Cell Cycle 12:18, 2933–2934; September 15, 2013; © 2013 Landes Bioscience

A new signaling paradigm to control the prenylation and trafficking of small GTPases

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Members of the Ras and Rho families of small GTPases regulate diverse signaling pathways that promote normal physiological processes as well as diseases such as cancer.[1,2] The localization of small GTPases in distinct subcellular regions defines which signaling pathways they activate, thus defining their participation in disease. The plasma membrane is viewed as the region of highest activity for small GTPases,[1,2] due to surface receptors that activate membrane-associated complexes consisting of small GTPases, their guanine nucleotide exchange factors, and their downstream effectors.

Inhibiting the membrane localization of small GTPases is a therapeutic strategy in cancer.[1,2] Clinical trials of lonafarnib and tipifarnib that block trafficking of newly synthesized small GTPases to the plasma membrane have met with limited success,[2] in part because we do not completely understand how newly synthesized small GTPases reach the plasma membrane, nor do we know how signaling is altered when small GTPases accumulate in regions away from the plasma membrane. Our studies of small GTPases interacting with the chaperone protein SmgGDS address these gaps in our knowledge.

Splice variants of SmgGDS, named SmgGDS-607 and SmgGDS-558, help newly synthesized small GTPases reach the plasma membrane.[3,4] An electronegative patch in SmgGDS binds the positively charged C-terminal polybasic region (PBR) in multiple Ras and Rho family members,[5] which may enhance the ability of SmgGDS to promote malignancy.[3] SmgGDS-607 binds small GTPases soon after their synthesis and helps escort them to cytosolic prenyltransferases that attach an isoprenoid to the CaaX motif adjacent to the PBR.[3] SmgGDS-558 intercepts prenylated small GTPases and may escort them to the endoplasmic reticulum for post-prenylation processing, followed by trafficking to the plasma membrane.[3] Prenylated small GTPases anchor at the plasma membrane by inserting their isoprenoid moiety into the phospholipid bilayer.[1,2]

The PBR-dependent binding of small GTPases to SmgGDS provides new insights into how the PBR promotes membrane localization of small GTPases. The PBR is thought to promote electrostatic interactions of prenylated small GTPases with negatively charged phospholipids at the plasma membrane.[1] We now know that the PBR also facilitates electrostatic interactions between non-prenylated small GTPases and SmgGDS-607, which regulates their entry into the prenylation pathway, and between prenylated small GTPases and SmgGDS-558, which promotes their trafficking to the plasma membrane.[3] These newly defined PBR functions suggest that signaling cascades that control the PBR-dependent interaction of small GTPases with SmgGDS will control the prenylation and membrane trafficking of small GTPases.

The paucity of known signaling cascades that regulate the prenylation of small GTPases supports the conventional view that newly synthesized small GTPases are constitutively prenylated without cellular regulation.[3,4] Constitutive prenylation will cause newly synthesized small GTPases to constitutively traffic to the plasma membrane, potentially restricting them from localizing in other subcellular compartments. Our recent discovery of an adenosine-mediated signaling pathway that suppresses small GTPase prenylation provides a mechanism to suppress membrane trafficking of newly synthesized small GTPases and promote their nuclear and cytosolic accumulation.[4]

We found that activation of A2B adenosine receptors (A2BR) causes protein kinase A to phosphorylate the PBR of newly synthesized Rap1B, which inhibits its interaction with SmgGDS-607 and suppresses Rap1B prenylation.[4] This lack of prenylation reduces Rap1B trafficking to the plasma membrane and diminishes Rap1B-mediated cell–cell adhesion, resulting in increased cell scattering and promoting the invasive phenotype[4] (**Fig. 1A**). We detected reduced Rap1B prenylation in human lung, breast, and pancreatic cancer cell lines and in rat mammary tumors, indicating that this pathway operates in multiple types of cancer.[4]

The adenosine-mediated suppression of Rap1B prenylation has broad implications. The high concentration of adenosine in the tumor microenvironment[6] could increase tumor metastasis by reducing Rap1B prenylation and promoting tumor cell dispersion. This pathway might explain why A2BR activation promotes tumor metastasis in animal models.[7,8] It also provides a rationale for testing A2BR antagonists as drugs to suppress metastasis. Our observation that suppressing Rap1B membrane localization promotes the invasive phenotype[4] indicates that therapeutically inhibiting the membrane localization of some small GTPases might unexpectedly promote, rather than suppress, malignancy.

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Submitted: 07/08/13; Accepted: 07/10/13
http://dx.doi.org/10.4161/cc.26230
Comment on: Ntantie E, et al. Sci Signal 2013; 6:ra39; PMID:23716716; http://dx.doi.org/10.1126/scisignal.2003374

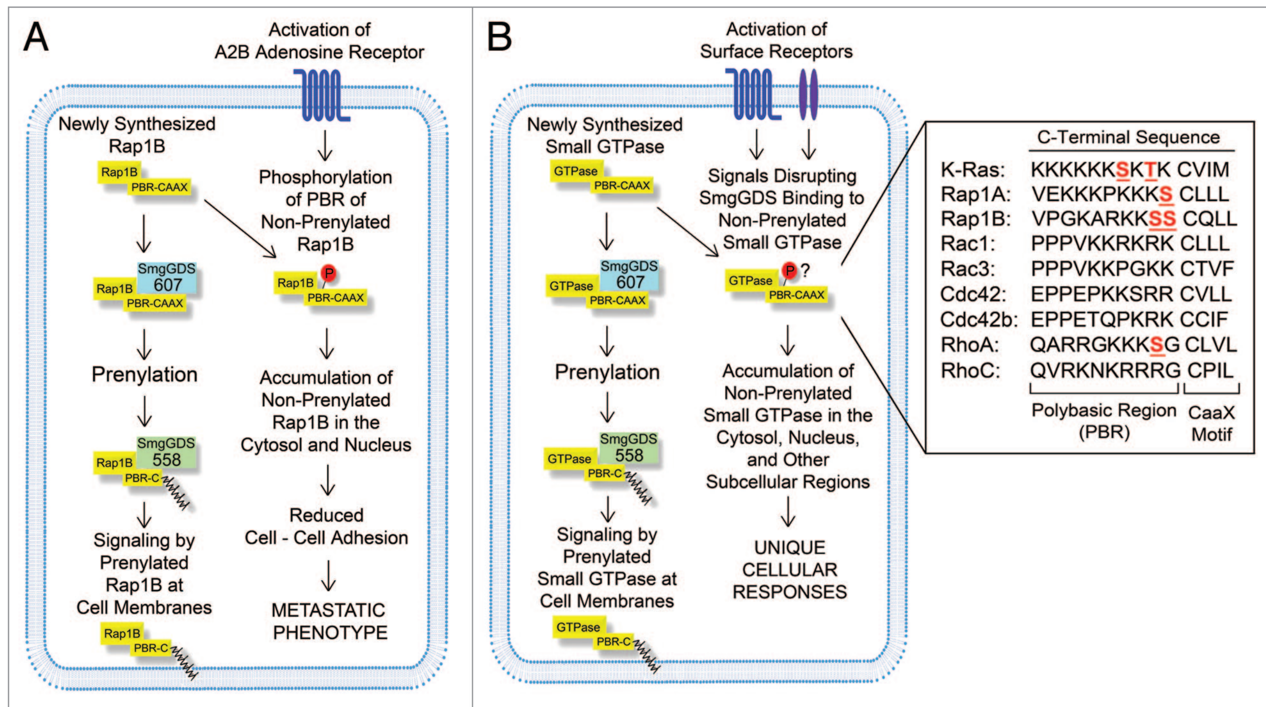

Figure 1. Signaling cascades that alter interactions of small GTPases with SmgGDS provide a novel mechanism to control the prenylation, trafficking, and activity of small GTPases.[3,4] (**A**) The PBR-dependent interaction of newly synthesized Rap1B with SmgGDS may help Rap1B become prenylated and traffic to the cell membrane, where Rap1B signals to promote cell–cell adhesion. A2BR activation phosphorylates the PBR of newly synthesized Rap1B, inhibiting its interactions with SmgGDS and reducing its prenylation and membrane trafficking. These events reduce cell–cell adhesion and promote the metastatic phenotype.[4] (**B**) SmgGDS splice variants may promote the prenylation and membrane trafficking of multiple PBR-containing small GTPases. Receptor-mediated signaling cascades could inhibit prenylation and membrane trafficking of newly synthesized small GTPases by disrupting their interactions with SmgGDS, either through phosphorylation of the PBR, or through other post-translational modifications of small GTPases and SmgGDS. These events may promote unique cellular responses due to small GTPases accumulating and signaling in regions other than the plasma membrane. The inset shows the C-terminal sequences of PBR-containing small GTPases that interact with SmgGDS. Reported phosphorylation sites are indicated by red, underlined residues.

This pathway defines a new signaling paradigm to regulate the prenylation and trafficking of small GTPases. Chronic receptor activation by agonists concentrated in the extracellular microenvironment (including A2BR activated by adenosine or β-adrenergic receptors activated by epinephrine[6]) could phosphorylate the PBRs of different small GTPases as they are synthesized, reducing their interaction with SmgGDS and suppressing their prenylation and trafficking to the plasma membrane. Additional signals that inhibit binding of SmgGDS to a small GTPase could similarly disrupt prenylation and trafficking of the small GTPase. These events could suppress membrane signaling and enhance cytosolic and nuclear signaling by the small GTPase, inducing unique cellular responses (**Fig. 1B**). These pathways would increase the signaling repertoire of small GTPases in different subcellular regions.

The discovery that prenylation of specific small GTPases can be regulated by signaling cascades activated by druggable surface receptors[4] greatly expands the therapeutic targets for manipulating the prenylation, trafficking, and activity of small GTPases in human disease. Future studies should address how these receptor-mediated pathways promote the participation of small GTPases in normal physiological processes, and examine methods to therapeutically target these pathways in cancer and other pathological conditions.

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