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SPOTLIGHT

ARF3 weights the balance for prostate cancer metastasis

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Patrick T. Caswell discusses work from Bryant and colleagues (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202206115) which shows how ARF3 impacts metastasis in prostate cancer by regulating a switch between modes of collective invasion.

Cell migration is fundamental to development, homeostasis, and disease progression. The mechanisms that cells use to move are still being uncovered, particularly in 3D and tissue contexts relevant to cancer metastasis (1). Cells can move both individually or as collective groups within organisms, and further variation and subtlety within each mode is thought to permit migration within correspondingly suitable environments (2). While both individual and collective cell migration are relevant to cancer metastasis, collective migration has been suggested to be a major route to dissemination in epithelial tumors (2). Collective migration involves the movement of cells as coordinated groups, where leader cells maintain some form of adhesion with followers that can promote more rapid and directional motility (3). Collective migration can broadly be divided into two modes, one in which cells maintain robust cell–cell contacts that physically couple the group and coordinate movement, and a second collective streaming mode where cells maintain transient/loose cell–cell contacts in a follow-the-leader fashion (3).

Vesicle trafficking pathways are important in determining signaling and adhesion in migrating cells (4), but very few modules of trafficking regulator-effector-cargo have been identified that control specific modes of migration. In this issue, Sandilands et al. (5) address this by investigating the ARFome—a cohort of ARF proteins, their upstream regulators, and downstream effectors—using high-throughput imaging and artificial intelligence to determine where ARF pathways determine key morphological behaviors in prostate cancer. By depleting each member of the ARFome in prostate cancer cells, Sandilands et al. reveal a new role for a vesicle trafficking module consisting of PH and Sec7 Domain (PSD)-ARF3-Rab11FIP4-N-cadherin in regulating collective motility behaviors to determine prostate cancer metastasis (Fig. 1).

The ARF small GTPases are a family of evolutionarily conserved regulators of membrane traffic in the secretory and endocytic pathways (6). Binding of GTP (facilitated by guanine nucleotide exchange factors, GEFs) and its hydrolysis to GDP (facilitated by GTPase activating proteins, GAPs) are both integral to the activation cycle that allows ARFs to interact with an overlapping group of effectors that form coat proteins, tethers, or control lipid modification (6, 7). The ARF family has been implicated in cell migration and invasion (8), with numerous studies focusing on ARF6 and trafficking of adhesion receptors, but because of the relative promiscuity of GEFs/GAPs for ARF family members (6), very few ARF pathways have been described. In particular, the function of ARF3 in migration and invasion was until now poorly understood.

With this in mind, Sandilands et al. embarked on the herculean task of determining the contribution of the ARFome (5 human ARF proteins, 17 GEFs, 23 GAPs, and 72 known interactors) to the morphology and migration of prostate cancer cells within 3D culture. The authors used high-throughput live imaging combined with machine learning–based classification of size, shape, and movement features of prostate cancer acini to define the frequency with which acini adopt specific phenotypes. They focused first on spherical, elongated, or locally invading features. This elegant approach allowed the classification of ARFome proteins based on the influence of knockdown on acini phenotype over time, establishing subsets of ARFome members with shared functionality.

The authors next chose to focus on the poorly characterized ARF3 and its closest homolog ARF1, which diverges by just four N-terminal and three C-terminal residues. In 2D culture, ARF1 or ARF3 depletion increased cell migration, and in 3D depletion of either promoted invasion of collective chains of cells following a leader cell. Using overexpression, the authors found that while ARF1 overexpression had little impact on motility, ARF3 overexpression promoted collective invasion of cell sheets, mapping this increased ability to support collective motility to the three divergent C-terminal residues in ARF3. This indicates that

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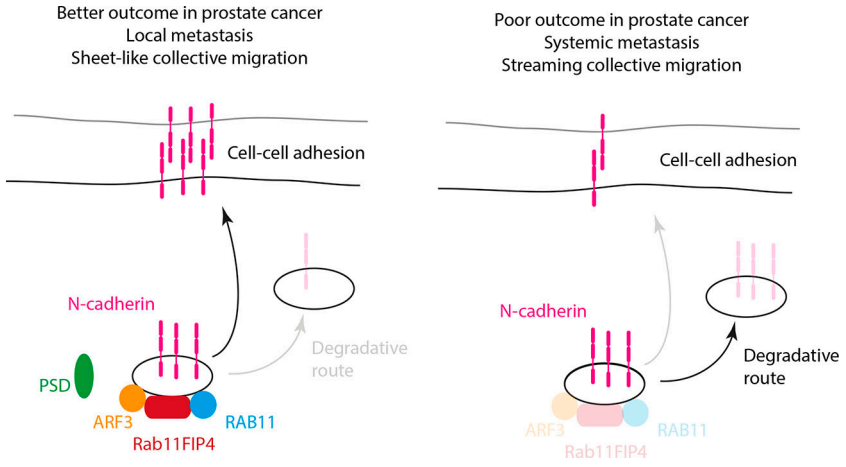

Figure 1. **ARF3 levels tune collective migration mode and metastasis.** The GEF PSD, ARF3, and Rab11FIP4 promote N-cadherin localization to Rab11-positive recycling endosomes, stabilizing N-cadherin. This leads to sheet-like collective migration in 3D matrix, local metastasis in mouse models, and patients with high ARF3 and N-cadherin levels have better prognosis. When ARF3 levels are depleted, N-cadherin is degraded. This promotes collective streaming of prostate cancer cells, systemic metastasis in mouse models, and in patients with high N-cadherin, low ARF3 levels predict poor prognosis.

modulating the level of ARF3 controls adhesion between migrating cells to switch between sheet-like and follow-the-leader modes of collective invasion in 3D matrix.

The ARFome screen suggested that the ARF GEF PSD3 and ARF effector Rab11FIP4 control similar collective motility behavior to ARF3, and indeed knockdown of PSD or Rab11FIP4 promoted invasion of collective strands of cells following a leader. The authors confirmed that PSD indeed acts as an ARF3 GEF, and that ARF3 associates with Rab11FIP4 on endosomes.

Cell–cell adhesion is a major regulator of collective migration, and the cadherins are key family of cell–cell adhesion molecules (2). Further investigation revealed a new link between ARF3 and cell–cell adhesion via N-cadherin. Through a series of elegant experiments, the authors were able to show that the PSD-ARF3-Rab11FIP4 module stabilizes N-cadherin protein levels by supporting the recruitment of N-cadherin to Rab11-FIP4 and Rab11 positive endosomes. Rab11FIP4 is in fact a dual effector of ARFs and the Rab11 recycling regulator, and it is therefore interesting to speculate that ARF3 recruits N-cadherin into an alternate recycling route that promotes N-cadherin stability, perhaps bypassing degradative route. Indeed, N-cadherin depletion was sufficient to induce follow-the-leader collective invasion irrespective of ARF3 levels. This suggests that the PSD-ARF3-Rab11FIP4-N-cadherin module boosts cell–cell adhesion to modulate collective invasion mode in prostate cancer cells, and that loss of ARF3 (and N-cadherin as a consequence) loosens cell–cell contacts to allow more flexibility and collective streaming (Fig. 1).

ARF3 levels were also found to modulate metastasis in a mouse model of prostate cancer metastasis. Overexpression of ARF3 led to an increase in macrometastases, but interestingly these were restricted to sites close to the orthotopic xenograft tumor. ARF3 depletion, however, resulted in metastasis in all cases, and these metastases were widespread within mice. This likely reflects the collective mode switching found in vitro, where ARF3 overexpression promotes cell–cell adhesion and movement of collective sheets, whereas ARF3 depletion increased the invasion of collective strands. Analysis of prostate cancer patients further associated low ARF3 levels with poor outcomes. For tumors where N-cadherin levels are high, correspondingly high ARF3 levels were associated with longer progression-free survival. However, in patients with N-cadherin^{high} ARF3^{low} tumors, progression-free survival was significantly decreased, and at a similar level to patients with N-cadherin^{low} tumors.

Sandilands et al. (5) beautifully demonstrate how systems biology approaches can be used to identify disease relevant pathways. They delineate these pathways through phenotypic analysis of cell collective invasion and apply this to develop our understanding of prostate cancer using relevant mouse models and patient data. The PSD-ARF3-Rab11FIP4-N-cadherin module they identify is likely to be important in disease and developmental contexts beyond prostate cancer, and their work raises new and interesting mechanistic questions around the role of endocytic recycling via ARF3-Rab11FIP3-Rab11 in controlling the stability of adhesion receptors. Moreover, Sandilands et al. (5) show how a relatively subtle shift in the mode of collective migration in 3D experiments in vitro can predict cell/tumor behavior in vivo and even predict patient outcomes. The ARF3 pathway is able to shift the balance of N-cadherin–based cell–cell adhesion to support more stable collective groups of migrating cells, but this limits the range of metastasis in mouse models and perhaps as a consequence correlates with improved survival of corresponding prostate cancer patient cohorts. Conversely, lower levels of ARF3 tune down cell–cell adhesion to favor collective streaming of prostate cancer cells, accompanied by systemic metastasis, which could explain the poor disease-free survival in ARF3^{low} prostate cancer patients. These findings demonstrate the power of their systematic approach, and provide a paradigm for the use of phenotypic cell biology in appropriate 3D contexts to infer cell behavior in vivo.

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