Année de publication : 2016

Tina Gruosso, Virginie Mieulet, Melissa Cardon, Brigitte Bourachot, Yann Kieffer, Flavien Devun, Thierry Dubois, Marie Dutreix, Anne Vincent-Salomon, Kyle Malcolm Miller, Fatima Mechta-Grigoriou (2016 Mar 24)

Chronic oxidative stress promotes H2AX protein degradation and enhances chemosensitivity in breast cancer patients.

EMBO molecular medicine : 527-49 : DOI : 10.15252/emmm.201505891

Résumé

Anti-cancer drugs often increase reactive oxygen species (ROS) and cause DNA damage. Here, we highlight a new cross talk between chronic oxidative stress and the histone variant H2AX, a key player in DNA repair. We observe that persistent accumulation of ROS, due to a deficient JunD-/Nrf2-antioxidant response, reduces H2AX protein levels. This effect is mediated by an enhanced interaction of H2AX with the E3 ubiquitin ligase RNF168, which is associated with H2AX poly-ubiquitination and promotes its degradation by the proteasome. ROS-mediated H2AX decrease plays a crucial role in chemosensitivity. Indeed, cycles of chemotherapy that sustainably increase ROS reduce H2AX protein levels in Triple-Negative breast cancer (TNBC) patients. H2AX decrease by such treatment is associated with an impaired NRF2-antioxidant response and is indicative of the therapeutic efficiency and survival of TNBC patients. Thus, our data describe a novel ROS-mediated regulation of H2AX turnover, which provides new insights into genetic instability and treatment efficacy in TNBC patients.

Christophe Couderc, Alizée Boin, Laetitia Fuhrmann, Anne Vincent-Salomon, Vinay Mandati, Yann Kieffer, Fatima Mechta-Grigoriou, Laurence Del Maestro, Philippe Chavrier, David Vallerand, Isabelle Brito, Thierry Dubois, Leanne De Koning, Daniel Bouvard, Daniel Louvard, Alexis Gautreau, Dominique Lallemand (2016 Jan 26)

AMOTL1 integrates Hippo signaling to promote breast cancer progression by inducing tumor cell proliferation and migration

Neoplasia (New York, N.Y.) : 10-24 : DOI : 10.1016/j.neo.2015.11.010

Résumé

The Hippo signaling network is a key regulator of cell fate. In the recent years, it was shown that its implication in cancer goes well beyond the sole role of YAP transcriptional activity and its regulation by the canonical MST/LATS kinase cascade. Here we show that the motin family member AMOTL1 is an important effector of Hippo signaling in breast cancer. AMOTL1 connects Hippo signaling to tumor cell aggressiveness. We show that both canonical and noncanonical Hippo signaling modulates AMOTL1 levels. The tumor suppressor Merlin triggers AMOTL1 proteasomal degradation mediated by the NEDD family of ubiquitin ligases through direct interaction. In parallel, YAP stimulates AMOTL1 expression. The loss of Merlin expression and the induction of Yap activity that are frequently observed in breast cancers

thus result in elevated AMOTL1 levels. AMOTL1 expression is sufficient to trigger tumor cell migration and stimulates proliferation by activating c-Src. In a large cohort of human breast tumors, we show that AMOTL1 protein levels are upregulated during cancer progression and that, importantly, the expression of AMOTL1 in lymph node metastasis appears predictive of the risk of relapse. Hence we uncover an important mechanism by which Hippo signaling promotes breast cancer progression by modulating the expression of AMOTL1.

Magali Michaut, Suet-Feung Chin, Ian Majewski, Tesa M Severson, Tycho Bismeijer, Leanne de Koning, Justine K Peeters, Philip C Schouten, Oscar M Rueda, Astrid J Bosma, Finbarr Tarrant, Yue Fan, Beilei He, Zheng Xue, Lorenza Mittempergher, Roelof J C Kluin, Jeroen Heijmans, Mireille Snel, Bernard Pereira, Andreas Schlicker, Elena Provenzano, Hamid Raza Ali, Alexander Gaber, Gillian O'Hurley, Sophie Lehn, Jettie J F Muris, Jelle Wesseling, Elaine Kay, Stephen John Sammut, Helen A Bardwell, Aurélie S Barbet, Floriane Bard, Caroline Lecerf, Darran P O'Connor, Daniël J Vis, Cyril H Benes, Ultan McDermott, Mathew J Garnett, Iris M Simon, Karin Jirström, Thierry Dubois, Sabine C Linn, William M Gallagher, Lodewyk F A Wessels, Carlos Caldas, Rene Bernards (2016 Jan 6)

Integration of genomic, transcriptomic and proteomic data identifies two biologically distinct subtypes of invasive lobular breast cancer.

Scientific reports : 18517 : DOI : 10.1038/srep18517

Résumé

Invasive lobular carcinoma (ILC) is the second most frequently occurring histological breast cancer subtype after invasive ductal carcinoma (IDC), accounting for around 10% of all breast cancers. The molecular processes that drive the development of ILC are still largely unknown. We have performed a comprehensive genomic, transcriptomic and proteomic analysis of a large ILC patient cohort and present here an integrated molecular portrait of ILC. Mutations in CDH1 and in the PI3K pathway are the most frequent molecular alterations in ILC. We identified two main subtypes of ILCs: (i) an immune related subtype with mRNA up-regulation of PD-L1, PD-1 and CTLA-4 and greater sensitivity to DNA-damaging agents in representative cell line models; (ii) a hormone related subtype, associated with Epithelial to Mesenchymal Transition (EMT), and gain of chromosomes 1q and 8q and loss of chromosome 11q. Using the somatic mutation rate and eIF4B protein level, we identified three groups with different clinical outcomes, including a group with extremely good prognosis. We provide a comprehensive overview of the molecular alterations driving ILC and have explored links with therapy response. This molecular characterization may help to tailor treatment of ILC through the application of specific targeted, chemo- and/or immune-therapies.

Année de publication : 2015

Elodie Manié, Tatiana Popova, Aude Battistella, Julien Tarabeux, Virginie Caux-Moncoutier, Lisa Golmard, Nicholas K Smith, Christopher R Mueller, Odette Mariani, Brigitte Sigal-Zafrani, Thierry

Dubois, Anne Vincent-Salomon, Claude Houdayer, Dominique Stoppa-Lyonnet, Marc-Henri Stern (2015 Aug 29)

Genomic hallmarks of homologous recombination deficiency in invasive breast carcinomas.

International journal of cancer : 891-900 : DOI : 10.1002/ijc.29829

Résumé

Therapeutic strategies targeting Homologous Recombination Deficiency (HRD) in breast cancer requires patient stratification. The LST (Large-scale State Transitions) genomic signature previously validated for triple-negative breast carcinomas (TNBC) was evaluated as biomarker of HRD in luminal (hormone receptor positive) and HER2-overexpressing (HER2+) tumors. The LST genomic signature related to the number of large-scale chromosomal breakpoints in SNP-array tumor profile was applied to identify HRD in in-house and TCGA sets of breast tumors, in which the status of BRCA1/2 and other genes was also investigated. In the in-house dataset, HRD was predicted in 5% (20/385) of sporadic tumors luminal or HER2+ by the LST genomic signature and the inactivation of BRCA1, BRCA2 or RAD51C confirmed this prediction in 75% (12/16) of the tested cases. In 14% (6/43) of tumors occurring in BRCA1/2 mutant carriers, the corresponding wild-type allele was retained emphasizing the importance of determining the tumor status. In the TCGA luminal and HER2+ subtypes HRD incidence was estimated at 5% (18/329, 95%CI: 5-8%) and 2% (1/59, 95%CI: 2-9%), respectively. In TNBC cisplatin-based neo-adjuvant clinical trials, HRD is shown to be a necessary condition for cisplatin sensitivity. This analysis demonstrates the high performance of the LST genomic signature for HRD detection in breast cancers, which suggests its potential as a biomarker for genetic testing and patient stratification for clinical trials evaluating platinum salts and PARP inhibitors.

Loredana Martignetti, Bruno Tesson, Anna Almeida, Andrei Zinovyev, Gordon C Tucker, Thierry Dubois, Emmanuel Barillot (2015 Jun 1)

Detection of miRNA regulatory effect on triple negative breast cancer transcriptome.

BMC genomics : S4 : DOI : 10.1186/1471-2164-16-S6-S4

Résumé

Identifying key microRNAs (miRNAs) contributing to the genesis and development of a particular disease is a focus of many recent studies. We introduce here a rank-based algorithm to detect miRNA regulatory activity in cancer-derived tissue samples which combines measurements of gene and miRNA expression levels and sequence-based target predictions. The method is designed to detect modest but coordinated changes in the expression of sequence-based predicted target genes. We applied our algorithm to a cohort of 129 tumour and healthy breast tissues and showed its effectiveness in identifying functional miRNAs possibly involved in the disease. These observations have been validated using an independent publicly available breast cancer dataset from The Cancer Genome Atlas. We focused on the triple negative breast cancer subtype to highlight potentially

relevant miRNAs in this tumour subtype. For those miRNAs identified as potential regulators, we characterize the function of affected target genes by enrichment analysis. In the two independent datasets, the affected targets are not necessarily the same, but display similar enriched categories, including breast cancer related processes like cell substrate adherens junction, regulation of cell migration, nuclear pore complex and integrin pathway. The R script implementing our method together with the datasets used in the study can be downloaded here (http://bioinfo-out.curie.fr/projects/targetrunningsum).

Céline Baldeyron, Amélie Brisson, Bruno Tesson, Fariba Némati, Stéphane Koundrioukoff, Elie Saliba, Leanne De Koning, Elise Martel, Mengliang Ye, Guillem Rigaill, Didier Meseure, André Nicolas, David Gentien, Didier Decaudin, Michelle Debatisse, Stéphane Depil, Francisco Cruzalegui, Alain Pierré, Sergio Roman-Roman, Gordon C Tucker, Thierry Dubois (2015 May 26)
TIPIN depletion leads to apoptosis in breast cancer cells.
Molecular oncology : 1580-98 : DOI : 10.1016/j.molonc.2015.04.010

Résumé

Triple-negative breast cancer (TNBC) is the breast cancer subgroup with the most aggressive clinical behavior. Alternatives to conventional chemotherapy are required to improve the survival of TNBC patients. Gene-expression analyses for different breast cancer subtypes revealed significant overexpression of the Timeless-interacting protein (TIPIN), which is involved in the stability of DNA replication forks, in the highly proliferative associated TNBC samples. Immunohistochemistry analysis showed higher expression of TIPIN in the most proliferative and aggressive breast cancer subtypes including TNBC, and no TIPIN expression in healthy breast tissues. The depletion of TIPIN by RNA interference impairs the proliferation of both human breast cancer and non-tumorigenic cell lines. However, this effect may be specifically associated with apoptosis in breast cancer cells. TIPIN silencing results in higher levels of single-stranded DNA (ssDNA), indicative of replicative stress (RS), in TNBC compared to non-tumorigenic cells. Upon TIPIN depletion, the speed of DNA replication fork was significantly decreased in all BC cells. However, TIPIN-depleted TNBC cells are unable to fire additional replication origins in response to RS and therefore undergo apoptosis. TIPIN knockdown in TNBC cells decreases tumorigenicity in vitro and delays tumor growth in vivo. Our findings suggest that TIPIN is important for the maintenance of DNA replication and represents a potential treatment target for the worst prognosis associated breast cancers, such as TNBC.

Sylvie Maubant, Bruno Tesson, Virginie Maire, Mengliang Ye, Guillem Rigaill, David Gentien, Francisco Cruzalegui, Gordon C Tucker, Sergio Roman-Roman, Thierry Dubois (2015 Apr 8)
Transcriptome analysis of Wnt3a-treated triple-negative breast cancer cells.
PloS one : e0122333 : DOI : 10.1371/journal.pone.0122333

Résumé

The canonical Wnt/β-catenin pathway is activated in triple-negative breast cancer (TNBC).

The activation of this pathway leads to the expression of specific target genes depending on the cell/tissue context. Here, we analyzed the transcriptome of two different TNBC cell lines to define a comprehensive list of Wnt target genes. The treatment of cells with Wnt3a for 6h up-regulated the expression (fold change > 1.3) of 59 genes in MDA-MB-468 cells and 241 genes in HCC38 cells. Thirty genes were common to both cell lines. Beta-catenin may also be a transcriptional repressor and we found that 18 and 166 genes were down-regulated in response to Wnt3a treatment for 6h in MDA-MB-468 and HCC38 cells, respectively, of which six were common to both cell lines. Only half of the activated and the repressed transcripts have been previously described as Wnt target genes. Therefore, our study reveals 137 novel genes that may be positively regulated by Wnt3a and 104 novel genes that may be negatively regulated by Wnt3a. These genes are involved in the Wnt pathway itself, and also in TGFβ, p53 and Hedgehog pathways. Thorough characterization of these novel potential Wnt target genes may reveal new regulators of the canonical Wnt pathway. The comparison of our list of Wnt target genes with those published in other cellular contexts confirms the notion that Wnt target genes are tissue-, cell line- and treatment-specific. Genes up-regulated in Wnt3a-stimulated cell lines were more strongly expressed in TNBC than in luminal A breast cancer samples. These genes were also overexpressed, but to a much lesser extent, in HER2+ and luminal B tumors. We identified 72 Wnt target genes higher expressed in TNBCs (17 with a fold change >1.3) which may reflect the chronic activation of the canonical Wnt pathway that occurs in TNBC tumors.

Année de publication : 2014

Sylvain Lefort, Carine Joffre, Yann Kieffer, Anne-Marie Givel, Brigitte Bourachot, Giulia Zago, Ivan Bieche, Thierry Dubois, Didier Meseure, Anne Vincent-Salomon, Jacques Camonis, Fatima Mechta-Grigoriou (2014 Nov 27)

Inhibition of autophagy as a new means of improving chemotherapy efficiency in high-LC3B triple-negative breast cancers.

Autophagy : 2122-42 : DOI : 10.4161/15548627.2014.981788

Résumé

The triple-negative breast cancer (TN BC) subtype is the most aggressive form of invasive BC. Despite intensive efforts to improve BC treatments, patients with TN BC continue to exhibit poor survival, with half developing resistance to chemotherapy. Here we identify autophagy as a key mechanism in the progression and chemoresistance of a subset of TN tumors. We demonstrate that LC3B, a protein involved in autophagosome formation, is a reliable marker of poor prognosis in TN BC, validating this prognostic value at both the mRNA and protein levels in several independent cohorts. We also show that LC3B has no prognostic value for other BC subtypes (Luminal or HER2 BC), thus revealing a specific impact of autophagy on TN tumors. Autophagy is essential for the proliferative and invasive properties in 3D of TN BC cells characterized by high LC3B levels. Interestingly, the activity of the transcriptional co-activator YAP1 (Yes-associated protein 1) is regulated by the autophagy process and we identify YAP1 as a new actor in the autophagy-dependent proliferative and invasive properties of high-LC3B TN BC. Finally, inhibiting autophagy by silencing ATG5 or ATG7 significantly impaired high-LC3B TN tumor growth in vivo. Moreover, using a patient-

derived TN tumor transplanted into mice, we show that an autophagy inhibitor, chloroquine, potentiates the effects of chemotherapeutic agents. Overall, our data identify LC3B as a new prognostic marker for TN BC and the inhibition of autophagy as a promising therapeutic strategy for TN BC patients.

Alizée Boin, Anne Couvelard, Christophe Couderc, Isabel Brito, Dan Filipescu, Michel Kalamarides, Pierre Bedossa, Leanne De Koning, Carine Danelsky, Thierry Dubois, Philippe Hupé, Daniel Louvard, Dominique Lallemand (2014 Feb 22)
Proteomic screening identifies a YAP-driven signaling network linked to tumor cell proliferation in human schwannomas.
Neuro-oncology : 1196-209 : DOI : 10.1093/neuonc/nou020

Résumé

Inactivation of the NF2 gene predisposes to neurofibromatosis type II and the development of schwannomas. In vitro studies have shown that loss of NF2 leads to the induction of mitogenic signaling mediated by receptor tyrosine kinases (RTKs), MAP kinase, AKT, or Hippo pathways. The goal of our study was to evaluate the expression and activity of these signaling pathways in human schwannomas in order to identify new potential therapeutic targets.

Année de publication : 2010

Aurore Toullec, Damien Gerald, Gilles Despouy, Brigitte Bourachot, Melissa Cardon, Sylvain Lefort, Marion Richardson, Guillem Rigaill, Maria-Carla Parrini, Carlo Lucchesi, Dorine Bellanger, Marc-Henri Stern, Thierry Dubois, Xavier Sastre-Garau, Olivier Delattre, Anne Vincent-Salomon, Fatima Mechta-Grigoriou (2010 Jun 11)
Oxidative stress promotes myofibroblast differentiation and tumour spreading.
EMBO molecular medicine : 211-30 : DOI : 10.1002/emmm.201000073

Résumé

JunD regulates genes involved in antioxidant defence. We took advantage of the chronic oxidative stress resulting from junD deletion to examine the role of reactive oxygen species (ROS) in tumour development. In a model of mammary carcinogenesis, junD inactivation increased tumour incidence and revealed an associated reactive stroma. junD-inactivation in the stroma was sufficient to shorten tumour-free survival rate and enhance metastatic spread. ROS promoted conversion of fibroblasts into highly migrating myofibroblasts through accumulation of the hypoxia-inducible factor (HIF)-1alpha transcription factor and the CXCL12 chemokine. Accordingly, treatment with an antioxidant reduced the levels of HIF and CXCL12 and numerous myofibroblast features. CXCL12 accumulated in the stroma of HER2- human breast adenocarcinomas. Moreover, HER2 tumours exhibited a high proportion of myofibroblasts, which was significantly correlated to nodal metastases. Interestingly, this

subset of tumours exhibited a significant nuclear exclusion of JunD and revealed an associated oxido-reduction signature, further demonstrating the relevance of our findings in human cancers. Collectively, our data uncover a new mechanism by which oxidative stress increases the migratory properties of stromal fibroblasts, which in turn potentiate tumour dissemination.

Année de publication : 2007

Julie Ménétrey, Mylène Perderiset, Jérome Cicolari, Thierry Dubois, Nadia Elkhatib, Fatima El Khadali, Michel Franco, Philippe Chavrier, Anne Houdusse (2007 Mar 10)
Structural basis for ARF1-mediated recruitment of ARHGAP21 to Golgi membranes.
The EMBO journal : 1953-62

Résumé

ARHGAP21 is a Rho family GTPase-activating protein (RhoGAP) that controls the Arp2/3 complex and F-actin dynamics at the Golgi complex by regulating the activity of the small GTPase Cdc42. ARHGAP21 is recruited to the Golgi by binding to another small GTPase, ARF1. Here, we present the crystal structure of the activated GTP-bound form of ARF1 in a complex with the Arf-binding domain (ArfBD) of ARHGAP21 at 2.1 A resolution. We show that ArfBD comprises a PH domain adjoining a C-terminal alpha helix, and that ARF1 interacts with both of these structural motifs through its switch regions and triggers structural rearrangement of the PH domain. We used site-directed mutagenesis to confirm that both the PH domain and the helical motif are essential for the binding of ArfBD to ARF1 and for its recruitment to the Golgi. Our data demonstrate that two well-known small GTPase-binding motifs, the PH domain and the alpha helical motif, can combine to create a novel mode of binding to Arfs.

Année de publication : 2005

Thierry Dubois, Philippe Chavrier (2005 Aug 24)
[ARHGAP10, a novel RhoGAP at the cross-road between ARF1 and Cdc42 pathways, regulates Arp2/3 complex and actin dynamics on Golgi membranes].
Médecine sciences : M/S : 692-4

Résumé

Thierry Dubois, Olivia Paléotti, Alexander A Mironov, Vincent Fraisier, Theresia E B Stradal, Maria Antonietta De Matteis, Michel Franco, Philippe Chavrier (2005 Mar 29)
Golgi-localized GAP for Cdc42 functions downstream of ARF1 to control Arp2/3

complex and F-actin dynamics.
Nature cell biology : 353-64

Résumé

The small GTP-binding ADP-ribosylation factor 1 (ARF1) acts as a master regulator of Golgi structure and function through the recruitment and activation of various downstream effectors. It has been proposed that members of the Rho family of small GTPases also control Golgi function in coordination with ARF1, possibly through the regulation of Arp2/3 complex and actin polymerization on Golgi membranes. Here, we identify ARHGAP10–a novel Rho GTPase-activating protein (Rho-GAP) that is recruited to Golgi membranes through binding to GTP-ARF1. We show that ARHGAP10 functions preferentially as a GAP for Cdc42 and regulates the Arp2/3 complex and F-actin dynamics at the Golgi through the control of Cdc42 activity. Our results establish a role for ARHGAP10 in Golgi structure and function at the crossroads between ARF1 and Cdc42 signalling pathways.

Année de publication : 2003

Magali Prigent, Thierry Dubois, Graça Raposo, Valérie Derrien, Danièle Tenza, Carine Rossé, Jacques Camonis, Philippe Chavrier (2003 Dec 10)
ARF6 controls post-endocytic recycling through its downstream exocyst complex effector.
The Journal of cell biology : 1111-21

Résumé

The small guanosine triphosphate (GTP)-binding protein ADP-ribosylation factor (ARF) 6 regulates membrane recycling to regions of plasma membrane remodeling via the endocytic pathway. Here, we show that GTP-bound ARF6 interacts with Sec10, a subunit of the exocyst complex involved in docking of vesicles with the plasma membrane. We found that Sec10 localization in the perinuclear region is not restricted to the trans-Golgi network, but extends to recycling endosomes. In addition, we report that depletion of Sec5 exocyst subunit or dominant inhibition of Sec10 affects the function and the morphology of the recycling pathway. Sec10 is found to redistribute to ruffling areas of the plasma membrane in cells expressing GTP-ARF6, whereas dominant inhibition of Sec10 interferes with ARF6-induced cell spreading. Our paper suggests that ARF6 specifies delivery and insertion of recycling membranes to regions of dynamic reorganization of the plasma membrane through interaction with the vesicle-tethering exocyst complex.

Florence Niedergang, Emma Colucci-Guyon, Thierry Dubois, Graca Raposo, Philippe Chavrier (2003 Jun 18)
ADP ribosylation factor 6 is activated and controls membrane delivery during phagocytosis in macrophages.

The Journal of cell biology : 1143-50

Résumé

Engulfment of particles by phagocytes is induced by their interaction with specific receptors on the cell surface, which leads to actin polymerization and the extension of membrane protrusions to form a closed phagosome. Membrane delivery from internal pools is considered to play an important role in pseudopod extension during phagocytosis. Here, we report that endogenous ADP ribosylation factor 6 (ARF6), a small GTP-binding protein, undergoes a sharp and transient activation in macrophages when phagocytosis was initiated via receptors for the Fc portion of immunoglobulins (FcRs). A dominant-negative mutant of ARF6 (T27N mutation) dramatically affected FcR-mediated phagocytosis. Expression of ARF6-T27N lead to a reduction in the focal delivery of vesicle-associated membrane protein 3+ endosomal recycling membranes at phagocytosis sites, whereas actin polymerization was unimpaired. This resulted in an early blockade in pseudopod extension and accumulation of intracellular vesicles, as observed by electron microscopy. We conclude that ARF6 is a major regulator of membrane recycling during phagocytosis.