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CELL MIGRATION

Invasion of the pseudopods

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> “…RAB25 directly interacts with and localizes α5β1 integrin at the pseudopodial tips of migrating cells…”

Rab proteins are members of the Ras GTPase superfamily and are known regulators of membrane-trafficking events. There is an increasing body of evidence that links Rab-regulated recycling pathways to cell polarization, migration and tumour metastasis, but the underlying mechanisms remain unclear. In *Developmental Cell*, Jim Norman and colleagues now report that RAB25 directly interacts with and localizes α5β1 integrin at the pseudopodial tips of migrating cells, thereby contributing to metastatic progression.

Integrins undergo continuous internalization from the plasma membrane into endosomal compartments where they are subsequently recycled. Because α5β1 integrin and RAB25 are both known to influence cancer aggressiveness, Norman and colleagues tested whether RAB25 affected α5β1 recycling and promoted invasive migration.

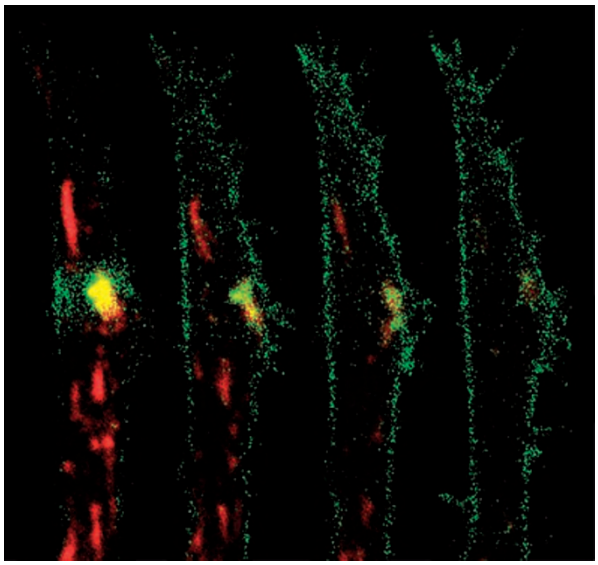
The authors first showed that GTP-bound RAB25 specifically interacted with the β1 subunit cytoplasmic tail of α5β1 in human ovarian A2780 cancer cells, and that binding was mediated by the hypervariable C-terminal domain of RAB25. Migration of A2780 cells on 2D matrices was unaffected by the presence of RAB25, suggesting that it is not involved in regulating the basic migration machinery. However, invasion of 3D matrices increased strongly on RAB25 expression if matrices were supplemented with the α5β1 ligand fibronectin (FN). Blocking α5β1–FN binding reduced migration, thus confirming the importance of α5β1 ligation in RAB25-mediated invasion. Using a chimeric RAB25 protein that was unable to bind α5β1 did not promote invasion, indicating that RAB25 promotes invasiveness through FN-rich matrices by directly binding to and regulating α5β1 integrin.

Analysis of cell migration on 3D matrices containing FN showed that RAB25-dependent invasiveness correlated to a change in cell morphology and type of migration. Cells expressing RAB25 initially extended longer pseudopods bilaterally, only to then retract one process and rapidly migrate in the direction of the persistent pseudopod. As a result, the persistence but not the speed of migration was increased.

The authors went on to investigate α5β1 integrin and RAB25 dynamics during pseudopod-driven invasive migration using high-resolution time-lapse fluorescence imaging and photoactivation experiments. In the absence of RAB25, vesicles containing α5β1 were rarely found at the cell front. Conversely, vesicles containing both RAB25 and α5β1 localized at the pseudopodial tip. α5β1 integrin in RAB25 vesicles was not static, but was continuously recycled to the pseudopodial tip plasma membrane. Furthermore, in cells migrating on cell-derived matrices, RAB25 expression prevented α5β1 integrin dispersion from the cell front, thus retaining a pool of integrin at the pseudopodial tip.

Taken together, these experiments demonstrate that RAB25 interacts directly with the β1 subunit of α5β1 to maintain a pool of actively cycling α5β1 integrin at the tips of extending pseudopodia. This results in the promotion of an invasive mode of cell migration through 3D matrices containing FN. The capacity of a cell to migrate through FN, which is abundant in connective tissues, is vital to metastatic progression, making the RAB25–β1 interaction a potential therapeutic target.

Kim Baumann, Editor,
Cell Migration Gateway



A photoactivatable integrin probe (green) shows exocytic delivery of α5β1 integrin from a RAB25 vesicle (red) to the tip of an advancing pseudopod during migration of an ovarian tumour cell on a 3D matrix. The time sequence (20 sec interval between images) is shown from left to right. Image provided by J.C. Norman, Beatson Institute for Cancer Research, Glasgow, UK.

ORIGINAL RESEARCH PAPER Caswell, P. *et al.* Rab25 associates with α5β1 integrin to promote invasive migration in 3D microenvironments. *Dev. Cell* **13**, 496–510 (2007)
FURTHER READING Jones, M. C. *et al.* Endocytic recycling pathways: emerging regulators of cell migration. *Curr. Opin. Cell Biol.* **18**, 549–557 (2006)