cells across fibronectin-coated surfaces and, therefore, was initially considered as a tumor cell motility receptor [100; reviewed in ref. 43]. $\alpha 5\beta 1$ also favors cell survival in vitro via upregulation of anti-aptoptotic Bcl-2 [182]. In contrast, an inverse correlation of $\alpha 5\beta 1$ expression and tumor cell growth was detected in vivo [183, 184], suggesting that interactions of fibronectin with $\alpha 5\beta 1$ suppress tumor development or progression. Recently, in mice lacking either the fibronectin or the $\alpha 5$ integrin gene, no change in tumor incidence or progression was detected in normal or p53-deficient animals, arguing against a function of $\alpha 5\beta 1$ integrin in tumorigenesis and metastasis [184]. The complexity of such diverse effects in different cell types and tumor models makes it difficult to predict the direct contribution of $\alpha v\beta 3$ and $\alpha 5\beta 1$ to tumor cell migration in the cascade of tumor progression events.

Genetic ablation experiments indicate that the contribution of specific receptor-ligand interactions to cell invasion and motility is more complex than predicted from antibody blocking experiments. For example, impaired angiogenesis was detected in $\beta 1$-deficient teratomas [185], whereas phenotypically normal blood vessels can be detected in αv-deficient mice [186]. It appears that the adhesive multiplicity of αv and $\beta 1$ integrins cross-compensate interactions to their respective ligands [185, 187]. Furthermore, tumor-associated tissue remodeling leads to protein degradation and the generation of new

ECM epitopes [188] which, in turn, may further contribute to unorthodox adhesive or antiadhesive interactions.

CD44 in cancer cell motility

CD44 has been proposed to play a major role in metastasis of different types of tumors [reviewed in ref. 189]. CD44 comprises a family of multifunctional adhesion receptors binding to diverse ECM ligands including HA, chondroitin sulfate, and osteopontin [reviewed in ref. 190]. The CD44 cytoplasmic domain binds to the actin-based cytoskeleton via ankyrin and members of the ezrin-radixin-moesin family of adapter proteins [191]. In vivo, administration of blocking anti-CD44 antibody reduces the metastatic potential of melanoma cells in the absence of changes in local tumor development [192], suggesting that CD44 plays a role in tumor dissemination. The function of CD44 in cell migration comes from haptokinetic models using HA as substrate. Migration across HA-coated surfaces is supported by CD44, while the blocking of CD44 by monoclonal antibody or disruption of receptor expression result in reduced migration [193, 194].

In highly invasive MV3 melanoma cells, the haptokinetic migration of which was previously shown to be CD44 dependent [194], only blocking of $\alpha 2\beta 1$ integrins but not of CD44 impairs migration if 3-D collagen lattices substituted with HA and the HA-cross-linker chondroitin sulfate are used [70]. Interestingly, the migratory insufficiency after loss of $\beta 1$ integrin function is not compensated by CD44-HA interactions [70]. Although the supramolecular assembly of HA in such 3-D migration models is not well understood, the promigratory function of CD44 remains in question [77]. The lack of CD44 function in tumor cell migration in 3-D multicomponent ECM is in line with data from CD44 gene disruption experiments in vivo: MDAY-D2 lymphosarcoma cells lacking CD44 expression develop completely intact invasive capacity into subcutaneous tissue and metastasis like MDAY-D2 wild-type cells [195]. Similarly, in highly metastatic pancreatic carcinoma cells, a CD44 variant binding to HA does not contribute to in vivo invasion and metastasis, as shown by hyaluronidase transfection resulting in undiminished metastasis despite a loss of the cellular capacity to bind HA [196].

Hence, although some adhesivity mediated by CD44 on isolated HA ligand may support haptokinetic migration across surfaces, migration in 3-D tissues may follow more complex principles. It was recently suggested that cell surface CD44 function promotes tumor cell survival in invaded tissue and that its suppression can induce apoptosis in tumor cells [197], providing an alternative mechanism for CD44 function in tumor progression. These data on CD44 suggest that further reevaluation of promigratory adhesion receptors that were initially established for 2-D migration models may yield a different order of hierarchy for 3-D migration models [70].

Migration-associated tissue remodeling

There is no 'cancer-specific' pattern of cell motility and tissue remodeling. Specific variants of tissue remodeling programs likely depend on the site from which the cancer originates. In 3-D matrix-based models, tumor cells (similar to fibroblasts) cause initial fiber traction at the leading edge followed by radial fiber alignment towards the cell which then favors persistent migration in direction of maximal traction (fig. 2) [55]. It is of interest that in 3-D collagen- [55] and 2-D fibronectin-based migration models [198], migrating cells, instead of diffusely lysing the substrate appear to bundle ECM components towards the edge of the cell thereby creating areas of matrix clearance in direct apposition to densely aligned strands (fig. 2B). The migratory action leads to the formation of tube-like matrix defects that further contain shed cell surface receptors such as $\alpha 2\beta 1$ integrin (fig. 2, arrowheads), CD44 [99], MMPs, and also cytoplasmic portions (C. Mayer and P. Friedl, unpublished data). Migration-associated matrix remodeling may represent a common strategy employed by large cells to overcome biophysical matrix resistance for their own migration. Such newly formed matrix defects facilitate the migration of following cells along these paths of least resistance [55] ultimately leading to the migration of aligned cell strands following each other [55, 95].

Many classes of matrix-degrading enzymes have been implicated in migration-associated matrix remodeling and tumor progression, including serine protases such as plasminogen activators, dipeptidylpeptidase IV, fibroblast-activating protein-1, cysteine proteases such as catepsin K [199], and the family of MMPs [136; reviewed in ref. 141, and by Johansson et al. in this issue]. In particular, the serine protease plasmin and a variety of MMPs may efficiently contribute to processes of tissue remodeling that might favor tumor penetration and dissemination. Interstitial types I and III fibrillar collagens are degraded by MMP-1, MMP-2, MMP-8, MMP-13, MT1-MMP, and MT2-MMP [134], while most other MMPs cleave basement membrane components including laminin and type IV collagen [143].

MMPs expressed at tumor sites can be produced by the tumor cells themselves, but also by nonneoplastic bystander cells such as fibroblasts or leukocytes present in the tumor-surrounding tissue [143, 145].

Tumor-cell derived remodeling

Tumor cells can either express endogenous MMPs, capture soluble MMPs by cell surface receptors, and/or release cell surface MMPs into the adjacent tissue [140]. Several MMPs including MMP-2, MMP-7, MMP-9, MMP-13, and MT1-MMP are expressed by tumor cells as both mRNA and proteins [12, 13, 141, 143]. The expression of MMPs on tumor cells is upregulated by integrin-ECM interactions and signaling, suggesting a substrate-driven expression mode [200]. MMPs and MT-MMPs are upregulated in fibroblasts and melanoma cells upon culture in 3-D collagen lattices depending on $\alpha 2\beta 1$ integrin-mediated outside-in signaling [174; C. Mauch, personal communication]. MMP-2 is further upregulated in melanoma cells by interaction with vitronectin, accompanied by enhanced invasiveness through Matrigel-coated filters [200]. Upon tissue penetration, invadopodia containing proteolytic activity are formed at the leading edge [201]. MT1-MMP is clustered in the membrane of polarized fibrosarcoma and glioma cells at substrate attachment and detachment sites [150], and it was speculated that integrin-mediated attachment to ECM may direct proteolysis at the subcellular level [130, 150, 199].

Soluble MMP-2 and other MMPs are captured by $\alpha V\beta 3$ integrins on the cell surface of tumor cells and become converted into the proteolytically active form. After binding to the cell surface, MMP-1, MMP-2, MMP-9, and uPA colocalize with integrins in close proximity to the invasion zone [130, 141]. In tissue sections from melanoma, MMP-2 is colocalized with $\alpha V\beta 3$ integrins at the site of invading melanoma cells [130]. Consistent with these results, MMP-2-deficient mice exhibit delayed tumor growth and experimental metastasis in a B16 melanoma and Lewis lung cell carcinoma model [151]. Although these studies suggest a role for MMPs in tumor invasion, the precise mechanism of MMP contribution to tumor progression is less clear.

The function of MMPs in tumor cell motility has been established in 2-D as well as 3-D migration models. Overexpression of MT1-MMP results in complex formation with TIMP-2 and activated MMP-2 at the surface of glioma cells followed by collagen degradation and a moderate increase in glioma cell migration across type I collagen [202]. Because the cells were migrating across a collagen-coated surface, the reduction of biophysical resistance by matrix degradation may be of minor importance in this model. The association of the uPA-uPAR complex with $\alpha v\beta 5$ integrins on the surface of carcinoma cells induces migration on vitronectin in these cells independent of altered adhesion [130]. In glioma cells, upregulation of MMP-2 results in increased invasiveness in vitro, as measured by Matrigel invasion and accelerated invasive remodeling of 3-D normal fetal brain explants [203]. Besides removal of physical barriers, as described above, several other effects resulting from proteolytic degradation of ECM may contribute to tumor cell invasion and migration.

First, some proteolytic ECM degradation products function as promigratory and/or chemotactic factors [reviewed in ref. 155]. Type I, II, or III collagen fragments act chemotactically on fibroblasts [147]. Similarly, the recruitment of previously nonmigrating breast epithelial cells is induced by MMP-2 degradation products of laminin-5 [204].

Second, proteases can remodel ECM components into substrates more suitable for migration. In some cases, a portion of ECM components is cross-linked rather than fully cleaved [205], forming graded deposition of ECM fragments that could favor cellular haptotaxis along the path of matrix remodeling [55]. The partial degradation of ECM also creates new functional epitopes resulting in the unmasking of previously hidden adhesion sites. The G1 domain degradation product of aggrecan generated by MMPs contains a neoepitope (VDIPEN) that is used for cell adhesion [188]. While $\alpha v\beta 3$ integrin fails to bind to native collagen, it does interact with cleaved collagen via a newly exposed cryptic RGD site providing adhesive contact to the substrate [178]. Additionally, new protease cleavage sites might be introduced allowing even more efficient degradation.

Third, partial degradation leads to the generation of epitopes that provide activatory signaling for cells to begin migration in the absence of altered adhesion. Specific cleavage of laminin-5 by soluble MMP-2 induces migration of breast epithelial cells on laminin-5, which is dependent on a newly formed putative signal-inducing cryptic site in the laminin $\gamma 2$ chain [204]. In contrast to MMP-2, other laminin-degrading proteases such as MMP-9 and plasmin show no such effect, suggesting a specific and tightly regulated process [204].

Finally, besides degrading ECM components, proteases may also cleave or mask adhesion receptors at the cell surface thereby promoting cell detachment and migration. MMP-2 interaction with $\alpha v\beta 3$ integrin interferes with the vitronectin-binding site thereby specifically reducing melanoma cell adhesion to vitronectin but not to laminin [130].

Hence, with respect to cell migration, it will be important to further define the specific mechanism by which a given proteinase contributes to tumor cell invasion in a particular model.

Stroma reaction

Whereas some MMPs are expressed by the tumor cells themselves, most of the components regulating proteolysis are produced by stroma cells, such as fibroblasts, endothelial cells, resident macrophages or inflammatory

cells reacting to a primary tumor [80, 145]. Soluble MMPs may then bind to tumor cells and angiogenic vessels. In several murine cancer models, MMP-1, MMP-2, MMP-3, MMP-11, MMP-14, and uPA are synthesized by fibroblast-like cells in the surrounding tissue stroma, and MMP-9 is predominantly produced by macrophages [145, 206, 207]. In epithelial cancer, expression of MT-MMPs is exclusively found in the tumor-associated stroma [207]. Such tissue reactions are assumed to follow a general program that is also active in nonneoplastic processes such as wound healing and inflammation, favoring not only matrix degradation and turnover but also facilitating the migration of diverse kinds of cells [137, 143].

Fibroblast-derived matrix proteinases play an important role in tumor invasion. Expression and activation of MMP-2 and other MMPs in normal fibroblasts is mediated by the interaction of $\beta 1$ integrins with collagen [174, 208]. In vitro, the presence of activated fibroblasts in 3-D collagen lattices has profound effects on adjacent tumor cells. Coculture of primary foreskin fibroblasts with a monolayer of transformed keratinocytes leads to the disorganization of cell-cell junctions and downregulation of $\beta 1$ integrin expression followed by keratinocyte invasion into the collagen matrix [209]. In the chick embryo, proMMP-2 bound by human melanoma cells at invasion zones is produced by the stroma of the host tissue but not the melanoma cells themselves [130]. There is strong evidence that stroma- but not tumor-derived uPA leads to plasminogen activation and to metastatic spread of mammary carcinoma cells independent of local tumor growth [210]. Although the mechanisms by which invasion is ultimately favored remain to be determined, these findings suggest that the stroma reaction has a major impact on proteolytic tissue remodeling and the motility of the cells therein. Although direct evidence for a promigratory effect are sparse, proteolytic cleavage may alter the biochemical and biophysical characteristics of ECM components thereby facilitating tissue penetration by disseminating tumor cells.

Concluding remarks

In addition to modulating invasion and migration of tumor cells, integrins and MMPs contribute to fundamental aspects of tumor-related angiogenesis as well as tumor cell proliferation and differentiation. In several primary tumor models in the mouse, MMP-zinc chelating agents efficiently inhibit growth, angiogenesis, and metastasis formation. How these diverse aspects of integrin- and MMP-mediated cell migration and tissue remodeling are integrated to contribute to tumor invasion and metastasis requires further investigation. In transgenic mice models, overexpression of some MMPs yields enhanced tumorigenesis [211] whereas deletion of MMP genes may delay tumor progression [13]. However, migratory processes do not always involve MMP function. A good example is the function of MMP-7 (matrilysin) in tumor progression. MMP-7 overexpression in a murine breast cancer model leads to drastic acceleration of the onset of tumor development, whereas once the tumor is established, the cellular capacity to metastasize does not differ from control mice [211]. These findings suggest a function for MMP-7 in the regulation of cell growth and differentiation rather than in the invasive cascade per se [211].

In conclusion, genetic and therapeutic in vivo studies deliver a complex picture of interdependent cell functions in the invasive and metastatic cascade. Such in vivo results are supplemented by 3-D ECM-based in vitro models for a more specific readout mimicking isolated aspects of cell-matrix and cell-cell interactions. Together, in vivo and 3-D in vitro culture models will help to further define cellular and molecular elements in tumor invasion and migration as well as reactive tissue remodeling.

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