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# Cell jamming: Collective invasion of mesenchymal tumor cells imposed by tissue confinement ☆

Anna Haeger [a,1], Marina Krause [a,1], Katarina Wolf [a,1], Peter Friedl [a,b,c,*]

[a] Radboud Institute for Molecular Life Sciences, Department of Cell Biology, Post 283, PO Box 9101, 6500HB Nijmegen, The Netherlands
[b] UT MD Anderson Cancer Center, Genitourinary Medical Oncology-Research, Houston TX, USA
[c] Cancer Genomics Network, The Netherlands

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ABSTRACT

*Background:* Cancer invasion is a multi-step process which coordinates interactions between tumor cells with mechanotransduction towards the surrounding matrix, resulting in distinct cancer invasion strategies. Defined by context, mesenchymal tumors, including melanoma and fibrosarcoma, develop both single-cell and collective invasion types, however, the mechanical and molecular programs underlying such plasticity of mesenchymal invasion programs remain unclear.
*Methods:* To test how tissue anatomy determines invasion mode, spheroids of MV3 melanoma and HT1080 fibrosarcoma cells were embedded into 3D collagen matrices of varying density and stiffness and analyzed for migration type and efficacy in the presence or absence of matrix metalloproteinase (MMP)-dependent collagen degradation.
*Results:* With increasing collagen density and dependent on proteolytic collagen breakdown and track clearance, but independent of matrix stiffness, cells switched from single-cell to collective invasion modes. Conversion to collective invasion included gain of cell-to-cell junctions, supracellular polarization and joint guidance along migration tracks.
*Conclusions:* The density of the ECM determines the invasion mode of mesenchymal tumor cells. Whereas fibrillar, high porosity ECM enables single-cell dissemination, dense matrix induces cell–cell interaction, leader–follower cell behavior and collective migration as an obligate protease-dependent process.
*General significance:* These findings establish plasticity of cancer invasion programs in response to ECM porosity and confinement, thereby recapitulating invasion patterns of mesenchymal tumors *in vivo*. The conversion to collective invasion with increasing ECM confinement supports the concept of cell jamming as a guiding principle for melanoma and fibrosarcoma cells into dense tissue. This article is part of a Special Issue entitled Matrix-mediated cell behavior and properties.



## 1. Introduction

Cancer invasion and dissemination into tissue is a multi-step process which balances mechanotransduction towards the ECM[2] with cell–cell interactions between tumor cells. Tumor cell movement may result from distinct migration strategies determined by both, molecular properties of the tumor cells as well as mechanical and signaling input from the tumor microenvironment. Mesenchymal single-cell migration results from stringent adhesion sites linked to high actomyosin-mediated traction force and the capability to proteolytically degrade or remodel ECM [1,2]. Conversely, amoeboid single-cell migration is mediated by a weak cell adhesion to ECM coupled to protrusive leading edge kinetics, including filopodia or blebs, and absence of structural ECM remodeling [3]. Distinct from single-cell movement, collective cell migration depends upon intact cell–cell junctions providing mechanical and signaling connection between tumor cells for supracellular polarization and coordination [4–6]. In collagen-rich 3D ECM, collective cell migration requires an integrin-based force generation and proteolytic cleavage of ECM to generate migration tracks that accommodate the moving cell group [1,7,8]. These cell migration programs are adaptive and interconvertible in response to cell-intrinsic and stroma-derived inputs [2].

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* Corresponding author at: Radboud Institute for Molecular Life Sciences, Department of Cell Biology, Post 283, PO Box 9101, 6500HB Nijmegen, The Netherlands. Tel.: +31 24 3610907.
*E-mail addresses:* a.haeger@ncmls.ru.nl (A. Haeger), p.friedl@ncmls.ru.nl (P. Friedl).
*URL:* http://www.cancergenomics.nl (P. Friedl).

[1] Tel.: +31 24 3610907.
[2] Extracellular matrix.

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As central mechanism for the conversion from multicellular to single-cell pattern, EMT[3] leads to the downregulation of stringent E-cadherin-based cell–cell junctions which releases individually migrating cells from multicellular epithelia [9–11]. Consistently, mesenchymal tumor cells migrate individually after experimental cell dissociation and exposure to 2D and 3D *in vitro* substrates, similar to migrating fibroblasts [12,13]. However, mesenchymal cells can also develop N-cadherin-based collective migration patterns in 3D models of multicellular invasion *in vitro* and mouse models of interstitial invasion [1,7,9,14–19]. Whereas molecular mechanisms of cell–cell junction regulation and cell patterning are well established, tissue determinants for single-cell *versus* collective migration modes remain unclear.

Depending on the type of tissue microenvironment, invading tumor cells are confronted with different extracellular structures and molecular patterns which jointly determine the biomechanics of cell–matrix interaction and migration efficacy. Physical, extracellular modulators of cell migration include: confinement, based on pore-size through which the cell migrates; geometry along which the cell-body aligns, determined by ECM alignment and dimensionality (2D vs. 3D); and stiffness which, depending on the composition, flexibility, density and cross-link status of ECM components, can vary greatly between tissue types and healthy or malignant tissue [20–22].

Collagen I, the main component of ECM in interstitial tissues, determines the spatial organization and stability of connective tissues. Natural patterns of collagen topography include low-density zones consisting of thick and thin collagen bundles forming a porous meshwork of random or aligned organization, or high-density zones, composed of tightly arranged and often aligned collagen bundles with micron-scale pore size. While loose connective tissue is usually located adjacent to epithelial layers, including the dermis or gut submucosa, densely packed collagen bundles dominate the desmoplastic peritumoral stroma [17,20,23,24]. To recapitulate such heterogeneity of ECM topography and density, multi-scale approaches were developed to predict how moving cells integrate varying tissue organization by adjusting migration mode and efficacy [25].

Several types of adaptation were identified in moving cells in response to ECM heterogeneity. Contact guidance enables cells to take the path of least resistance when confronted with discontinuous environments, which supports preferential migration along ECM interfaces or aligned structures [17,25–30]. As a fundamental biomechanical determinant, deformation of the cell body and nucleus maintains individual cell movement through narrow pores of mechanically challenging environments [31,32]. To overcome tissue constraints, cell deformation is further complemented by a pericellular cleavage of ECM proteins through cell-derived MMPs,[4] which increases space for facilitated single-cell and collective cell migration [1,8,20,33–35]. In addition, pericellular functionalization of ECM is achieved by paracrine deposition of ECM components which may increase ligand density and stiffness and thereby modulate mechanocoupling during migration [2,21,25,36–38]. These cellular responses cooperate and support a repertoire of adaptation responses to cope with heterogeneous tissue organization during migration.

Depending on the experimental model, mesenchymal cells, including fibroblasts, neural crest cells, fibrosarcoma and melanoma cells, migrate either individually or as collective cell groups [1,7,12,18], however the environmental conditions enabling such diversity of migration mode are poorly understood. Using a systematic approach to modulate ECM density, stiffness and availability of MMP-dependent pericellular proteolysis, we here address how mechanical and molecular requirements govern the balance between single-cell and collective invasion of mesenchymal melanoma and fibrosarcoma cells. The data show mesenchymal patterning and migration mode as a function of matrix density and support proteolytic track clearance followed by cell jamming as key steps to collective mesenchymal migration in confining environments.

## 2. Material and methods

### 2.1. Cell culture

Human wild-type HT1080 fibrosarcoma (ACC315; DSMZ Braunschweig) [39] and human wild-type MV3 melanoma (provided by G. van Muijen, Dept. of Pathology, RadboudUMC Nijmegen, The Netherlands) [40] cells were cultured (37 °C at 5% $CO_2$ humidified atmosphere) in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) supplemented with 10% FCS (Sigma-Aldrich), penicillin (100 U/ml) and streptomycin (100 μg/ml; both PAA), L-glutamine (2 mM) and sodium pyruvate (1 mM; both Invitrogen). MMP function was inhibited by the broad-spectrum inhibitor GM6001 (ilomastat; EMD Millipore) at non-toxic concentration (20 μM) [13].

### 2.2. 3D spheroid culture

Cells from subconfluent culture were detached with EDTA (1 mM) and trypsin (0.075%; Invitrogen), and multicellular spheroids were generated using the hanging-drop method [41]. In brief, cells were suspended in medium supplemented with methylcellulose (20%; Sigma) and incubated as droplets (25 μl) containing 7000 (MV3) or 4000 (HT1080) cells for 24 h to ensure multicellular aggregation.

For 3D culture in collagen, spheroids were washed (PBS) and mixed with collagen solution consisting of non-pepsinized rat-tail collagen (BD Biosciences/Corning) at different concentrations (2.5 mg/ml to 8.0 mg/ml). Collagen–spheroid mixtures were either incorporated into a custom chamber or pipetted as a drop-matrix and polymerized at 37 °C [31]. To generate collagen lattices with both high ligand and porosity, collagen polymerization in a custom chamber was performed at low temperature (21 °C) which delayed polymerization and increased both fiber caliber and pore dimensions, as described [23,31]. (For reconstituting high-density matrices, collagen solution was concentrated to 12.0 mg/ml using a Speed Vac Concentrator (Savant) prior to reconstitution to a final concentration of 6.0 or 8.0 mg/ml).

Spheroid-containing collagen lattices were maintained at 37 °C for 24 h (HT1080 cells) or 48 h (MV3 cells).

### 2.3. Time-lapse microscopy and cell tracking

Emigration from 3D spheroid cultures in 3D fibrillar collagen was monitored at 37 °C using digital time-lapse, bright-field microscopy (20×/0.30 NA air objective; Leica) connected to CCD cameras (Sentech) and Vistek software for up to 72 h at 4 min frame interval.

Cell tracking and quantification of the migration index was performed manually, using tracking plugin, area selection- and measurement tools in Fiji/Image J software (v1.48) [42].

### 2.4. Confocal fluorescence microscopy and quantification of matrix porosity and 3D invasion

Spheroids in 3D collagen matrices were fixed (4% PB-buffered PFA), washed and stained using the following reagents: mouse anti-ALCAM mAb (AZN-L50; IgG2A; Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences (RIMLS), The Netherlands [43]); polyclonal rabbit anti-COL23/4C Ab (collagen I cleavage site) (Immunoglobe); secondary Alexa-Fluor-488-conjugated goat anti-rabbit or anti-mouse IgG (Invitrogen); Alexa-Fluor-488- or Alexa-Fluor-568-conjugated phalloidin (Invitrogen); DAPI (Roche). For COL23/4C staining, samples were pre-incubated with murine serum IgG (Sigma) to reduce non-specific background adsorption, followed by addition of primary antibody prior to fixation.

[3] Epithelial–mesenchymal transition.
[4] Matrix metalloproteinases.



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