Année de publication : 2020

Decaudin Didier, Frisch Dit Leitz Estelle, Nemati Fariba, Tarin Malcy, Naguez Adnan, Zerara Mohamed, Marande Benjamin, Vivet-Noguer Raquel, Halilovic Ensar, Fabre Claire, Jochemsen Aart, Roman-Roman Sergio, Alsafadi Samar. (2020 Jan 9)
Preclinical evaluation of drug combinations identifies co-inhibition of Bcl-2/XL/W and MDM2 as a potential therapy in uveal melanoma.
European Journal of CancerEuropean Journal of Cancer : DOI : 10.1016/j.ejca.2019.12.012

Résumé

Année de publication : 2019

Vivet-Noguer R, Tarin M, Roman-Roman S, Alsafadi S. (2019 Jul 20)
Emerging Therapeutic Opportunities Based on Current Knowledge of Uveal Melanoma Biology.
Cancers

Résumé

Année de publication : 2018

Lynn Quek, Muriel D. David, Alison Kennedy, Marlen Metzner, Michael Amatangelo, Alan Shih, Bilyana Stoilova, Cyril Quivoron, Maël Heiblig, Christophe Willekens, Véronique Saada, Samar Alsafadi, M. S. Vijayabaskar, Andy Peniket, Oliver A. Bernard, Sam Agresta, Katharine Yen, Kyle MacBeth, Eytan Stein, George S. Vassiliou, Ross Levine, Stephane De Botton, Anjan Thakurta, Virginie Penard-Lacronique & Paresh Vyas (2018 Jul 16)
Clonal heterogeneity of acute myeloid leukemia treated with the IDH2 inhibitor enasidenib.
Nature Medicine : 24(8):1167-117 : DOI : 10.1038/s41591-018-0115-6

Résumé

Mutations in the gene encoding isocitrate dehydrogenase 2 (IDH2) occur in several types of cancer, including acute myeloid leukemia (AML). In model systems, mutant IDH2 causes hematopoietic differentiation arrest. Enasidenib, a selective small-molecule inhibitor of mutant IDH2, produces a clinical response in 40% of treated patients with relapsed/refractory AML by promoting leukemic cell differentiation. Here, we studied the clonal basis of response and acquired resistance to enasidenib treatment. Using sequential patient samples, we determined the clonal structure of hematopoietic cell populations at different stages of differentiation. Before therapy, IDH2-mutant clones showed variable differentiation arrest. Enasidenib treatment promoted hematopoietic differentiation from either terminal or ancestral mutant clones; less frequently, treatment promoted

differentiation of nonmutant cells. Analysis of paired diagnosis/relapse samples did not identify second-site mutations in IDH2 at relapse. Instead, relapse arose by clonal evolution or selection of terminal or ancestral clones, thus highlighting multiple bypass pathways that could potentially be targeted to restore differentiation arrest. These results show how mapping of clonal structure in cell populations at different stages of differentiation can reveal the response and evolution of clones during treatment response and relapse.

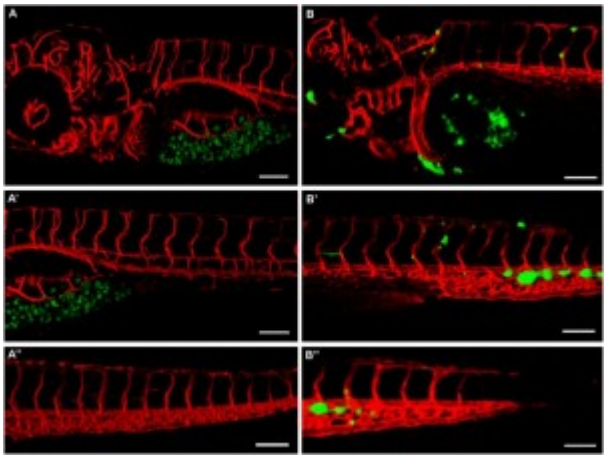
Giulia Fornabaio, Raymond L. Barnhill, Claire Lugassy, Laurent A. Bentolila, Nathalie Cassoux, Sergio Roman-Roman, Samar Alsafadi & Filippo Del Bene (2018 Jul 11)

Angiotropism and extravascular migratory metastasis in cutaneous and uveal melanoma progression in a zebrafish model.

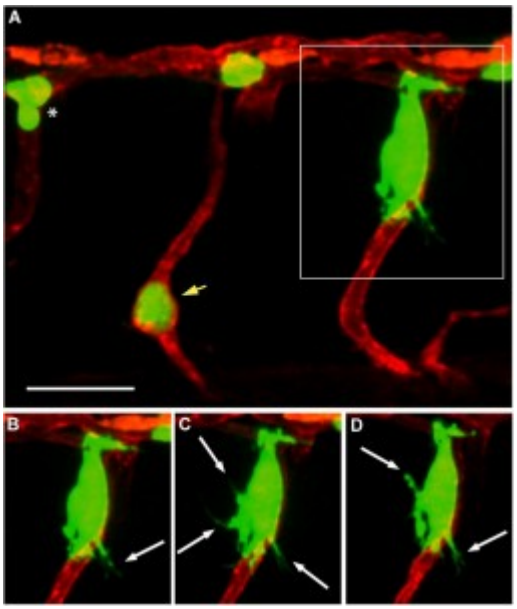
Scientific Reports : 8(1):10448 : DOI : 10.1038/s41598-018-28515-6

Résumé

Cutaneous melanoma is a highly aggressive cancer with a propensity for distant metastasis to various organs. In contrast, melanoma arising in pigmented uveal layers of the eye metastasizes mostly in the liver. The mechanisms of these metastases, which are ultimately resistant to therapy, are still unclear. Metastasis via intravascular dissemination of tumour cells is widely accepted as a central paradigm. However, we have previously described an alternative mode of tumour dissemination, extravascular migratory metastasis, based on clinical and experimental data. This mechanism is characterised by the interaction of cancer cells with the abluminal vascular surface, which defines angiotropism. Here, we employed our 3D co-culture approach to monitor cutaneous and uveal human melanoma cells dynamics in presence of vascular tubules. Using time-lapse microscopy, we evaluated angiotropism, the migration of tumour cells along vascular tubules and the morphological changes occurring during these processes. Cutaneous and uveal melanoma cells were injected in zebrafish embryos in order to develop xenografts. Employing *in vivo* imaging coupled with 3D reconstruction, we monitored the interactions between cancer cells and the external surface of zebrafish vessels. Overall, our results indicate that cutaneous and uveal melanoma cells spread similarly along the abluminal vascular surfaces, *in vitro* and *in vivo*.



Cutaneous melanoma cells and non-malignant melanocytes show different migratory properties in zebrafish. (A), (A') and (A") Different images of a 3 dpi larva injected with Hermes-GFP cells, showing no melanocytes outside the yolk cavity. (B), (B') and (B") Different images of a 2 dpi embryo injected with C8161-GFP cells, showing numerous melanoma cells spread all over the body of the fish. Pictures were taken with a 10 × dry objective, employing a Zeiss LSM 700 confocal microscope. Scale bar is 50 µm, green shows melanocytes, red shows zebrafish blood vessels.



Angiotropism in zebrafish xenograft of uveal melanoma. (A) A larva injected with OMM 2.3-GFP cells, displaying a micrometastasis of angiotropic cells (in the square) cuffing the external surface of an intersegmental vessel. (B–D) are time-lapse images of the same angiotropic cells taken at time 0, 4 and 8 hours after the beginning of the imaging. The images were obtained employing a Zeiss LSM 880 confocal microscope (40 × water objective), starting from 30 hours post injection. Scale bar is 20 µm, green shows melanoma cells, red shows zebrafish blood vessels, white arrows show pseudopodial protrusions formed by angiotropic cells, white asterisk shows intravascular melanoma cells, yellow arrow shows melanoma cells trapped in an

intersegmental vessel.

Samar Alsafadi, Lenha Mobuchon, Manuel Rodrigues, Marc-Henri Stern (2018 Feb 1)
Uveal melanoma, a model disease for splicing alterations and oncogenesis
médecine/sciences : DOI : 10.1051/medsci/20183402013

Résumé

Uveal melanoma is a rare cancer in adults, whose highly stereotyped oncogenic events have been decrypted over the last decade. Its epidemiological, genetic and transcriptional features make it a remarkable model of oncogenesis. Malignant transformation involves almost mutually exclusive alteration of fundamental biologic pathways, including chromatin regulation with inactivation of *BAP1*, splicing with mutations of *SF3B1* or translation with mutations of *EIF1AX*. Uveal melanoma analyses unraveled the splicing defect due to *SF3B1* mutations. Understanding the link between these alterations and malignant transformation will be a key step to define novel therapeutic targets.

Année de publication : 2016

Guillaume Carita, Estelle Frisch-Dit-Leitz, Ahmed Dahmani, Chloé Raymondie, Nathalie Cassoux, Sophie Piperno-Neumann, Fariba Némati, Cécile Laurent, Leanne De Koning, Ensar Halilovic, Sebastien Jeay, Andrew Wylie, Caroline Emery, Sergio Roman-Roman, Marie Schoumacher, Didier Decaudin, (2016 Jun 7)
Dual inhibition of protein kinase C and p53-MDM2 or PKC and mTORC1 are novel efficient therapeutic approaches for uveal melanoma.
Oncotarget : DOI : 10.18632/oncotarget.9552

Résumé

Uveal melanoma (UM) is the most common cancer of the eye in adults. Many UM patients develop metastases for which no curative treatment has been identified. Novel therapeutic approaches are therefore urgently needed. UM is characterized by mutations in the genes *GNAQ and GNA11* which activate the PKC pathway, leading to the use of PKC inhibitors as a rational strategy to treat UM tumors. Encouraging clinical activity has been noted in UM patients treated with PKC inhibitors. However, it is likely that curative treatment regimens will require a combination of targeted therapeutic agents. Employing a large panel of UM patient-derived xenograft models (PDXs), several PKC inhibitor-based combinations were tested *in vivo* using the PKC inhibitor AEB071. The most promising approaches were further investigated *in vitro* using our unique panel of UM cell lines. When combined with AEB071, the two agents CGM097 (p53-MDM2 inhibitor) and RAD001 (mTORC1 inhibitor) demonstrated greater activity than single agents, with tumor regression observed in several UM PDXs. Follow-up studies in UM cell lines on these two drug associations confirmed their combination

activity and ability to induce cell death. While no effective treatment currently exists for metastatic uveal melanoma, we have discovered using our unique panel of preclinical models that combinations between PKC/mTOR inhibitors and PKC/p53-MDM2 inhibitors are two novel and very effective therapeutic approaches for this disease. Together, our study reveals that combining PKC and p53-MDM2 or mTORC1 inhibitors may provide significant clinical benefit for UM patients.

Nabil Amirouchene-Angelozzi, Estelle Frisch-Dit-Leitz, Guillaume Carita, Ahmed Dahmani, Chloé Raymondie, Géraldine Liot, David Gentien, Fariba Némati, Didier Decaudin, Sergio Roman-Roman, Marie Schoumacher (2016 Apr 26)

The mTOR inhibitor Everolimus synergizes with the PI3K inhibitor GDC0941 to enhance anti-tumor efficacy in uveal melanoma.

Oncotarget : DOI : 10.18632/oncotarget.8054

Résumé

Uveal melanoma (UM) is the most frequent malignant ocular tumor in adults. While the primary tumor is efficiently treated by surgery and/or radiotherapy, about one third of UM patients develop metastases, for which no effective treatment is currently available. The PKC, MAPK and PI3K/AKT/mTOR signaling cascades have been shown to be associated with tumor growth. However, none of the compounds against those pathways results in tumor regression when used as single agents. To identify more effective therapeutic strategies for UM patients, we performed a combination screen using seven targeted agents inhibiting PKC, MEK, AKT, PI3K and mTOR in a panel of ten UM cell lines, representative of the UM disease. We identified a strong synergy between the mTOR inhibitor Everolimus and the PI3K inhibitor GDC0941. This combination resulted in an increase in apoptosis in several UM cell lines compared to monotherapies and enhanced the anti-tumor effect of each single agent in two patient-derived xenografts. Furthermore, we showed that the synergism between the two drugs was associated with the relief by GDC0491 of a reactivation of AKT induced by Everolimus. Altogether, our results highlight a novel and effective combination strategy, which could be beneficial for UM patients.

Samar Alsafadi, Alexandre Houy, Aude Battistella, Tatiana Popova, Michel Wassef, Emilie Henry, Franck Tirode, Angelos Constantinou, Sophie Piperno-Neumann, Sergio Roman-Roman, Martin Dutertre, Marc-Henri Stern (2016 Feb 4)

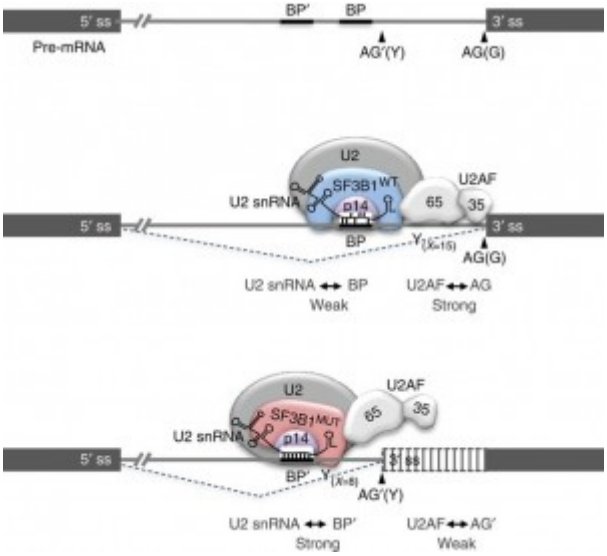
Cancer-associated SF3B1 mutations affect alternative splicing by promoting alternative branchpoint usage.

Nature Communications : DOI : 10.1038/ncomms10615

Résumé

Hotspot mutations in the spliceosome gene *SF3B1* are reported in ~20% of uveal melanomas. SF3B1 is involved in 3′-splice site (3′ss) recognition during RNA splicing;

however, the molecular mechanisms of its mutation have remained unclear. Here we show, using RNA-Seq analyses of uveal melanoma, that the *SF3B1*$^{R625/K666}$ mutation results in deregulated splicing at a subset of junctions, mostly by the use of alternative 3′ss. Modelling the differential junctions in *SF3B1*^{WT} and *SF3B1*$^{R625/K666}$ cell lines demonstrates that the deregulated splice pattern strictly depends on *SF3B1* status and on the 3'ss-sequence context. SF3B1^{WT} knockdown or overexpression do not reproduce the *SF3B1*$^{R625/K666}$ splice pattern, qualifying *SF3B1*$^{R625/K666}$ as change-of-function mutants. Mutagenesis of predicted branchpoints reveals that the *SF3B1*$^{R625/K666}$-promoted splice pattern is a direct result of alternative branchpoint usage. Altogether, this study provides a better understanding of the mechanisms underlying splicing alterations induced by mutant SF3B1 in cancer, and reveals a role for alternative branchpoints in disease.



A model for alternative splicing dysregulation induced by SF3B1 hotspot mutations.
The 3′ss contains a segment, which is rich in pyrimidines (Y), a well-conserved AG dinucleotide and a branchpoint (BP) sequence recognized by the U2 snRNP. The U2 snRNP complex binds to the intron through base-pairing interactions between the BP sequence and the U2 snRNA, and through interactions between intron sequences, SF3B1 and p14. The HEAT repeats of SF3B1 form helical structures that occlude the surface of RNA recognition motif of p14. U2 snRNP containing SF3B1WT recognizes the canonical U2AF-dependant BP. The hotspot mutations of SF3B1 targeting the HEAT repeats occur on the inner surface of the structure and might induce a conformational change in the U2 snRNP complex altering its selectivity for BPs. U2 snRNP containing SF3B1MUT has more stringent requirement for BP

sequences and less for U2AF-dependent sequences, leading to the binding of alternative branchpoints (BP') with high potential of base-pairing with U2 snRNP. AG, canonical 3′ss; AG', alternative 3′ss; x, average number of pyrimidines; Y, pyrimidine.

Année de publication : 2014

Nabil Amirouchene-Angelozzi, Fariba Nemati, David Gentien, André Nicolas, Amaury Dumont, Guillaume Carita, Jacques Camonis, Laurence Desjardins, Nathalie Cassoux, Sophie Piperno-Neumann, Pascale Mariani, Xavier Sastre, Didier Decaudin, Sergio Roman-Roman (2014 Jun 13)
Establishment of novel cell lines recapitulating the genetic landscape of uveal melanoma and preclinical validation of mTOR as a therapeutic target.
Molecular Oncology : DOI : 10.1016/j.molonc.2014.06.004

Résumé

Uveal melanoma (UM) is the most common primary tumor of the eye in adults. There is no standard adjuvant treatment to prevent metastasis and no effective therapy in the metastatic setting. We have established a unique panel of 7 UM cell lines from either patient's tumors or patient-derived tumor xenografts (PDXs). This panel recapitulates the molecular landscape of the disease in terms of genetic alterations and mutations. All the cell lines display GNAQ or GNA11 activating mutations, and importantly four of them display BAP1 (BRCA1 associated protein-1) deficiency, a hallmark of aggressive disease. The mTOR pathway was shown to be activated in most of the cell lines independent of AKT signaling. mTOR inhibitor Everolimus reduced the viability of UM cell lines and significantly delayed tumor growth in 4 PDXs. Our data suggest that mTOR inhibition with Everolimus, possibly in combination with other agents, may be considered as a therapeutic option for the management of uveal melanoma.