Année de publication : 2019

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 Thebaud, Marion Gambart, Dominique Valteau-Couanet, Jean Michon, Caroline Louis-Brennetot, Isabelle Janoueix-Lerosey, Anne-Sophie Defachelles, Franck Bourdeaut, Olivier Delattre, Gudrun Schleiermacher (2019 Apr 25)

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

International journal of cancer : DOI : 10.1002/ijc.32361

Résumé

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.

Année de publication : 2018

Forget Antoine, Martignetti Loredana, Puget Stéphanie, Calzone Laurence, Brabetz Sebastian, Picard Daniel, Montagud Arnau, Liva Stéphane, Sta Alexandre, Dingli Florent, Arras Guillaume, Rivera Jaime, Loew Damarys, Besnard Aurore, Lacombe Joëlle, Pagès Mélanie, Varlet Pascale, Dufour Christelle, Yu Hua, L. Mercier Audrey, Indersie Emilie, Chivet Anaïs, Leboucher Sophie, Sieber Laura, Beccaria Kevin, Gombert Michael, D. Meyer Frauke, Qin Nan, Bartl Jasmin, Chavez Lukas, Okonechnikov Konstantin, Sharma Tanvi, Thatikonda Venu, Bourdeaut Franck, Puponnot

Celio, Ramaswamy Vijay, Korshunov Andrey, Borkhardt Arndt, Reifenberger Guido, Poullet Patrick, D. Taylor Michael, Kool Marcel, M. Pfister Stefan, Kawauchi Daisuke, Barillot Emmanuel, Remke Marc, Ayrault Olivier (2018 Sep 10)
Aberrant ERBB4-SRC Signaling as a Hallmark of Group 4 Medulloblastoma Revealed by Integrative Phosphoproteomic Profiling
Cancer Cell : 34 : 379-395 : DOI : 10.1016/j.ccell.2018.08.002

Résumé

The current consensus recognizes four main medulloblastoma subgroups (wingless, Sonic hedgehog, group 3 and group 4). While medulloblastoma subgroups have been characterized extensively at the (epi-)genomic and transcriptomic levels, the proteome and phosphoproteome landscape remain to be comprehensively elucidated. Using quantitative (phospho)-proteomics in primary human medulloblastomas, we unravel distinct posttranscriptional regulation leading to highly divergent oncogenic signaling and kinase activity profiles in groups 3 and 4 medulloblastomas. Specifically, proteomic and phosphoproteomic analyses identify aberrant ERBB4-SRC signaling in group 4. Hence, enforced expression of an activated SRC combined with p53 inactivation induces murine tumors that resemble group 4 medulloblastoma. Therefore, our integrative proteogenomics approach unveils an oncogenic pathway and potential therapeutic vulnerability in the most common medulloblastoma subgroup.

Irene Jiménez, Mathieu Chicard, Léo Colmet-Daage, Nathalie Clément, Adrien Danzon, Eve Lapouble, Gaelle 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](DOI : 10.1002/ijc.31620)

Résumé

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.

Résumé

Motivation:

In cancer, clonalevolution is assessed basedon 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 clonalpopulations 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 cancerclones using most reliable variants of high depth of coverage and assigning functionalmutationsto the detected clones. The key element of our framework is QuantumClone, a methodfor variant clustering into clones basedon VAFs, genotypes of corresponding regions and information about tumor purity. We validated QuantumCloneand 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. Functionalmutational 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.

Année de 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

Résumé

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.

Céline Chauvin, Amaury Leruste, Arnault Tauziede-Espariat, Mamy Andrianteranagna, Didier Surdez, Aurianne Lescure, Zhi-Yan Han, Elodie Anthony, Wilfrid Richer, Sylvain Baulande, Mylène Bohec, Sakina Zaidi, Marie-Ming Aynaud, Laetitia Maillot, Julien Masliah-Planchon, Stefano Cairo, Sergio Roman-Roman, Olivier Delattre, Elaine Del Nery, Franck Bourdeaut (2017 Nov 16)
High-Throughput Drug Screening Identifies Pazopanib and Clofilium Tosylate as Promising Treatments for Malignant Rhabdoid Tumors.
Cell reports : 1737-1745 : DOI : S2211-1247(17)31539-5

Résumé

Rhabdoid tumors (RTs) are aggressive tumors of early childhood characterized by SMARCB1 inactivation. Their poor prognosis highlights an urgent need to develop new therapies. Here, we performed a high-throughput screening of approved drugs and identified broad inhibitors of tyrosine kinase receptors (RTKs), including pazopanib, and the potassium channel inhibitor

clofilium tosylate (CfT), as SMARCB1-dependent candidates. Pazopanib targets were identified as PDGFRα/β and FGFR2, which were the most highly expressed RTKs in a set of primary tumors. Combined genetic inhibition of both these RTKs only partially recapitulated the effect of pazopanib, emphasizing the requirement for broad inhibition. CfT perturbed protein metabolism and endoplasmic reticulum stress and, in combination with pazopanib, induced apoptosis of RT cells in vitro. In vivo, reduction of tumor growth by pazopanib was enhanced in combination with CfT, matching the efficiency of conventional chemotherapy. These results strongly support testing pazopanib/CfT combination therapy in future clinical trials for RTs.

Richer W, Masliah-Planchon J, Clement N, Jimenez I, Maillot L, Gentien D, Albaud B, Chemlali W, Galant C, Larousserie F, Boudou-Rouquette P, Leruste A, Chauvin C1, Han ZY, Coindre JM, Varlet P, Freneaux P, Ranchère-Vince D, Delattre O, Bourdeaut F (2017 May 23)
Embryonic signature distinguishes pediatric and adult rhabdoid tumors from other SMARCB1-deficient cancers.

Résumé

Extra-cranial rhabdoid tumors (RT) are highly aggressive malignancies of infancy, characterized by undifferentiated histological features and loss of SMARCB1 expression. The diagnosis is all the more challenging that other poorly differentiated cancers lose SMARCB1 expression, such as epithelioid sarcomas (ES), renal medullary carcinomas (RMC) or undifferentiated chordomas (UC). Moreover, late cases occurring in adults are now increasingly reported, raising the question of differential diagnoses and emphasizing nosological issues. To address this issue, we have analyzed the expression profiles of a training set of 32 SMARCB1-deficient tumors (SDT), with ascertained diagnosis of RT (n = 16, all < 5 years of age), ES (n = 8, all > 10 years of age), UC (n = 3) and RMC (n = 5). As compared with other SDT, RT are characterized by an embryonic signature, and up-regulation of key-actors of de novo DNA methylation processes. Using this signature, we then analysed the expression profiling of 37 SDT to infer the appropriate diagnosis. Thirteen adult onset tumors showed strong similarity with pediatric RT, in spite of older age; by exome sequencing, these tumors also showed genomic features indistinguishable from pediatric RT. In contrary, 8 tumors were reclassified within carcinoma, ES or UC categories, while the remaining could not be related to any of those entities. Our results demonstrate that embryonic signature is shared by all RT, whatever the age at diagnosis; they also illustrate that many adult-onset SDT of ambiguous histological diagnosis are clearly different from RT. Finally, our study paves the way for the routine use of expression-based signatures to give accurate diagnosis of SDT.

A Bertrand, C Rondenet, J Masliah-Planchon, P Leblond, A de la Fourchardière, D Pissaloux, K Aït-Raïs, D Lequin, A Jouvet, P Freneaux, H Sevestre, D Ranchere-Vince, A Tauziede-Espariat, C-A Maurage, K Silva, G Pierron, O Delattre, P Varlet, D Frappaz, F Bourdeaut (2017 Jan 6)

Rhabdoid component emerging as a subclonal evolution of paediatric glioneuronal tumours.

Neuropathology and applied neurobiology : DOI : 10.1111/nan.12379

Résumé

Atypical teratoid/rhabdoid tumors (AT/RT) are high-grade tumors partially composed of rhabdoid cells (1). The 1-year overall survival rate is 41% (2). Rhabdoid cells have large eccentric nuclei, a single prominent nucleolus, and abundant cytoplasm with eosinophilic inclusions. The immunohistochemical profile of these cells frequently includes loss of nuclear BAF47 expression due to loss of the SMARCB1 locus combined with a mutation of the other allele (3). This article is protected by copyright. All rights reserved.

Gudrun Schleiermacher, Olivier Delattre (2017 Jan 2)

Kids Enter the MATCH.

Journal of the National Cancer Institute : DOI : djw305

Résumé

Année de publication : 2016

Thomas Pincez, Nathalie Clément, Eve Lapouble, Gaëlle Pierron, Maud Kamal, Ivan Bieche, Virginie Bernard, Paul Fréneaux, Jean Michon, Daniel Orbach, Isabelle Aerts, Hélène Pacquement, Franck Bourdeaut, Irene Jiménez, Estelle Thébaud, Caroline Oudot, Cécile Vérité, Sophie Taque, Cormac Owens, François Doz, Christophe Le Tourneau, Olivier Delattre, Gudrun Schleiermacher (2016 Nov 30)

Feasibility and clinical integration of molecular profiling for target identification in pediatric solid tumors.

Pediatric blood & cancer : DOI : 10.1002/pbc.26365

Résumé

The role of tumor molecular profiling in directing targeted therapy utilization remains to be defined for pediatric tumors. We aimed to evaluate the feasibility of a sequencing and molecular biology tumor board (MBB) program, and its clinical impact on children with solid tumors.

Mathieu Chicard, Sandrine Boyault, Leo Colmet Daage, Wilfrid Richer, David Gentien, Gaelle Pierron, Eve Lapouble, Angela Bellini, Nathalie Clement, Isabelle Iacono, Stéphanie Bréjon, Marjorie Carrere, Cécile Reyes, Toby Hocking, Virginie Bernard, Michel Peuchmaur, Nadège Corradini, Cécile Faure-Conter, Carole Coze, Dominique Plantaz, Anne Sophie Defachelles, Estelle

Thebaud, Marion Gambart, Frédéric Millot, Dominique Valteau-Couanet, Jean Michon, Alain Puisieux, Olivier Delattre, Valérie Combaret, Gudrun Schleiermacher (2016 Jul 22)

Genomic copy number profiling using circulating free tumor DNA highlights heterogeneity in neuroblastoma.

Clinical cancer research : an official journal of the American Association for Cancer Research :

DOI : clincanres.0500.2016

Résumé

The tumor genomic copy number profile is of prognostic significance in neuroblastoma (NB) patients. We have studied the genomic copy number profile of cell free DNA (cfDNA) and compared this to primary tumor aCGH at diagnosis.

Navin Pinto, Jodi R Mayfield, Gordana Raca, Mark A Applebaum, Alexandre Chlenski, Madina Sukhanova, Rochelle Bagatell, Meredith S Irwin, Anthony Little, Jawhar Rawwas, Yasmin Gosiengfiao, Olivier Delattre, Isabelle Janoueix-Lerosey, Eve Lapouble, Gudrun Schleiermacher, Susan L Cohn (2016 Feb 12)

Segmental Chromosomal Aberrations in Localized Neuroblastoma Can be Detected in Formalin-Fixed Paraffin-Embedded Tissue Samples and Are Associated With Recurrence.

Pediatric blood & cancer : 1019-23 : DOI : 10.1002/pbc.25934

Résumé

Array comparative genomic hybridization (CGH) analyses of frozen tumors have shown strong associations between the pattern of chromosomal aberrations and outcome in patients with advanced-stage neuroblastoma. New platforms for analyzing chromosomal aberrations using formalin-fixed paraffin-embedded (FFPE) tissue have recently been developed. We sought to determine whether chromosomal microarray analysis (CMA) using FFPE tumors is feasible and if segmental chromosomal aberrations were prognostic of recurrence in localized neuroblastoma.

Zhi-Yan Han, Wilfrid Richer, Paul Fréneaux, Céline Chauvin, Carlo Lucchesi, Delphine Guillemot, Camille Grison, Delphine Lequin, Gaelle Pierron, Julien Masliah-Planchon, André Nicolas, Dominique Ranchère-Vince, Pascale Varlet, Stéphanie Puget, Isabelle Janoueix-Lerosey, Olivier Ayrault, Didier Surdez, Olivier Delattre, Franck Bourdeaut (2016 Jan 29)

The occurrence of intracranial rhabdoid tumours in mice depends on temporal control of Smarcb1 inactivation.

Nature communications : 10421 : DOI : 10.1038/ncomms10421

Résumé

Rhabdoid tumours (RTs) are highly aggressive tumours of infancy, frequently localized in the central nervous system (CNS) where they are termed atypical teratoid/rhabdoid tumours (AT/RTs) and characterized by bi-allelic inactivation of the SMARCB1 tumour suppressor gene. In this study, by temporal control of tamoxifen injection in Smarcb1(flox/flox);Rosa26-Cre(ERT2) mice, we explore the phenotypes associated with Smarcb1 inactivation at different developmental stages. Injection before E6, at birth or at 2 months of age recapitulates previously described phenotypes including embryonic lethality, hepatic toxicity or development of T-cell lymphomas, respectively. Injection between E6 and E10 leads to high penetrance tumours, mainly intra-cranial, with short delays (median: 3 months). These tumours demonstrate anatomical, morphological and gene expression profiles consistent with those of human AT/RTs. Moreover, intra- and inter-species comparisons of tumours reveal that human and mouse RTs can be split into different entities that may underline the variety of RT cells of origin.

Année de publication : 2015

Eleveld TF, Oldridge DA, Bernard V, Koster J, Colmet-Daage L, Diskin SJ, Schild L, Bentahar NB, Bellini A, Chicard M, Lapouble E, Combaret V, Legoix-Né P, Michon J, Pugh TJ, Hart LS, Rader J, Attiyeh EF, Wei JS, Zhang S, Naranjo A, Gastier-Foster JM, Hogarty MD, Asgharzadeh S, Smith MA, Guidry Auvil JM, Watkins TB, Zwijnenburg DA, Ebus ME, van Sluis P, Hakkert A, van Wezel E, van der Schoot CE, Westerhout EM, Schulte JH, Tytgat GA, Dolman ME, Janoueix-Lerosey I, Gerhard DS, Caron HN, Delattre O, Khan J, Versteeg R, Schleiermacher G, Molenaar JJ, Maris JM (2015 Jun 29)

Relapsed neuroblastomas show frequent RAS-MAPK pathway mutations
Nature Genetics : 47(8) : 864,871 : DOI : 10.1038/ng.3333

Résumé

The majority of patients with neuroblastoma have tumors that initially respond to chemotherapy, but a large proportion will experience therapy-resistant relapses. The molecular basis of this aggressive phenotype is unknown. Whole-genome sequencing of 23 paired diagnostic and relapse neuroblastomas showed clonal evolution from the diagnostic tumor, with a median of 29 somatic mutations unique to the relapse sample. Eighteen of the 23 relapse tumors (78%) showed mutations predicted to activate the RAS-MAPK pathway. Seven of these events were detected only in the relapse tumor, whereas the others showed clonal enrichment. In neuroblastoma cell lines, we also detected a high frequency of activating mutations in the RAS-MAPK pathway (11/18; 61%), and these lesions predicted sensitivity to MEK inhibition in vitro and in vivo. Our findings provide a rationale for genetic characterization of relapse neuroblastomas and show that RAS-MAPK pathway mutations may function as a biomarker for new therapeutic approaches to refractory disease.

Angela Bellini, Virginie Bernard, Quentin Leroy, Thomas Rio Frio, Gaelle Pierron, Valérie Combaret, Eve Lapouble, Nathalie Clement, Herve 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

Résumé

Purpose: 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.

Experimental Design: In a clinically representative series of 276 diagnostic neuroblastoma samples, exons 23 and 25 of the *ALK* gene, containing the F1174 and R1275 mutation hotspots, respectively, were resequenced with an extremely high depth of coverage.

Results: At the F1174 hotspot (exon 23), mutations were observed in 15 of 277 samples (range of fraction of mutated allele per sample: 0.562%–40.409%). At the R1275 hotspot (exon 25), *ALK* mutations were detected in 12 of 276 samples (range of fraction of mutated allele: 0.811%–73.001%). Altogether, subclonal events with a mutated allele fraction below 20% were observed in 15/27 *ALK*-mutated samples. The presence of an *ALK* mutation was associated with poorer 5-year overall survival (OS: 75% vs. 57%, *P* = 0.0212 log-rank test), with a strong correlation between *F1174 ALK* mutations and *MYCN* amplification being observed.

Conclusions: In this series, deep sequencing allows the detection of F1174 and R1275 *ALK* mutational events at diagnosis in 10% of cases, with subclonal events in more than half of these, which would have gone undetected by Sanger sequencing. These findings are of clinical importance given the potential role of *ALK* mutations in clonal evolution and relapse. These findings also demonstrate the importance of deep sequencing techniques for the identification of patients especially when considering targeted therapy