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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

β2-Chimaerin in Cancer Signaling

Connecting Cell Adhesion and MAP Kinase Activation

Stephen P. Bruinsma

Thomas J. Baranski*

Department of Medicine and Department of Molecular Biology and Pharmacology; Washington University School of Medicine; St. Louis, Missouri USA

*Correspondence to: Thomas J. Baranski; Washington University School of Medicine; 660 South Euclid Avenue; Campus Box 8127; St. Louis 63110 Missouri; Tel.: 314.747.3997; Fax: 314.362.7641; Email: baranski@wustl.edu;

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KEY WORDS

GAP, MAP kinase, Rac, Rho, EGF, chimerin, chimaerin

ABBREVIATIONS

GAP	GTPase-activating protein
GEF	guanine nucleotide exchange factor
DAG	diacylglycerol
EGF	epidermal growth factor
EGFR	EGF receptor

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 adenocarcinomas[10] and malignant gliomas.[11] This phenomenon has been investigated by

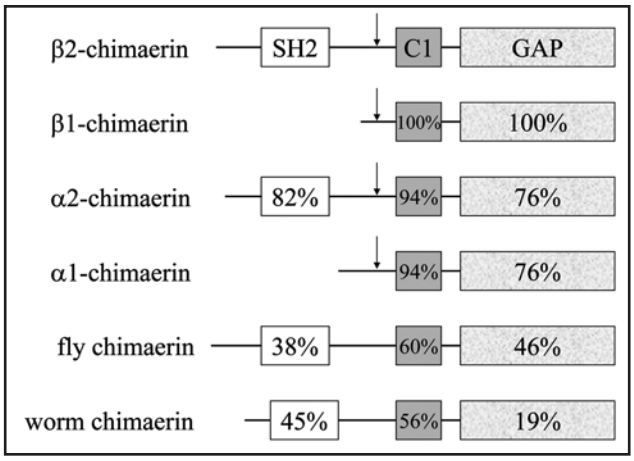

Figure 1. Chimaerin structure and homology. The structure of human β2-chimaerin is shown and compared to the other known human isoforms and the fly (RhoGAP5A) and worm (CE39208) chimaerin homologs. All numbers represent percent amino acid identity with human β2-chimaerin within the indicated domain. The splice site, which is identical at both the α- and β-chimaerin loci, is indicated on the human chimaerin isoforms.

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,[10] 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.[12] 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;[13] 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.[14] 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,[15-17] including medullary thyroid[18] and ovarian carcinomas.[19] 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.[20,21] 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.[22-26] 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.[27,28] 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.[29] 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 localizes specifically to the plasma membrane at cell-cell contacts between interommatidial pigment cells, those cells that endogenously express highest levels of RhoGAP5A. Thus, at least in the fly eye, chimaerin plays a constitutive role in shutting down ERK signaling such that a loss of chimaerin causes an apparent build up of activated ERK at the plasma membrane.

ERK localization is tightly linked to its function: activated ERK phosphorylates different targets in the nucleus and the cytoplasm,[30,31] leading to distinct physiologic consequences.[32,33] 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.[34,35] Disruption of ERK activity at specific membranes by the loss of scaffold activity can result in altered amplitude[36] or duration[37] of signaling.

Table 1 **Rho family GAPs and GEFs that are mutated or differentially expressed in human cancers**

GAPs	Putative Function	Evidence	GEFs	Putative Function	Evidence
β2-chimaerin	tumor suppressor	Decreased protein levels; overexpression[10, 11]	VAV1	oncogene	Protein expression levels[23, 56]
DLC1		mRNA expression, locus deletion, overexpression[57]	Trio		Alt. transcript, amplification, over expression[58, 59]
DLC2		mRNA expression, overexpression[60, 61]	LARG		Fused w/MLL, overexpression[62, 63]
GRAF		Fusion, mutation in leukemia[64]	GEF-H1		Microarray, overexpression[65, 66]
RacGAP1	oncogene	Microarray[67]	Dbl		Oncogenic rearrangement, expression level[68, 69]
ARHGAP21		Differential display, microarray[70]	Ect2		Overexpression, protein expression level[71, 72]
p190B RhoGAP		Differential display, in situ staining of rat tumors[73]	Ost		Overexpression[74, 75]
			Tiam1	tumor suppressor/ oncogene?	Protein expression level, mutants[76, 77]

What is the significance of the location of activated ERK at the plasma membrane in chimaerin-deficient cells? Restriction of ERK to the plasma membrane could be seen as inhibitory, sequestering ERK at a 'dead end' location, away from nuclear or cytosolic targets. For instance, in breast cancer and glioblastoma cell lines, expression of the scaffold PEA-15 sequesters ERK in the cytoplasm, preventing nuclear signaling and reducing tumor cell invasion.[38] This does not appear to be the case in the fly eye: in addition to resulting in a large increase in activated ERK at the plasma membrane, chimaerin-deficiency suppresses the phenotype of impaired EGFR signaling and also increases the levels of Argos, a transcriptional target of nuclear ERK signaling in the fly. Alternatively, retention of ERK at the plasma membrane could lead to the phosphorylation of distinct (currently unknown) membrane targets, resulting in an increase in signaling through their corresponding pathways.

Disruption of adherens junctions in response to aberrant chimaerin and Rac signaling. Adherens junctions are formed by clusters of cadherins on the plasma membrane of neighboring cells binding to one another in trans. These junctions play a critical dual role in epithelial maintenance, both structurally attaching cells to the epithelium and acting as 'signaling centers' for multiple intracellular signaling pathways, thereby coupling cell-cell adhesion to proliferative signals.[39,40] The vast majority of human cancers arise from epithelia, and the down regulation of adherens junctions appears to be critical for metastatic transformation of epithelial tumors.[25,41] Temporally, ERK build up at the plasma membrane in chimaerin-deficient cells occurs in a short burst, approximately 28 hours after puparium formation-a time at which adherens junctions between the eye epithelial cells are in flux. In wild-type eyes, this timing corresponds to a period of cell movement as the pigment cells establish their final position, weakening contacts between neighboring cells, and subsequently establishing cell-cell contacts at their final location. In chimaerin-deficient fly eyes, this final step in pigment cell positioning and the reestablishment of adherens junctions 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[42,43] or too little[44,45] 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.[46,47] 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 Raf[48,49] 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.

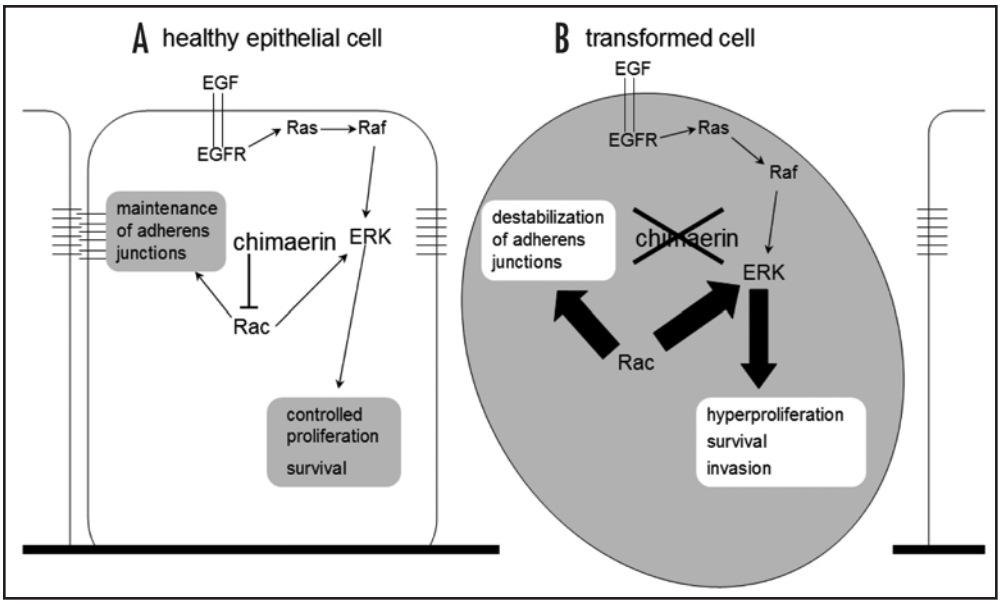

Figure 2. A model for chimaerin signaling in epithelia. In healthy epithelial tissue (A), tightly regulated intracellular signaling ensures the formation of stable contacts between neighboring cells, regulated by Rac activity, and appropriate proliferation and survival, regulated by EGF receptor/ERK and other signaling pathways. In many cell types, Rac is required for full activation of ERK as well. In the fly retinal epithelium, both of these pathways are down regulated by fly chimaerin (RhoGAP5A). When this equilibrium is disturbed by decreased expression of chimaerin (B), increased Rac signaling leads to destabilization of adherens junctions, while increased EGF receptor/ERK signaling permits hyperproliferation and aberrant survival-hallmarks of malignant transformation.

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