Collective invasion of cancer cells plays an important role in the lethal metastasis of tumors [1, 2]. However, the mechanisms that coordinate 3D collective cell motility is not fully understood. A number of mechanisms have been shown to coordinate multicellular invasion. For instance, cell-cell adhesion leads to group invasion as cell clusters [3, 4]. Strands of cells may follow each other along microtracks created by leader cells [5, 6]. Communications mediated by diffusive factors have also been shown to coordinate the collective motility of tumor cells by establishing leader-follower phenotypes [7].

Here we propose and test a distinct mechanism for 3D collective invasion that only depends on the physical interactions between cancer cells and their ECM (extracellular matrix). We show both experimentally and computationally that the reconfigurability of ECM, as well as the force generation and force sensation of cancer cells maintain a mechanical conversation between invading tumor cells in 3D tissue space. As a result, the collective invasion is coordinated by collective micromechanical remodeling of the ECM, which leads to organoid-geometry dependent dissemination of the tumor cells. Our results suggest that ECM-mediated physical interactions between invasive cells may play a powerful role in determining the metastatic potential of malignant tumors.

Geometry controls tumor organoid invasiveness and collective micromechanical remodeling of ECM. To study the 3D collective invasion of cancer cells, we use tumor organoid models created with DIGME technique [8]. Each organoid consists of approximately 1000 GFP-labeled MDA-MB-231 cells molded into various shapes in 3D type I collagen ECM (Fig. S1). Confocal imaging starts immediately after the gelation process completes (time zero).

To test this hypothesis, we first examine if the ECM deformation caused by tumor-generated mechanical forces depends on the tumor geometry. We embed 1-µm di-
ameter fluorescently-labeled polystyrene particles in the collagen ECM and use particle image velocimetry (PIV) to quantify the ECM deformation field. Fig. 1(e-f) show the radial velocity \(v_r\) of the ECM by averaging over symmetric locations (dotted points in the insets of Fig. 1(e-f)). A circular tumor first pushes out the ECM \(\Delta d > 0\) due to cell spreading upon seeding (leading to overall expansion of the organoid), then pulls in the ECM \(\Delta d < 0\) with their traction force. As a result, after 2 days the ECM shows net inward deformation while some of the cells have already been disseminated from the tumor (Fig. 1(b) insets). On the other hand, we find that a triangular tumor mostly pushes out the ECM, albeit with a diminishing rate. As a result, the ECM surrounding the triangular tumor maintains a radially outward net deformation (Fig. 1).

**Computational modeling of collective micromechanical remodeling of ECM and tumor invasion.**

To gain further insights on how collective ECM remodeling modulates the collective cancer invasion, we devise a multi-scale computational model that takes into account the fibrous microstructure of the ECM [10], nonlinear ECM mechanics [11], as well as cell motility directed by contact guidance cues [12, 13].

Based on the experimental observations, we consider the dissemination of tumor organoids to start from an expansion phase, where cells spread and push out the ECM. This is followed by an invasion phase, where cells pull in the ECM and migrate. We model tumor cells as polarized active particles with coupled force generation and locomotion [12] (Fig. S2-S7). Cells deform the ECM fibers in their vicinity, which in turn alters the migration and polarization of the cells. Explicitly accounting for the reciprocal interactions between cells and ECM allows us to investigate the collective migration regulated by the non-local mechanical dialogues among the cells mediated by the ECM.

We first employ the computational model to simulate and calculate the remodeling of the ECM surrounding circular and triangular tumor organoids. Fig. 2(a-b) show the relative magnitudes of displacement fields in the ECM, where the maximum value is normalized to 1. Here we measure the ECM properties at 5 hours and 50 hours, two time points empirically determined to represent the expansion and invasion phases respectively. The magnitude of displacement decays roughly as \(1/r^3\) as one moves away from the organoids. After the expansion phase, individual cells migrate away from the original organoid. The right panels of Fig. 2(a-b) show locations of the invaded cells in a typical simulation run. Cell invasion is accompanied with continuous ECM deformation. As shown in Fig. 2(c-d), our simulated ECM radial velocity agree well (qualitatively for circular organoids and quantitatively for triangular organoids) with experimental measurements. Importantly, the contractile deformation is much more pronounced near circular tumors compared with triangular tumors.

To better reveal the structural remodeling of the ECM, we have calculated the average orientation of the ECM fibers with respect to the tangential direction of the tumor surfaces (Fig. 2(e-f)). During the expansion phase, organoids push the fibers to be aligned parallel to the
tumor boundary, which bias the cell polarization accordingly. Later on, the pulling forces from the cells reorient the fibers. For circular tumors, the collective cellular traction force is sufficient to align the ECM fibers radially (Fig. 2e), contributing to the accelerated dissemination. In contrast, the fibers remain tangentially aligned along the flat edges of the triangular tumors (Fig. 2f).

The structural remodeling of the ECM significantly reconfigures the micromechanics of the ECM. We find that for both circular and triangular tumors ECM is consistently stiffer in the direction of fiber alignment, and softer in the direction perpendicular to the fibers. Such mechanical cues may further regulate the dynamics and functions of cells through mechanosensing pathways [14].

Our simulations show that the circular tumors exhibit larger invasion depths compared with the triangular tumors (Fig. S9), which is consistent with the experimental observations. To further explore the mechanisms behind the geometry-dependent collective migration, we modified the simulation parameters to steer the cell-ECM interactions. We find that when the cell polarization and ECM fiber orientations become uncorrelated, the invasion depth of tumors drastically reduces (Fig. S10). This is consistent with the experimental results that reducing the level of contact guidance diminished the advantage of circular tumors in dissemination (Fig. S11). We also find that reducing the cellular traction forces leads to weaker ECM remodeling, and thus weaker dependence of tumor invasion dynamics on the organoid geometry (Fig. S12).

Collective invasion of tumor organoids with complex shapes. Having tested our simulation model against experimental results for circular and triangular tumors, we ask if the mechanical principles considered in our model are sufficient to predict the invasiveness of tumors with more complex geometries. We focus on two particular shapes of tumors: semicircle and star. The boundary of a semicircle contains regions of both positive and zero curvature, therefore can be considered to be a hybrid of circular and triangular tumors. A star shape, on the other hand, contains both convex and concave surfaces.

Taking into account of the asymmetric shape of semicircle diskoids, we divide the ECM space into cap, corner and flat regions (Fig. 3b). We manually identify all disseminated cells and their locations after 10 days from seeding the original tumor. To help visualize the spatial distribution of the cells we compute the normalized cell density $\rho_N$ (Methods), which represents a dilution factor: if $\rho_N = 1$ then the local cell density is the same as in the original tumor, where all cells are presumably uniformly distributed in the original tumor.

We find the cell density is almost uniform surrounding the original semicircle diskoid, and decreases rapidly at larger distance. However, the flat region contains fewer cells that migrate deeply into the ECM space from the diskoid boundary. To further quantify the relation between invasiveness and local geometry of tumors, we calculate the ranked average invasion depths (RAID) $\Delta D(f)$. In particular, we first measure the invasion depth $\Delta d_i$ of each cell $i$ as the distance of the cell from the original tumor boundary (arrows in Fig. 3a,b). We then compute $\Delta D(f)$ as the average invasion depth of cells in the top $f$ percentile ranked by $\Delta d_i$. Using the metric RAID, we compare the invasiveness of cells in each of the three regions of the ECM surrounding semicircle diskoids. As shown in Figure. 3f, cells in the cap region are leading the dissemination. For instance, the top 10% invaders in the cap region have an average invasion depth of 672 µm, while the top 10% invaders in the corner and flat region have an average invasion depth of 470 µm. At a percentile of 5%, cells in the cap region have a lead of 200 µm than cells in the flat region.

Consistent with the previous observations in Fig. 3
the invasiveness of semicircular diskoids provides strong evidence that local geometry regulate cancer cell dissemination. In particular, a positive curvature in the tumor surface accelerates the overall invasion [15].

We have also quantified the invasiveness of star-shaped diskoids after 3 days of seeding the tumor. In particular, we divide the ECM space into regions that are in the direction of the tips (positive curvature), and regions that are in the direction of valleys (negative curvature). Cells in the buffer regions (black dots in Fig. 3b) are excluded from the analysis.

By measuring RAID we find that overall cells in the valley region possess larger invasion depth (Fig. 3d), suggesting that negative curvature accelerates cell dissemination even more than positive curvature. Of note, at 10 days, the disseminated cells become uniformly distributed in all directions. This is due to the proximity of the ridge and valley regions as well as the mixing caused by lateral motion of the cells.

These experimental results agree well with the predictions of our simulations as shown in Fig. 3c and f. Furthermore, our simulations also reveal the ECM remodeling by the tumor organoids. Fig. 3(g-h) shows the average fiber orientation and microscopic anisotropy in the expansion and invasion phases. For semicircle organoids, fibers in the flat region remain tangentially aligned to the tumor boundary through the whole process; whereas fibers in the cap region are re-oriented radially by cellular traction force during the invasion phase. For star-shaped organoids, fiber orientation in the ridge region rotates from tangential of the tumor boundary to random alignment; whereas fibers in the valley region are pulled normal to the tumor boundary during the invasion phase. The structural anisotropy translates directly to the micromechanical anisotropy, such that the ECM is stiffer in the direction parallel to the fiber alignment. These results confirm that local geometry program collective force generation and ECM remodeling by the cancer cells, which modulates the rate of dissemination of the tumors.

In this letter, we demonstrate a previously unrecognized mechanism of cell-cell interaction that coordinates multicellular dynamics. This mechanism does not require direct contact between cells such as cadherin-based adhesion [8, 9] or contact inhibition [16, 17], nor it relies on the cooperation of leader-follower phenotypes [5, 6, 18, 19]. Instead, we show both experimentally and computationally that cells collectively apply forces to their ECM [20], which in turn provides mechanical cues to bias cell motility. Because collective force generation can be controlled by geometry, we find dissemination of cancer cells from tumor organoids are dependent on tumor geometry.

Our results provide physical insights for processes in cancer biology and morphogenesis. Clinical studies have
shown that collagen fibers aligned tangentially and normally to tumor boundary correspond to opposite prognosis \cite{21,22}. While the origin of tumor-associated ECM misalignment is unclear, our results suggest that tumor geometry is an important contributing factor. On the other hand, mesenchymal cell migration is often considered as a single-cell process during development and diseases \cite{23,24}. Our model system show that underlying multicellular coordination may take place in the form of collective force generation and ECM remodeling. Together, we find that 3D collective cell migration may exploit the mechanical feedback between force-generating cells and reconfigurable ECM as an indirect yet effective channel of communication.

Finally, our results show that 3D migrating cells represent a distinct class of active particles which actively re-sculpture their microenvironment and respond to the cues generated by themselves and others. Future research is needed to systematically investigate the collective dynamics of such active particles as a route to understand general living systems.

Methods

Sample preparation. See Supplementray Information for details.

Image analysis. Confocal images were taken using Leica SPE at a rate of 30 min per frame for continuous imaging or at days 0, 1, 3, 5, 10 for discrete imaging. Cell locations were projected onto the $x-y$ plane, whereas movement in the $z$ direction is relatively small \cite{8}. The invasion depths were manually measured with the help of NIH ImageJ. The deformation of the ECM was measure using PIVlab implemented on Matlab. To calculate the velocity field in the ECM we perform PIV analysis on image pairs with 2-hour delay.

To approximate the cell density from the scattered cell locations we use a gaussian kernel:

$$\rho_N(r) = \frac{A_0}{m} \sum_{i=1}^{m} \frac{1}{2\pi\sigma^2} e^{-\frac{(r-r_i)^2}{2\sigma^2}}$$

Here we choose the kernel width $\sigma$ to be 80 $\mu$m, approximately twice the size of a cell. $m$ is the total number of disseminated cells, and $A_0$ is the area of the original tumor diskoid. To calculate the ranked average invasion depth (RAID) we used the Matlab `quantile()` function to select the data for averaging.

Computation. See supplementary information for details.

Data Availability

All data and computer codes are available from the authors upon reasonable request.

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Author Contributions

B. S. and Y. J. designed the research and oversaw the experimental and computational studies respectively. J. K., Y. Z., A. A., H. N., J. T. collected data. All authors analyzed data and wrote the manuscript. Y. Z. and H. N. thank Arizona State University for the University Graduate Fellowship.

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