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.

ALK mutation dynamics and clonal evolution in a neuroblastoma model exhibiting two ALK mutations.

Summary

The gene is a major oncogene of neuroblastoma cases exhibiting ALK activating mutations. Here, we characterized two neuroblastoma cell lines established from a stage 4 patient at diagnosis either from the primary tumor (PT) or from the bone marrow (BM). Both cell lines exhibited similar genomic profiles. All cells in the BM-derived cell line exhibited an ALK F1174L mutation, whereas this mutation was present in only 5% of the cells in the earliest passages of the PT-derived cell line. The BM-derived cell line presented with a higher
proliferation rate and injections in Nude mice resulted in tumor formation only for the BM-derived cell line. Next, we observed that the F1174L mutation frequency in the PT-derived cell line increased with successive passages. Further Whole Exome Sequencing revealed a second ALK mutation, L1196M, in this cell line. Digital droplet PCR documented that the allele fractions of both mutations changed upon passages, and that the F1174L mutation reached 50% in late passages, indicating clonal evolution. Treatment of the PT-derived cell line exhibiting the F1174L and L1196M mutations with the alectinib inhibitor resulted in an enrichment of the L1196M mutation. Using xenografts, we documented a better efficacy of alectinib compared to crizotinib on tumor growth and an enrichment of the L1196M mutation at the end of both treatments. Finally, single-cell RNA-seq analysis was consistent with both mutations resulting in ALK activation. Altogether, this study provides novel insights into ALK mutation dynamics in a neuroblastoma model harbouring two ALK mutations.

Laura Romero-Pérez, Didier Surdez, Erika Brunet, Olivier Delattre, Thomas G P Grünewald (2019 Aug 19)

STAG Mutations in Cancer.
Trends in cancer : 506-520 : DOI : S2405-8033(19)30138-4

Summary

Stromal Antigen 1 and 2 (STAG1/2) are key subunits of the cohesin complex that mediate sister chromatid cohesion, DNA repair, transcriptional regulation, and genome topology. Genetic alterations comprising any of the 11 cohesin-associated genes possibly occur in up to 26% of patients included in The Cancer Genome Atlas (TCGA) studies. STAG2 shows the highest number of putative driver truncating mutations. We provide a comprehensive review of the function of STAG1/2 in human physiology and disease and an integrative analysis of available omics data on STAG alterations in a wide array of cancers, comprising 53,691 patients and 1067 cell lines. Lastly, we discuss opportunities for therapeutic intervention.


Study of chromatin remodeling genes implicates SMARCA4 as a putative player in oncogenesis in neuroblastoma.
International journal of cancer : DOI : 10.1002/ijc.32361

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.

Year of publication 2018


**Genome-wide association study identifies multiple new loci associated with Ewing sarcoma susceptibility.**

*Nature communications* : 3184 : [DOI : 10.1038/s41467-018-05537-2](https://doi.org/10.1038/s41467-018-05537-2)

**Summary**

Ewing sarcoma (EWS) is a pediatric cancer characterized by the EWSR1-FLI1 fusion. We performed a genome-wide association study of 733 EWS cases and 1346 unaffected individuals of European ancestry. Our study replicates previously reported susceptibility loci at 1p36.22, 10q21.3 and 15q15.1, and identifies new loci at 6p25.1, 20p11.22 and 20p11.23. Effect estimates exhibit odds ratios in excess of 1.7, which is high for cancer GWAS, and striking in light of the rarity of EWS cases in familial cancer syndromes. Expression quantitative trait locus (eQTL) analyses identify candidate genes at 6p25.1 (RREB1) and
20p11.23 (KIZ). The 20p11.22 locus is near NKX2-2, a highly overexpressed gene in EWS. Interestingly, most loci reside near GGAA repeat sequences and may disrupt binding of the EWSR1-FLI1 fusion protein. The high locus to case discovery ratio from 733 EWS cases suggests a genetic architecture in which moderate risk SNPs constitute a significant fraction of risk.

Kathleen I Pishas, Christina D Drenberg, Cenny Taslim, Emily R Theisen, Kirsten M Johnson, Ranajeet S Saund, Ioana L Pop, Brian D Crompton, Elizabeth R Lawlor, Franck Tirode, Jaume Mora, Olivier Delattre, Mary C Beckerle, David F Callen, Sunil Sharma, Stephen L Lessnick (2018 Jul 13)

**Therapeutic Targeting of KDM1A/LSD1 in Ewing Sarcoma with SP-2509 Engages the Endoplasmic Reticulum Stress Response.**

*Molecular cancer therapeutics*: 1902-1916 : [DOI: 10.1158/1535-7163.MCT-18-0373]

**Summary**

Multi-agent chemotherapeutic regimes remain the cornerstone treatment for Ewing sarcoma, the second most common bone malignancy diagnosed in pediatric and young adolescent populations. We have reached a therapeutic ceiling with conventional cytotoxic agents, highlighting the need to adopt novel approaches that specifically target the drivers of Ewing sarcoma oncogenesis. As KDM1A/lysine-specific demethylase 1 (LSD1) is highly expressed in Ewing sarcoma cell lines and tumors, with elevated expression levels associated with worse overall survival ( = 0.033), this study has examined biomarkers of sensitivity and mechanisms of cytotoxicity to targeted inhibition using SP-2509 (reversible inhibitor). We report, that innate resistance to SP-2509 was not observed in our Ewing sarcoma cell line cohort ( = 17; IC range, 81 -1,593 nmol/L), in contrast resistance to the next-generation irreversible inhibitor GSK-LSD1 was observed across multiple cell lines (IC > 300 μmol/L). Although status and basal KDM1A mRNA and protein levels did not correlate with SP-2509 response, induction of KDM1B following SP-2509 treatment was strongly associated with SP-2509 hypersensitivity. We show that the transcriptional profile driven by SP-2509 strongly mirrors genetic depletion. Mechanistically, RNA-seq analysis revealed that SP-2509 imparts robust apoptosis through engagement of the endoplasmic reticulum stress pathway. In addition, were specifically induced/repressed, respectively following SP-2509 treatment only in our hypersensitive cell lines. Together, our findings provide key insights into the mechanisms of SP-2509 cytotoxicity as well as biomarkers that can be used to predict inhibitor sensitivity in Ewing sarcoma.

Thomas G P Grünewald, Florencia Cidre-Aranaz, Didier Surdez, Eleni M Tomazou, Enrique de Álava, Heinrich Kovar, Poul H Sorensen, Olivier Delattre, Uta Dirksen (2018 Jul 7)

**Ewing sarcoma.**

*Nature reviews. Disease primers*: 5 : [DOI: 10.1038/s41572-018-0003-x]

**Summary**
Ewing sarcoma is the second most frequent bone tumour of childhood and adolescence that can also arise in soft tissue. Ewing sarcoma is a highly aggressive cancer, with a survival of 70-80% for patients with standard-risk and localized disease and ~30% for those with metastatic disease. Treatment comprises local surgery, radiotherapy and polychemotherapy, which are associated with acute and chronic adverse effects that may compromise quality of life in survivors. Histologically, Ewing sarcomas are composed of small round cells expressing high levels of CD99. Genetically, they are characterized by balanced chromosomal translocations in which a member of the FET gene family is fused with an ETS transcription factor, with the most common fusion being EWSR1-FLI1 (85% of cases). Ewing sarcoma breakpoint region 1 protein (EWSR1)-Friend leukaemia integration 1 transcription factor (FLI1) is a tumour-specific chimeric transcription factor (EWSR1-FLI1) with neomorphic effects that massively rewires the transcriptome. Additionally, EWSR1-FLI1 reprogrammes the epigenome by inducing de novo enhancers at GGAA microsatellites and by altering the state of gene regulatory elements, creating a unique epigenetic signature. Additional mutations at diagnosis are rare and mainly involve STAG2, TP53 and CDKN2A deletions. Emerging studies on the molecular mechanisms of Ewing sarcoma hold promise for improvements in early detection, disease monitoring, lower treatment-related toxicity, overall survival and quality of life.

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)
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.

Genevieve C Kendall, Sarah Watson, Lin Xu, Collette A LaVigne, Whitney Murchison, Dinesh Rakheja, Stephen X Skapek, Franck Tirode, Olivier Delattre, James F Amatruda (2018 Jun 6) transgenic zebrafish models identify as a mediator of rhabdomyosarcoma tumorigenesis. 
*eLife*: DOI: 10.7554/eLife.33800

**Summary**

Alveolar rhabdomyosarcoma is a pediatric soft-tissue sarcoma caused by fusion oncogenes and is characterized by impaired skeletal muscle development. We developed human-driven zebrafish models of tumorigenesis and found that exhibits discrete cell lineage susceptibility and transformation. Tumors developed by 1.6-19 months and were primitive neuroectodermal tumors or rhabdomyosarcoma. We applied this transgenic zebrafish model to study how leverages early developmental pathways for oncogenesis and found that is a unique target. Ectopic expression of the human ortholog, , inhibits myogenesis in zebrafish and mammalian cells, recapitulating the arrested muscle development characteristic of rhabdomyosarcoma. In patients, is overexpressed in fusion-positive versus fusion-negative tumors. Finally, overexpression is associated with reduced survival in patients in the context of the fusion. Our novel zebrafish rhabdomyosarcoma model identifies a new target, /, that contributes to impaired myogenic differentiation and has prognostic significance in human disease.


**Summary**

**Motivation:**

In cancer, clonalevolution is assessed based on information coming from single nucleotide variants and copy number alterations. Nonetheless, existing methods often fail to accurately combine information from both sources to truthfully reconstruct clonal populations in a given tumor sample or in a set of tumor samples coming from the same patient. Moreover, previously published methods detect clones from a single set of variants. As a result, compromises have to be done between stringent variant filtering [reducing dispersion in variant allele frequency estimates (VAFs)] and using all biologically relevant variants.
Results:
We present a framework for defining cancer clones using most reliable variants of high depth of coverage and assigning functional mutations to the detected clones. The key element of our framework is QuantumClone, a method for variant clustering into clones based on VAFs, genotypes of corresponding regions and information about tumor purity. We validated QuantumClone on our framework on simulated data. We then applied our framework to whole genome sequencing data for 19 neuroblastoma trios each including constitutional, diagnosis and relapse samples. We confirmed an enrichment of damaging variants within such pathways as MAPK (mitogen-activated protein kinases), neuritogenesis, epithelial-mesenchymal transition, cell survival and DNA repair. Most pathways had more damaging variants in the expanding clones compared to shrinking ones, which can be explained by the increased total number of variants between these two populations. Functional mutational rate varied for ancestral clones and clones shrinking or expanding upon treatment, suggesting changes in clone selection mechanisms at different time points of tumor evolution.

Franck Bourdeaut, Olivier Delattre (2018 May 14)
Genetic predisposition to medulloblastomas: just follow the tumour genome.
The Lancet. Oncology : 722-723 : DOI : S1470-2045(18)30289-4

Summary

The ALK receptor in sympathetic neuron development and neuroblastoma
Cell and Tissue Research : 325, 337 : DOI : https://doi.org/10.1007/s00441-017-2784-8

Summary

The ALK gene encodes a tyrosine kinase receptor characterized by an expression pattern mainly restricted to the developing central and peripheral nervous systems. In 2008, the discovery of ALK activating mutations in neuroblastoma, a tumor of the sympathetic nervous system, represented a breakthrough in the understanding of the pathogenesis of this pediatric cancer and established mutated ALKs as a tractable therapeutic target for precision medicine. Subsequent studies addressed the identity of ALK ligands, as well as its physiological function in the sympathoadrenal lineage, its role in neuroblastomad evelopment and the signaling pathways triggered by mutated ALK. This review focuses on these different aspects of the ALK biology and summarizes the various therapeutic strategies relying on ALK inhibition in neuroblastoma, either as monotherapies or combinatorial treatments.

Julien Masliah-Planchon, Dominique Lévy, Delphine Héron, Fabienne Giuliano, Catherine Badens, Paul Fréneaux, Louise Galmiche, Jean-Marc Guinebretiere, Cécile Cellier, Joshua J Waterfall, Khadija Aït-Rais, Gaëlle Pierron, Christophe Glorion, Isabelle Desguerre, Christine Soler, Anne
Devile, Olivier Delattre, Jean Michon, Franck Bourdeaut (2018 May 1)

**Does ATRX germline variation predispose to osteosarcoma? Three additional cases of osteosarcoma in two ATR-X syndrome patients.**

*European journal of human genetics : EJHG* : 1217-1221 : [DOI : 10.1038/s41431-018-0147-x](https://doi.org/10.1038/s41431-018-0147-x)

**Summary**

Osteosarcoma is the most common malignant bone tumor in adolescents and young adults. Most osteosarcomas are sporadic but the risk of osteosarcoma is also increased by germline variants in TP53, RB1 and RECQL4 genes. ATRX germline variations are responsible for the rare genetic disorder X-linked alpha-thalassemia mental retardation (ATR-X) syndrome characterized by severe developmental delay and alpha-thalassemia but no obvious increased risk of cancer. Here we report two children with ATR-X syndrome who developed osteosarcoma. Notably, one of the children developed two osteosarcomas separated by 10 years. Those two cases raise the possibility that ATRX germline variant could be associated with an increased risk of osteosarcoma.


**Transcriptomic definition of molecular subgroups of small round cell sarcomas**


**Summary**

Sarcoma represents a highly heterogeneous group of tumors. We report here the first unbiased and systematic search for gene fusions combined with unsupervised expression analysis of a series of 184 small round cell sarcomas. Fusion genes were detected in 59% of samples, with half of them being observed recurrently. We identified biologically homogeneous groups of tumours such as the **CIC**-fused (to **DUX4**, **FOXO4**or **NUTM1**) and **BCOR**-rearranged (**BCOR**–**CCNB3**, **BCOR**–**MAML3**, **ZC3H7B**–**BCOR**, and **BCOR**internal duplication) tumour groups. **VGLL2**-fused tumours represented a more biologically and pathologically heterogeneous group. This study also refined the characteristics of some entities such as **EWSR1**–**PATZ1**spindle cell sarcoma or **FUS**–**NFATC2**bone tumours that are different from **EWSR1**–**NFATC2**tumours and transcriptionally resemble **CIC**-fused tumour entities. We also describe a completely novel group of epithelioid and spindle-cell rhabdomyosarcomas characterized by **EWSR1**–or **FUS**–**TFCP2**fusions. Finally, expression data identified some potentially new therapeutic targets or pathways. Copyright © 2018 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Gregorio J Petrirena, Julien Masliah-Planchon, Quentin Sala, Bertrand Pourroy, Didier Frappaz, Emeline Tabouret, Thomas Graillon, Jean-Claude Gentet, Olivier Delattre, Olivier Chinot, Laetitia Padovani (2018 Mar 9)
Recurrent extraneural sonic hedgehog medulloblastoma exhibiting sustained response to vismodegib and temozolomide monotherapies and inter-metastatic molecular heterogeneity at progression.

_Oncotarget_ : 10175-10183 : [DOI : 10.18632/oncotarget.23699](https://doi.org/10.18632/oncotarget.23699)

**Summary**

Response to targeting and non-targeting agents is variable and molecular information remains poorly described in patients with recurrent sonic-hedgehog-driven medulloblastoma (SHH-MB).