β2-Chimaerin in cancer signaling: Connecting cell adhesion and MAP kinase activation

Stephen P. Bruinsma
Washington University School of Medicine in St. Louis

Thomas J. Baranski
Washington University School of Medicine in St. Louis

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Extra View

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Connecting Cell Adhesion and MAP Kinase Activation

ABSTRACT

The chimaerins are Rac GTPase-activating proteins that bind diacylglycerol. Emerging evidence implicates β2-chimaerin in tumor progression. Here, we discuss our recent work in Drosophila melanogaster in the context of previous studies performed in human cancer cell lines that together lend new mechanistic insight into the role of chimaerins in cancer.

The chimaerins are a family of GTPase-activating proteins (GAPs) that are regulated by the lipid diacylglycerol (DAG). Conserved homologs from worms to humans (Fig. 1) exhibit a characteristic tripartite structure: an N-terminal SH2 domain, a C1 domain that binds DAG and phorbol esters,1,2 and a C-terminal GAP domain that selectively inactivates Rac.3,4 Mammalian genomes contain two chimaerin loci, each of which produces at least two splice variants: a full-length transcript (α2- and β2-chimaerin, respectively) and a truncated transcript (α1- and β1-) that lacks the SH2 domain.3,4 For a recent review on the structure and function of chimaerins, please see ref. 5.

Rho-family GTPases such as Rac function as molecular switches, failing to bind and activate effectors when bound to GDP, but interacting with various downstream targets to promote signaling in the GTP-bound state. GAPs such as the chimaerins catalyze an increase in the slow endogenous rate of GTP hydrolysis to GDP, causing a conformational shift to the inactive state, while guanine nucleotide exchange factors (GEFs) catalyze the release of GDP (subsequently replaced by the binding of GTP) thereby shifting the equilibrium toward activation.

Spatial and temporal regulation of Rac activity is of critical importance to cells, as Rac signaling has been shown to regulate virtually every aspect of cell biology including cell motility, adhesion, proliferation, apoptosis, and cytoskeletal organization.5,6 Activators and inhibitors of Rac signaling, then, are important not simply for ‘flipping the switch’, but also for imposing specificity of action by localizing Rac activity to the appropriate place at the appropriate time. Mechanistically, it is thought that the cell accomplishes this by expressing a relatively large number of distinct GAPs and GEFs, each with a unique set of binding partners, regulatory domains, and functional domains. The fruit fly genome, for example, encodes six Rho family genes but at least 21 Rho family GAPs and an approximately equal number of Rho GEFs. Systematic knockdown of each fruit fly RhoGAP by siRNA revealed that while the knockdown of most GAPs failed to show an obvious phenotype, several GAPs were required for normal development and survival.7 Similarly, although there are only about 22 Rho family genes in the human genome, three or four of which are Racs, there are approximately 70 Rho family GEFs and 80 Rho family GAPs that individually modulate the activity of Rac or other Rho family GTPases in the context of specific cellular events.8 The physiologic function of most of these regulators is unknown, although interestingly, many GEFs have been identified as oncogenes (see Table 1). As proteins that act in opposition to GEFs, GAPs might be expected to function as tumor suppressors in some contexts. While fewer studies linking GAPs to cancer have been published, some examples have recently been reported in ref. 9 (Table 1).

β2-CHIMAERIN AS A TUMOR SUPPRESSOR

Emerging evidence implicates β2-chimaerin as a tumor suppressor. Levels of β2-chimaerin are reduced in multiple types of cancer including breast cancer, duodenal adenocarcinomas10 and malignant gliomas.11 This phenomenon has been investigated by
the overexpression (or restoration of expression) of β2-chimaerin in cell lines that are models for these cancers. For example, overexpression of β2-chimaerin in a breast cancer cell line inhibits proliferation,\(^1\) while overexpression of the β2-chimaerin GAP domain in a mouse mammary cancer cell line reduced the growth rate and metastatic potential of tumors in vivo.\(^2\) These data suggest that the down regulation of β2-chimaerin in cancers is not merely incidental; rather, β2-chimaerin constitutes one of the multiple targets of metastatic transformation, and restoration of its activity in tumor cells could potentially induce more 'normal' cellular behavior.

Conversely, a prediction of this hypothesis is that in healthy epithelial tissue, reducing β2-chimaerin levels could bias toward tumorigenesis. Unfortunately, there is currently no chimaerin knockout mouse model, while knockdown of the α2-chimaerin homolog in zebrafish results in the death of most embryos by day five due to major morphologic defects;\(^3\) thus, work in these model organisms has not yet evaluated the above prediction. Recently, we utilized the fruit fly Drosophila melanogaster to investigate the role of endogenous chimaerin in a native epithelium.\(^4\) The eye of the fruit fly is a simple neuroepithelium with defined cell types present in stereotypic number and morphology, making it amenable to analysis of cell number and cell-cell contacts. It has been described as a model system for the study of cancers,\(^5\) including medullary thyroid\(^6\) and ovarian carcinomas.\(^7\) The fly genome contains a single chimaerin gene, RhoGAP5A, whose gene product is expressed from early embryo to adulthood in multiple tissues, including the pupal eye.\(^8\) Strikingly, reduction of RhoGAP5A levels in the fly eye results in an increase in cell number and aberrant cell-cell adhesion, consistent with a progression to a more ‘tumor-like’ phenotype.

**MOLECULAR MECHANISMS OF β2-CHIMAERIN SIGNALING IN CANCER**

Although our understanding of β2-chimaerin’s role in tumor progression is incomplete, the data accumulated from fruit fly and cancer cell models provide several clues as to the mechanism. General principles, discussed in more detail below, are: (1) the effects of chimaerin are mediated, at least in part, by interactions with Rac; (2) this interaction occurs downstream of growth factor receptors (possibly in response to the generation of DAG) and affects the activation state of ERK MAP kinase; and (3) chimaerin modulates the effects of Rac on cell-cell adhesion.

Chimaerin functions by modulating Rac activity. The interplay between Rac and chimaerin in cancer signaling is somewhat intuitive, based on the biochemical characterization of chimaerins as inhibitors of Rac coupled with the observation that Rac itself shows increased activity in a variety of human carcinomas including breast, colorectal, and pancreatic cancers.\(^9\) The data bear this out: in addition to inhibiting proliferation, β2-chimaerin expression in breast cancer cell lines reduces the amount of active Rac in the cell. Furthermore, coexpression of a constitutively active Rac mutant (unable to be inactivated by chimaerin) abolishes this effect,\(^10\) as expected if β2-chimaerin is regulating proliferation through Rac. Similarly, in the fly eye, the phenotype of loss of RhoGAP5A is both mimicked and enhanced by overexpression of wild-type Rac1. Expression of dominant negative Rac alleles or the use of Rac null mutants also modifies the cell number and cell-cell contacts of the same cells affected by RhoGAP5A loss.

Activation of chimaerin and Rac downstream of EGFR. Growth factors signal through receptor tyrosine kinases such as the EGF receptor (EGFR) to activate Rac. Activation of EGFR also recruits β2-chimaerin to the membrane via generation of DAG, thereby providing feedback inhibition of Rac.\(^11\) Accordingly, overexpression of β2-chimaerin in breast cancer cells suppresses the growth factor-dependent activation of Rac, leading to a reduction in proliferation;\(^10\) interestingly, inactivation of Rac by β2-chimaerin also suppresses the activation of ERK in these cells.\(^12\) Similarly, loss of chimaerin in the fly eye rescues the effects of impaired EGFR signaling, suppressing the mutant phenotypes associated with mutations in EGFR, ERK MAP kinase, or upstream inhibition of the pathway by Argos. Thus, data from cancer cell models and the fly eye indicate that chimaerin acts downstream of growth factor receptors to inactivate Rac and modulate growth factor signaling through ERK.

ERK is activated and retained at the plasma membrane in the absence of chimaerin. Experiments in the fly eye reveal an additional layer of complexity at the intersection between chimaerin and ERK signaling: decreasing the levels of RhoGAP5A results in increased levels of activated (dual-phosphorylated) ERK, and this ERK localization is tightly linked to its function: activated ERK phosphorylates different targets in the nucleus and the cytoplasm,\(^13\) leading to distinct physiologic consequences.\(^14\) Although in the steady state ERK typically shows nuclear or cytoplasmic localization, its activation is thought to occur at membranes, mediated by binding to scaffolding proteins that bring ERK into proximity with upstream activating kinases such as Raf.\(^15\) Disruption of ERK activity at specific membranes by the loss of scaffold activity can result in altered amplitude\(^16\) or duration\(^17\) of signaling.

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**Figure 1. Chimaerin structure and homology.** The structure of human β2-chimaerin is shown and compared to the other known human isoforms and the fly (RhoGAP5A) and worm (CE39208) chimaerin homologs. All numbers represent percent amino acid identity with human β2-chimaerin within the indicated domain. The splice site, which is identical at both the α- and β-chimaerin loci, is indicated on the human chimaerin isoforms.
junctures is impaired, resulting in aberrant contacts. This phenotype is mimicked by the overexpression of Rac. When overexpression of Rac is combined with down regulation of fly chimaerin, presumably hyperactivating Rac, a synergistic effect is observed, and most adherens junctions between interommidial pigment cells are eliminated.

The level of Rac activity is tightly regulated to properly maintain adherens junctions: too much or too little Rac activity can disrupt adherens junctions in mammalian epithelial cells. The modulation of Rac-mediated disruption of adherens junctions by RhoGAP5A suggests that chimaerins fine-tune Rac activity in the context of cell-cell adhesion.

### TYING TOGETHER PATHWAYS INVOLVED IN TUMOR PROGRESSION

Taken together, experiments in human cancer cell lines and the model organism *Drosophila melanogaster* now link β2-chimaerin to two distinct tumorigenic pathways (Fig. 2): (1) activation of ERK, which can confer growth factor independence, leading to increased cell proliferation and aberrant survival, and (2) adherens junction stability, a critical regulator of epithelial homeostasis. Tumors develop from healthy tissue in a complicated, multi-step process resulting from multiple changes in cell biology. Molecules such as β2-chimaerin, then, that coregulate two or more processes important in tumor progression make likely targets for down regulation in human cancers and potentially promising drug targets for anti-cancer therapies.

How are ERK activation and adherens junction stability coregulated by chimaerin? It is likely that the effects are mediated downstream of Rac. In some cell types, Rac is required for the activation of ERK through activation of p21-activated kinase (PAK), which phosphorylates both MEK and Raf98,49 and can bind directly to ERK.50,51 Rac is also known to regulate adherens junctions by mechanisms that are not fully understood but appear to involve antagonism of Rho via p120-catenin and p190RhoGAP.52 Given the membrane localization of ERK in chimaerin-deficient cells, it is tempting to speculate that ERK phosphorylates targets responsible for adherens junction stability. Activation of ERK signaling disrupts adherens junctions in some cell lines,53,54 while impairment of ERK signaling by loss of its activator, prohibitin, leads to an increase in the strength of adherens junctions.55

Many important questions remain: if β2-chimaerin is a target for down regulation in cancer, how many other Gaps are also involved in cancer signaling? Is the down regulation of β2-chimaerin a general principle in tumor progression from epithelia, or is this phenomenon limited to a small subset of tumors? Can small molecules be found that specifically modulate the activity of β2-chimaerin, and would they be useful as cancer therapeutics? Given recent progress in the field, we anticipate that answers to these and other questions will be elucidated in the near future as the role of the chimaerins in cancer signaling is explored in more detail.
References


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