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AUTHOR'S VIEWS

C3G self-regulatory mechanism revealed: implications for hematopoietic malignancies

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ABSTRACT

Abnormally increased signaling by the GTPase RAP1 favors progression of diverse tumors. We have characterized the auto-regulation and activation of C3G (RAPGEF1), an activator of RAP1. This led us to discover mutations in non-Hodgkin's lymphomas that activate C3G-RAP1 constitutively, suggesting that deregulation of C3G may favor the dissemination of tumor cells.

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RAP1 (RAS-associated protein-1) proteins are small guanosine triphosphatases (GTPases) of the RAS family. RAP1 are molecular switches; they are activated by guanine nucleotide exchange factors (GEFs) that promote the active form bound to guanosine triphosphate (GTP), and are brought to the inactive state bound to guanosine diphosphate (GDP) by GTPase-activating proteins (GAPs) that stimulate GTP hydrolysis. In normal tissues RAP1 are key regulators of cell adhesion, motility, and polarity.[1] Abnormally increased activation of RAP1 has been implicated in tumor progression.[2]

RAPGEF1, best known as C3G (CRK SRC homology 3 (SH3) domain-binding GEF) is a ubiquitously expressed GEF of RAP1. C3G activates integrin-mediated cell adhesion through RAP1;[3] for example, using a platelet-specific C3G knock-out mouse model, we have recently shown that C3G is required for RAP1B-mediated activation of integrin αIIbβ3 in platelets after stimulation with phorbol 12-myristate 13-acetate (PMA) or thrombin.[4] C3G also regulates migration, actin remodeling, apoptosis, proliferation, differentiation and exocytosis.[5] C3G may act as a tumor suppressor or promoter depending on the type of cancer.

In our recent work[6] we have characterized the mechanisms of C3G regulation and their potential alterations in diseases. C3G has a C-terminal GEF catalytic region that contains a RAS exchanger motif (REM) and a Cdc25 homology domain (Cdc25HD), the latter harbors the GEF activity. The non-catalytic regions include the N-terminal domain (NTD) and the central SH3-binding (SH3b) domain. The SH3b contains five Pro-rich motifs (P0-P4) that are binding sites for proteins with SH3 domains. We have shown that the final part of the SH3b binds to the Cdc25HD and blocks the GEF activity; thus, we named this segment the autoinhibitory region (AIR) (Figure 1a). We identified two sub-regions within the AIR. The first part is sufficient and necessary for binding to Cdc25HD, but does not repress the GEF activity; hence, we named it Cdc25HD-binding region (AIR-CBR). A second part, named the inhibitory tail (AIR-IT), is required for blocking the GEF activity; even though it was not sufficient for binding to the Cdc25HD. Collectively, the AIR/Cdc25HD autoinhibition resembles a lock-and-lid mechanism; the AIR-IT acts as a lid that probably blocks the GTPase-binding site, while the AIR-CBR functions as a lock that maintains the lid in place.

We also studied the mechanisms of physiological activation of C3G by signals that induce tyrosine phosphorylation of proteins. Upon stimulation, CRK (CT10 (chicken tumor virus number 10) regulator of kinase) adaptor proteins CRKII and CRK-like (CRKL) recruit C3G to signaling sites, where it is phosphorylated by SRC and other tyrosine kinases (Figure 1a). CRK proteins also stimulate the GEF activity of C3G directly. We showed that *in vitro* binding of CRKL or phosphorylation by SRC activated C3G independently. CRKL binding to the P3 motif, adjacent to the AIR-CBR, displaces the AIR/Cdc25HD interaction and releases the autoinhibition. Phosphorylation of the AIR by SRC did not prevent binding to the Cdc25HD. Nonetheless, the phospho-AIR blocked the Cdc25HD slightly less efficiently than unphosphorylated AIR, suggesting that phosphorylation acts on a regulatory element outside the AIR-CBR. When combined, CRKL and SRC induced a higher activation than each of them individually, indicating that they are independent and additive stimuli.

We also identified three residues in the AIR-CBR (M551, Y554, and M555) that are essential for binding to the Cdc25HD. Individual mutation of these residues in the full-length protein induced constitutive activation, both *in vitro*, and in HEK293T

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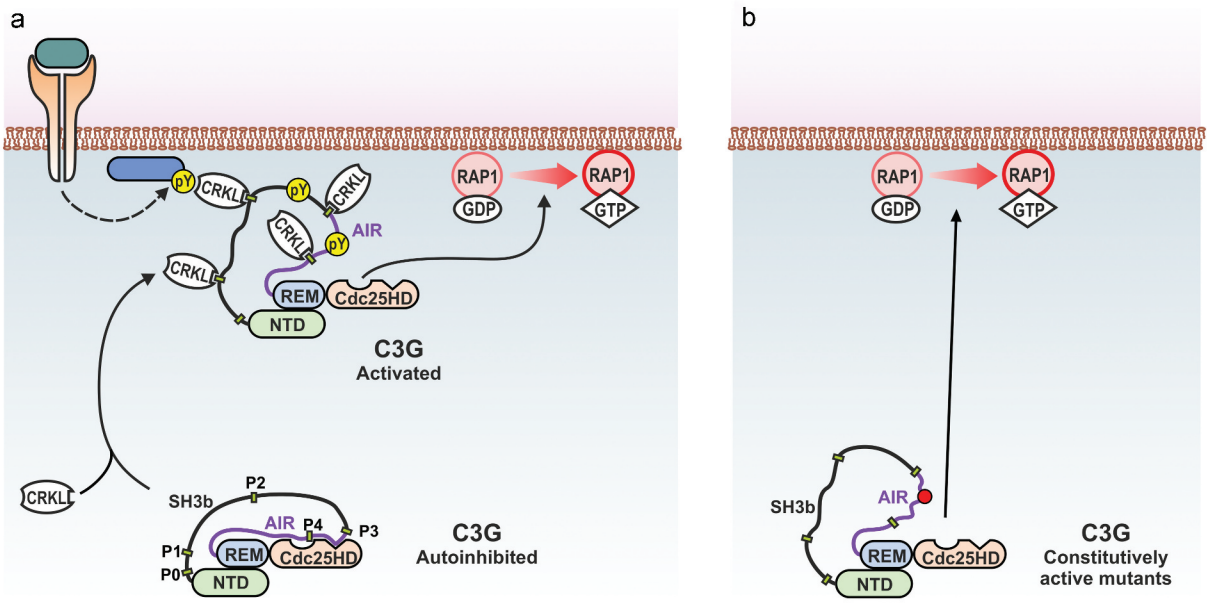

Figure 1. Mechanisms of C3G activation and deregulation. (a) The guanine nucleotide exchange (GEF) activity of unstimulated C3G (RAPGEF1) is self-repressed by an interaction between the autoinhibitory region (AIR) and the Cdc25 homology domain (Cdc25HD). C3G is activated by signals that induce tyrosine phosphorylation. The adaptor protein CRK-like (CRKL) recognizes phospho-sites and recruits C3G to signaling sites. C3G is then activated by the combined action of CRKL binding and tyrosine phosphorylation, which displace the autoinhibition, allowing the access of RAP1 to the catalytic site in the Cdc25HD. (b) Mutations that disrupt the AIR/Cdc25HD interaction, such as Y554H and M555K found in non-Hodgkin's lymphomas, cause constitutive activation of C3G. Those mutants bypass the requirement of CRKL and tyrosine-phosphorylation for direct stimulation of C3G resulting in aberrant activation of RAP1.

and Ba/F3 cells (Figure 1b). The activity of some of these mutants (e.g. Y554R) was similar to that of the wild type C3G activated by CRKL and SRC combined; supporting the notion that the AIR/Cdc25HD interaction is the main autoinhibitory mechanism.

The activation of C3G by point mutations was a crucial observation because such changes can be caused by single-nucleotide variants (SNVs) in the *RAPGEF1* gene. This led us to identify two missense mutations detected in non-Hodgkin's lymphomas (Y554H and M555K) that cause constitutive activation of the C3G-RAP1 signaling axis, leading to the activation of integrin LFA-1 (lymphocyte function-associated antigen-1) in Ba/F3 cells.

The impact of abnormal activation of C3G in tumors had been explored using an engineered farnesylated form of C3G (C3G-F) that is targeted to the membrane, causing constitutive activation of RAP1. Mice transplanted with hematopoietic progenitor cells expressing C3G-F and lacking SIPA1 (signal-induced proliferation-associated 1, also known as SPA-1), which is a GAP of RAP1, develop T-cell acute lymphoblastic leukemia,[7] which suggests a tumor-promoting role of sustained C3G stimulation.

The C3G-activating SNVs that we have identified provide a novel mechanism for the acquisition of overstimulated RAP1 signaling during the somatic evolution of tumors, which might favor the progression and dissemination of some hematological malignancies. For example, high expression of CD38 in chronic lymphocytic leukemia cells leads to abnormal stimulation of the RAP1 GEF RASGRP2 and activation of RAP1, which results in increased adhesion and migration of cancer cells.[8]

Additionally, we have identified a second intramolecular interaction between the NTD and REM domains, which was not required for autoinhibition of C3G. On the contrary, the NTD/REM contact positively regulates the GEF activity of C3G, both before and after activation by CRKL and SRC. These observations suggest that the Cdc25HD might be allosterically regulated by the NTD through the REM, which is reminiscent of the allosteric activation of the GEF Son of Sevenless (SOS) by RAS-GTP.[9] Noteworthy, chronic myeloid leukemia (CML) cells mainly overexpress the variant p87C3G that lacks the NTD;[10] the attenuated GEF activity of p87C3G might be linked to its overexpression in CML cells.

In summary, the detailed characterization of the autoregulation and activation of C3G has been fundamental to identify gain-of-function mutations present in lymphomas, which confer potentially pro-migratory characteristics. C3G-activating mutations might serve as predictive biomarkers, and when present in tumors, the inhibition of the GEF activity of C3G might be beneficial.

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Disclosure of Potential Conflicts of Interest

The authors report no conflict of interest.

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