Summary

Deficiencies in genome maintenance genes result in increased mutagenesis and genome rearrangements that impact cell viability, species adaptation, and evolvability. The accumulation of somatic mutations is also a landmark of most tumor cells but it remains difficult to retrospectively determine their mechanistic origin(s). Here, we conducted a prospective reciprocal approach to inactivate evolutionary conserved genes involved in various genome maintenance processes and characterize de novo mutations in diploid S. cerevisiae mutation accumulation lines. Our results revealed the diversity, trajectory, complexity, and ultimate uniqueness of the clonal mutational landscapes. Some mutational signatures resemble those found in human tumors.

Summary

A subset of cancer-associated fibroblasts (FAP+/CAF-S1) mediates immunosuppression in breast cancers, but its heterogeneity and its impact on immunotherapy response remain unknown. Here, we identify 8 CAF-S1 clusters by analyzing more than 19,000 single CAF-S1 fibroblasts from breast cancer. We validate the five most abundant clusters by flow cytometry and in silico analyses in other cancer types, highlighting their relevance. Myofibroblasts from clusters 0 and 3, characterized by extracellular matrix proteins and TGFβ signaling, respectively, are indicative of primary resistance to immunotherapies. Cluster 0/ecm-myCAF upregulates PD-1 and CTLA4 protein levels in regulatory T lymphocytes (Tregs), which, in turn, increases CAF-S1 cluster 3/TGFβ-myCAF cellular content. Thus, our study highlights a positive feedback loop between specific CAF-S1 clusters and Tregs and uncovers their role in immunotherapy resistance.
Transcriptional Programs Define Intratumoral Heterogeneity of Ewing Sarcoma at Single-Cell Resolution.

**Summary**

EWSR1-FLI1, the chimeric oncogene specific for Ewing sarcoma (EwS), induces a cascade of signaling events leading to cell transformation. However, it remains elusive how genetically homogeneous EwS cells can drive the heterogeneity of transcriptional programs. Here, we combine independent component analysis of single-cell RNA sequencing data from diverse cell types and model systems with time-resolved mapping of EWSR1-FLI1 binding sites and of open chromatin regions to characterize dynamic cellular processes associated with EWSR1-FLI1 activity. We thus define an exquisitely specific and direct enhancer-driven EWSR1-FLI1 program. In EwS tumors, cell proliferation and strong oxidative phosphorylation metabolism are associated with a well-defined range of EWSR1-FLI1 activity. In contrast, a subpopulation of cells from below and above the intermediary EWSR1-FLI1 activity is characterized by increased hypoxia. Overall, our study reveals sources of intratumoral heterogeneity within EwS tumors.

Cancer-associated fibroblast heterogeneity in axillary lymph nodes drives metastases in breast cancer through complementary mechanisms

**Summary**

Although fibroblast heterogeneity is recognized in primary tumors, both its characterization in and its impact on metastases remain unknown. Here, combining flow cytometry, immunohistochemistry and RNA-sequencing on breast cancer samples, we identify four Cancer-Associated Fibroblast (CAF) subpopulations in metastatic lymph nodes (LN). Two myofibroblastic subsets, CAF-S1 and CAF-S4, accumulate in LN and correlate with cancer cell invasion. By developing functional assays on primary cultures, we demonstrate that these subsets promote metastasis through distinct functions. While CAF-S1 stimulate cancer cell migration and initiate an epithelial-to-mesenchymal transition through CXCL12 and TGFβ pathways, highly contractile CAF-S4 induce cancer cell invasion in 3-dimensions via NOTCH signaling. Patients with high levels of CAFs, particularly CAF-S4, in LN at diagnosis are prone to metastasis.
to develop late distant metastases. Our findings suggest that CAF subset accumulation in LN is a prognostic marker, suggesting that CAF subsets could be examined in axillary LN at diagnosis.

Rafael Galupa, Elphège Pierre Nora, Rebecca Worsley-Hunt, Christel Picard, Chris Gard, Joke Gerardavann Bemmel, Nicolas Servant, Yinxiu Zhan, Fatima El Marjou, Colin Johanneau, Patricia Diabangouaya, Agnès Le Saux, Sonia Lameiras, Juliana Pipoli da Fonseca, Friedemann Loos, Joost Gribnau, Sylvain Baulande, Uwe Ohler, Luca Giorgetti, Edith Heard (2020 Jan 16)

**A Conserved Noncoding Locus Regulates Random Monoallelic Xist Expression across a Topological Boundary**  
*Molecular Cell* : 77 n°2 : 352-367.e8 : [DOI : 10.1016/j.molcel.2019.10.030]

**Summary**

cis-Regulatory communication is crucial in mammalian development and is thought to be restricted by the spatial partitioning of the genome in topologically associating domains (TADs). Here, we discovered that the Xist locus is regulated by sequences in the neighboring TAD. In particular, the promoter of the noncoding RNA Linx (LinxP) acts as a long-range silencer and influences the choice of X chromosome to be inactivated. This is independent of Linx transcription and independent of any effect on Tsix, the antisense regulator of Xist that shares the same TAD as Linx. Unlike Tsix, LinxP is well conserved across mammals, suggesting an ancestral mechanism for random monoallelic Xist regulation. When introduced in the same TAD as Xist, LinxP switches from a silencer to an enhancer. Our study uncovers an unsuspected regulatory axis for X chromosome inactivation and a class of cis-regulatory effects that may exploit TAD partitioning to modulate developmental decisions.

Robert Ragazzini, Raquel Pérez-Palacios, Irem H Baymaz, Seynabou Diop, Katia Ancelin, Dina Zielinski, Audrey Michaud, Maëlle Givelet, Mate Borsos, Setareh Aflaki, Patricia Legoix, Pascal W T C Jansen, Nicolas Servant, Maria-Elena Torres-Padilla, Deborah Bourc'his, Pierre Fouchet, Michiel Vermeulen, Raphaël Margueron (2019 Aug 26)

**EZHIP constrains Polycomb Repressive Complex 2 activity in germ cells.**  
*Nature communications* : 10 : 1-18 : [DOI : 10.1038/s41467-019-11800-x]

**Summary**

The Polycomb group of proteins is required for the proper orchestration of gene expression due to its role in maintaining transcriptional silencing. It is composed of several chromatin modifying complexes, including Polycomb Repressive Complex 2 (PRC2), which deposits H3K27me2/3. Here, we report the identification of a cofactor of PRC2, EZHIP (EZH1/2 Inhibitory Protein), expressed predominantly in the gonads. EZHIP limits the enzymatic activity of PRC2 and lessens the interaction between the core complex and its accessory
subunits, but does not interfere with PRC2 recruitment to chromatin. Deletion of Ezhip in mice leads to a global increase in H3K27me2/3 deposition both during spermatogenesis and at late stages of oocyte maturation. This does not affect the initial number of follicles but is associated with a reduction of follicles in aging. Our results suggest that mature oocytes Ezhip-/- might not be fully functional and indicate that fertility is strongly impaired in Ezhip-/- females. Altogether, our study uncovers EZHIP as a regulator of chromatin landscape in gametes.

Kevin Grosselin, Adeline Durand, Justine Marsolier, Adeline Poitou, Elisabetta Marangoni, Fariba Nemati, Ahmed Dahmani, Sonia Lameiras, Fabien Reyal, Olivia Frenoy, Yannick Pousse, Marcel Reichen, Adam Woolfe, Colin Brenan, Andrew D. Griffiths*, Céline Vallot* & Annabelle Gérard* (2019 May 31)

High-throughput single-cell ChIP-seq identifies heterogeneity of chromatin states in breast cancer

Nature Genetics : 51 : 1060–1066 : DOI : 10.1038/s41588-019-0424-9

Summary

Modulation of chromatin structure via histone modification is a major epigenetic mechanism and regulator of gene expression. However, the contribution of chromatin features to tumor heterogeneity and evolution remains unknown. Here we describe a high-throughput droplet microfluidics platform to profile chromatin landscapes of thousands of cells at single-cell resolution. Using patient-derived xenograft models of acquired resistance to chemotherapy and targeted therapy in breast cancer, we found that a subset of cells within untreated drug-sensitive tumors share a common chromatin signature with resistant cells, undetectable using bulk approaches. These cells, and cells from the resistant tumors, have lost chromatin marks—H3K27me3, which is associated with stable transcriptional repression—for genes known to promote resistance to treatment. This single-cell chromatin immunoprecipitation followed by sequencing approach paves the way to study the role of chromatin heterogeneity, not just in cancer but in other diseases and healthy systems, notably during cellular differentiation and development.

Joke G van Bemmel, Rafael Galupa, Chris Gard, Nicolas Servant, Christel Picard, James Davies, Anthony James Szempruch, Yinxiu Zhan, Jan J Żylicz, Elphège P Nora, Sonia Lameiras, Elzo de Wit, David Gentien, Sylvain Baulande, Luca Giorgetti, Mitchell Gutttman, Jim R Hughes, Douglas R Higgs, Joost Gribnau, Edith Heard (2019 May 29)

The bipartite TAD organization of the X-inactivation center ensures opposing developmental regulation of Tsix and Xist.

Nature genetics : 51 : 1024-34 : DOI : 10.1038/s41588-019-0412-0

Summary

The mouse X-inactivation center (Xic) locus represents a powerful model for understanding
the links between genome architecture and gene regulation, with the non-coding genes Xist and Tsix showing opposite developmental expression patterns while being organized as an overlapping sense/antisense unit. The Xic is organized into two topologically associating domains (TADs) but the role of this architecture in orchestrating cis-regulatory information remains elusive. To explore this, we generated genomic inversions that swap the Xist/Tsix transcriptional unit and place their promoters in each other’s TAD. We found that this led to a switch in their expression dynamics: Xist became precociously and ectopically upregulated, both in male and female pluripotent cells, while Tsix expression aberrantly persisted during differentiation. The topological partitioning of the Xic is thus critical to ensure proper developmental timing of X inactivation. Our study illustrates how the genomic architecture of cis-regulatory landscapes can affect the regulation of mammalian developmental processes.

Angela Bellini, Nadia Bessoltane-Bentahar, Jaydutt Bhalshankar, Nathalie Clement, Virginie Raynal, Sylvain Baulande, Virginie Bernard, Adrien Danzon, Mathieu Chicard, Léo Colmet-Daage, Gaelle Pierron, Laura Le Roux, Julien Masliah Planchon, Valérie Combaret, Eve Lapouble, Nadège Corradini, Estelle Thebaut, Marion Gambart, Dominique Valteau-Couanet, Jean Michon, Caroline Louis-Brennetot, Isabelle Janoueix-Lerosey, Anne-Sophie Defachelles, Franck Bourdeaut, Olivier Delatte, Gudrun Schleiermacher (2019 Apr 25)

Study of chromatin remodeling genes implicates SMARCA4 as a putative player in oncogenesis in neuroblastoma.

Summary

In neuroblastoma (NB), genetic alterations in chromatin remodeling (CRGs) and epigenetic modifier genes (EMGs) have been described. We sought to determine their frequency and clinical impact. Whole exome (WES)/whole genome sequencing (WGS) data and targeted sequencing (TSCA®) of exonic regions of 33 CRGs/EMGs were analyzed in tumor samples from 283 NB patients, with constitutional material available for 55 patients. The frequency of CRG/EMG variations in NB cases was then compared to the Genome Aggregation Database (gnomAD). The sequencing revealed SNVs/small InDels or focal CNAs of CRGs/EMGs in 20% (56/283) of all cases, occurring at a somatic level in 4 (7.2%), at a germline level in 12 (22%) cases, whereas for the remaining cases, only tumor material could be analyzed. The most frequently altered genes were ATRX (5%), SMARCA4 (2.5%), MLL3 (2.5%) and ARID1B (2.5%). Double events (SNVs/small InDels/CNAs associated with LOH) were observed in SMARCA4 (n=3), ATRX (n=1) and PBRM1 (n=1). Among the 60 variations, 24 (8.4%) targeted domains of functional importance for chromatin remodeling or highly conserved domains but of unknown function. Variations in SMARCA4 and ATRX occurred more frequently in the NB as compared to the gnomAD control cohort (OR=4.49, 95%CI:1.63-9.97, P=0.038; OR 3.44, 95%CI:1.46-6.91, P=0.043, respectively). Cases with CRG/EMG variations showed a poorer overall survival compared to cases without variations. Genetic variations of CRGs/EMGs with likely functional impact were observed in 8.4% (24/283) of NB. Our case-control approach suggests a role of SMARCA4 as a player of NB oncogenesis. This article is protected by copyright. All rights reserved.
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*: 10: 1-15 : [DOI: 10.1038/s41467-018-08255-x]

**Summary**

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.

**Year of publication 2018**

Irene Jiménez, Mathieu Chicard, Léo Colmet-Daage, Nathalie Clément, Adrien Danzon, Eve Lapouble, Gaëlle Pierron, Mylène Bohec, Sylvain Baulande, Dominique Berrebi, Paul Fréneau, Aurore Coulomb, Louise Galmiche-Rolland, Sabine Sarnacki, Georges Audry, Pascale Philippe-Chomette, Hervé J Brisse, François Doz, Jean Michon, Olivier Delattre, Gudrun Schleiermacher (2018 Jun 21)

**Circulating tumor DNA analysis enables molecular characterization of pediatric renal tumors at diagnosis.**
*International journal of cancer*: [DOI: 10.1002/ijc.31620]

**Summary**

Circulating tumor DNA (ctDNA) is a powerful tool for the molecular characterization of cancer. The most frequent pediatric kidney tumors (KT) are Wilms' tumors (WT), but other diagnoses may occur. According to the SIOP strategy, in most countries pediatric KT have a presumptive diagnosis of WT if they are clinically and radiologically compatible. The histologic confirmation is established after post-chemotherapy nephrectomy. Thus, there is a risk for a small fraction of patients to receive neoadjuvant chemotherapy that is not adapted to the disease. The aim of this work is to perform molecular diagnosis of pediatric KT by tumor genetic characterization based on the analysis of ctDNA. We analyzed ctDNA extracted from plasma samples of 18 pediatric patients with KT by whole-exome sequencing and compared the results to their matched tumor and germline DNA. Copy number
alterations (CNAs) and single nucleotide variations (SNVs) were analyzed. We were able to detect tumor cell specific genetic alterations-CNAs, SNVs or both-in ctDNA in all patients except in one (for whom the plasma sample was obtained long after nephrectomy). These results open the door to new applications for the study of ctDNA with regards to the molecular diagnosis of KT, with a possibility of its usefulness for adapting the treatment early after diagnosis, but also for disease monitoring and follow up.

Irene Jiménez, Mathieu Chicard, Léo Colmet-Daage, Nathalie Clément, Adrien Danzon, Eve Lapouble, Gaëlle Pierron, Mylène Bohec, Sylvain Baulande, Dominique Berrebi, Paul Fréneaux, Aurore Coulomb, Louise Galmiche-Rolland, Sabine Sarnacki, Georges Audry, Pascale Philippe-Chomette, Hervé J Brisse, François Doz, Jean Michon, Olivier Delattre, Gudrun Schleiermacher (2018 Jun 21)

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**Year of publication 2017**

Mathieu Chicard, Leo Colmet-Daage, Nathalie Clement, Adrien Danzon, Mylène Bohec, Virginie Bernard, Sylvain Baulande, Angela Bellini, Paul Deveau, Gaëlle Pierron, Eve Lapouble, Isabelle Janoueix-Lerosey, Michel Peuchmaur, Nadège Corradini, Anne Sophie Defachelles, Dominique Valteau-Couanet, Jean Michon, Valérie Combaret, Olivier Delattre, Gudrun Schleiermacher (2017 Dec 2)
Whole-Exome Sequencing of Cell-Free DNA Reveals Temporo-spatial Heterogeneity and Identifies Treatment-Resistant Clones in Neuroblastoma.

*Clinical cancer research*: 939-949 : [DOI: 10.1158/1078-0432.CCR-17-1586]

**Summary**

**Purpose:** Neuroblastoma displays important clinical and genetic heterogeneity, with emergence of new mutations at tumor progression. **Experimental Design:** To study clonal evolution during treatment and follow-up, an innovative method based on circulating cell-free DNA (cfDNA) analysis by whole-exome sequencing (WES) paired with target sequencing was realized in sequential liquid biopsy samples of 19 neuroblastoma patients. **Results:** WES of the primary tumor and cfDNA at diagnosis showed overlap of single-nucleotide variants (SNV) and copy number alterations, with 41% and 93% of all detected alterations common to the primary neuroblastoma and cfDNA. CfDNA WES at a second time point indicated a mean of 22 new SNVs for patients with progressive disease. Relapse-specific alterations included genes of the MAPK pathway and targeted the protein kinase A signaling pathway. Deep coverage target sequencing of intermediate time points during treatment and follow-up identified distinct subclones. For 17 seemingly relapse-specific SNVs detected by cfDNA WES at relapse but not tumor or cfDNA WES at diagnosis, deep coverage target sequencing detected these alterations in minor subclones, with relapse-emerging SNVs targeting genes of neuritogenesis and cell cycle. Furthermore a persisting, resistant clone with concomitant disappearance of other clones was identified by a mutation in the ubiquitin protein ligase HERC2. **Conclusions:** Modelization of mutated allele fractions in cfDNA indicated distinct patterns of clonal evolution, with either a minor, treatment-resistant clone expanding to a major clone at relapse, or minor clones collaborating toward tumor progression. Identification of treatment-resistant clones will enable development of more efficient treatment strategies.

**Year of publication**: 2015

María Elena Fernández-Sánchez, Sandrine Barbier, Joanne Whitehead, Gaëlle Béalle, Aude Michel, Heldmuth Latorre-Ossa, Colette Rey, Laura Fouassier, Audrey Claperon, Laura Brullé, Elodie Girard, Nicolas Servant, Thomas Rio-Frio, Hélène Marie, Sylviane Lesieur, Chantal Houssset, Jean-Luc Gennisson, Mickaël Tanter, Christine Ménager, Silvia Fre, Sylvie Robine, Emmanuel Farge (2015 Jul 2)

**Mechanical induction of the tumorigenic β-catenin pathway by tumour growth pressure.**

*Nature*: 92-5 : [DOI: 10.1038/nature14329]

**Summary**

The tumour microenvironment may contribute to tumorigenesis owing to mechanical forces such as fibrotic stiffness or mechanical pressure caused by the expansion of hyper-proliferative cells. Here we explore the contribution of the mechanical pressure exerted by tumour growth onto non-tumorous adjacent epithelium. In the early stage of mouse colon tumour development in the Notch(+)Apc(+/1638N) mouse model, we observed mechanistic
pressure stress in the non-tumorous epithelial cells caused by hyper-proliferative adjacent crypts overexpressing active Notch, which is associated with increased Ret and β-catenin signalling. We thus developed a method that allows the delivery of a defined mechanical pressure in vivo, by subcutaneously inserting a magnet close to the mouse colon. The implanted magnet generated a magnetic force on ultra-magnetic liposomes, stabilized in the mesenchymal cells of the connective tissue surrounding colonic crypts after intravenous injection. The magnetically induced pressure quantitatively mimicked the endogenous early tumour growth stress in the order of 1,200 Pa, without affecting tissue stiffness, as monitored by ultrasound strain imaging and shear wave elastography. The exertion of pressure mimicking that of tumour growth led to rapid Ret activation and downstream phosphorylation of β-catenin on Tyr654, impairing its interaction with the E-cadherin in adherens junctions, and which was followed by β-catenin nuclear translocation after 15 days. As a consequence, increased expression of β-catenin-target genes was observed at 1 month, together with crypt enlargement accompanying the formation of early tumorous aberrant crypt foci. Mechanical activation of the tumorigenic β-catenin pathway suggests unexplored modes of tumour propagation based on mechanical signalling pathways in healthy epithelial cells surrounding the tumour, which may contribute to tumour heterogeneity.

Angela Bellini, Virginie Bernard, Quentin Leroy, Thomas Rio Frio, Gaëlle Pierron, Valérie Combaret, Eve Lapouble, Nathalie Clement, Hervé Rubie, Estelle Thebaud, Pascal Chastagner, Anne Sophie Defachelles, Christophe Bergeron, Nimrod Buchbinder, Sophie Taque, Anne Auvrignon, Dominique Valteau-Couanet, Jean Michon, Isabelle Janoueix-Lerosey, Olivier Delattre, Gudrun Schleiermacher (2015 Feb 20)

**Deep Sequencing Reveals Occurrence of Subclonal ALK Mutations in Neuroblastoma at Diagnosis.**

*Clinical cancer research : an official journal of the American Association for Cancer Research* : 4913-21 : [DOI : 10.1158/1078-0432.CCR-15-0423]

**Summary**

In neuroblastoma, activating ALK receptor tyrosine kinase point mutations play a major role in oncogenesis. We explored the potential occurrence of ALK mutations at a subclonal level using targeted deep sequencing.