Année de publication : 2021

Zhou J, Gelot C, Pantelidou C, Li A, Yücel H, Davis RE, Farkkila A, Kochupurakkal B, Syed A, Shapiro GI, Tainer JA, Blagg BSJ, Ceccaldi R*, D'Andrea AD*. * co-last and co-corresponding authors. (2021 Jun 2)

A first-in-class Polymerase Theta Inhibitor selectively targets Homologous Recombination-Deficient Tumors.

Nature Cancer : 598-610 : DOI : 10.1038/s43018-021-00203-xs

Résumé

DNA polymerase theta (POLθ or POLQ) is synthetic lethal with homologous recombination (HR) deficiency and is thus a candidate target for HR-deficient cancers. Through high-throughput small-molecule screens, we identified the antibiotic novobiocin (NVB) as a specific POLθ inhibitor that selectively kills HR-deficient tumor cells in vitro and in vivo. NVB directly binds to the POLθ ATPase domain, inhibits its ATPase activity and phenocopies POLθ depletion. NVB kills HR-deficient breast and ovarian tumors in genetically engineered mouse models and xenograft and patient-derived xenograft models. Increased POLθ levels predict NVB sensitivity, and HR-deficient tumor cells with acquired resistance to poly(ADP-ribose) polymerase (PARP) inhibitors
(PARPi) are sensitive to NVB in vitro and in vivo. Mechanistically, NVB-mediated cell death in PARPi-resistant cells arises from increased double-strand break end resection, leading to accumulation of single-stranded DNA intermediates and nonfunctional foci of the recombinase RAD51. Our results demonstrate that NVB may be useful alone or in combination with PARPi for treating HR-deficient tumors, including those with acquired PARPi resistance.

Peter Peneder, Adrian M Stütz, Didier Surdez, Manuela Krumbholz, Sabine Semper, Mathieu Chicard, Nathan C Sheffield, Gaelle Pierron, Eve Lapouble, Marcus Tötzl, Bekir Ergüner, Daniele Barreca, André F Rendeiro, Abbas Agaimy, Heidrun Boztug, Gernot Engstler, Michael Dworzak, Marie Bernkopf, Sabine Taschner-Mandl, Inge M Ambros, Ola Myklebost, Perrine Marec-Bérard, Susan Ann Burchill, Bernadette Brennan, Sandra J Strauss, Jeremy Whelan, Gudrun Schleiermacher, Christiane Schaefer, Uta Dirksen, Caroline Hutter, Kjetil Boye, Peter F Ambros, Olivier Delattre, Markus Metzler, Christoph Bock, Eleni M Tomazou (2021 May 29)

Multimodal analysis of cell-free DNA whole-genome sequencing for pediatric cancers with low mutational burden.

Nature communications : 3230 : DOI : 10.1038/s41467-021-23445-w

Résumé

Sequencing of cell-free DNA in the blood of cancer patients (liquid biopsy) provides attractive opportunities for early diagnosis, assessment of treatment response, and minimally invasive disease monitoring. To unlock liquid biopsy analysis for pediatric tumors with few genetic aberrations, we introduce an integrated genetic/epigenetic analysis method and

demonstrate its utility on 241 deep whole-genome sequencing profiles of 95 patients with Ewing sarcoma and 31 patients with other pediatric sarcomas. Our method achieves sensitive detection and classification of circulating tumor DNA in peripheral blood independent of any genetic alterations. Moreover, we benchmark different metrics for cell-free DNA fragmentation analysis, and we introduce the LIQUORICE algorithm for detecting circulating tumor DNA based on cancer-specific chromatin signatures. Finally, we combine several fragmentation-based metrics into an integrated machine learning classifier for liquid biopsy analysis that exploits widespread epigenetic deregulation and is tailored to cancers with low mutation rates. Clinical associations highlight the potential value of cfDNA fragmentation patterns as prognostic biomarkers in Ewing sarcoma. In summary, our study provides a comprehensive analysis of circulating tumor DNA beyond recurrent genetic aberrations, and it renders the benefits of liquid biopsy more readily accessible for childhood cancers.

Ashley L Arthur, Amy Crawford, Anne Houdusse, Margaret A Titus (2021 May 27)

VASP mediated actin dynamics activate and recruit a filopodia myosin.

eLife : DOI : 10.7554/eLife.68082

Résumé

Filopodia are thin, actin-based structures that cells use to interact with their environments. Filopodia initiation requires a suite of conserved proteins but the mechanism remains poorly understood. The actin polymerase VASP and a MyTH-FERM (MF) myosin, DdMyo7 in amoeba, are essential for filopodia initiation. DdMyo7 is localized to dynamic regions of the actin-rich cortex. Analysis of VASP mutants and treatment of cells with anti-actin drugs shows that myosin recruitment and activation in requires localized VASP-dependent actin polymerization. Targeting of DdMyo7 to the cortex alone is not sufficient for filopodia initiation; VASP activity is also required. The actin regulator locally produces a cortical actin network that activates myosin and together they shape the actin network to promote extension of parallel bundles of actin during filopodia formation. This work reveals how filopodia initiation requires close collaboration between an actin binding protein, the state of the actin cytoskeleton and MF myosin activity.

Anne Houdusse, Margaret A Titus (2021 May 25)

The many roles of myosins in filopodia, microvilli and stereocilia.

Current biology : CB : R586-R602 : DOI : S0960-9822(21)00518-2

Résumé

Filopodia, microvilli and stereocilia represent an important group of plasma membrane protrusions. These specialized projections are supported by parallel bundles of actin filaments and have critical roles in sensing the external environment, increasing cell surface area, and acting as mechanosensors. While actin-associated proteins are essential for actin-filament elongation and bundling in these protrusions, myosin motors have a surprising role in the formation and extension of filopodia and stereocilia and in the organization of

microvilli. Actin regulators and specific myosins collaborate in controlling the length of these structures. Myosins can transport cargoes along the length of these protrusions, and, in the case of stereocilia and microvilli, interactions with adaptors and cargoes can also serve to anchor adhesion receptors to the actin-rich core via functionally conserved motor-adaptor complexes. This review highlights recent progress in understanding the diverse roles myosins play in filopodia, microvilli and stereocilia.

Silvia Benito-Martinez, Laura Salavessa, Graça Raposo, Michael S Marks, Cédric Delevoye (2021 May 22)
Melanin transfer and fate within keratinocytes in human skin pigmentation.
Integrative and comparative biology : DOI : icab094

Résumé

Human skin and hair pigmentation play important roles in social behavior but also in photoprotection from the harmful effects of ultraviolet light. The main pigments in mammalian skin, the melanins, are synthesized within specialized organelles called melanosomes in melanocytes, which sit at the basal layer of the epidermis and the hair bulb. The melanins are then transferred from melanocytes to keratinocytes, where they accumulate perinuclearly in membrane-bound organelles as a « cap » above the nucleus. The mechanism of transfer, the nature of the pigmented organelles within keratinocytes, and the mechanism governing their intracellular positioning are all debated and poorly understood, but likely play an important role in the photoprotective properties of melanin in the skin. Here, we detail our current understanding of these processes and present a guideline for future experimentation in this area.

Linh Le, Julia Sirés-Campos, Graça Raposo, Cédric Delevoye, Michael S Marks (2021 May 22)
Melanosome biogenesis in the pigmentation of mammalian skin.
Integrative and comparative biology : DOI : icab078

Résumé

Melanins, the main pigments of the skin and hair in mammals, are synthesized within membrane-bound organelles of melanocytes called melanosomes. Melanosome structure and function are determined by a cohort of resident transmembrane proteins, many of which are expressed only in pigment cells, that localize specifically to melanosomes. Defects in the genes that encode melanosome-specific proteins or components of the machinery required for their transport in and out of melanosomes underlie various forms of ocular or oculocutaneous albinism, characterized by hypopigmentation of the hair, skin and eyes and by visual impairment. We review major components of melanosomes, including the enzymes that catalyze steps in melanin synthesis from tyrosine precursors, solute transporters that allow these enzymes to function, and structural proteins that underlie melanosome shape and melanin deposition. We then review the molecular mechanisms by which these components are biosynthetically delivered to newly forming melanosomes-many of which

are shared by other cell types that generate cell type-specific lysosome-related organelles. We also highlight unanswered questions that need to be addressed by future investigation.

Hai-Feng Zhang, Christopher S Hughes, Wei Li, Jian-Zhong He, Didier Surdez, Amal M El-Naggar, Hongwei Cheng, Anna Prudova, Alberto Delaidelli, Gian Luca Negri, Xiaojun Li, Maj Sofie Orum-Madsen, Michael M Lizardo, Htoo Zarni Oo, Shane Colborne, Taras Shyp, Renata Scopim-Ribeiro, Colin A Hammond, Anne-Chloe Dhez, Sofya Langman, Jonathan Km Lim, Sonia Hy Kung, Amy Li, Anne Steino, Mads Daugaard, Seth J Parker, Ramon I Klein Geltink, Rimas J Orentas, Li-Yan Xu, Gregg B Morin, Olivier Delattre, Dimiter S Dimitrov, Poul H Sorensen (2021 May 22)

Proteomic screens for suppressors of anoikis identify IL1RAP as a promising surface target in Ewing sarcoma.

Cancer discovery : DOI : candisc.1690.2020

Résumé

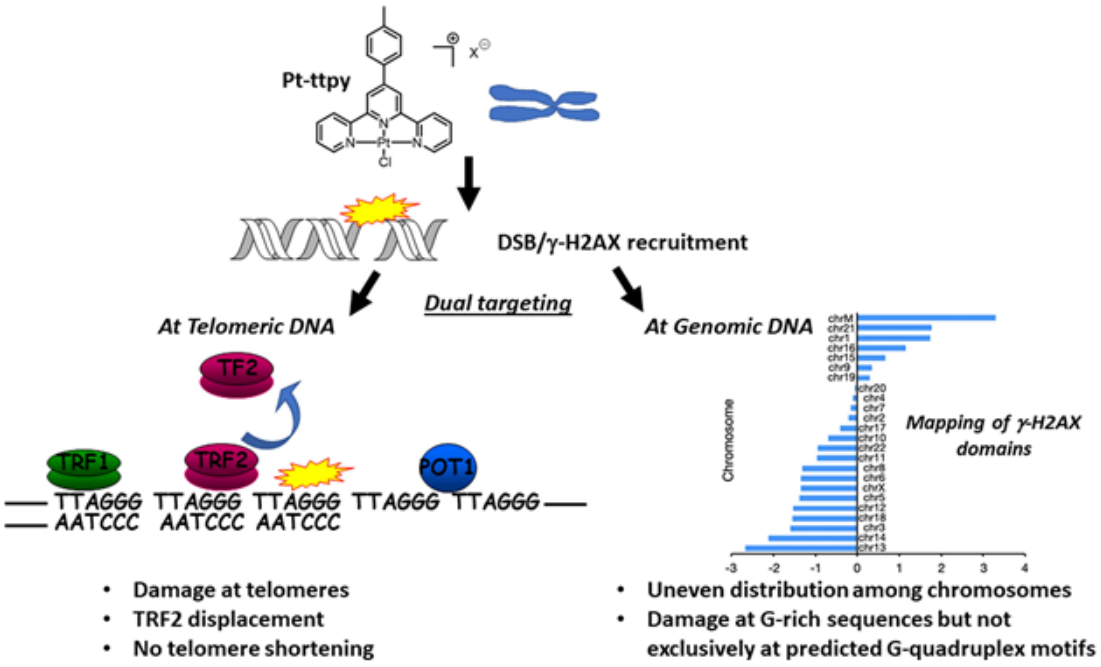
Cancer cells must overcome anoikis (detachment-induced death) to successfully metastasize. Using proteomic screens, we found that distinct oncoproteins upregulate IL-1 receptor accessory protein (IL1RAP) to suppress anoikis. IL1RAP is directly induced by oncogenic fusions of Ewing sarcoma (EwS), a highly metastatic childhood sarcoma. IL1RAP inactivation triggers anoikis and impedes metastatic dissemination of EwS cells. Mechanistically, IL1RAP binds the cell surface system Xc- transporter to enhance exogenous cystine uptake, thereby replenishing cysteine and the glutathione antioxidant. Under cystine depletion, IL1RAP induces cystathionine gamma lyase (CTH) to activate the transsulfuration pathway for de novo cysteine synthesis. Therefore IL1RAP maintains cyst(e)ine and glutathione pools which are vital for redox homeostasis and anoikis resistance. IL1RAP is minimally expressed in pediatric and adult normal tissues, and human anti-IL1RAP antibodies induce potent antibody-dependent cellular cytotoxicity of EwS cells. Therefore, we define IL1RAP as a new cell surface target in EwS, which is potentially exploitable for immunotherapy.

Samar Ali, Emilia Puig Lombardi, Deepanjan Ghosh, Tao Jia, Géraldine Vitry, Lina Saker, Joël Poupon, Marie-Paule Teulade-Fichou, Alain Nicolas, Arturo Londono-Vallejo, Sophie Bombard (2021 May 22)

Pt-ttpy, a G-quadruplex binding platinum complex, induces telomere dysfunction and G-rich regions DNA damage.

Metallomics : integrated biometal science : 13 : mfab029 : DOI : 10.1093/mtomcs/mfab029

Résumé



Pt-ttpy (tolyl terpyridin-Pt complex) covalently binds to G-quadruplex (G4) structures in vitro and to telomeres in cellulo via its Pt moiety. Here, we identified its targets in the human genome, in comparison to Pt-tpy, its derivative without G4 affinity, and cisplatin. Pt-ttpy, but not Pt-tpy, induces the release of the shelterin protein TRF2 from telomeres concomitantly to the formation of DNA damage foci at telomeres but also at other chromosomal locations. γ-H2AX chromatin immunoprecipitation (ChIP-seq) after treatment with Pt-ttpy or cisplatin revealed accumulation in G- and A-rich tandemly repeated sequences, but not particularly in potential G4 forming sequences. Collectively, Pt-ttpy presents dual targeting efficiency on DNA, by inducing telomere dysfunction and genomic DNA damage at specific loci.

Catalina Lodillinsky, Laetitia Fuhrmann, Marie Irondelle, Olena Pylypenko, Xiao-Yan Li, Hélène Bonsang-Kitzis, Fabien Reyal, Sophie Vacher, Claire Calmel, Olivier De Wever, Ivan Bièche, Marie-Lise Lacombe, Ana Maria Eiján, Anne Houdusse, Anne Vincent-Salomon, Stephen J Weiss, Philippe Chavrier, Mathieu Boissan (2021 May 20)

Metastasis-suppressor NME1 controls the invasive switch of breast cancer by regulating MT1-MMP surface clearance.

Oncogene : DOI : 10.1038/s41388-021-01826-1

Résumé

Membrane Type 1 Matrix Metalloprotease (MT1-MMP) contributes to the invasive progression of breast cancers by degrading extracellular matrix tissues. Nucleoside diphosphate kinase,

NME1/NM23-H1, has been identified as a metastasis suppressor; however, its contribution to local invasion in breast cancer is not known. Here, we report that NME1 is up-regulated in ductal carcinoma in situ (DCIS) as compared to normal breast epithelial tissues. NME1 levels drop in microinvasive and invasive components of breast tumor cells relative to synchronous DCIS foci. We find a strong anti-correlation between NME1 and plasma membrane MT1-MMP levels in the invasive components of breast tumors, particularly in aggressive histological grade III and triple-negative breast cancers. Knockout of NME1 accelerates the invasive transition of breast tumors in the intraductal xenograft model. At the mechanistic level, we find that MT1-MMP, NME1 and dynamin-2, a GTPase known to require GTP production by NME1 for its membrane fission activity in the endocytic pathway, interact in clathrin-coated vesicles at the plasma membrane. Loss of NME1 function increases MT1-MMP surface levels by inhibiting endocytic clearance. As a consequence, the ECM degradation and invasive potentials of breast cancer cells are enhanced. This study identifies the down-modulation of NME1 as a potent driver of the in situ-to invasive transition during breast cancer progression.

Olivier Saulnier, Katia Guedri-Idjouadiene, Marie-Ming Aynaud, Alina Chakraborty, Jonathan Bruyr, Joséphine Pineau, Tina O'Grady, Olivier Mirabeau, Sandrine Grossetête, Bartimée Galvan, Margaux Claes, Zahra Al Oula Hassoun, Benjamin Sadacca, Karine Laud, Sakina Zaïdi, Didier Surdez, Sylvain Baulande, Xavier Rambout, Franck Tirode, Martin Dutertre, Olivier Delattre, Franck Dequiedt (2021 May 19)

ERG transcription factors have a splicing regulatory function involving RBFOX2 that is altered in the EWS-FLI1 oncogenic fusion.

Nucleic acids research : DOI : 10.1093/nar/gkab305

Résumé

ERG family proteins (ERG, FLI1 and FEV) are a subfamily of ETS transcription factors with key roles in physiology and development. In Ewing sarcoma, the oncogenic fusion protein EWS-FLI1 regulates both transcription and alternative splicing of pre-messenger RNAs. However, whether wild-type ERG family proteins might regulate splicing is unknown. Here, we show that wild-type ERG proteins associate with spliceosomal components, are found on nascent RNAs, and induce alternative splicing when recruited onto a reporter minigene. Transcriptomic analysis revealed that ERG and FLI1 regulate large numbers of alternative spliced exons (ASEs) enriched with RBFOX2 motifs and co-regulated by this splicing factor. ERG and FLI1 are associated with RBFOX2 via their conserved carboxy-terminal domain, which is present in EWS-FLI1. Accordingly, EWS-FLI1 is also associated with RBFOX2 and regulates ASEs enriched in RBFOX2 motifs. However, in contrast to wild-type ERG and FLI1, EWS-FLI1 often antagonizes RBFOX2 effects on exon inclusion. In particular, EWS-FLI1 reduces RBFOX2 binding to the ADD3 pre-mRNA, thus increasing its long isoform, which represses the mesenchymal phenotype of Ewing sarcoma cells. Our findings reveal a RBFOX2-mediated splicing regulatory function of wild-type ERG family proteins, that is altered in EWS-FLI1 and contributes to the Ewing sarcoma cell phenotype.

Laura Fourmois, Florent Poyer, Aude Sourdon, Delphine Naud-Martin, Sounderya Nagarajan,

Rahima Chennoufi, Eric Deprez, Marie-Paule Teulade-Fichou, Florence Mahuteau-Betzer (2021 May 19)

Modulation of cellular fate of vinyl triarylamines through structural fine tuning: to stay or not to stay in the mitochondria?

Chembiochem : a European journal of chemical biology : Accepted Article : DOI : 10.1002/cbic.202100168

Résumé

Mitochondria is involved in many cellular pathways and dysfunctional mitochondria are linked to various diseases. Hence efforts have been driven to design mitochondria-targeted fluorophores for monitoring the mitochondria status. However, the factors that govern the mitochondria-targeted potential of dyes are not well-understood. In this context, we synthesized analogues of the TP-2Bzim probe belonging to the vinyltriphenylamine (TPA) class and already described for its capacity to bind nuclear DNA in fixed cells and mitochondria in live cells. These analogues (TP-1Bzim, TP n -2Bzim, TP 1+ -2Bzim, TN-2Bzim) differ by the cationic charge, the number of vinylbenzimidazolium branches and the nature of the triaryl core. Using microscopy, we demonstrated that the cationic derivatives accumulate in mitochondria but do not reach mtDNA. Under depolarisation of the mitochondrial membrane, TP-2Bzim and TP 1+ -2Bzim translocate to the nucleus in direct correlation with their strong DNA affinity. This reversible phenomenon emphasizes that these probes can be used to monitor ΔΨ m variations.

Yolanda Gutiérrez, Sergio López-García, Argentina Lario, Silvia Gutiérrez-Eisman, Cédric Delevoye, José A Esteban (2021 May 17)

KIF13A drives AMPA receptor synaptic delivery for long-term potentiation via endosomal remodeling.

The Journal of cell biology : DOI : e202003183

Résumé

The regulated trafficking of AMPA-type glutamate receptors (AMPARs) from dendritic compartments to the synaptic membrane in response to neuronal activity is a core mechanism for long-term potentiation (LTP). However, the contribution of the microtubule cytoskeleton to this synaptic transport is still unknown. In this work, using electrophysiological, biochemical, and imaging techniques, we have found that one member of the kinesin-3 family of motor proteins, KIF13A, is specifically required for the delivery of AMPARs to the spine surface during LTP induction. Accordingly, KIF13A depletion from hippocampal slices abolishes LTP expression. We also identify the vesicular protein centaurin-α1 as part of a motor transport machinery that is engaged with KIF13A and AMPARs upon LTP induction. Finally, we determine that KIF13A is responsible for the remodeling of Rab11-FIP2 endosomal structures in the dendritic shaft during LTP. Overall, these results identify specific kinesin molecular motors and endosomal transport machinery that catalyzes the dendrite-to-synapse translocation of AMPA receptors during synaptic plasticity.

Mamy Andrianteranagna, Joanna Cyrta, Julien Masliah-Planchon, Karolina Nemes, Alice Corsia, Amaury Leruste, Dörthe Holdhof, Uwe Kordes, Daniel Orbach, Nadège Corradini, Natacha Entz-Werle, Gaëlle Pierron, Marie-Pierre Castex, Anne Brouchet, Noëlle Weingertner, Dominique Ranchère, Paul Fréneaux, Olivier Delattre, Jonathan Bush, Alexandra Leary, Michael C Frühwald, Ulrich Schüller, Nicolas Servant, Franck Bourdeaut (2021 May 17)

SMARCA4-deficient rhabdoid tumours show intermediate molecular features between SMARCB1-deficient rhabdoid tumours and small cell carcinomas of the ovary, hypercalcaemic type.

The Journal of pathology : DOI : 10.1002/path.5705

Résumé

Extracranial rhabdoid tumours (ECRT) are an aggressive malignancy of infancy and early childhood. The vast majority of cases demonstrate inactivation of SMARCB1 (ECRT) on a background of a remarkably stable genome, a low mutational burden, and no other recurrent mutations. Rarely, ECRT can harbour the alternative inactivation of SMARCA4 (ECRT) instead of SMARCB1. However, very few ECRT cases have been published to date, and a systematic characterization of ECRT is missing from the literature. In this study, we report the clinical, pathological, and genomic features of additional cases of ECRT , and show that they are comparable to those of ECRT We also assess whether ECRT , ECRT and small cell carcinomas of the ovary, hypercalcaemic type (SCCOHT) represent distinct or overlapping entities at a molecular level. Using DNA methylation and transcriptomics-based tumour classification approaches, we demonstrate that ECRT display molecular features intermediate between SCCOHT and ECRT ; however, ECRT appear to be more closely related to SCCOHT by DNA methylation. Conversely, both transcriptomics and DNA methylation show a larger gap between SCCOHT and ECRT , potentially supporting their continuous separate classification. Lastly, we show that ECRT display concomitant lack of SMARCA4 (BRG1) and SMARCA2 (BRM) expression at the protein level, similar to what is seen in SCCOHT. Overall, our results expand the knowledge on this rare tumour type and explore the similarities and differences among entities from the 'rhabdoid tumour' spectrum. This article is protected by copyright. All rights reserved.

Anne Houdusse (2021 May 14)

Biological nanomotors, driving forces of life.

Comptes rendus biologies : 53-78 : DOI : 10.5802/crbiol.45

Résumé

Life is driven by awe-inspiring coordinated movements observed in cells and tissues. In each cell, nm-size molecular motor proteins contribute to these movements as they power numerous mechanical processes with precision and complex orchestration. For the multiple functions that an eukaryotic cell accomplish, motility is essential both at molecular and cellular scales. Tissue morphogenesis, cell migration, cell division or cell differentiation are all controlled by the precise action of such nanomotors that work on cytoskeletal tracks using ATP as fuel. The study of motility has a long history and scientists of all disciplines have

contributed to its understanding. The first part of this review compares myosin and kinesin motors to describe the principles underlying how motors convert chemical energy into mechanical movement. In a second part, I will describe how sequence differences selected through evolution can lead to distinct force production output despite a common mechanism. Motors within a superfamily can thus carry out distinct functions in cells. Such differences give rise to their individual, specific motility properties, including reversal of directionality or ability to organize cytoskeletal tracks. The power of structural biology to reveal unexpected and surprising structures, with certainty when visualized at atomic resolution, has been a great advantage for this field. The critical insights gained from the structures can be carefully tested with functional experiments, leading to progress in defining the role motors play in cells. Last, I will describe how targeting these motors can be beneficial for human health. Allosteric sites for specific small molecules can act as activators or inhibitors of the force produced by these nanomotors. While frequent sites of mutations in these motors can lead to disease phenotypes, high therapeutic potential of allosteric effectors is now established for heart muscle diseases and should be extended to treat other pathologies.

Didier Surdez, Sakina Zaidi, Sandrine Grossetête, Karine Laud-Duval, Anna Sole Ferre, Lieke Mous, Thomas Vourc'h, Franck Tirode, Gaelle Pierron, Virginie Raynal, Sylvain Baulande, Erika Brunet, Véronique Hill, Olivier Delattre (2021 Apr 30)

STAG2 mutations alter CTCF-anchored loop extrusion, reduce cis-regulatory interactions and EWSR1-FLI1 activity in Ewing sarcoma.

Cancer cell : DOI : 10.1016/j.ccell.2021.04.001

Résumé

STAG2, a cohesin family gene, is among the most recurrently mutated genes in cancer. STAG2 loss of function (LOF) is associated with aggressive behavior in Ewing sarcoma, a childhood cancer driven by aberrant transcription induced by the EWSR1-FLI1 fusion oncogene. Here, using isogenic Ewing cells, we show that, while STAG2 LOF profoundly changes the transcriptome, it does not significantly impact EWSR1-FLI1, CTCF/cohesin, or acetylated H3K27 DNA binding patterns. In contrast, it strongly alters the anchored dynamic loop extrusion process at boundary CTCF sites and dramatically decreases promoter-enhancer interactions, particularly affecting the expression of genes regulated by EWSR1-FLI1 at GGAA microsatellite neo-enhancers. Down-modulation of cis-mediated EWSR1-FLI1 activity, observed in STAG2-LOF conditions, is associated with enhanced migration and invasion properties of Ewing cells previously observed in EWSR1-FLI1 cells. Our study illuminates a process whereby STAG2-LOF fine-tunes the activity of an oncogenic transcription factor through altered CTCF-anchored loop extrusion and cis-mediated enhancer mechanisms.