New and Notable

Switching from Protease-Independent to Protease-Dependent Cancer Cell Invasion

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Metastasis of tumor cells, starting from their infiltration of local tissues, is responsible for the vast majority of cancer-related deaths (1). Understanding the molecular mechanisms of cancer cell invasion and migration during metastasis is critical for the development of novel therapies for cancer treatment. Cells have been proposed to employ either protease-dependent or protease-independent modes for migration and invasion (2). In response to matrix properties, moving cells degrade the extracellular matrix (ECM) molecules by upregulating or activating specific enzymes, such as matrix metalloproteinases (MMPs), in cell protrusions confronting the dense mesh of the ECM (e.g., lamellipodia for cells on 2D substrates (3), dendritic protrusions of cells in a 3D matrix (4), and invadopodia for cells crossing the basement membrane (5)). Cells that lack ECM proteases or those treated with protease inhibitors use mechanical forces to physically reorganize the matrix, while coordinately deforming their cell body to migrate through the space in an amoeboid-like manner (6).

Both proteolytic and nonproteolytic cell invasion/migration require traction forces generated by actomyosin contractility to contract the cell body, which is then transmitted to the ECM as traction stresses (forces per unit area) (7,8). Traction stresses not only play pivotal roles in driving cell movement but also facilitate the mechanical interactions between cell and ECM. The past two decades have witnessed the development of traction force microscopy (TFM) methods, which allow for the quantitative determination of cellular traction forces (7–10). In TFM, the local deformation of the matrix is monitored by tracing the movements of fluorescent particles embedded in the matrix. Then the traction stress is calculated by solving a boundary-value problem, assuming that cell-generated strains are small enough to be within the linear elastic range, so that Hooke's law can be applied. TFM is relatively straightforward to set up and is compatible with the measurement of spatially resolved forces over a wide range of force and length scales. Despite these advantages, previous TFM methods all require a zero-stress reference image of the matrix in a stress-free condition. If the matrix experiences permanent remodeling during the protease-dependent invasion, the zero-stress reference of the matrix network will be different from the original status and will continuously evolve as the matrix is remodeled. This renders the investigation of the switch from protease-independent to protease-dependent invasion challenging.

The work presented by Aung et al. in this issue (11) introduces an elegant quantitative single-cell assay to calculate the 3D traction stresses generated during cancer cell invasion. Employing a model that simulates the measured indentation profiles generated by the invading cells, they show that cells invading the matrix utilize a stress-focusing mechanism to sense the mechanical resistance by the matrix and to promote invasion. The 3D traction stresses obtained from this reference-free method agree well with the values obtained from the full 3D TFM method of del Álamo et al. (10). Without the necessity to image the undeformed condition for each invading cell in the matrix network subjected to deformation or degradation, the authors are able to determine the transition from a protease-independent (low traction stresses) to a protease-dependent (high traction stresses) mode of invasion, at compressive traction stresses >~165 Pa.

Perhaps the most exciting aspect of this work is that it contributes to our knowledge the first quantitative evidence for a direct transition from protease-independent to protease-dependent invasion within a single cell. In a recent clinical trial, MMP inhibitors failed to prevent cancer progression, which suggests the physiological relevance of a protease-independent invasion mode; however, it remains unclear when pure mechanical deformation is sufficient and when proteolytic matrix widening is required for the penetration of tumor cells through the basement membrane. The study by Aung et al. provides invaluable insights to address this question. When the tumor cell invades the ECM at small deformation, such that the traction stress exerted by the matrix on the cell is below a certain threshold (165 Pa for MDA-MB-231 cells in Matrigel), cell invasion is independent of protease activity and largely relies on cellular deformation, such as membrane blebbing (Fig. 1 *A*). Continuing its invasion into the matrix, which results in larger matrix deformation and increased traction stresses, the cell switches to a protease-dependent mode of invasion and relies on cell protrusions, such as invadopodia, which are rich in active MMPs and capable of enzymatically cleaving the matrix to pave the way for cell invasion (Fig. 1 *B*).

Aung et al. show that the critical traction stress that triggers the mode switching of the invading cell is independent of the thickness and the apparent Young's modulus of the matrix. Cells on thinner gels with higher

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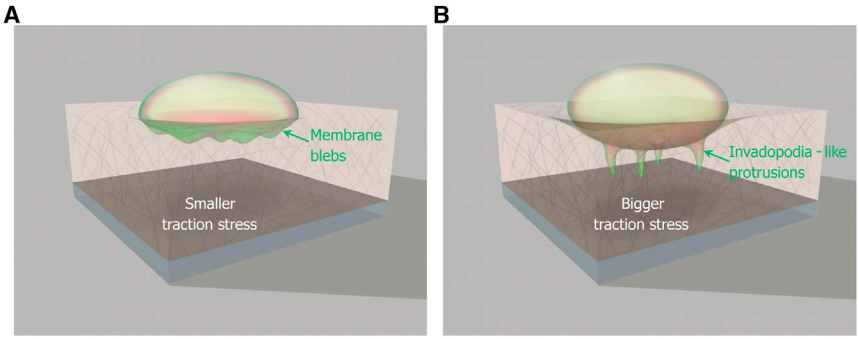

FIGURE 1 Illustrative figure showing two modes of cancer cell invasion. (*A*) Protease-independent invasion occurs when the traction stress of the matrix generated by cell invasion is small (<165 Pa for MDA-MB-231 cells in Matrigel in this study by Aung et al.). The invading cell exhibits membrane blebs at the leading edge, where there is no MT1-MMP. (*B*) The cell switches to protease-dependent invasion when the traction stress increases (to >165 Pa for MDA-MB-231 cells in Matrigel in the work by Aung et al.) and develops invadopodia-like protrusions that are rich in MT1-MMP. Actin is shown in green and MT1-MMP in red. To see this figure in color, go online.

Young's modulus start to degrade and permanently deform the matrix at shallower indentation angles and smaller matrix deformations. This is consistent with previous findings, which have indicated that cells preferably adopt proteolytic migration at relatively high matrix stiffness and migrate in nonproteolytic mode at low matrix stiffness (12). It also supports the notion that cells exhibit higher MMP activity on substrates of higher rigidity (5).

The study by Aung et al. uses MDA-MB-231 breast cancer cells as a model for invading cells and Matrigel to represent the ECM. The quantitative approach presented here can be easily applied to other cancer cells and other ECM systems, as long as the indentation profiles conform to the experimental observations and physical considerations, as the authors carefully validated in their work. In addition to stiffness of the matrix, the effect of which is addressed in this work, the physical parameters of the ECM also include pore size and fiber alignment. Previous study has shown that an ECM with large pores or gaps, aligned fibers, or tracks will facilitate cell movement without protease activity. How these ECM parameters regulate the invasion mode of cancer cells requires future investigation. Another interesting use of this novel assay would be in isolating the roles of cell mechanical properties in this invasion-mode switch. Furthermore, the reference-free approach in this work can be utilized in a high-throughput manner to identify molecular pathways responsible for cancer cell invasion or the switch between different invasion modes, thereby facilitating the development of new therapies for cancer metastasis treatment.

Despite these exciting discoveries and possible future advancements, there is room to improve this novel quantitative method. For example, the matrix was assumed to be homogeneous and isotropic. The impact of protease activity on the mechanical properties of the matrix was not taken into consideration in the model presented here. Although 165 Pa was identified as the compression stress that enables the switch of invasion mode, whether this value can be translated to in vivo cancer cell invasion requires further investigation. The development of a quantitative TFM assay that considers the inhomogeneous and anisotropic ECM and the change in the modulus of the matrix due to protease-induced digestion will provide more physiologically relevant predictions of critical traction stresses.

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