Année de publication : 2019

CAMPAGNE Antoine, LEE Ming-Kang, ZIELINSKI Dina, MICHAUD Audrey, LE CORRE Stéphanie, DINGLI Florent, CHEN Hong, SHAHIDIAN Lara Z, SERVANT Nicolas, LOEW Damarys, PASMANT Eric, PISTEL-VINAY Sophie, WASSEF Michel, MARGUERON Raphaël (2019 Jan 21)

BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation

Nature Communications : DOI : 10.1038/s41467-018-08255-x

Résumé

In Drosophila, a complex consisting of Calypso and ASX catalyzes H2A deubiquitination and has been reported to act as part of the Polycomb machinery in transcriptional silencing. The mammalian homologs of these proteins (BAP1 and ASXL1/2/3, respectively), are frequently mutated in various cancer types, yet their precise functions remain unclear. Using an integrative approach based on isogenic cell lines generated with CRISPR/Cas9, we uncover an unanticipated role for BAP1 in gene activation. This function requires the assembly of an enzymatically active BAP1-associated core complex (BAP1.com) containing one of the redundant ASXL proteins. We investigate the mechanism underlying BAP1.com-mediated transcriptional regulation and show that it does not participate in Polycomb-mediated silencing. Instead, our results establish that the function of BAP1.com is to safeguard transcriptionally active genes against silencing by the Polycomb Repressive Complex 1.

Année de publication : 2017

Lucie Hebert, Dorine Bellanger, Chloé Guillas, Antoine Campagne, Florent Dingli, Damarys Loew, Alice Fievet, Virginie Jacquemin, Tatiana Popova, Didier Jean, Fatima Mechta-Grigoriou, Raphaël Margueron, Marc-Henri Stern (2017 Oct 27)

Modulating BAP1 expression affects ROS homeostasis, cell motility and mitochondrial function.

Oncotarget : 72513-72527 : DOI : 10.18632/oncotarget.19872

Résumé

The tumor suppressor BAP1 associates with ASXL1/2 to form the core Polycomb complex PR-DUB, which catalyzes the removal of mono-ubiquitin from several substrates including histone H2A. This complex also mediates the poly-deubiquitination of HCFC1, OGT and PCG1-α, preventing them from proteasomal degradation. Surprisingly, considering its role in a Polycomb complex, no transcriptional signature was consistently found among BAP1-inactivated tumor types. It was hypothesized that BAP1 tumor suppressor activity could reside, at least in part, in stabilizing proteins through its poly-deubiquitinase activity. Quantitative mass spectrometry and gene expression arrays were used to investigate the consequences of BAP1 expression modulation in the NCI-H226 mesothelioma cell line. Analysis of differentially expressed proteins revealed enrichment in cytoskeleton organization, mitochondrial activity and ROS management, while gene expression analysis

revealed enrichment in the epithelial-to-mesenchymal transition pathway. Functional assessments in BAP1 inactivated, BAP1 wild-type and BAP1 catalytically dead-expressing NCI-H226 and QR mesothelioma cell lines confirmed alteration of these pathways and demonstrated that BAP1 deubiquitinase activity was mandatory to maintain these phenotypes. Interestingly, monitoring intracellular ROS levels partly restored the morphology and the mitochondrial activity. Finally, the study suggests new tumorigenic and cellular functions of BAP1 and shows for the first time the interest of studying the proteome as readout of BAP1 inactivation.

M Wassef, A Luscan, A Battistella, S Le Corre, H Li, M R Wallace, M Vidaud, R Margueron (2017 May 14)

Versatile and precise gene-targeting strategies for functional studies in mammalian cell lines.

Methods (San Diego, Calif.) : DOI : S1046-2023(16)30269-9

Résumé

The advent of programmable nucleases such as ZFNs, TALENs and CRISPR/Cas9 has brought the power of genetic manipulation to widely used model systems. In mammalian cells, nuclease-mediated DNA double strand break is mainly repaired through the error-prone non-homologous end-joining (NHEJ) repair pathway, eventually leading to accumulation of small deletions or insertions (indels) that can inactivate gene function. However, due to the variable size of the indels and the polyploid status of many cell lines (e.g., cancer-derived cells), obtaining a knockout usually requires lengthy screening and characterization procedures. Given the more precise type of modifications that can be introduced upon homology-directed repair (HDR), we have developed HDR-based gene-targeting strategies that greatly facilitate the process of knockout generation in cell lines. To generate reversible knockouts (R-KO), a selectable promoter-less STOP cassette is inserted in an intron, interrupting transcription. Loss-of-function can be validated by RT-qPCR and is removable, enabling subsequent restoration of gene function. A variant of the R-KO procedure can be used to introduce point mutations. To generate constitutive knockouts (C-KO), an exon is targeted, which makes use of HDR-based gene disruption together with NHEJ-induced indels on non-HDR targeted allele(s). Hence the C-KO procedure greatly facilitates simultaneous inactivation of multiple alleles. Overall these genome-editing tools offer superior precision and efficiency for functional genetic approaches. We provide detailed protocols guiding in the design of targeting vectors and in the analysis and validation of gene targeting experiments.

Daniel Holoch, Raphaël Margueron (2017 May 10)

Mechanisms Regulating PRC2 Recruitment and Enzymatic Activity.

Trends in biochemical sciences : DOI : S0968-0004(17)30072-5

Résumé

Polycomb repressive complex 2 (PRC2) and its histone H3 lysine-27 methylation activity are crucial for multicellular development by virtue of their role in maintaining transcriptional repression patterns. The recruitment and enzymatic activity of PRC2 are controlled by a series of intricate mechanisms whose molecular details have been emerging at a rapid pace. Recent studies have uncovered intriguing modes of PRC2 regulation by facultative PRC2 subunits, PRC1, and specific features of the chromatin environment. Together, these findings have produced a rich and fast-evolving picture of the biochemical signals that govern PRC2 function, with many exciting questions still remaining.

Manuela Portoso, Roberta Ragazzini, Živa Brenčič, Arianna Moiani, Audrey Michaud, Ivaylo Vassilev, Michel Wassef, Nicolas Servant, Bruno Sargueil, Raphaël Margueron (2017 Feb 8)
PRC2 is dispensable for HOTAIR-mediated transcriptional repression.
The EMBO journal : DOI : e201695335

Résumé

Long non-coding RNAs (lncRNAs) play diverse roles in physiological and pathological processes. Several lncRNAs have been suggested to modulate gene expression by guiding chromatin-modifying complexes to specific sites in the genome. However, besides the example of Xist, clear-cut evidence demonstrating this novel mode of regulation remains sparse. Here, we focus on HOTAIR, a lncRNA that is overexpressed in several tumor types and previously proposed to play a key role in gene silencing through direct recruitment of Polycomb Repressive Complex 2 (PRC2) to defined genomic loci. Using genetic tools and a novel RNA-tethering system, we investigated the interplay between HOTAIR and PRC2 in gene silencing. Surprisingly, we observed that forced overexpression of HOTAIR in breast cancer cells leads to subtle transcriptomic changes that appear to be independent of PRC2. Mechanistically, we found that artificial tethering of HOTAIR to chromatin causes transcriptional repression, but that this effect does not require PRC2. Instead, PRC2 recruitment appears to be a consequence of gene silencing. We propose that PRC2 binding to RNA might serve functions other than chromatin targeting.

Daniel Holoch, Raphaël Margueron (2017 Jan 31)
Chromatin biology: Breaking into the PRC2 cage.
Nature chemical biology : DOI : 10.1038/nchembio.2313

Résumé

Année de publication : 2016

M Wassef, R Margueron (2016 Oct 16)
The multiple facets of PRC2 alterations in cancers.
Journal of molecular biology : DOI : S0022-2836(16)30427-2

Résumé

Genome sequencing of large cohorts of tumors has revealed that mutations in genes encoding chromatin regulators are frequent in cancer. However, the precise contribution of these mutations to tumor development often remains elusive. Here, we review the current knowledge concerning alterations of the Polycomb machinery in cancer, with a particular focus on the Polycomb Repressive Complex 2 (PRC2), a key chromatin modifier involved in the maintenance of transcriptional silencing. A broad variety of alterations can impair PRC2 activity yet, overall, only one type of alteration is found in a given class of tumor. We discuss the potential impact of the various types of PRC2 alterations on gene expression. We propose that the distinct set of genes regulated by PRC2 depending on tumor etiology constrain the type of alteration of PRC2 that can fuel tumor development. Beyond this specificity, we propose that PRC2 and more generally chromatin regulators act as gatekeepers of transcriptional integrity, a role that often confers a tumor-suppressive function.

M Wassef, A Michaud, R Margueron (2016 Jul 16)
Association between EZH2 expression, silencing of tumor suppressors and disease outcome in solid tumors.
Cell cycle (Georgetown, Tex.) : 0

Résumé

EZH2, the main catalytic component of the Polycomb Repressive Complex 2 (PRC2) is apparently upregulated in most solid tumors. Furthermore its expression generally associates with poor prognosis. It was proposed that this correlation reflects a causal event, EZH2 mediating the silencing of key tumor suppressor loci. In contrast, we recently showed that EZH2 is dispensable for solid tumor development and that its elevated expression reflects the abnormally high proliferation rate of cancer cells. Here, we investigate the functional association between EZH2 expression and silencing of key tumor suppressor loci and further illustrate the confounding effect of proliferation on EZH2's association to outcome.

Marie Schoumacher, Stéphanie Le Corre, Alexandre Houy, Eskeatnaf Mulugeta, Marc-Henri Stern, Sergio Roman-Roman, Raphaël Margueron (2016 Jun 9)
Uveal melanoma cells are resistant to EZH2 inhibition regardless of BAP1 status.
Nature medicine : 577-8 : DOI : 10.1038/nm.4098

Résumé

Année de publication : 2015

Michel Wassef, Veronica Rodilla, Aurélie Teissandier, Bruno Zeitouni, Nadege Gruel, Benjamin Sadacca, Marie Irondelle, Margaux Charruel, Bertrand Ducos, Audrey Michaud, Matthieu Caron, Elisabetta Marangoni, Philippe Chavrier, Christophe Le Tourneau, Maud Kamal, Eric Pasmant, Michel Vidaud, Nicolas Servant, Fabien Reyal, Dider Meseure, Anne Vincent-Salomon, Silvia Fre, Raphaël Margueron (2015 Dec 6)

Impaired PRC2 activity promotes transcriptional instability and favors breast tumorigenesis.

Genes & development : 2547-62 : DOI : 10.1101/gad.269522.115

Résumé

Alterations of chromatin modifiers are frequent in cancer, but their functional consequences often remain unclear. Focusing on the Polycomb protein EZH2 that deposits the H3K27me3 (trimethylation of Lys27 of histone H3) mark, we showed that its high expression in solid tumors is a consequence, not a cause, of tumorigenesis. In mouse and human models, EZH2 is dispensable for prostate cancer development and restrains breast tumorigenesis. High EZH2 expression in tumors results from a tight coupling to proliferation to ensure H3K27me3 homeostasis. However, this process malfunctions in breast cancer. Low EZH2 expression relative to proliferation and mutations in Polycomb genes actually indicate poor prognosis and occur in metastases. We show that while altered EZH2 activity consistently modulates a subset of its target genes, it promotes a wider transcriptional instability. Importantly, transcriptional changes that are consequences of EZH2 loss are predominantly irreversible. Our study provides an unexpected understanding of EZH2's contribution to solid tumors with important therapeutic implications.

Wassef M1, Rodilla V1, Teissandier A2, Zeitouni B2, Gruel N3, Sadacca B3, Irondelle M3, Charruel M1, Ducos B4, Michaud A1, Caron M1, Marangoni E3, Chavrier P3, Le Tourneau C5, Kamal M6, Pasmant E7, Vidaud M7, Servant N2, Reyal F3, Meseure D8, Vincent-Salomon A3, Fre S1, Margueron R1. (2015 Dec 4)

Impaired PRC2 activity promotes transcriptional instability and favors breast tumorigenesis.

Genes & DevelopmentImpaired PRC2 activity promotes transcriptional instability and favors breast tumorigenesis. : DOI : 10.1101/gad.269522.115

Résumé

Abstract

Alterations of chromatin modifiers are frequent in cancer, but their functional consequences often remain unclear. Focusing on the Polycomb protein EZH2 that deposits the H3K27me3 (trimethylation of Lys27 of histone H3) mark, we showed that its high expression in solid tumors is a consequence, not a cause, of tumorigenesis. In mouse and human models, EZH2

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Stéphanie Maupetit-Méhouas, Bertille Montibus, David Nury, Chiharu Tayama, Michel Wassef, Satya K Kota, Anne Fogli, Fabiana Cerqueira Campos, Kenichiro Hata, Robert Feil, Raphael Margueron, Kazuhiko Nakabayashi, Franck Court, Philippe Arnaud (2015 Sep 25)

Imprinting control regions (ICRs) are marked by mono-allelic bivalent chromatin when transcriptionally inactive.

Nucleic acids research : 621-35 : DOI : 10.1093/nar/gkv960

Résumé

Parental allele-specific expression of imprinted genes is mediated by imprinting control regions (ICRs) that are constitutively marked by DNA methylation imprints on the maternal or paternal allele. Mono-allelic DNA methylation is strictly required for the process of imprinting and has to be faithfully maintained during the entire life-span. While the regulation of DNA methylation itself is well understood, the mechanisms whereby the opposite allele remains unmethylated are unclear. Here, we show that in the mouse, at maternally methylated ICRs, the paternal allele, which is constitutively associated with H3K4me2/3, is marked by default by H3K27me3 when these ICRs are transcriptionally inactive, leading to the formation of a bivalent chromatin signature. Our data suggest that at ICRs, chromatin bivalency has a protective role by ensuring that DNA on the paternal allele remains unmethylated and protected against spurious and unscheduled gene expression. Moreover, they provide the proof of concept that, beside pluripotent cells, chromatin bivalency is the default state of transcriptionally inactive CpG island promoters, regardless of the developmental stage, thereby contributing to protect cell identity.

Serena Sanulli, Neil Justin, Aurélie Teissandier, Katia Ancelin, Manuela Portoso, Matthieu Caron, Audrey Michaud, Berangère Lombard, Simao T da Rocha, John Offer, Damarys Loew, Nicolas Servant, Michel Wassef, Fabienne Burlina, Steve J Gamblin, Edith Heard, Raphaël Margueron (2015 Mar 5)

Jarid2 Methylation via the PRC2 Complex Regulates H3K27me3 Deposition during Cell Differentiation.

Molecular cell : 769-83 : DOI : 10.1016/j.molcel.2014.12.020

Résumé

Polycomb Group (PcG) proteins maintain transcriptional repression throughout development, mostly by regulating chromatin structure. Polycomb Repressive Complex 2 (PRC2), a component of the Polycomb machinery, is responsible for the methylation of histone H3 lysine 27 (H3K27me2/3). Jarid2 was previously identified as a cofactor of PRC2, regulating PRC2 targeting to chromatin and its enzymatic activity. Deletion of Jarid2 leads to impaired orchestration of gene expression during cell lineage commitment. Here, we reveal an unexpected crosstalk between Jarid2 and PRC2, with Jarid2 being methylated by PRC2. This modification is recognized by the Eed core component of PRC2 and triggers an allosteric activation of PRC2's enzymatic activity. We show that Jarid2 methylation is important to promote PRC2 activity at a locus devoid of H3K27me3 and for the correct deposition of this mark during cell differentiation. Our results uncover a regulation loop where Jarid2 methylation fine-tunes PRC2 activity depending on the chromatin context.

Année de publication : 2014

Steven Zuryn, Arnaud Ahier, Manuela Portoso, Esther Redhouse White, Marie-Charlotte Morin, Raphaël Margueron, Sophie Jarriault (2014 Aug 16)
Transdifferentiation. Sequential histone-modifying activities determine the robustness of transdifferentiation.
Science (New York, N.Y.) : 826-9 : DOI : 10.1126/science.1255885

Résumé

Natural interconversions between distinct somatic cell types have been reported in species as diverse as jellyfish and mice. The efficiency and reproducibility of some reprogramming events represent unexploited avenues in which to probe mechanisms that ensure robust cell conversion. We report that a conserved H3K27me3/me2 demethylase, JMJD-3.1, and the H3K4 methyltransferase Set1 complex cooperate to ensure invariant transdifferentiation (Td) of postmitotic Caenorhabditis elegans hindgut cells into motor neurons. At single-cell resolution, robust conversion requires stepwise histone-modifying activities, functionally partitioned into discrete phases of Td through nuclear degradation of JMJD-3.1 and phase-specific interactions with transcription factors that have conserved roles in cell plasticity and terminal fate selection. Our results draw parallels between epigenetic mechanisms underlying robust Td in nature and efficient cell reprogramming in vitro.

Simão Teixeira da Rocha, Valentina Boeva, Martin Escamilla-Del-Arenal, Katia Ancelin, Camille Granier, Neuza Reis Matias, Serena Sanulli, Jen Chow, Edda Schulz, Christel Picard, Syuzo Kaneko, Kristian Helin, Danny Reinberg, A Francis Stewart, Anton Wutz, Raphaël Margueron, Edith Heard (2014 Jan 23)
Jarid2 Is Implicated in the Initial Xist-Induced Targeting of PRC2 to the Inactive

X Chromosome.
Molecular cell : 301-16 : DOI : 10.1016/j.molcel.2014.01.002

Résumé

During X chromosome inactivation (XCI), the Polycomb Repressive Complex 2 (PRC2) is thought to participate in the early maintenance of the inactive state. Although Xist RNA is essential for the recruitment of PRC2 to the X chromosome, the precise mechanism remains unclear. Here, we demonstrate that the PRC2 cofactor Jarid2 is an important mediator of Xist-induced PRC2 targeting. The region containing the conserved B and F repeats of Xist is critical for Jarid2 recruitment via its unique N-terminal domain. Xist-induced Jarid2 recruitment occurs chromosome-wide independently of a functional PRC2 complex, unlike at other parts of the genome, such as CG-rich regions, where Jarid2 and PRC2 binding are interdependent. Conversely, we show that Jarid2 loss prevents efficient PRC2 and H3K27me3 enrichment to Xist-coated chromatin. Jarid2 thus represents an important intermediate between PRC2 and Xist RNA for the initial targeting of the PRC2 complex to the X chromosome during onset of XCI.