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.

Année de publication : 2018

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

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.

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.

Année de 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 Housset, 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

Résumé

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

Résumé

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.

Thomas F Eleveld, Derek A Oldridge, Virginie Bernard, Jan Koster, Leo Colmet Daage, Sharon J Diskin, Linda Schild, Nadia Bessoltane Bentahar, Angela Bellini, Mathieu Chicard, Eve Lapouble, Valérie Combaret, Patricia Legoix-Né, Jean Michon, Trevor J Pugh, Lori S Hart, JulieAnn Rader, Edward F Attiyeh, Jun S Wei, Shile Zhang, Arlene Naranjo, Julie M Gastier-Foster, Michael D Hogarty, Shahab Asgharzadeh, Malcolm A Smith, Jaime M Guidry Auvil, Thomas B K Watkins, Danny A Zwijnenburg, Marli E Ebus, Peter van Sluis, Anne Hakkert, Esther van Wezel, C Ellen van der Schoot, Ellen M Westerhout, Johannes H Schulte, Godelieve A Tytgat, M Emmy M Dolman, Isabelle Janoueix-Lerosey, Daniela S Gerhard, Huib N Caron, Olivier Delattre, Javed Khan, Rogier Versteeg, Gudrun Schleiermacher, Jan J Molenaar, John M Maris (2015 Jan 15)
Relapsed neuroblastomas show frequent RAS-MAPK pathway mutations.
Nature genetics : 864-71 : 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.

Année de publication : 2014

Ronald Lebofsky, Charles Decraene, Virginie Bernard, Maud Kamal, Anthony Blin, Quentin Leroy, Thomas Rio Frio, Gaëlle Pierron, Céline Callens, Ivan Bieche, Adrien Saliou, Jordan Madic, Etienne Rouleau, François-Clément Bidard, Olivier Lantz, Marc-Henri Stern, Christophe Le Tourneau, Jean-Yves Pierga (2014 Aug 14)

Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types.

Molecular oncology : 783-90 : DOI : 10.1016/j.molonc.2014.12.003

Résumé

Cell-free tumor DNA (ctDNA) has the potential to enable non-invasive diagnostic tests for personalized medicine in providing similar molecular information as that derived from invasive tumor biopsies. The histology-independent phase II SHIVA trial matches patients with targeted therapeutics based on previous screening of multiple somatic mutations using metastatic biopsies. To evaluate the utility of ctDNA in this trial, as an ancillary study we performed de novo detection of somatic mutations using plasma DNA compared to metastasis biopsies in 34 patients covering 18 different tumor types, scanning 46 genes and more than 6800 COSMIC mutations with a multiplexed next-generation sequencing panel. In 27 patients, 28 of 29 mutations identified in metastasis biopsies (97%) were detected in matched ctDNA. Among these 27 patients, one additional mutation was found in ctDNA only. In the seven other patients, mutation detection from metastasis biopsy failed due to inadequate biopsy material, but was successful in all plasma DNA samples providing three more potential actionable mutations. These results suggest that ctDNA analysis is a potential alternative and/or replacement to analyses using costly, harmful and lengthy tissue biopsies of metastasis, irrespective of cancer type and metastatic site, for multiplexed mutation detection in selecting personalized therapies based on the patient's tumor genetic content.