CELL MIGRATION

An ABC of the RHO subfamily

The idea that RHOA, RHOB and RHOC could differ in their functions seems at odds with the 84% sequence similarity shared by these RHO subfamily members, but a report by Vega *et al.* in the *Journal of Cell Biology* now provides definitive evidence that RHOA and RHOC have distinct roles in cell migration and invasion, and indicates that this difference arises through the use of different effectors.

Vega *et al.* used RNA interference (RNAi) to explore the potentially different roles of the RHOA and RHOC isoforms in cell morphology and migration in cancer cells. RHOA depletion caused cells to elongate by extending several narrow protrusions, frequently from both ends and along the lateral sides. On a two-dimensional substrate, RHOA-deficient cells showed reduced overall net movement and migrated persistently only following exposure to a chemoattractant; however, they showed enhanced cell invasion within Matrigel. So, RHOA normally functions to restrict the number of protrusions. By contrast, silencing RHOC increased cell spreading, giving rise to cells with broad lamellipodia that often failed to polarize. Consequently, chemotaxis and invasion in Matrigel were inefficient. The normal function of RHOC, therefore, is to inhibit lamellipodial broadening.

Given these changes in cell shape, a concomitant change in the expression levels or activity of RAC1 might have been expected, but was not observed. Instead, RHOA and RHOC affected the localization of active RAC1. The results indicate that RHOA usually suppresses the activity of RAC1 to limit the number of protrusions, whereas RHOC restricts lamellipodial broadening by inhibiting the spreading of RAC1 activity along the plasma membrane.

To investigate how RHOA and RHOC mediate these effects, Vega *et al.* initially depleted the known downstream effectors RHO kinase 1 (ROCK1) and ROCK2. Knockdown of each individual ROCK isoform induced a similar, but not identical, effect to RHOA depletion. However, simultaneous depletion generated a phenotype strongly resembling that of RHOA deficiency, including an enhanced ability to invade Matrigel. This indicates a specific role for each ROCK isoform downstream of RHOA in morphology and migration. Further analysis suggested that ROCK1 is required for tail retraction, whereas ROCK2 limits the number of protrusions and is implicated in directional migration.

As neither ROCK1 nor ROCK2 depletion resulted in phenotypes similar to those generated

by loss of RHOC, the authors turned their search for RHOC effectors to the formin-like (FMNL) family of proteins, and again used RNAi to target individual members. Depletion of FMNL1 or FMNL3 produced a phenotype that resembled that of RHOC-knockdown cells, but the results of simultaneous depletion experiments implied that RHOC and FMNL3 function in the same pathway. And, as FMNL3 bound to constitutively active recombinant RHOC, localized to cell protrusions and could prevent lamellipodial broadening in RHOC-depleted cells, it seems to be a bona fide RHOC-specific target.

So, despite being closely related, RHOA and RHOC function through different downstream targets to elicit distinct effects on cell morphology, migration and invasion. Their different effectors and distinct effects could explain why RHOA reportedly inhibits cancer cell invasion whereas RHOC expression is often associated with a metastatic phenotype.

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ORIGINAL RESEARCH PAPER Vega, F. M. *et al.* RhoA and RhoC have distinct roles in migration and invasion by acting through different targets. *J. Cell Biol.* **193**, 655–665 (2011)

Compared with control cells (a), RHOA-depleted PC3 cells (b) become elongated and display increased numbers of protrusions (red staining indicates phalloidin-labelled actin), whereas RHOC-depleted cells (c) show broad lamellipodia. Images courtesy of A. Ridley, King's College London, UK.