Year of publication 2018

Bettoun, A., Joffre, C., Zago, G., Surdez, D., Vallerand, D., Gundogdu, R., Sharif, A.A.D., Gomez, M., Cascone, I., Meunier, B., White, M.A., Codogno, P., Parrini, M.C., Camonis, J.H., and Hergovich, A (2018 Apr 27)

Correction: Mitochondrial clearance by the STK38 kinase supports oncogenic Ras-induced cell transformation

Oncotarget : 9 : 22870 , 22870 : DOI : 10.18632/oncotarget.9875.]

Summary

Costa, A., Kieffer, Y., Scholer-Dahirel, A., Pelon, F., Bourachot, B., Cardon, M., Sirven, P., Magagna, I., Fuhrmann, L., Bernard, C., Bonneau, C., Kondratova, M., Kuperstein, I., Zinovyev, A., Givel, A.-M., Parrini, M.-C., Soumelis, V., Vincent-Salomon, A., and Mechta-Grigoriu, F (2018 Mar 12)

Fibroblast Heterogeneity and Immunosuppressive Environment in Human Breast Cancer

Cancer Cell : 33 : 463,479 : DOI : doi.org/10.1016/j.ccell.2018.01.011

Summary

Carcinoma-associated fibroblasts (CAF) are key players in the tumor microenvironment. Here, we characterize four CAF subsets in breastcancerwith distinct properties and levels of activation. Two myofibroblastic subsets (CAF-S1, CAF-S4) accumulate differentially in triple-negative breastcancers (TNBC). CAF-S1 fibroblasts promote an immunosuppressiveenvironmentthrough a multi-step mechanism. By secreting CXCL12, CAF-S1 attracts $CD4^{+}CD25^{+}$T lymphocytes and retains them by OX40L, PD-L2, and JAM2. Moreover, CAF-S1 increases T lymphocyte survival and promotes their differentiation into $CD25^{High}FOXP3^{High}$, through B7H3, CD73, and DPP4. Finally, in contrast to CAF-S4, CAF-S1 enhances the regulatory T cell capacity to inhibit T effector proliferation. These data are consistent with FOXP3+ T lymphocyte accumulation in CAF-S1-enriched TNBC and show how a CAF subset contributes to immunosuppression.

Year of publication 2017

Remorino A, De Beco S, Cayrac F, Di Federico F, Cornilleau G, Gautreau A, Parrini MC, Masson JB, Dahan M, Coppey M. (2017 Nov 14)

Gradients of Rac1 Nanoclusters Support Spatial Patterns of Rac1 Signaling

Cell Reports : 21 : 1922,1935 : DOI : 10.1016

Summary

Rac1is a small RhoGTPase switch that orchestrates actin branching in space and time and protrusion/retraction cycles of the lamellipodia at the cell front during mesenchymal migration. Biosensor imaging has revealed a graded concentration of active GTP-loaded Rac1in protruding regions of the cell. Here, using single-molecule imaging and super-resolution microscopy, we show an additional supramolecular organization of Rac1. We find that Rac1partitions and is immobilized into nanoclustersof 50-100 molecules each. These nanoclustersassemble because of the interaction of the polybasic tail of Rac1with the phosphoinositide lipids PIP2 and PIP3. The additional interactions with GEFs and possibly GAPs, downstream effectors, and other partners are responsible for an enrichment of Rac1nanoclustersin protruding regions of the cell. Our results show that subcellular patternsof Rac1activity are supported by gradientsof signalingnanodomains of heterogeneous molecular composition, which presumably act as discrete signalingplatforms.

Zago, G., Biondini, M., Camonis, J., and Parrini, M.C (2017 May 12)
A family affair: A Ral-exocyst-centered network links Ras, Rac, Rho signaling to control cell migration
Small GTPases : 1,8 : DOI : 10.1080/21541248.2017.1310649

Summary

Cellmigrationis central to many developmental, physiologic and pathological processes, including cancer progression. The Ral GTPases (RalA and RalB) which act down-stream the Rasoncogenes, are key players in the coordination between membrane trafficking and actin polymerization. A major direct effector of Ral, the exocyst complex, works in polarized exocytosis and is at the center of multiple protein-protein interactions that support cellmigrationby promoting protrusion formation, front-rear polarization, and extra-cellular matrix degradation. In this review we describe the recent advancements in deciphering the molecular mechanisms underlying this role of Ral via exocyst on cellmigration. Among others, we will discuss the recently identified cross-talk between Ral and Rac1 pathways: exocyst binds to a negative regulator (the RacGAP SH3BP1) and to the major effector (the Wave Regulatory Complex, WRC) of Rac1, the master regulator of protrusions. Next challenge will be to better characterize the dynamics in space and in time of these molecular interplays, to better understand the pleiotropic functions of Ral in both normal and cancer cells.

Year of publication 2016

Yamada, A., Renault, R., Chikina, A., Venzac, B., Pereiro, I., Coscoy, S., Verhulsel, M., Parrini, M.C., Villard, C., Viovy, J.-L., and Descroix, S (2016 Nov 29)
Transient microfluidic compartmentalization using actionable microfilaments for biochemical assays, cell culture and organs-on-chip
Lab ChipLab on a Chip : 4691,4701 : DOI : 10.1039/c6lc01143h

Summary

We report here a simple yet robust transientcompartmentalizationsystem for microfluidicplatforms. Cylindrical microfilamentsmade of commercially available fishing lines are embedded in a microfluidicchamber and employed as removable walls, dividing the chamber into several compartments. These partitions allow tight sealing for hours, and can be removed at any time by longitudinal sliding with minimal hydrodynamic perturbation. This allows the easy implementation of various functions, previously impossible or requiring more complex instrumentation. In this study, we demonstrate the applications of our strategy, firstly to trigger chemical diffusion, then to make surface co-coating or cellco-cultureon a two-dimensional substrate, and finally to form multiple cell-laden hydrogel compartments for three-dimensional cellco-culturein a microfluidicdevice. This technology provides easy and low-cost solutions, without the use of pneumatic valves or external equipment, for constructing well-controlled microenvironments for biochemicaland cellular assays.

Marco Biondini, Amel Sadou-Dubourgnoux, Perrine Paul-Gilloteaux, Giulia Zago, Melis D Arslanhan, François Waharte, Etienne Formstecher, Maud Hertzog, Jinchao Yu, Raphael Guerois, Alexis Gautreau, Giorgio Scita, Jacques Camonis, Maria Carla Parrini (2016 Sep 4)

Direct interaction between Exocyst and Wave complexes promotes cell protrusions and motility.

Journal of cell science : DOI : jcs.187336

Summary

Coordination between membrane trafficking and actin polymerization is fundamental in cell migration, but a dynamic view of the underlying molecular mechanisms is still missing. The Rac1 GTPase controls actin polymerization at protrusions by interacting with its effector, the Wave Regulatory Complex (WRC). The Exocyst complex, which functions in polarized exocytosis, has been involved in regulation of cell motility. Here we show a physical and functional connection between Exocyst and WRC. Purified components of Exocyst and WRC complexes directly associate in vitro and interactions interfaces are identified. The Exocyst/WRC interaction is confirmed in cells by co-immunoprecipitation and is shown to occur independently of the Arp2/3 complex. Disruption of the Exocyst/WRC interaction leads to impaired migration. By time-lapse microscopy coupled to image correlation analysis, we visualize the traffic of WRC toward the front in nascent protrusions. Exocyst is necessary for WRC recruitment at the leading edge and for resulting cell edge movements. This direct link between Exocyst and WRC complexes provides a novel mechanistic insight into the spatio-temporal regulation of cell migration.

Bettoun A, Joffre C, Zago G, Surdez D, Vallerand D, Gundogdu R, Sharif AA, Gomez M, Cascone I, Meunier B, White MA, Codogno P, Parrini MC, Camonis JH, Hergovich A. (2016 Jul 12)

Mitochondrial clearance by the STK38 kinase supports oncogenic Ras-induced cell transformation

Oncotarget : DOI : 10.18632

Summary

OncogenicRas signalling occurs frequently in many human cancers. However, no effective targeted therapies are currently available to treat patients suffering from Ras-driven tumours. Therefore, it is imperative to identify downstream effectors of Ras signalling that potentially represent promising new therapeutic options. Particularly, considering that autophagy inhibition can impair the survival of Ras-transformed cellsin tissue culture and mouse models, an understanding of factors regulating the balance between autophagy and apoptosis in Ras-transformed human cellsis needed. Here, we report critical roles of the STK38protein kinasein oncogenicRas transformation. STK38knockdown impaired anoikis resistance, anchorage-independent soft agar growth, and in vivo xenograft growth of Ras-transformed human cells. Mechanistically, STK38supportsRas-driven transformationthrough promoting detachment-induced autophagy. Even more importantly, upon celldetachment STK38is required to sustain the removal of damaged mitochondriaby mitophagy, a selective autophagic process, to prevent excessive mitochondrialreactive oxygen species production that can negatively affect cancer cellsurvival. Significantly, knockdown of PINK1 or Parkin, two positive regulators of mitophagy, also impaired anoikis resistance and anchorage-independent growth of Ras-transformed human cells, while knockdown of USP30, a negative regulator of PINK1/Parkin-mediated mitophagy, restored anchorage-independent growth of STK38-depleted Ras-transformed human cells. Therefore, our findings collectively reveal novel molecular players that determine whether Ras-transformed human cellsdie or survive upon celldetachment, which potentially could be exploited for the development of novel strategies to target Ras-transformed cells.

O Santos A, Parrini MC, Camonis J. (2016 May 5)

RalGPS2 Is Essential for Survival and Cell Cycle Progression of Lung Cancer Cells Independently of Its Established Substrates Ral GTPases.

PLoS One : DOI : 10.1371/journal.pone.0154840

Summary

The human genome contains six genes coding for proteins validated in vitro as specific activators of the small GTPases “Ras-related protein Ral-A” and “Ras-related protein Ral-B”, generically named Ral-guanine nucleotide exchange factors (RalGEF). Ral proteins are important contributors to Ras oncogenic signaling, and RAS oncogenes are important in human Non-Small Cell Lung Carcinoma (NSCLC). Therefore in this work, RalGEF contribution to oncogenic and non-oncogenic features of human NSCLC cell lines, as anchorage-dependent and independent growth, cell survival, and proliferation, was investigated. Among all human RalGEF, silencing of RGL1 and RALGPS1 had no detectable effect. However, silencing of either RGL2, RGL3, RALGDS or, to a larger extent, RALGPS2 inhibited cell population growth in anchorage dependent and independent conditions (up to 90 and 80%, respectively). RALGPS2 silencing also caused an increase in the number of apoptotic cells, up to 45% of the cell population in transformed bronchial BZR cells. In H1299 and A549, two NSCLC cell lines, RALGPS2 silencing caused an arrest of cells in the G0/G1-phase of cell

cycle. Furthermore, it was associated with the modulation of important cell cycle regulators: the E3 Ubiquitin Protein Ligase S-phase kinase-associated protein 2 (Skp2) was strongly down-regulated (both at mRNA and protein levels), and its targets, the cell cycle inhibitors p27 and p21, were up-regulated. These molecular effects were not mimicked by silencing RALA, RALB, or both. However, RALB silencing caused a modest inhibition of cell cycle progression, which in H1299 cells was associated with Cyclin D1 regulation. In conclusion, RALGPS2 is implicated in the control of cell cycle progression and survival in the in vitro growth of NSCLC cell lines. This function is largely independent of Ral GTPases and associated with modulation of Skp2, p27 and p21 cell cycle regulators.

Fatéméh Dubois, Maureen Keller, Olivier Calvayrac, Fabrice Soncin, Lily Hoa, Alexander Hergovich, Maria-Carla Parrini, Julien Mazières, Mélissa Vaisse-Lesteven, Jacques Camonis, Guénaëlle Levallet and Gérard Zalcman (2016 Mar 15)

G. RASSF1A Suppresses the Invasion and Metastatic Potential of Human Non-Small Cell Lung Cancer Cells by Inhibiting YAP Activation through the GEF-H1/RhoB Pathway

Cancer Research : 76 : DOI : 10.1158/0008-5472

Summary

Inactivation of the tumor suppressor gene RASSF1A by promoter hypermethylation represents a key event underlying the initiation and progression of lung cancer. RASSF1A inactivation is also associated with poor prognosis and may promote metastatic spread. In this study, we investigated how RASSF1A inactivation conferred invasive phenotypes to human bronchial cells. RNAi-mediated silencing of RASSF1A induced epithelial-to-mesenchymal transition (EMT), fomenting a motile and invasive cellular phenotype *in vitro*and increased metastatic prowess *in vivo*. Mechanistic investigations revealed that RASSF1A blocked tumor growth by stimulating cofilin/PP2A–mediated dephosphorylation of the guanine nucleotide exchange factor GEF-H1, thereby stimulating its ability to activate the antimetastatic small GTPase RhoB. Furthermore, RASSF1A reduced nuclear accumulation of the Hippo pathway transcriptional cofactor Yes-associated protein (YAP), which was reinforced by RhoB activation. Collectively, our results indicated that RASSF1 acts to restrict EMT and invasion by indirectly controlling YAP nuclear shuttling and activation through a RhoB-regulated cytoskeletal remodeling process, with potential implications to delay the progression of RASSF1-hypermethylated lung tumors. *Cancer Res; 76(6); 1627–40. ©2016 AACR*.

Carine Joffre, Patrice Codogno, Manolis Fanto, Alexander Hergovich, Jacques Camonis (2016 Feb 19)

STK38 at the crossroad between autophagy and apoptosis.

Autophagy : 594-5 : DOI : 10.1080/15548627.2015.1135283

Summary

We describe the STK38 protein kinase as a conserved regulator of autophagy. We discovered STK38 as a novel binding partner of Beclin1, a key regulator of autophagy. By combining molecular, cell biological and genetic approaches, we show that STK38 promotes autophagosome formation in human cells and in Drosophila. Furthermore, we also provide evidence demonstrating that STK38 with the small GTPase RalB, assist the co-ordination between autophagic and apoptotic events upon autophagy induction, hence proposing a role for STK38 in determining cellular fate in response to autophagic conditions.

Joffre C, Codogno P, Fanto M, Hergovich A, Camonis J. (2016 Feb 18)
STK38 at the crossroad between autophagy and apoptosis
Autophagy : DOI : 10.1080/15548627.2015.1135283

Summary

We describe the STK38protein kinase as a conserved regulator of autophagy. We discovered STK38as a novel binding partner of Beclin1, a key regulator of autophagy. By combining molecular, cell biological and genetic approaches, we show that STK38promotes autophagosome formation in human cells and in Drosophila. Furthermore, we also provide evidence demonstrating that STK38with the small GTPase RalB, assist the co-ordination between autophagic and apoptotic events upon autophagyinduction, hence proposing a role for STK38in determining cellular fate in response to autophagic conditions.

Fatéméh Dubois, Maureen Keller, Olivier Calvayrac, Fabrice Soncin, Lily Hoa, Alexander Hergovich, Maria-Carla Parrini, Julien Mazières, Mélissa Vaisse-Lesteven, Jacques Camonis, Guénaëlle Levallet, Gérard Zalcman (2016 Jan 14)
RASSF1A Suppresses the Invasion and Metastatic Potential of Human Non-Small Cell Lung Cancer Cells by Inhibiting YAP Activation through the GEF-H1/RhoB Pathway.
Cancer research : 1627-40 : DOI : 10.1158/0008-5472.CAN-15-1008

Summary

Inactivation of the tumor suppressor gene RASSF1A by promoter hypermethylation represents a key event underlying the initiation and progression of lung cancer. RASSF1A inactivation is also associated with poor prognosis and may promote metastatic spread. In this study, we investigated how RASSF1A inactivation conferred invasive phenotypes to human bronchial cells. RNAi-mediated silencing of RASSF1A induced epithelial-to-mesenchymal transition (EMT), fomenting a motile and invasive cellular phenotype in vitro and increased metastatic prowess in vivo. Mechanistic investigations revealed that RASSF1A blocked tumor growth by stimulating cofilin/PP2A-mediated dephosphorylation of the guanine nucleotide exchange factor GEF-H1, thereby stimulating its ability to activate the antimetastatic small GTPase RhoB. Furthermore, RASSF1A reduced nuclear accumulation of the Hippo pathway transcriptional cofactor Yes-associated protein (YAP), which was reinforced by RhoB activation. Collectively, our results indicated that RASSF1 acts to restrict

EMT and invasion by indirectly controlling YAP nuclear shuttling and activation through a RhoB-regulated cytoskeletal remodeling process, with potential implications to delay the progression of RASSF1-hypermethylated lung tumors.

Year of publication 2015

Frédéric Canal, Elodie Anthony, Aurianne Lescure, Elaine Del Nery, Jacques Camonis, Franck Perez, Bruno Ragazzon, Christine Perret (2015 Dec 31)

A kinome siRNA screen identifies HGS as a potential target for liver cancers with oncogenic mutations in CTNNB1.

BMC cancer : 1020 : DOI : 10.1186/s12885-015-2037-8

Summary

Aberrant activation of the Wnt/β-catenin pathway is a major and frequent event in liver cancer, but inhibition of oncogenic β-catenin signaling has proven challenging. The identification of genes that are synthetically lethal in β-catenin-activated cancer cells would provide new targets for therapeutic drug design.

Joffre C., Dupont N., Hoa L., Gomez V., Pardo R., Gonc calves-Pimentel C., Achard P., Bettoun A., Meunier B., Bauvy C., Cascone I., Codogno P., Fanto M., Hergovich A., Camonis J. (2015 Oct 5)

The Pro-apoptotic STK38 Kinase Is a New Beclin1 Partner Positively Regulating Autophagy.

Current biology : CB : 25 : 2479-92 : DOI : 10.1016/j.str.2010.05.013

Summary

Autophagy plays key roles in development, oncogenesis, cardiovascular, metabolic, and neurodegenerative diseases. Hence, understanding how autophagy is regulated can reveal opportunities to modify autophagy in a disease-relevant manner. Ideally, one would want to functionally define autophagy regulators whose enzymatic activity can potentially be modulated. Here, we describe the STK38 protein kinase (also termed NDR1) as a conserved regulator of autophagy. Using STK38 as bait in yeast-two-hybrid screens, we discovered STK38 as a novel binding partner of Beclin1, a key regulator of autophagy. By combining molecular, cell biological, and genetic approaches, we show that STK38 promotes autophagosome formation in human cells and in Drosophila. Upon autophagy induction, STK38-depleted cells display impaired LC3B-II conversion; reduced ATG14L, ATG12, and WIPI-1 puncta formation; and significantly decreased Vps34 activity, as judged by PI3P formation. Furthermore, we observed that STK38 supports the interaction of the exocyst component Exo84 with Beclin1 and RalB, which is~required to initiate autophagosome formation. Upon studying the activation of STK38 during autophagy induction, we found that STK38 is stimulated in a MOB1- and exocyst-dependent manner. In contrast, RalB depletion triggers hyperactivation of STK38, resulting in STK38-dependent apoptosis under prolonged autophagy conditions. Together, our data establish STK38 as a conserved regulator of

autophagy in human cells and flies. We also provide evidence demonstrating that STK38 and RalB assist the coordination between autophagic and apoptotic events upon autophagy induction, hence further proposing a role for STK38 in determining cellular fate in response to autophagic conditions.

Marco Biondini, Guillaume Duclos, Nathalie Meyer-Schaller, Pascal Silberzan, Jacques Camonis, Maria Carla Parrini (2015 Mar 31)

Akirin specifies NF-κB selectivity of Drosophila innate immune response via chromatin remodeling

Scientific reports : 11759 : DOI : 10.1038/srep11759

Summary

RalA and RalB proteins are key mediators of oncogenic Ras signaling in human oncogenesis. Herein we investigated the mechanistic contribution of Ral proteins to invasion of lung cancer A549 cells after induction of epithelial-mesenchymal transition (EMT) with TGFβ. We show that TGFβ-induced EMT promotes dissemination of A549 cells in a 2/3D assay, independently of proteolysis, by activating the Rho/ROCK pathway which generates actomyosin-dependent contractility forces that actively remodel the extracellular matrix, as assessed by Traction Force microscopy. RalB, but not RalA, is required for matrix deformation and cell dissemination acting via the RhoGEF GEF-H1, which associates with the Exocyst complex, a major Ral effector. Indeed, uncoupling of the Exocyst subunit Sec5 from GEF-H1 impairs RhoA activation, generation of traction forces and cell dissemination. These results provide a novel molecular mechanism underlying the control of cell invasion by RalB via a cross-talk with the Rho pathway.