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SPOTLIGHT

# Exchangeable leaders of collectively migrating glioma abuse N-cadherin trafficking

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**Collectively migrating cells consist of leaders and followers with different features. In this issue, Kim et al. (https://doi.org/10.1083/jcb.202401057) characterize the leader and follower cells in collective glioma migration and uncover important roles of YAP1/TAZ-mediated regulation of N-cadherin in the leader cells.**

Cell migration has pivotal roles in various cellular events, and dysregulation of cell migration leads to cancer invasion and metastasis. While many cells move individually, cells in tissue morphogenesis, wound healing, and cancer metastasis often migrate as a cohesive group whereby they retain cell-to-cell adhesion with neighboring migrating cells (1, 2). This collective cell migration involves leader and follower cells with different characteristics. At the front, the leader cells form protrusion and determine the migration direction and speed according to extracellular environmental cues, whereas the followers form strong cell-to-cell adhesion and modulate the migration behaviors (1, 2).

In spite of these distinct features, Kim et al. show that the leader and follower cells of collectively migrating pediatric high-grade glioma (PHGG) are exchangeable and exhibit quite similar gene expression patterns, except for YAP-response genes and wound healing–related genes (3). In other words, YAP signaling defines the leader- and follower-cell properties in collective cell migration. This work also indicates that YAP signaling regulates N-cadherin cell surface levels and its endocytic recycling in the leader cells and promotes collective glioma migration.

## Different features in the leader and follower cells in collective glioma migration

PHGGs are difficult to treat, and the 2-year survival rate of patients is ∼30% (4). PHGG cells basically exhibit collective migration, which may confer aggressive invasion properties compared with single-cell migration. In fact, intercellular connections between glioma cells, including microtubule-associated gap junctions or N-cadherin– and p120-catenin–mediated filamentous junctions, contribute to the infiltration into brain tissues and/or radioresistance (5, 6). Kim et al. reveal that the leader cells of a collectively migrating PHGG cell line (PHGG leaders) are connected via filamentous junctions, similar to adult glioma, whereas the follower cells form epithelial adherens junction–like circumferential adhesion (3, 5) (Fig. 1 A). Interestingly, not only the filamentous junctions but also epithelial-like adhesion are mediated by N-cadherin, but not E-cadherin, suggesting that the migration pattern of the PHGG cells may be close to mesenchymal, rather than epithelial, collective migration (2), possibly due to non-epithelial origin of PHGG cells.

## N-cadherin endocytic recycling during collective glioma migration

A unique feature of the PHGG leader cells is that N-cadherin is actively internalized and recycled to the plasma membrane, although its cell surface levels are constantly higher than the followers (3) (Fig. 1 A). In addition to the plasma membrane, N-cadherin is observed in perinuclear vesicles and colocalized with Rab5 or Rab11, early and recycling endosome markers, respectively. This endocytic trafficking of N-cadherin may be important for proper collective glioma migration, because an essential role of Rab5- and Rab11-mediated N-cadherin endocytic recycling has been reported in a long-distance migration of immature neurons in normal embryonic cerebral cortex (7) (Fig. 1 B).

Rab5 is a general regulator for clathrin-mediated endocytic pathways and therefore it is not specific to N-cadherin. Interestingly, however, the number of vesicles containing transferrin receptor, a marker for clathrin-mediated endocytic pathways, is comparable between the leader and follower cells. Although a possibility of involvement of clathrin-independent endocytosis in N-cadherin internalization cannot be excluded (8), endocytosis specific for N-cadherin, rather than general endocytic machinery, may be upregulated in the leader cells.

## YAP1/TAZ specifically regulates N-cadherin trafficking in the PHGG leader cells

Although the leader and follower cells exhibit different behaviors, these cells can be interchanged during collective migration

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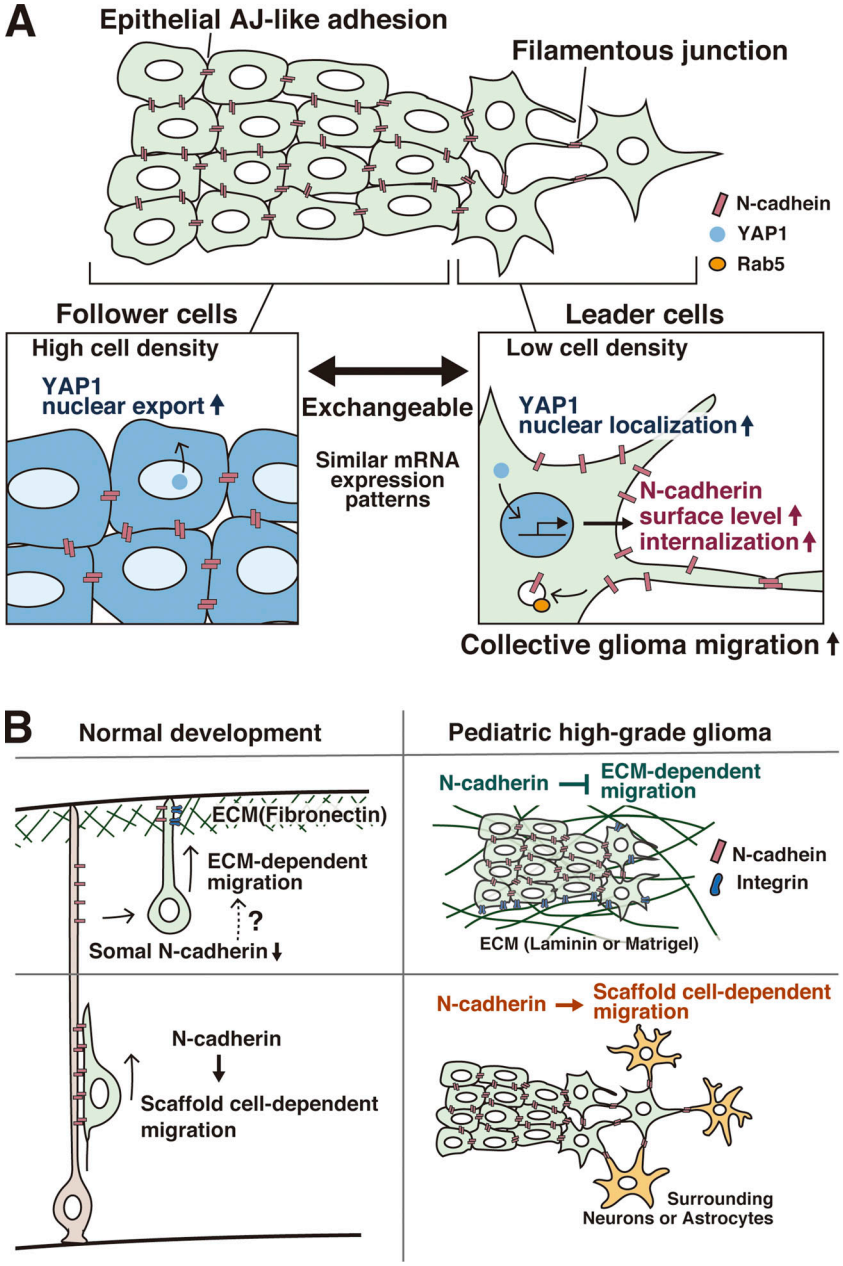


Figure 1. **Multiple roles of N-cadherin in collective glioma migration. (A)** Collectively migrating PHGG cells show two types of cell adhesion. The leader cells are connected via N-cadherin–mediated filamentous junctions, whereas the follower cells form epithelial adherens junction (AJ)–like adhesion, which also depends on N-cadherin, but not E-cadherin. YAP1 exhibits different localizations: cytoplasmic in high-density followers and nuclear in low-density leaders. Under the control of YAP1/TAZ, N-cadherin is internalized into Rab5-positive endosomes and recycled to the plasma membrane, which may be required for collective glioma migration. **(B)** Pediatric glioma cells migrate on scaffold cells, such as neurons or astrocytes, which is similar to scaffold cell–dependent migration of immature neurons in normally developing cerebral cortex. Scaffold cell–dependent migration of both the PHGG and the normal immature neurons requires N-cadherin. In contrast, N-cadherin suppresses ECM-dependent migration of the PHGG cells. In the developing cerebral cortex, somal N-cadherin levels are decreased during ECM-dependent neuronal migration, although it is unclear whether N-cadherin plays a negative role in the ECM-dependent migration in normally developing brains.

(3). A similar exchange of the leader and follower cells has been reported in other collectively migrating cells, such as border cells in the oogenesis of *Drosophila melanogaster* (9), but the position of the leaders is relatively stable in general (1). In the PHGG cells, 40–60% of the leader cells exchange their places with the followers during the time, moving 35–40 µm, which implies that the leaders and followers may share common features. In fact, RNA sequencing analyses by Kim et al. reveal that the gene expression patterns of the PHGG leader and follower cells are almost the same. Thus, very small sets of mRNAs may cause large differences in the morphologies and behaviors between the leader and follower cells.

A part of YAP-response genes and wound-healing genes only shows higher expression in the leader cells (3). YAP1 is a transcriptional coactivator that acts downstream of cell density (10). In response to low cell density, YAP1 accumulates in the nucleus in the PHGG leaders, whereas it disperses throughout the cytoplasm in the crowded follower cells, suggesting that YAP1 is activated only in the leader cells (Fig. 1 A). Interestingly, when the positions of the leader and follower cells are exchanged, YAP1 localization is also changed; YAP1 becomes concentrated in the nuclei of the new leaders. These data suggest that YAP1 activation in the nucleus is important for characterizing the leader cells.

Knockdown of YAP1 or its related protein, TAZ, decreases cell surface N-cadherin and its internalization in the PHGG leaders, but not in followers, and results in the suppression of collective glioma migration. Because N-cadherin mRNA levels are similar between the PHGG leader and follower cells, YAP1/TAZ may indirectly regulate N-cadherin protein dynamics. Importantly, YAP1 or TAZ depletion does not affect transferrin uptake, indicating that YAP1/TAZ specifically regulates N-cadherin trafficking in the PHGG leader cells, which may determine the leader cell–specific behavior and characteristics during collective glioma migration (Fig. 1 A).

## Similarities between PHGG cell migration and normal embryonic neuronal migration

Glioma cells migrate not only on the extracellular matrix (ECM) but also on other scaffold cells, such as neurons and astrocytes, which may contribute to the widespread and rapid invasion of glioma. The PHGG leader cells form N-cadherin–mediated filamentous junctions with the scaffold neurons or astrocytes (3). Knockdown of N-cadherin in the PHGG cells migrating on the scaffold cells

disturbs the migration directionality and eventually suppresses the overall migration speed. Interestingly, however, N-cadherin knockdown promotes the overall migration speed of the PHGG cells migrating on ECM (laminin or Matrigel) (3). This may also be similar to migrating neurons in normally developing cerebral cortex (Fig. 1 B). During cerebral cortical development, immature neurons mainly show scaffold cell–dependent migration, which requires N-cadherin and its proper trafficking, whereas at the final phase of the migration, these neurons decrease N-cadherin expression at the cell soma to detach from the scaffold cells and exhibit migration depending on α5β1-integrin, a receptor for fibronectin, an ECM molecule (7). Thus, pediatric glioma cells may partly abuse the regulatory systems of scaffold cell–dependent and ECM-dependent migration of neurons in normal developing brain, which may explain the variety of migration patterns of glioma.

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