Steady-state turnover is a hallmark of epithelial tissues throughout adult life. Intestinal epithelial turnover is marked by continuous cell migration, which is assumed to be driven by mitotic pressure from the crypts. However, the balance of forces in renewal remains ill-defined. Combining biophysical modeling and quantitative three-dimensional tissue imaging with genetic and physical manipulations, we revealed the existence of an actin-related protein 2/3 complex-dependent active migratory force, which explains quantitatively the profiles of cell speed, density, and tissue tension along the villi. Cells migrate collectively with minimal rearrangements while displaying dual-apicobasal and front-back-polarity characterized by actin-rich basal protrusions oriented in the direction of migration. We propose that active migration is a critical component of gut epithelial turnover.

In the early stages of metastasis, cancer cells exit the primary tumor and enter the vasculature. Although most studies have focused on the tumor invasive front, cancer cells from the tumor core can also potentially metastasize. To address cell motility in the tumor core, we imaged tumor explants from spontaneously forming tumors in mice in real time using long-term two-photon microscopy. Cancer cells in the tumor core are remarkably dynamic and exhibit correlated migration patterns, giving rise to local ‘currents’ and large-scale tissue dynamics. Although cells exhibit stop-and-start migration with intermittent pauses, pausing does not appear to be required during division. Use of pharmacological inhibitors indicates that migration patterns in tumors are actively driven by the actin cytoskeleton. Under these conditions, we also observed a relationship between migration speed and correlation length, suggesting that cells in tumors are near a jamming transition. Our study provides new insight into the dynamics of cancer cells in the tumor core, opening new avenues of research in understanding the migratory properties of cancer cells and later metastasis. This article has an associated First Person interview with the first author of the paper.
Année de publication : 2018

Jorge Barbazán, Danijela Matic Vignjevic (2018 Oct 12)

**Cancer associated fibroblasts: is the force the path to the dark side?**

*Current opinion in cell biology*: 71-79 : [DOI: 10.1093/coibiober/ber078](https://doi.org/10.1093/coibiober/ber078)

**Résumé**

The most abundant cell type in the tumor microenvironment are cancer-associated fibroblasts (CAFs). CAFs play an important role in tumor growth and progression. Besides direct communication with cancer cells via secreted molecules or cell-cell adhesions, CAFs also indirectly affect cancer cell behavior by remodeling the extracellular matrix (ECM). Here, we summarize recent findings on the distinct mechanisms that CAFs use to modify ECM, specifically, their proteolytic versus force-dependent activity. We then review the consequences of CAF force transmission on the physico-chemical properties of the matrix, focusing on the deposition of new matrix components, and the alteration of the organization and stiffness of the ECM. CAFs promote tumor invasion by creating the roads cancer cells use to escape the tumor mass. However, there is also evidence that CAFs can prevent invasion, possibly by forming a physical barrier around the tumor edge. We discuss the controversial role of CAFs in tumor progression.


**A new biomimetic assay reveals the temporal role of matrix stiffening in cancer cell invasion.**

*Molecular biology of the cell*: 2979-2988 : [DOI: 10.1091/mbc.E18-01-0068](https://doi.org/10.1091/mbc.E18-01-0068)

**Résumé**

Tumor initiation and growth is associated with significant changes in the surrounding tissue. During carcinoma progression, a global stiffening of the extracellular matrix is observed and is interpreted as a signature of aggressive invasive tumors. However, it is still unknown whether this increase in matrix rigidity promotes invasion and whether this effect is constant along the course of invasion. Here we have developed a biomimetic in vitro assay that enabled us to address the question of the importance of tissue rigidity in the chronology of tumor invasion. Using low concentrations of the sugar threose, we can effectively stiffen reconstituted collagen I matrices and control the stiffening in time with no direct effect on residing cells. Our findings demonstrate that, depending on the timing of its stiffening, the extracellular matrix could either inhibit or promote cancer cell invasion and subsequent metastasis: while matrix stiffening after the onset of invasion promotes cancer cell migration and tumor spreading, stiff matrices encapsulate the tumor at an early stage and prevent cancer cell invasion. Our study suggests that adding a temporal dimension in in vitro models to analyze biological processes in four dimensions is necessary to fully capture their complexity.
Frustrated endocytosis controls contractility-independent mechanotransduction at clathrin-coated structures.

**Résumé**

It is generally assumed that cells interrogate the mechanical properties of their environment by pushing and pulling on the extracellular matrix (ECM). For instance, acto-myosin-dependent contraction forces exerted at focal adhesions (FAs) allow the cell to actively probe substrate elasticity. Here, we report that a subset of long-lived and flat clathrin-coated structures (CCSs), also termed plaques, are contractility-independent mechanosensitive signaling platforms. We observed that plaques assemble in response to increasing substrate rigidity and that this is independent of FAs, actin and myosin-II activity. We show that plaque assembly depends on αvβ5 integrin, and is a consequence of frustrated endocytosis whereby αvβ5 tightly engaged with the stiff substrate locally stalls CCS dynamics. We also report that plaques serve as platforms for receptor-dependent signaling and are required for increased Erk activation and cell proliferation on stiff environments. We conclude that CCSs are mechanotransduction structures that sense substrate rigidity independently of cell contractility.

**Résumé**

Chemotaxis is an important biological process involved in the development of multicellular organisms, immune response and cancer metastasis. In order to better understand how cells follow chemical cues in their native environments, we recently developed a microfluidics-based chemotaxis device that allows for observation of cells or cell aggregates in 3D networks in response to tunable chemical gradients (Aizel et al., 2017). Here, we describe the methods required for fabrication of this device as well as its use for live imaging experiments and subsequent analysis of imaging data. This device can be adapted to study a number of different cell arrangements and chemical gradients, opening new avenues of research in 3D chemotaxis.
Cell Migration in Tissues: Explant Culture and Live Imaging.

**Résumé**

Cell migration is a process that ensures correct cell localization and function in development and homeostasis. In disease such as cancer, cells acquire an upregulated migratory capacity that leads to their dissemination throughout the body. Live imaging of cell migration allows for better understanding of cell behaviors in development, adult tissue homeostasis and disease. We have optimized live imaging procedures to track cell migration in adult murine tissue explants derived from: (1) healthy gut; (2) primary intestinal carcinoma; and (3) the liver, a common metastatic site. To track epithelial cell migration in the gut, we generated an inducible fluorescent reporter mouse, enabling us to visualize and track individual cells in unperturbed gut epithelium. To image intratumoral cancer cells, we use a spontaneous intestinal cancer model based on the activation of Notch1 and deletion of p53 in the mouse intestinal epithelium, which gives rise to aggressive carcinoma. Interaction of cancer cells with a metastatic niche, the mouse liver, is addressed using a liver colonization model. In summary, we describe a method for long-term 3D imaging of tissue explants by two-photon excitation microscopy. Explant culturing and imaging can help understand dynamic behavior of cells in homeostasis and disease, and would be applicable to various tissues.

Alexandros Glentis, Philipp Oertle, Pascale Mariani, Aleksandra Chikina, Fatima El Marjou, Youmna Attieh, Francois Zaccarini, Marick Lae, Damarys Loew, Florent Dingli, Philemon Sirven, Marie Schoumacher, Basile Gurchenkov, Marija Plodinec, Danijela Matic Vignjevic (2018 Mar 9)

**Author Correction:** Cancer-associated fibroblasts induce metalloprotease-independent cancer cell invasion of the basement membrane.
*Nature communications* : 1036 : [DOI : 10.1038/s41467-018-03304-x]

**Résumé**

In the original version of this Article, financial support and contributions in manuscript preparation were not fully acknowledged. The PDF and HTML versions of the Article have now been corrected to include the following:"M.P. and P.O. would like to thank Prof. Roderick Y.H. Lim for advice during manuscript preparation and for providing the laboratory and microscopy infrastructure.... [We also thank] the NanoteraProject, awarded to the PATLiSciill Consortium (M.P and P.O)...’.

Année de publication : 2017

Grégory Beaune, Guillaume Duclos, Nada Khalifat, Tomita Vasilica Stirbat, Danijela Matic Vignjevic, Françoise Brochard-Wyart (2017 Nov 2)

**Reentrant wetting transition in the spreading of cellular aggregates.**
Résumé

We study spreading on soft substrates of cellular aggregates using CT26 cells that produce an extracellular matrix (ECM). Compared to our previous work on the spreading of S180 cellular aggregates, which did not secrete ECMs, we found that the spreading velocity of the precursor film is also maximal for intermediate rigidities, but new striking features show up. First, we observed a cascade of liquid-gas-liquid (L/G/L) transitions of the precursor film as the substrate rigidity is decreased. We attribute the L/G transition to a decrease of cell/cell adhesion resulting from the weakening of the cell/substrate adhesion. We attribute the reentrant liquid phase (G/L) observed on soft substrates to the slow spreading of the aggregates on ultra-soft substrates, which gives time to the cells to secrete more ECM proteins and stick together. Second, a nematic order appears in the cohesive (liquid) states of the precursor film, attributed to the gradient of cell’s velocities.

Alexandros Glentis, Philipp Oertle, Pascale Mariani, Aleksandra Chikina, Fatima El Marjou, Youmna Attieh, Francois Zaccarini, Marick Lae, Damarys Loew, Florent Dingli, Philemon Sirven, Marie Schoumacher, Basile Gurchenkov, Marija Plodinec, Danijela Matic Vignjevic (2017 Oct 15)
Cancer-associated fibroblasts induce metalloprotease-independent cancer cell invasion of the basement membrane.
Nature communications : 924 : DOI : 10.1038/s41467-017-00985-8

Résumé

At the stage of carcinoma in situ, the basement membrane (BM) segregates tumor cells from the stroma. This barrier must be breached to allow dissemination of the tumor cells to adjacent tissues. Cancer cells can perforate the BM using proteolysis; however, whether stromal cells play a role in this process remains unknown. Here we show that an abundant stromal cell population, cancer-associated fibroblasts (CAFs), promote cancer cell invasion through the BM. CAFs facilitate the breaching of the BM in a matrix metalloproteinase-independent manner. Instead, CAFs pull, stretch, and soften the BM leading to the formation of gaps through which cancer cells can migrate. By exerting contractile forces, CAFs alter the organization and the physical properties of the BM, making it permissive for cancer cell invasion. Blocking the ability of stromal cells to exert mechanical forces on the BM could therefore represent a new therapeutic strategy against aggressive tumors. Stromal cells play various roles in tumor establishment and metastasis. Here the authors, using an ex-vivo model, show that cancer-associated fibroblasts facilitate colon cancer cells invasion in a matrix metalloproteinase-independent manner, likely by pulling and stretching the basement membrane to form gaps.

Koceila Aizel, Andrew G Clark, Anthony Simon, Sara Geraldo, Anette Funfak, Pablo Vargas, Jérôme Bibette, Danijela Matic Vignjevic, Nicolas Bremond (2017 Oct 13)
A tuneable microfluidic system for long duration chemotaxis experiments in a
Résumé

In many cell types, migration can be oriented towards a chemical stimulus. In mammals, for example, embryonic cells migrate to follow developmental cues, immune cells migrate toward sites of inflammation, and cancer cells migrate away from the primary tumour and toward blood vessels during metastasis. Understanding how cells migrate in 3D environments in response to chemical cues is thus crucial to understanding directed migration in normal and disease states. To date, chemotaxis in mammalian cells has been primarily studied using 2D migration models. However, it is becoming increasingly clear that the mechanisms by which cells migrate in 2D and 3D environments dramatically differ, and cells in their native environments are confronted with a complex chemical milieu. To address these issues, we developed a microfluidic device to monitor the behaviour of cells embedded in a 3D collagen matrix in the presence of complex concentration fields of chemoattractants. This tuneable microsystem enables the generation of (1) homogeneous, stationary gradients set by a purely diffusive mechanism, or (2) spatially evolving, stationary gradients, set by a convection-diffusion mechanism. The device allows for stable gradients over several days and is large enough to study the behaviour of large cell aggregates. We observe that primary mature dendritic cells respond uniformly to homogeneous diffusion gradients, while cell behaviour is highly position-dependent in spatially variable convection-diffusion gradients. In addition, we demonstrate a directed response of cancer cells migrating away from tumour-like aggregates in the presence of soluble chemokine gradients. Together, this microfluidic device is a powerful system to observe the response of different cells and aggregates to tuneable chemical gradients.

Youmna Attieh, Andrew G Clark, Carina Grass, Sophie Richon, Marc Pocard, Pascale Mariani, Nadia Elkhatab, Timo Betz, Basile Gurchenkov, Danijela Matic Vignjevic (2017 Sep 22) Cancer-associated fibroblasts lead tumor invasion through integrin-β3-dependent fibronectin assembly. The Journal of cell biology: DOI: jcb.201702033

Résumé

Cancer-associated fibroblasts (CAFs) are the most abundant cells of the tumor stroma. Their capacity to contract the matrix and induce invasion of cancer cells has been well documented. However, it is not clear whether CAFs remodel the matrix by other means, such as degradation, matrix deposition, or stiffening. We now show that CAFs assemble fibronectin (FN) and trigger invasion mainly via integrin-αvβ3. In the absence of FN, contractility of the matrix by CAFs is preserved, but their ability to induce invasion is abrogated. When degradation is impaired, CAFs retain the capacity to induce invasion in an FN-dependent manner. The level of expression of integrins αv and β3 and the amount of assembled FN are directly proportional to the invasion induced by fibroblast populations. Our results highlight FN assembly and integrin-αvβ3 expression as new hallmarks of CAFs that promote tumor invasion.


**Microfluidic-Based Generation of 3D Collagen Spheres to Investigate Multicellular Spheroid Invasion.**

Methods in molecular biology (Clifton, N.J.) : 269-279 : DOI : 10.1007/978-1-4939-7021-6_20

**Résumé**

During tumor progression, cancer cells acquire the ability to escape the primary tumor and invade adjacent tissues. They migrate through the stroma to reach blood or lymphatics vessels that will allow them to disseminate throughout the body and form metastasis at distant organs. To assay invasion capacity of cells in vitro, multicellular spheroids of cancer cells, mimicking primary tumor, are commonly embedded in collagen I extracellular matrix, which mimics the stroma. However, due to their higher density, spheroids tend to sink at the bottom of the collagen droplets, resulting in the spreading of the cells on two dimensions. We developed an innovative method based on droplet microfluidics to embed and control the position of multicellular spheroids inside spherical droplets of collagen. In this method cancer cells are exposed to a uniform three-dimensional (3D) collagen environment resulting in 3D cell invasion.

**Liver metastasis is facilitated by the adherence of circulating tumor cells to vascular fibronectin deposits.**


**Résumé**

The interaction between circulating tumor cells (CTC) and endothelial cells during extravasation is a critical process during metastatic colonization, but its mechanisms remain poorly characterized. Here we report that the luminal side of liver blood vessels contains fibronectin deposits that are enriched in mice bearing primary tumors and are also present in vessels from human livers affected with metastases. Cancer cells attached to endothelial fibronectin deposits via talin1, a major component of focal adhesions. Talin1 depletion impaired cancer cell adhesion to the endothelium and transendothelial migration, resulting in reduced liver metastasis formation in vivo. Talin1 expression levels in patient CTC’s correlated with prognosis and therapy response. Together, our findings uncover a new mechanism for liver metastasis formation involving an active contribution of hepatic vascular fibronectin and talin1 in cancer cells.
Année de publication : 2016

Youmna Attieh, Danijela Matic Vignjevic (2016 Aug 31)

The hallmarks of CAFs in cancer invasion.

Résumé

The ability of cancer cells to move out of the primary tumor and disseminate through the circulation to form metastases is one of the main contributors to poor patient outcome. The tumor microenvironment provides a niche that supports cancer cell invasion and proliferation. Carcinoma-associated fibroblasts (CAFs) are a highly enriched cell population in the tumor microenvironment that plays an important role in cancer invasion. However, it remains unclear whether CAFs directly stimulate cancer cell invasion or they remodel the extracellular matrix to make it more permissive for invasion. Here we discuss paracrine communication between cancer cells and CAFs that promotes tumor invasion but also stimulates CAFs to remodel the matrix increasing cancer dissemination.