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Nuclear and Cellular Plasticity: Nuclear RAC1 Takes Center Stage

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Navarro-Lérida et al. (2015) report in this issue of *Developmental Cell* that RAC1 nuclear accumulation causes actin-dependent deformation of the nuclear envelope and increases nuclear plasticity. It further leads to depletion of cytoplasmic, active RAC1 with a concomitant increase in RHOA signaling driving actomyosin-mediated cell shape changes. These two properties combine to enhance tumor cells invasiveness.

RAC1, a member of the RHO family of small GTPases, controls a wide array of cellular functions, including actin remodeling for cell ruffling, adherens junction formation, cell motility, and polarity. RAC1 has also been implicated in cellular transformation and cancer progression via its role in cell invasion (Bosco et al., 2009). A key function of RAC1 is the control of ARP2/3-mediated branched actin polymerization, which is required for the formation of membrane protrusions that drive mesenchymal motility in virtually all cells, including cancer cells (Bisi et al., 2013). Notably, metastatic cancer cells negotiate the diverse microenvironmental conditions they encounter by adopting flexible invasive strategies (Wolf and Friedl, 2011). In “mesenchymal motility,” invasion is achieved by coupling polarized actin-based protrusions with spatially restricted pericellular proteolytic activity in both migrating cells and reactive stromal cells (Wolf and Friedl, 2011). In “amoeboid motility,” by contrast, cancer cells use actomyosin-based mechanical forces to displace matrix fibrils while adopting a rounded cell shape. Whereas mesenchymal motility depends on RAC1, amoeboid migration involves RHOA (Sanz-Moreno et al., 2011). Thus, a tight interplay between these GTPases dictates individual tumor cells’ invasion strategy. Furthermore, these cells must constantly deform and plastically adapt their nuclei to squeeze through narrow gaps of interstitial tissues (Wolf and Friedl, 2011). In this issue of *Developmental Cell*, Navarro-Lérida et al. (2015) show that nucleocytoplasmic shuttling of RAC1 impacts both cell shape—by regulating the balance of cytoplasmic RAC1/RHOA activities—and nuclear plasticity and deformability, thus contributing to the regulation of cell invasion.

Numerous guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), combined with a variety of downstream effectors, enable RAC1 to produce a diverse array of signaling outputs (Bosco et al., 2009). However, the differential regulation of signaling by RAC1 in different contexts remains poorly understood. Furthermore, increasing evidence suggests that subcellular compartmentalization plays a major role in regulating the signaling output of RAC1. In addition to the cytosol and plasma membrane, RAC1 also localizes to endosomes (Palamidessi et al., 2008) and the nucleus (Kraynov et al., 2000). RAC1 activation on early endosomes and subsequent recycling of RAC1 to the plasma membrane ensure polarized signaling, leading to localized actin-based migratory protrusions (Palamidessi et al., 2008) and spatial restriction of RAC1 motogenic signals, which promotes mesenchymal motility. In the nucleus, accumulation of RAC1 has been linked to regulation of RAC1 proteosomal degradation (Lanning et al., 2004). Additionally, cell-cycle-dependent accumulation of nuclear RAC1 promotes mitotic progression (Michaelson et al., 2008). These findings suggest that nucleocytoplasmic shuttling is important for the spatial control of specific RAC1 functions.

An elegant study by the Del Pozo group now examines the processes that contribute to, and are impacted by, RAC1 nucleocytoplasmic shuttling (Navarro-Lérida et al., 2015). The authors, through a proteomic approach, initially identified nucleophosmin (nucleolar phosphoprotein B23) as a nuclear binding partner of RAC1. B23 is a multifunctional phosphoprotein involved in ribosome biogenesis. It shuttles between the nucleus and the cytoplasm, interacting with a variety of proteins and acting as a bona fide chaperone (Colombo et al., 2011). Indeed, the authors show that RAC1 utilizes B23 for nucleocytoplasmic shuttling and that the

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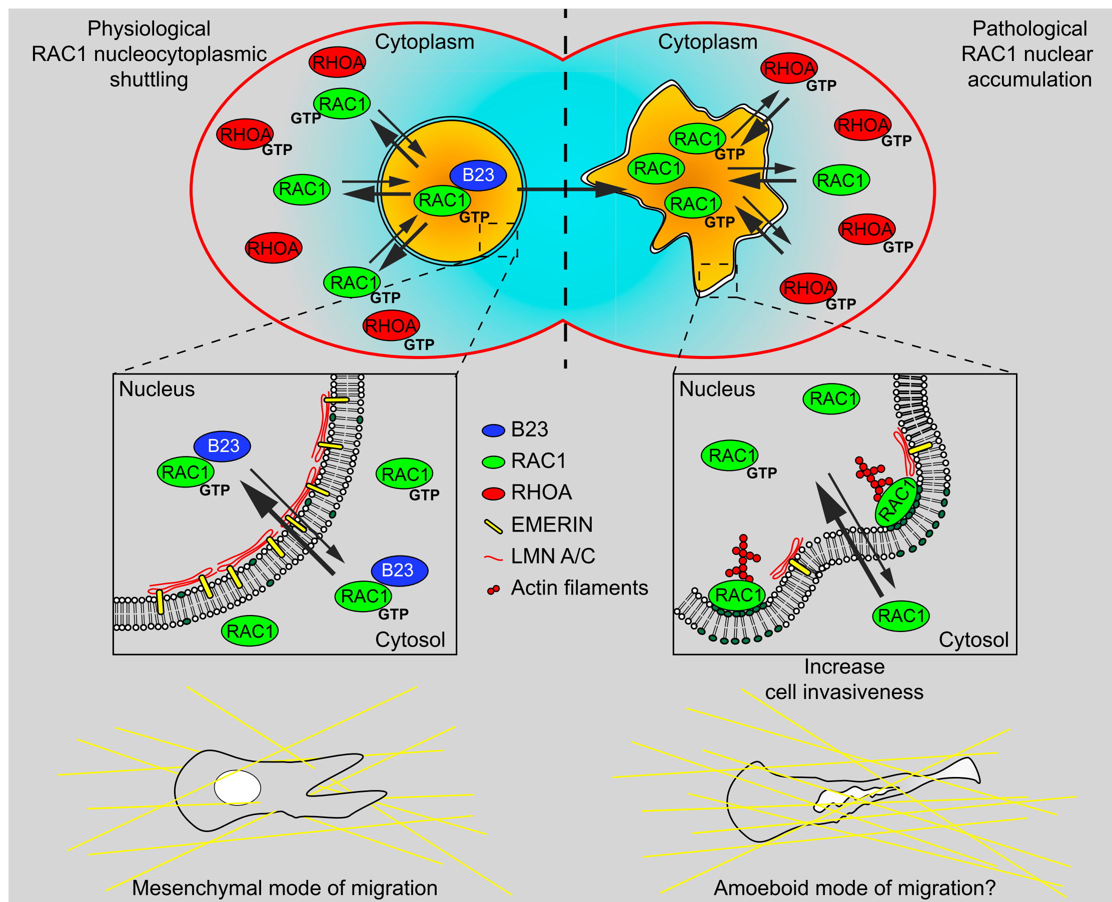

Figure 1. RAC1 Shuttling and Cell Invasion

Nucleocytoplasmic shuttling of RAC1 controls the organization of the nuclear membrane and the amount of RAC1 available outside the nucleus, leading to actin-dependent changes of nuclear and cell shape linked to invasiveness. In cells, a fraction of RAC1 shuttles in and out of the nucleus. Whereas RAC1 nuclear entry is mediated by canonical nuclear import signaling (not shown), RAC1 exit is promoted by its association with nucleophosmin (nucleolar phosphoprotein B23), a multi-functional phosphoprotein that shuttles between the nucleus and the cytoplasm, acting as a chaperone. Nucleocytoplasmic shuttling buffers the amounts and activity of cytoplasmic RAC1; it further controls the balance between cytosolic RAC1 and RHOA activity and signaling. Accumulation of RAC1 in the nucleus, which is detected in aggressive cancer cells, leads to actin polymerization-dependent alterations in nuclear membrane organization, including aberrant distribution of Lamin A/C, major structural proteins of the nuclear lamina, and Emerin, an integral nuclear transmembrane protein. These alterations correlate with dramatic changes in nuclear shape and deformability. The increased cytoplasmic RHOA signaling and nuclear deformability, caused by nuclear accumulation of RAC1, combine to enhance tumor cell migratory plasticity, possibly promoting a switch from a mesenchymal to an amoeboid mode of motility, thus increasing invasiveness.

interaction with B23 depends on the N-terminal 88 amino acids of RAC1. Notably, the same region is also contacted by RHOGDI, a guanine nucleotide dissociation inhibitor that binds cytoplasmic RAC1, preventing its membrane association. This latter finding suggests that competition between RHOGDI and B23 for RAC1 binding determines its cytoplasmic/nucleoplasmic distribution. In addition, the authors discovered two nuclear export sequences (NES1-2) in RAC1, indicating that canonical nuclear export routes and B23 chaperone activity cooperate to control the rate of RAC1 exit from the nucleus.

Functionally, B23 expression promoted, whereas B23 silencing impaired, RAC1 nuclear export. Interestingly, B23 interacted primarily with GTP-bound RAC1, suggesting that nuclear RAC1 might be active. The use of a FRET-RAC1 sensor confirmed this prediction. More strikingly, forced nuclear accumulation of RAC1, by ectopic expression either of the wild-type or of NES mutants, dramatically deformed nuclear shape. Under these conditions, RAC1 displayed a nearly vesicular nuclear distribution, with obvious accumulation in discrete patches. These alterations were accompanied by the aberrant redistribution of Lamin A/C, major structural proteins of the nuclear lamina, and Emerin, an integral nuclear transmembrane protein. Activated nuclear RAC1 also partitioned into nuclear membrane microdomains, similar to liquid-ordered regions of the plasma membrane. This suggested that RAC1 might act as a nuclear membrane organizer for the assembly of signaling platforms. These platforms contain filamentous actin and the ARP2/3 complex. Additionally, both nuclear deformation and altered nuclear shape depended on RAC1-driven ARP2/3 activation and actin polymerization. Thus, the first functional consequence of accumulated nuclear RAC1 is to promote nuclear envelope-localized actin dynamics that drive nuclear deformation and plasticity (Figure 1).

RAC1 nuclear accumulation was mirrored by a decrease in active,

cytoplasmic RAC1, with a concomitant elevation of RHOA-GTP. This shift in the cytoplasmic ratio of RAC1 to RHOA activity led to RHOA-dependent activation of ROCK and increased actomyosin contractility, mimicking biochemical changes known to promote a switch from a mesenchymal to an amoeboid mode of migration. Additionally, pericellular proteolytic activity increased in cells depleted for B23 and depended on RHOA-ROCK activity. Functionally, accumulation of nuclear RAC1 enabled cells to invade a 3D matrix and to migrate through transwell filters of limited pore size, where nuclear deformability becomes a critical factor. Thus, nuclear sequestration of RAC1 results in elevated RHOA signaling, actomyosin contractility, and increased nuclear plasticity that combine to promote tumor cell invasiveness (Figure 1). Not surprisingly, the authors found nuclear accumulation of RAC1 in aggressive lung and prostate carcinoma and in an acute myeloid leukemia caused by B23 mutations that result in inappropriate cytoplasmic relocalization of the protein.

Outstanding questions remain. For example, how is nuclear RAC1 activated? What are the key RAC1 effectors promoting ARP2/3-dependent nuclear actin polymerization? Is nuclear accumulation of RAC1 sufficient to promote amoeboid motility in 3D? What causes the accumulation of RAC1 in the nucleus of aggressive tumors? Although future studies will be required to address these issues, the study from Navarro-Lérida et al. provides insight into how nucleocytoplasmic shuttling of RAC1 controls nuclear and cellular plasticity in tumors by spatially restricting RAC1 activity.

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