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SPOTLIGHT

A collective strategy to promote the dissemination of single cancer cells

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The transition from collective to single-cell invasion in metastatic tumors has been regarded as the consequence of oncogenic drivers in concert with extracellular triggers received from the tumor microenvironment. In this issue, Yoon and colleagues (https://doi.org/10.1083/jcb.202308080) have identified an epigenetic program by which collective niches release laminin-332 and thereby cause the detachment and invasion of fully individualized tumor cells.

Invading cancer cells exhibit astounding plasticity of invasion strategies in response to environmental, physical, and chemical signals, including single-cell and collective invasion. Consequently, cell culture and cell isolation methods to approach collective versus single-cell invasion have identified underlying molecular differences, including the regulation of cell-cell adhesion, cell-matrix interaction, and cytoskeletal regulation by Rho GTPases (1). While being typically investigated in isolation, broadly used collective invasion models including murine triple-negative 4T1 mammary carcinoma cells develop a notable cell-intrinsic diversity of invasion strategies, with >95% of the cells invading collectively, and a smaller subset detaching and developing single-cell invasion. However, whether collective and single-cell invasion modes in the same tumor differ in their epigenetic status and even cooperate to enhance or limit invasion remained unclear.

Yoon and coworkers performed single-cell RNA sequencing and other analyses on isolated subsets and identified laminin-332 (LN-332) to be upregulated in 4T1 breast cancer groups, but not in single cells (2). The photoconversion approach to isolate individually moving from collectively moving subsets was previously developed by the same team, based on expression of H2B-Dendra2, which converts from green to red fluorescence after excitation with a blue laser (3). When applied to individualized cells in 3D invasion culture to mark and isolate green and red cells followed by sample digestion and cell sorting, a sufficient number of single cells was obtained for RNA sequencing, DNA methylation analysis, and proteomic analysis. Strongly differentially regulated mRNA expression between collectively invading and single cells correlated with—in single cells—increased methylation at the promoter regions and repressed mRNA levels of key genes implicated in controlling the collective migration mode, including *Cdh1*, *Krt14*, *Esrp1*, *Cldn4*, *Tacstd2*, and *Lama3*. *Lama3* encodes the α chain of the LN-332 molecule, and also the β and γ chain of LN-332 were highly expressed in collective cells, indicating expression of mature LN-332. This indicates that single cells differ from collectively invading cells in their epigenetic program, with altered cell-cell and cell-matrix interactions (Fig. 1).

In a series of functional in vitro analyses, including deletion of the *Lama3* gene, they show that LN-332 expression in cell collectives and Rac1 activation in single cells are associated with individualization and required to induce single-cell detachment from collective invasion strands. The results indicate a juxtacrine mechanism by which collectively invading cells secrete LN-332 to give rise to or alter the biology of their single-cell companions. The identified chromatin hypermethylation in individualized cells indicates a relatively stable epigenetic program with a distinct molecular repertoire. The gene ontology data delineating the transcriptomic and epigenetic differences between collective and single-cell phenotypes will be a valuable resource for researchers to cross-reference additional pathways potentially implicated in cell-intrinsic invasion plasticity.

LN-332 is an epithelial basement membrane component, including in skin and stratified squamous mucosa, which maintains epithelial cohesion and mechanical tissue stability. In epithelial tumors, LN-332 accumulates at the interface of the tumor with the surrounding stroma and its expression predicts invasiveness and poor clinical outcome (4). The new data presented here indicate that LN-332 can act in a juxtacrine manner between tumor cells to enable single-cell detachment from more bulky, collective invasion niches by inducing Rac1 activation. Consistently, Yoon et al. show that after seeding on LN-332–coated plates, single-cell but not the collective cell subset developed Rac1 activation, suggesting a haptokinetic direct mechanism of inducing single-cell invasion that depends on cell interaction with an ECM-deposited or -anchored LN-332.

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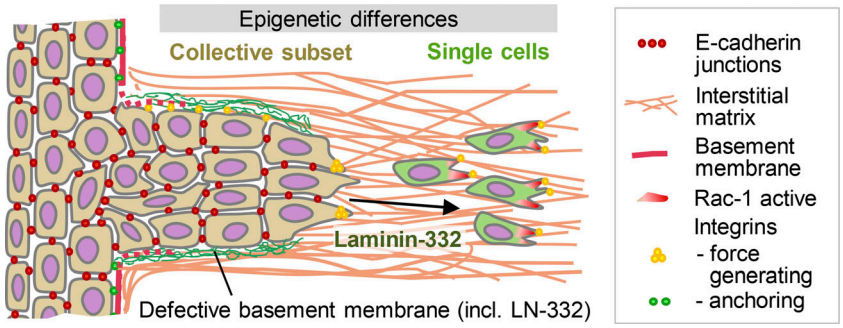

Figure 1. **Distinct transcriptomic states in collective subsets in mammary carcinoma cells reveal LN 332 as mediator of single-cell migration and dissemination.** LN-332 induces Rac-1 activation and consecutively enhances migration in single cells but not the collective subset.

On the other hand, LN-332 staining in the 3D spheroid cultures revealed predominantly cytosolic intracellular localization but no interstitial deposition, leaving open questions of where and by which mechanism LN-332 triggers single-cell migration in the 3D model. LN-332 could act on cancer cells directly, as a soluble or haptokinetic factor deposited in low amounts in the tumor microenvironment, below the limits of microscopic detection in the spheroid model. Alternatively, or in addition, LN-332 is proteolytically processed by matrix metalloproteinases (5) and other enzymes (4), and then can exert invasion-inducing activity (5, 6). Thus, depending on proteolytic competence of the tumor model and stromal composition, proteolytic processing of LN-332 into functional fragments may modulate (i) its bioactivity toward cell anchorage or migration induction as well as (ii) ECM binding and solubility, and thereby affect detection in 3D culture by immunofluorescence. Lastly, currently unappreciated activities of LN-332 may be at work, such as direct chemotactic activity by low molecular weight fragments of LN-332 or activation of integrin and non-integrin receptors, which may cooperate with cytokine or chemokine signaling to enhance single-cell migration (7).

Whether this mechanism of plasticity induction is druggable and can be exploited therapeutically is difficult to predict. Given the range of known LN-332 processing options and activities, ranging from stable cell anchorage to induction of invasive migration and increased cell survival, it will be important to identify the required proteolytic processing steps and subsequent receptor-ligand interactions underlying LN-332–dependent dissemination. Further, it will be important to detect how long evading single cells remain dependent on LN-332, or whether alternative signals take over and mediate subsequent steps of the metastatic cascade. ESRP1, which mediates alternative splicing of many Rho GTPase regulators and was identified in this study as down-regulated in individually moving cells (2), may be involved in sustained differential activation of Rac1 (8). In addition, reduced ESRP1 expression may de-repress the generation of Rac1B, the only known Rac1 splice variant that is self-activating and associated with aggressive breast cancer (9, 10). To this end, it will be important to further investigate the plethora of LN-332–mediated effects on cancer invasion plasticity and metastasis, beyond its immediate function as a basement membrane component. This may consolidate rationales for developing integrating biomarker concepts and intervention strategies against metastasis-driving mechanisms, including LN-332 and beyond.

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