Rigid tumors contain soft cancer cells

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Article

Keywords: rigid tumors, soft cells, soft motile cells

Posted Date: December 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1114106/v1>

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Abstract

Palpation, as already mentioned in the ancient Egyptian medical text Ebers Papyrus, utilizes that solid tumors are stiffer than the surrounding tissue. However, cancer cell lines tend to soften, which may intuitively foster invasion by enhancing the ability of cancer cells to squeeze through dense tissue. This paradox raises questions besides the oxymoron itself: Does softness emerge from adaptation to the external microenvironment? Or are soft cells already present inside a rigid primary tumor mass to support cancer cell unjamming? We investigate primary tumor explants from patients with breast and cervix carcinomas on multiple length scales from the tissue level down to single cells. We find that primary tumors are highly heterogeneous in their mechanical properties. From the tissue level this heterogeneity persists down to the scale of individual cells in cancer cell clusters, resulting in a broad distribution of cell rigidities with a higher fraction of softer, more squeezable cells. Plus, squeezed cell shapes correlate with cancer cell motility. Mechanical modelling based on patient data reveals that a tumor mass as a whole is able to maintain a rigid, solid behavior even when it contains a significant fraction of very soft cells. Cell softening induced cancer cell unjamming generates heterogeneous cancer cell clusters with a solid backbone of rigid cells surrounded by soft motile cells.

Main Text

Rigidity and softness are both advantageous for tumor progression

Since the early days of tumor biology, it has been recognized that cancer cells undergo dedifferentiation towards a more disordered and thus softer cytoskeleton than healthy cells.¹ While studies suggest that soft cells may already exist inside a primary tumor, the evidence is only circumstantial.^{2–5} Several measurements have been performed on cell lines, but these are mechanically and physiologically distinct from clinical samples. In carcinomas, cell softening is associated with down-regulation of keratin after the cancer cells have partially or completely transformed through the EMT,^{6,7} resulting in cells that migrate more efficiently through dense environments,⁸ until nuclear jamming becomes the limiting factor, and the soft cells are stuck as well.^{9,10} Other measurements on clinical samples investigate already metastatic cells extracted from extracellular fluids, such as pleural effusions that contain soft cells and correlate with clinical pathology.¹¹ Similarly, cytobrushes of the mouth indicate cancer cell softening in oral cancer.¹² In contrast, circulating breast cancer cells are slightly stiffer than the surrounding white blood cells.¹³ Fine needle aspirations of breast tumors show that a solid tumor contains well-defined soft regions,^{14,15} although it remains unclear if soft areas are composed of cancer cells or extracellular matrix.

On a larger, collective cell, scale our recent research indicates that breast and cervix tumors may contain areas of solid cell clusters of jammed cancer cells and fluid areas of unjammed, motile cancer cells.¹⁶ Moreover, despite that cell proliferation is only possible when the cell cluster is resistant enough to divide against a typically firm surrounding tumor stroma,^{17,18} cell proliferation fluidizes tissues.^{19,20} Our

measurements of tumor mechanical heterogeneity, moreover, provide a deeper understanding about when the unordered cancer cell mass behaves more solid-like or fluid-like, as we have already found in different regions of carcinomas.¹⁶ Tumor cell spheroids can spread like a fluid droplet²¹, and yet their shapes and sorting behaviors are not solely governed by surface tension as expected for fluids²² indicating that cell jamming plays a central role. Many cell aggregates exhibit features of glassiness or jamming, suggesting that they are near a solid-fluid transition.^{23,24} Because fluid-like and solid-like tissues have different mechanisms for proliferation,²⁵ migration, self-organization, and cohesion, these uncertainties prevent us from fully understanding the mechanical barriers to metastasis and invasion.

On the other hand, a breast tumor is undoubtedly a rigid mass. Neoplastic tissue such as in carcinoma, which is composed of cancer cell clusters surrounded by enhanced, stiff stroma, appears as a rigid mass²⁶ with respect to the healthy surrounding tissue. The tumor stimulates fibrosis and the enhanced extra cellular matrix (ECM) may be the origin of tumor stiffness. Pathologists use excessive ECM deposition, rigidifying the stroma, as a marker for poor prognosis, since it is a strong tumor promoter.²⁷ Tumor progression seems to require rigid and soft properties of tumor tissue as well as cancer cells. This paradox remains unresolved as discussed in a recent Nature Outlook article.¹⁸

All this illustrates that cancer cells are highly mechanically adaptive. Mechanical changes in cancer cells may be induced by the microenvironment directly through mechanosensitive responses of the cytoskeleton or through expression changes by cellular mechanotransduction. It may cause cell stiffening after the cancer cell has left the tumor cell mass into the stiff ECM, as well as fluid cancer cell clusters may induce cancer cell softening. The question remains if these mechanical changes already exist in the cancer clusters of a tumor mass or rather occur only when they leave these cell aggregates into the stroma.

Multiscale tumor mechanics

We perform multiscale mechanical measurements with live tumor explants starting at the centimeter scale - the range where palpation works - down to individual cells embedded in their microenvironment. We also study statistically significant large numbers of isolated single cells unstimulated by their surroundings. This study analyses carcinomas of breast cancer and cervical cancer patients as two typical solid tumor types. Magnetic resonance elastography (MRE) and atomic force microscopy (AFM) measurements, as well as collective cancer cell tracking are performed within hours after tissue resection on vital human tumor explants. By dissolving another piece of the tumor explant into vital single suspended cells individual cell properties are examined with reasonably high throughput to measure a large, statistically relevant number of cells to obtain the broad distributions of the cells' biomechanical behavior. A device for this task is the optical stretcher (OS).⁴ An inherent limitation of these single cell measurements is that they have to be conducted *in vitro*, i.e. under non-physiological conditions. The OS probes the mechanics of single cells in suspension, which means that no interactions with the microenvironment stimulate mechanosensitive cellular responses. Consequently, the cells are probed in

their mechanical “ground state”. We then use these quantitative patient data to infer the mechanical stability of the tumor mass, using a mechanical model based on cancer cell unjamming.

Carcinomas are not necessarily stiffer than the surrounding tissue matrix

Starting on the bulk tissue level, we performed tabletop MRE^{28–30} on centimeter sized vital tumor explants, from resected tissue obtained from cervical and mammary carcinomas to quantify the macroscopic average viscoelastic properties of the tumor (**Figure 1**). Soft tissues and cells often exhibit a distinctive power-law viscoelastic response arising from a broad distribution of time-scales present in their complex internal structure.³¹ A promising approach to accurately describe the rheological behavior of these materials is complex shear modulus from the fractional Maxwell model (see **Extended Data**). By fitting our data to this model, we extract two parameters, representing stiffness/elastic resistance (μ) and its power law exponent (α) that serves as a measure for fluidity/dissipation. Cells, as well as tissues, are highly complex compound materials. No analytic constitutive model derived from first principles of soft matter physics exists to describe their multiscale mechanical behavior. Thus, as recently investigated by a consortium of scientists published in Nature Methods, the model dependent values of mechanical constants measured with diverse techniques on different scales cannot be aligned for a quantitative comparison.³² Nevertheless, the measured mechanical behaviors can be compared and correlated.

As expected from palpation, the breast tumor explants ($n = 5$) with an median stiffness, i.e. mechanical resistance, $\mu = (2.9 \pm 1.9) \cdot 10^5$ Pa are clearly ($p = 0.021$ KS-Test) stiffer than healthy breast tissue $\mu = (163 \pm 77) \cdot \text{Pa}$ ($n = 3$), while the fluidity α was similar for both samples with 0.576 ± 0.061 and 0.615 ± 0.042 , respectively (**Figure 1b**). So the MRE data confirm the medical practice that breast cancer can be identified by palpation. In the tumor explants fibrotic stroma may contribute to increase the stiffness, while healthy epithelial breast tissue is surrounded by connective tissue and very soft fat tissue, which may dominate the averaged bulk stiffness measured by MRE.

In contrast, for the cervix samples, tumors $(4.4 \pm 1.4) \cdot 10^4$ Pa are not significantly ($p = 0.53$ KS-Test) stiffer than healthy tissue $(1.4 \pm 1.3) \cdot 10^5$ Pa and also have similar fluidity 0.4882 ± 0.0083 vs 0.538 ± 0.069 (both $n = 4$). Cervical epithelium is primarily surrounded by rigid connective tissue and smooth muscle cells. For effective cancer cell proliferation, it is sufficient if the mechanical resistance of the tumor mass just matches or slightly exceeds the rigidity of the surrounding microenvironment.¹⁷ Moreover, cervical tissue is highly active showing functional and structural changes, e.g. during the menstrual circle, which is also reflected in variably altered viscoelastic properties.³³ This demonstrates that carcinomas do not have to be drastically stiffer than the healthy surrounding tissue.³⁴

Cell contribution to the mechanical heterogeneity of carcinoma

We proceeded to measure high resolution elasticity maps of the same live tumor explants with AFM, to capture the local heterogeneous distribution of tumor stiffness with cellular resolution. The deformation curves have been evaluated using a standard Hertz model. By using simultaneous fluorescence

microscopy of DNA-stained cell nuclei, we distinguished tumor areas with cancer cell clusters from the surrounding ECM in our maps. We chose a large measurement area up to $1 \times 1 \text{ mm}^2$ combined with $10 \text{ }\mu\text{m}$ pixel spacing. At this resolution we see an extremely wide distribution in local stiffness with a long tail typical for log-normal distributions (**Figure 1e**), also seen in single cell AFM measurements.^{35,36} For breast cancer we observe a stiffening in the median Young's Modulus from $E_{\text{BN}} = 132 \text{ Pa}$ ($n=16$) to $E_{\text{BC}} = 288 \text{ Pa}$ ($n=13$) moving from healthy to cancerous tissue. In the cervix we see a drop in median stiffness from $E_{\text{CN}} = 570 \text{ Pa}$ ($n=5$) for healthy cervix to $E_{\text{CC}} = 385 \text{ Pa}$ ($n=7$) in cervix cancer. This integrates over the differences between stroma and cancer cell clusters. Yet even within the cancer cell clusters, we observe stiffer regions surrounded by softer areas, both spanning several $100 \text{ }\mu\text{m}$ (**Figure 1c**). It appears that the rigid cancer cells may form a collective scaffold throughout the tumor mass surrounded by soft cancer cells. The situation can be best described as islands or backbone of rigid cells surrounded by a sea of soft cells. Since we have only sections of the tumor it remains unclear whether the rigid regions are percolated or remain islands. The log-normal distribution suggests the existence of very soft cancer cells. The rigid backbone permits that not solely the fibrotic stroma contributes to the rigidity of a solid tumor.

Solid tumors contain cancer cells that are softer than healthy cells

As already mentioned the cytoskeleton of cancer cells is highly adaptable to its microenvironment by direct immediate mechanosensitive cytoskeletal responses and by cellular mechanotransduction changing expression³⁷. This raises the question whether the observed mechanical heterogeneity in cancer cells is caused by the self-regulatory abilities of the cytoskeleton, or by changes in protein expression, e.g. by dedifferentiation. Are these mechanical changes quickly reversible, e.g. via regulation of the cytoskeleton, or much longer lived, e.g. due to changes in expression? To capture these mechanics of individual cells and to determine which changes are long-lived, the bulk samples were dissolved. Suspended cells lose all stimuli from their microenvironment and enter an unperturbed ground state, so that changes in the mechanical behavior must be due to expression changes. This ground state was characterized in step stress, i.e. creep, experiments with the OS³⁸ (see **Figure 2** and **Methods**). The preparation of cells for the optical stretcher suppressed fat cells and fibroblasts. This study analyses carcinomas of 13 breast cancer and 4 cervical cancer patients. For cervical tumors, neighboring normal epithelial tissue was used as a reference. For breast cancer, benign lesions known as fibroadenomas (FA) obtained by fine needle aspirations and primary human mammary epithelia cells (HMEpC) from breast reductions (PromoCell, Heidelberg, Germany) served as reference. All samples were in culture for a short time (3-17 days), we find that primary cells soften with increasing time on rigid cell culture dishes. For the breast cancer samples, we find an average increase of $(33 \pm 12) \%$ in the relative deformation from first to second passage (see **Extended Data Figure 1**). As primary cells are mechanosensitive,^{39,40} their adaptability to the microenvironment must be considered.⁴⁰ Therefore, all data was obtained from cells that have not been passaged, except HMEpC cells, as they were only provided in second passage. Since cell culture leads to cell softening, the observed differences between HMEpC controls and tumor samples underestimate the relative softening of cancer cells.

Since, as already mentioned, there is no fundamental model that can be applied across scales to all our biomechanical measurement techniques, we used simply the relative cell deformation at the end of the optical stretch as a measure of the cells' mechanical resistance. Single cell deformation measurements in the optical stretcher reveal that cells isolated from solid tumors show a propensity to more deformable cells than their healthy counterparts (**Figure 2, Extended Data Figure 3**). The distributions also follow a log-normal distribution. The log normal distributions for solid tumors display a pronounced shift towards soft cells. From the cumulated distribution of 13 breast cancer samples ($n = 6526$), compared to 2 fibroadenoma samples ($n = 186$) and one HMEpC sample ($n = 358$) we find that the breast cancer distribution contains softer cells leading to a median relative deformation of $MD_{BC} = 0.024$. The benign lesions, the fibroadenomas, have a median relative deformation of $MD_{FA} = 0.015$, and the healthy HMEpC cells from breast reductions have a median relative deformation of $MD_{HMEpC} = 0.018$. The significance of these differences was tested with pairwise Mann-Whitney-tests of the raw data, and t-tests on the logarithm of the data, both tests confirmed that the differences between the cancer cells and the controls are significant with a $p = 0.001$ level, for both tests. The width of the distribution measured as the difference between 1st and 3rd quartile is larger for the cancer cells, $width_{BC} = 0.016$, $width_{FA} = 0.009$, and $width_{HMEpC} = 0.012$, which demonstrates the broad mechanical heterogeneity for breast cancer cells, even in their unstimulated ground state.

For the cervix carcinomas, we find an even more pronounced softening effect of the primary cancer cells ($MD_{CC} = 0.020$) with respect to the median deformation of cells from the surrounding primary healthy tissue ($MD_{CN} = 0.011$). This difference is significant at the $p = 0.001$ level. The distribution widened for the cancer cells from the $width_{CN} = 0.010$ for cells from healthy tissue to the $width_{CC} = 0.013$ as a measure of increasing mechanical heterogeneity for unstimulated cancer cells in the ground state from cervix carcinoma (**Figure 2, Extended Data Figure 4**).

An increase in soft cancer cells with respect to healthy cells – visible by the shift of the distribution to higher relative deformations – characterizes both breast and cervical tumors. Thereby cell softening is more pronounced in cervical tumors compared to mamma carcinoma (see **Extended Data Table 1**). Cells from tumors are more heterogeneous, i.e. display a broad log-normal distribution, with a large fraction of cells that are just as stiff as those in normal tissues. The stiffening of breast tissue felt by palpation, can be also attributed to the different composition of healthy and cancerous breast tissue, as soft fat cells get replaced by large volumes of stiffer cancer cells. Yet also soft cancer cells emerge that distinguishes pathological tissue from healthy epithelial tissue.

Cancer cell unjamming modulates stiffness of cell clusters

In vital patient tumor explants for breast and cervix tumors we have found that cellular regions of a tumor contain unjammed as well as jammed regions. To better understand how such regions affect global tumor behavior, we use cell aggregates as a model system to carefully quantify features of jammed and unjammed tissues, as cell spheroids permit us a detailed look at the mechanics of cell aggregates with a

defined population of cells. We have recently shown that MDA-MB-436 spheroids, a model for cancer cells, consist of unjammed cells that can move within the cell cluster, while MCF-10A spheroids, a model for epithelial cells, consist of jammed, non-moving, cells.¹⁶ Spheroid-fusion experiments demonstrate that the spheroids with motile cells behave like a fluid and the jammed cell clusters have the properties of an amorphous solid.¹⁶ This suggested that tissue fluidity, as an emergent collective cell behavior, is a key modulator of tissue stiffness. As a complement to those previous studies, we investigate the changes of mechanical properties between single cells and cell aggregates using AFM. We grew multicellular spheroids from 10000 cells and performed force-indentation experiments with an AFM. Spheroids grown for 24 h from MCF-10A have an elastic modulus of $88(\pm 63)$ Pa compared to $135(\pm 38)$ Pa for the single cells. MDA-MB-436 spheroids dropped from a single cell elastic modulus of $570(\pm 300)$ Pa to $111(\pm 72)$ Pa for the spheroid (**Figure 3 a,d**). The motile cells in the MDA-MB-436 spheroids oppose much less external loads compared to individual cells, much less than the jammed MCF-10A cells. The MCF-10A spheroids also lose some of their individual strength but only 36 % compared to the 80 % of the MDA-MB-436s. This illustrates that it is more tissue fluidity than individual cell stiffness that directly impacts the mechanical stability of cell clusters. Individual cell stiffness may be more a determinant of tissue fluidity. Already the two cell lines show broad log-normal distributions for their cell stiffness. However, the primary tissue samples are even more heterogeneous. Our AFM-based cell elasticity maps of cervix and breast carcinoma display rigid and soft regions, which suggests that the soft areas are unjammed and that the rigid backbone is jammed. Computer simulations help to understand the function of soft cancer cells and mechanical heterogeneity in the tumor mass.

Simulations of biomechanical heterogeneity in tumor progression

To assess the influence of cellular mechanical heterogeneity on tissue mechanics, we use a theoretical vertex-based model, which simulates cell aggregates with no free space in between the cells, and incorporated the measured stiffness distribution from patient samples (see **Figure 4**).⁴¹ These models have been previously used to describe the cell unjamming transition^{42–44} in cell aggregates with a homogenous mechanical stiffness and has provided reliable predictions for cell lines in cell monolayers. For a mechanically homogeneous tissue, the collective rigidity of the tissue is controlled by the cell shape index P_0 . P_0 is a single cell mechanical measure that describes the interaction between cellular cortical tension (i.e. active cellular stiffness) and cell adhesion, which are strongly co-regulated, that governs cell shape. In cells types where this interaction leads to large interfacial tension between cells, cells tend towards a non-squeezable round shape, whereas when the interfacial tension is low, cells tend towards a squeezable, elongated shape.^{16,45,46} Thus, round, stiff cells with a critical shape parameter smaller than P_0 collectively assume the state of an amorphous, jammed solid, while elongated, soft cells with larger P_0 are motile in a cooperative fluid, unjammed state.

A solid backbone of rigid cancer cells surrounded by motile, soft cancer cells

How is it possible that a tumor can remain solid despite the prevalence of soft cells within its bulk? Can the simulations demonstrate how the measured broad log-normal stiffness distribution of cancer cells

results in the observed stiff and soft cellular regions in carcinoma? To assess the influence of cellular heterogeneity on tissue mechanics, we have incorporated in our theoretical vertex-based model⁴¹ the measured stiffness profiles from our patient samples. Since carcinoma show a mixed epithelial and mesenchymal phenotype⁷ and different cadherins can bind to each other,⁴⁷ we assume that differences in P_0 between the cancer cells are predominately caused by changes in the cancer cell's cortical tension measured by the optical stretcher. Thus, in our theoretical model the shape parameter P_0 can be used to encode for the measured stiffness of the cancer cells and we have recently shown that the elastic modulus of a single cell is linearly proportional to P_0 for small deformations. The patient-derived log-normal distributions of measured deformability profiles (**Figure 2 b, d**) are directly mapped onto a corresponding log-normal distribution of P_0 values in our vertex model simulation. In our vertex model, the mechanical bulk property of the tissue can be determined by computing the shear modulus G .⁴⁸ In terms of mechanical stability, $G > 0$ in a fully jammed, solid tissue but vanishes in a completely unjammed, fluid one. We computed G as a function of the mean and standard deviation of P_0 and found three distinct mechanical phases (**Figure 4a**): a fully unjammed (fluid) phase where the shear modulus of the tissue remained zero, a partially jammed, heterogeneous phase where tension percolation gives a finite bulk stiffness and a fully jammed solid phase. Representative simulation snapshots are shown in **Figure 4b**. Stiff cancer cells ($P_0 < 3.812$) and soft cells ($P_0 > 3.812$) are shown in light blue and dark blue, respectively. The stiff cells form the jammed regions, while the soft cells are responsible for fluid regions. In the heterogeneous solid phase, the tension network self-organizes into a percolated (tissue-spanning) structure yet the rigid, jammed cells do not percolate. This means that already a small fraction of jammed islands in a fluid sea are sufficient to cause a finite shear modulus as found in solid materials. In the fully solid phase, both the tension and the contacts between the jammed cells form percolating networks throughout the bulk. The tension network in the heterogeneous solid phase, which is also a measure of local cell stiffness, is highly reminiscent of the spatial maps of Young's moduli obtained by AFM (**Figure 1**). The drastic effect of heterogeneity becomes even more evident by computing the fraction of rigid cells in the tissue, f_r . The pure fluid phase only exists for $f_r < 0.24$. The heterogeneous solid already perseveres for $0.24 < f_r < 0.48$, and the fully solid phase for $f_r > 0.48$. As shown in **Figure 4c**, in a plot of the shear modulus as a function of f_r at semilog scale, the fluid-solid rigidity transition already occurred at $f_r = 0.24$. Moreover, we find that the tumor heterogeneity, i.e. the width of the log-normal stiffness distribution, described by the variance σ_{P_0} fosters rigidifying of a tumor, which can be seen by the positive slope of the phase boundaries between heterogeneous solid and fluid (thick black line in the phase diagram of **Figure 4a**). Counterintuitively, cancer cells can rigidify a tissue by only increasing the heterogeneity as observed in our optical stretcher measurements of individual cancer cell stiffness. We can now position our patient-derived stiffness data within the phase diagram by calculating the fraction of rigid cells f_r based on the measured deformation distributions. Only the relative value of P_0 with respect to the cell stiffness of healthy cells can be inferred from the experimental data, but not the actual value of P_0 for each cell. For the breast and the cervix, the mean deformation value of the non-cancer cells (**Figure 2**) is used to define the single cell rigidity threshold to divide into rigid and soft cell for both the non-cancer and cancer phenotypes. Then, f_r is calculated by computing the fraction of cells that deformation values are smaller

than the mean deformation value of their non-cancerous counterpart. This permits to categorize the breast and cervix tumor samples with respect to the solid-fluid nexus as predicted by the vertex model. The relative positions of the tissues from the patient samples are shown in **Figure 4d**. We find that breast and cervix cancer samples are located within the heterogeneous solid phase while healthy cells from breast reductions, cell from healthy cervix tissue and benign fibroadenomas are in the solid, jammed phase.

The spatial organization of cancer cells with soft and rigid regions within a tumor causes the counterintuitive result that many soft cells can exist within the solid mass without destroying its mechanical stability as a solid that resists the microenvironment. Even for mixtures with less than 50 % stiff cancer cells, where no backbone of stiff cells can permeate the whole bulk, the tissue spontaneously self-organizes a percolating tension network that maintains tissue rigidity. Of special interest is the heterogeneous solid phase, as this phase forms already at low fractions of rigid cells. The heterogeneous solid phase perfectly meets the demands of a tumor simultaneously providing mechanical stability through the solid bulk behavior and cancer cell motility through the presence of soft, unjammed cells.

Soft cancer cells induce multicellular streaming

The region of stiff cancer cells that allows a tumor mass to remain solid in the presence of large quantities of soft cells may also facilitate the spontaneous directed motility of cancer cells. To understand the effect of heterogeneity on cell migration in tumors, we use a dynamic vertex model^{43,49} (**see Extended Data**) to simulate a single active cell in a heterotypic microenvironment. Highly intermittent migration dynamics for the invading cell has been observed in a similar theoretical approach,⁴¹ which is consistent with experimental evidence that heterogeneity can drive pulsating cancer cell migration in epithelial monolayers.⁵⁰ By calculating the velocity correlation between the invading cell and surrounding cells, we characterize the collective cellular streaming behavior in the tissue. Color maps of the velocity correlations are shown in **Figure 5a**. For a fluid state at $f_r = 0.11$, directly behind the invading cancer cell, the correlations are long ranged indicating that up to 3-4 cancer cells tend to “follow” the moving cell. However, lateral to the invading cell, the correlations are weaker and vanish at around a single cell diameter. This directional anisotropy is characteristic for the formation of a cellular stream of cancer cell, which can be seen in snapshots of cell velocity field in **Figure 5b** taken from the corresponding states in **Figure 5a**. The correlation length in the directions from are calculated. The stream anisotropy parameter is defined as the ratio and plotted as a function of f_r in **Extended Data Figure 5**.⁴⁹ The stream anisotropy is decreasing with f_r and disappears for the solid states at $f_r > 0.48$. Stiff cancer cells show a small jiggling motion as isotropic displacements, soft cancer cells strongly move anisotropically in the same direction as their soft unjammed neighbors.

Such a predicted multicellular stream can, in fact, be observed *ex vivo* in time-lapse movies of small patient tumor explants of primary tumors stained with SiR-DNA. We have investigated the vital explants (12 cervix and 4 mamma carcinoma). In half of the samples, we find unjammed as well as jammed regions in cancer clusters, while the others were completely jammed, independent of staging, grading, and

nodal status. In agreement with the heterogeneity, i.e. soft and stiff regions in cancer cell clusters detected by AFM, we find areas of jammed cells and areas of collective motile, unjammed cells. **Figure 5** and **Extended Data Movie 2** show the collective motility of individual tumor cells surrounded by areas of jammed cells in a piece of cervix as well as in an explant of breast tumor. The rigid jammed cancer cell clusters act as dynamic obstacles that lead to percolated tension networks and transiently channel soft, unjammed cells into parallel streams that wind through the cellular tissue (see **Figure 5**).

Soft and stiff – heterogeneity simultaneously facilitates solid tumor behavior and a large fraction of motile cancer cells

Despite the fact that tumor progression is a systemic disease, and in particular that metastasis quintessentially depend on biomechanical changes on the cell and tissue level,^{51–53} the black-and-white characterization of tumor masses as stiff and cancer cells as soft demonstrates a lack of a comprehensive, detailed picture of mechanics in tumor biology. For the development of a malignant tumor, cancer cells have to move, proliferate and displace dense healthy tissue. Previously, the importance of cellular mechanical changes has been recognized when cancer cells leave the tumor cell mass and enter the surrounding stroma. We show that unjamming induced motility transitions already occur in the primary cancer cell clusters. Therefore, mechanical heterogeneity, which we have detected by AFM and vital cell tracking, permits tumors to simultaneously take advantage of both soft and stiff cancer cells. It becomes clear that the mechanical rigidity of cell clusters in a solid tumor is not directly determined by single cell elasticity. A percolated tension network caused by regions of jammed rigid cells provides mechanical support to the tumor, while a simultaneously present, considerable pool of collectively unjammed soft cancer cells are associated with regions of motility and proliferation. The fraction of solid areas determines the bulk rigidity, while the individual cancer cell stiffness distribution determines the jammed and unjammed areas.

The collective concept of jamming explains how soft and stiff cancer cells influence tissue rigidity. As long as the tumor contains a significant fraction of rigid cells, which can be as low as 24 %, this population can give rise to a finite elastic modulus due to tension percolation in a heterogeneous solid phase emerges. Starting at 48 % rigid cells the tissue can be jammed in a completely solid phase (see **Figure 4**). A finite elastic storage modulus allows the tumor to maintain stability and rigidity, independent of the surrounding tissue and against external forces. In addition, jamming induced tissue solidification is a primary mechanism holding the tumor mass together, as a cell that tries to leave the tumor has to unjam. This is in accordance with studies that show that tumor boundaries are not predominately stabilized by differential adhesion,²² in contrast to tissue boundaries in embryonic development⁵⁴.

Multicellular streaming, which is frequently observed in stroma surrounding the tumor,^{55–57} self-organizes aligned linear collagen associated with the cellular cancer tissue,⁵⁸ which facilitates the aligned streaming of softened cancer cells into the stroma. Streaming in the bulk of the cell clusters causes a 3D volume flow of cancer cells out of the depth of the cell mass to the tumor boundary, which is much more efficient than just 2D dissociation of cancer cells from the boundary of the cell aggregate

boundary. Moreover, the multicellular streams themselves allow cells to express stemcell associated programs and exit the tumor with the ability to form large clusters, enhancing their ability to survive outside the tumor mass.^{59,60}

The features induced by cancer cell softening that may play a critical role in cancer invasiveness are emergent cooperative properties of a heterogeneous tissue, and cannot be understood from studying individual metastatic cells. The interplay of mechanical heterogeneity and cancer cell unjamming regulates the stiffness of cancer cell aggregates and simultaneously permits cell motility. To our knowledge the tumor mass forms a truly novel state of active matter. Deregulation and dedifferentiation, which are part of any malignant transformation, cause most likely a broad mechanical heterogeneity together with a shift to softer cells. Thus, it is to expect that the observed mechanical changes occur inherently with early neoplasm.

The ability for cancer cell unjamming (e.g. through cell softening) may be the initial difference between benign tumors that grow locally and malignant, invasive tumors. To overcome the complexity and heterogeneity, the universal physics underlying the mechanical processes in the progression of solid tumors may provide a more general perspective on cancer development as a systemic disease than the molecular cell perspective alone. Since the described processes relate to the initial steps of cancer cell spreading, they may become important predictors of patient outcome complementary to genetic signatures. As described here, pathological mechanical changes driven by emergent effects, which cannot be directly related to a simple molecular cause, are a missing link in understanding cancer and will ultimately lead to new diagnostics as well as therapy.

Declarations

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements

We would like to thank Andrea Bruckert, Jana Schnedemann and S. Klingowski from the pathology Hamburg-West, as well as Felicia Juliano from Montefiore Hospital, for the preparation of tissue samples. This work was funded by the German Science Foundation (DFG, KA1116/9-1 and KA1116/17-1), as well as project “FORCE” within the EU's Horizon 2020 research and innovation program. Graduate students A.W.F., T.R.K., S.P. and F.W. were funded by fellowships of the DFG Excellence graduate school BuildMoNa. The research with A.N. was part of the joint project EXPRIMAGE funded by the German Federal Ministry for Education and Research (BMBF 13N9366). T.F. was funded by ERC Advanced Grant “HoldCancerBack” (741350), D.B. would like to acknowledge the Center for Studies in Physics and Biology at Rockefeller University and funding from the Raymond and Beverly Sackler foundation. MO and JC were funded by CA255153. The authors would also like to acknowledge the Syracuse University HTC Campus Grid, NSF award ACI-1341006.

Author Contributions

J.A.K planned and directed this study. T.F. and J.A.K wrote the manuscript. All authors discussed the results and commented on the manuscript. F.W. carried out experiments on breast tumor cells and controls. A.W.F. and S.P. were responsible for measurements of the cervical samples. R.S., T.R.K., S.P., A.W.F., E.M. and F.W. developed a sample preparation and measurement routine. T.F., M.Z., and S.F. carried out the AFM measurements. F.S. carried out the MRE measurements, S.G., and J.L. performed experiments on MTS, F.R. performed experiments on tumor explants. B.A., L.C.H., M.H., K.B., S.B., B.W., M.O., J.C. and A.N. provided patient samples and scientific/medical advice for the interpretation of the data. D.B., M.C.M. and M.L.M designed the theoretical and simulation models. D.B. and X.L. developed, executed and analyzed simulation results with oversight from M.L.M. and J.A.K.

Author Information

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Figures

Figure 1

Viscoelasticity of cancer tissue a) HE-stain of invasive breast cancer growth, regions of desmoplastic reaction (1), and intraductal tumor growth (2) and fatty tissue (3) can be identified. b) Magnetic resonance elastography (MRE) of breast tumors. Centimeter sized vital pieces of primary breast tumors (n=5) and healthy breast tissue (n=3) were measured. The bulk stiffness of tumors is higher compared to healthy tissue as expected from palpation c, f) AFM maps of local tissue elasticity for breast tumor and healthy breast tissue. Tissue elasticity maps measured for 0.5 x 0.5 mm² areas in vital tissue explants with 10 µm resolution reveals domains of several 100 µm size with distinctively higher or lower local elasticity leading to a heterogeneous structure. d) Median breast tissue stiffness, the median Young's Modulus rises from EBN = 132 Pa (n=16) to EBC = 288 Pa (n=13) moving from healthy to cancerous

tissue. e) Distribution of local Young's moduli of breast cancer tissue (dark blue) and control tissue (light blue) from the maps shown in c) and f). Both tissues show a log-normal distribution in stiffness, with the tumor showing a much wider and such more heterogeneous distribution in stiffness. g) HE Stain of Squamous cell carcinoma of the uterine cervix, the tumor (1) is confined to the cervix. The vaginal cuff (3) and ectocervix (2) are not affected. h) MRE of cervix tumors. Centimeter sized pieces of vital primary tumor explants of cervix tumors (n=4) and healthy cervix tissue (n=4) were measured. On the bulk tissue level, tumors are similar compared to healthy tissue. The tumor is not significantly stiffer than the surrounding tissue. i, l) AFM-based map of local tissue elasticity of cervix carcinoma and healthy control tissue. Tissue elasticity measured for patches of 1x1 mm² with 10 µm resolution from vital tissue explants reveals that the cancer cell clusters are heterogeneously divided into domains of several 100 µm with high or low local elastic strength. j) Median cervix tissue stiffness, in the cervix we see a drop in median stiffness from ECN = 570 Pa (n=5) for healthy cervix to ECC = 385 Pa (n=7) in cervix cancer. k) Histogram of the distribution of local Young's moduli from AFM measurements of cervix cancer tissue and control tissue, from the maps shown in i) and l). Both tissues show a log-normal distribution in stiffness, with the tumor only displaying softer cells than the cells from the healthy tissue.

Figure 2

Single cell mechanics of carcinomas: a) Relative deformation and relaxation curves of single measured cells from a breast carcinoma (Tumor, n = 613) compared to primary HMEpC cells from healthy epithelial breast tissue from breast reductions (n = 358). b) Cumulated distribution over 13 breast cancer samples (n = 6526), compared to 2 fibroadenoma samples (FA, n = 186) and one HMEpC sample (n = 358). A log-normal distribution was successfully fitted. The distribution is clearly shifted towards softer cancer cells. Nevertheless, the extreme width of the distribution means that also stiff cancer cells exist within the tumor mass. c) Relative deformation and relaxation behavior of single cells from a cervical carcinoma (n = 111) compared to normal cervical epithelial (n=38) tissue from the same patient. d) Cumulated distribution over 4 pairs of cervix samples (nCT = 1481, nCN = 262). A log-normal distribution fits very well the data. The cancer cell softening effect visible by the shift of the distribution towards larger relative deformations is for the cervix carcinoma even more pronounced than for breast tumors.

Figure 3

Multicellular spheroids of MCF 10A cells (a-c) represent healthy breast epithelium and MDA MB 436 cells (d-f) malignant breast cancer cells. a) Elastic Modulus of MCF 10A spheroid compared to single cells measured by AFM-indentation experiments based on the Hertz model. The spheroids formed by MCF-10A cells soften to 88 Pa with respect to 135 Pa for single cells, d) Spheroids formed by MDA-MB-436 soften by a factor of 5 from 570 Pa (single cells) to 111 Pa (MTS). The cells freely move within the cluster. This fluid behavior leads to the significant softening of the spheroid. b, c) MCF 10A spheroids don't fuse

completely, the fusion arrests at smaller θ ($\theta \sim 50^\circ$) e, f) MDA-MB 436 spheroids fuse almost completely and θ approaches 90° ($\theta \sim 80^\circ$).

Figure 4

2D Vertex Model Simulations of mixtures of soft and rigid cancer cells a) Mechanical Phase Diagram of Cancer Cell Aggregates. This phase diagram distinguishes between solid, heterogeneous-solid, and fluid tissues as a function of the mean value of P_0 and its distribution width. P_0 values of each cell ($N = 100$) are randomly assigned from a log-normal distribution. Here the fraction of rigid cells f_r is determined by $\text{mean}(P_0)$ and $\text{S.D.}(P_0)$ (Extended Data). At $f_r = 0.24$, a rigidity transition occurs which is indicated by the thick black line. Below $f_r = 0.24$, the tissue behaves as a fluid that lacks mechanical rigidity due to the absence of an intercellular tension network. Above $f_r = 0.24$, the tension network percolation generates solid behavior despite that no backbone of jammed, stiff cells spans the bulk. The red dashed curve represents the onset of contact percolation transition at $f_r = 0.48$ for stiff, jammed cells. The mismatch between rigidity and contact percolations results in an intermediate heterogeneous solid state as an unexpected novel mechanical state of tissues. The broad heterogeneity in cancer cells mechanical properties is the key determinant of cellular tumor tissue. b) Representative snapshots of different mechanical states corresponding to fluid, heterogeneous solid and solid tissues. Rigid cancer cells are shown in light blue while soft ones are in dark blue. The intercellular tension network is indicated by red lines drawn at cell junctions. These snapshots are taken from $N=400$ tissues. c) Tumor rigidity is plotted as a function of the fraction of rigid cancer cells. Rigidity is expressed by the elastic shear modulus. The data are plotted as function of the fraction of rigid cells in a semi-log scale. The red curve represents the median of the scattered points at each f_r value and shows a rigidity transition at $f_r = 0.24$ from finite to vanishing shear modulus. d) Fraction of rigid cells extracted from relative deformations of various tissue types. For each cell type, the mean deformation value of the non-cancer cells (Figure 2) is used to define the single cell rigidity threshold for both the non-cancer and cancer phenotypes. f_r for each cell type is then calculated by computing the fraction of cells that deformation values smaller than the mean deformation value of their non-cancer counterpart. This allows mapping each cell type to the categorization of tumor to the solid-fluid nexus as predicted by the vertex model.

Figure 5

Emergent Cellular Stream caused by an invading cell inside a heterogeneous tumor. a) Simulated velocity correlations for fluid (top), heterogeneous solid (middle) and solid (bottom) tissues. In the simulated $N = 100$ cell system, one actively invading cell is with propulsive force $v_0 = 0.4$ and rotational noise $Dr = 0.01$ is introduced. Here the x-axis is always oriented along the direction of motion of the invading cell and we choose a frame of reference in which the invading cell is always located at $(x=0, y=0)$. Colors indicate the value of correlations $C_{vv}(x,y)$ between the velocity vector of the invading cell and surrounding cells located at relative position (x,y) . Positions are in units of the mean cell diameter. When $f_r=0.11$, directly

behind the invading cell, the correlations are long ranged indicating that up to 3-4 cells tend to “follow” the invading cell. However, lateral to the invading cell the correlations are weaker and decay beyond 1-cell diameter. This directional anisotropy is indicative of a cellular stream forming behind the invading cell. With the increasing of f_r , the anisotropy disappears. b) Snapshots of cell velocity field taken from the corresponding states of a). The invading cell is colored with green edges. Light blue and dark blue represent rigid and soft cells respectively. c) Tracks from time-lapse image series of a stream of cells in a cervix tumor sample, ex vivo. A stream of cells is visible in the upper right part of the piece. (enlarged in d) Surrounding cells are basically immobile, i.e. jammed. (see e) The tracks are colored according to maximal velocity. (see Extended Data Movie M1, M2).

Supplementary Files

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