Année de publication : 2020

Julie U Bertrand, Eirikur Steingrimsson, Fanélie Jouenne, Brigitte Bressac-de Paillerets, Lionel Larue (2020 Apr 30)

Melanoma Risk and Melanocyte Biology.

Acta dermato-venereologica : adv00139 : DOI : 10.2340/00015555-3494

Résumé

Cutaneous melanoma arises from melanocytes following genetic, epigenetic and allogenetic (i.e. other than epi/genetic) modifications. An estimated 10% of cutaneous melanoma cases are due to inherited variants or de novo mutations in approximately 20 genes, found using linkage, next-generation sequencing and association studies. Based on these studies, 3 classes of predisposing melanoma genes have been defined based on the frequency of the variants in the general population and lifetime risk of developing a melanoma: (i) ultra-rare variants with a high risk, (ii) rare with a moderate risk, and (iii) frequent variants with a low risk. Most of the proteins encoded by these genes have been shown to be involved in melanoma initiation, including proliferation and senescence bypass. This paper reviews the role(s) of these genes in the transformation of melanocytes into melanoma. It also describes their function in the establishment and renewal of melanocytes and the biology of pigment cells, if known.

Année de publication : 2019

Amina Boussouar, Antonin Tortereau, Ambroise Manceau, Andrea Paradisi, Nicolas Gadot, Jonathan Vial, David Neves, Lionel Larue, Maxime Battistella, Christophe Leboeuf, Celeste Lebbé, Anne Janin, Patrick Mehlen (2019 Dec 7)

Netrin-1 and Its Receptor DCC Are Causally Implicated in Melanoma Progression.

Cancer research : 747-756 : DOI : 10.1158/0008-5472.CAN-18-1590

Résumé

Deleted in colorectal cancer (DCC), the receptor for the multifunctional cue netrin-1, acts as a tumor suppressor in intestinal cancer and lung metastasis by triggering cancer cell death when netrin-1 is lowly expressed. Recent genomic data highlighted that DCC is the third most frequently mutated gene in melanoma; we therefore investigated whether DCC could act as a melanoma tumor suppressor. Reexpressing DCC in human melanoma cell lines promoted tumor cell death and tumor growth inhibition in xenograft mouse models. Genetic silencing of DCC prodeath activity in a BRAF mouse model increased the proportion of mice with melanoma, further supporting that DCC is a melanoma tumor suppressor. Netrin-1 expression was elevated in melanoma compared with benign melanocytic lesions. Upregulation of netrin-1 in the skin cells of a BRAF-mutated murine model reduced cancer cell death and promoted melanoma progression. Therapeutic antibody blockade of netrin-1 combined with dacarbazine increased overall survival in several mouse melanoma models.

Together, these data support that interfering with netrin-1 could be a viable therapeutic approach in patients with netrin-1-expressing melanoma. SIGNIFICANCE: Netrin-1 and its receptor DCC regulate melanoma progression, suggesting therapeutic targeting of this signaling axis as a viable option for melanoma treatment.

Zackie Aktary, Andre Corvelo, Camille Estrin, Lionel Larue (2019 Nov 4)
Sequencing two Tyr::CreER transgenic mouse lines.
Pigment cell & melanoma research : 426-434 : DOI : 10.1111/pcmr.12842

Résumé

The Cre/loxP system is a powerful tool that has allowed the study of the effects of specific genes of interest in various biological settings. The Tyr::CreER system allows for the targeted expression and activity of the Cre enzyme in the melanocyte lineage following treatment with tamoxifen, thus providing spatial and temporal control of the expression of specific target genes. Two independent transgenic mouse models, each containing a Tyr::CreER transgene, have been generated and are widely used to study melanocyte transformation. In this study, we performed whole genome sequencing (WGS) on genomic DNA from the two Tyr::CreER mouse models and identified their sites of integration in the C57BL/6 genome. Based on these results, we designed PCR primers to accurately, and efficiently, genotype transgenic mice. Finally, we discussed some of the advantages of each transgenic mouse model.

Anca G Radu, Sakina Torch, Florence Fauvelle, Karin Pernet-Gallay, Anthony Lucas, Renaud Blervaque, Véronique Delmas, Uwe Schlattner, Laurence Lafanechère, Pierre Hainaut, Nicolas Tricaud, Véronique Pingault, Nadège Bondurand, Nabeel Bardeesy, Lionel Larue, Chantal Thibert, Marc Billaud (2019 Jul 23)
LKB1 specifies neural crest cell fates through pyruvate-alanine cycling.
Science advances : eaau5106 : DOI : 10.1126/sciadv.aau5106

Résumé

Metabolic processes underlying the development of the neural crest, an embryonic population of multipotent migratory cells, are poorly understood. Here, we report that conditional ablation of the tumor suppressor kinase in mouse neural crest stem cells led to intestinal pseudo-obstruction and hind limb paralysis. This phenotype originated from a postnatal degeneration of the enteric nervous ganglia and from a defective differentiation of Schwann cells. Metabolomic profiling revealed that pyruvate-alanine conversion is enhanced in the absence of . Mechanistically, inhibition of alanine transaminases restored glial differentiation in an mTOR-dependent manner, while increased alanine level directly inhibited the glial commitment of neural crest cells. Treatment with the metabolic modulator AICAR suppressed mTOR signaling and prevented Schwann cell and enteric defects of mutant mice. These data uncover a link between pyruvate-alanine cycling and the specification of glial cell fate with potential implications in the understanding of the

molecular pathogenesis of neural crest diseases.

Valérie Petit, Jeremy Raymond, Christophe Alberti, Marie Pouteaux, Stuart J Gallagher, Mai Q Nguyen, Andrew E Aplin, Véronique Delmas, Lionel Larue (2019 Jun 29)
C57BL/6 congenic mouse NRAS melanoma cell lines are highly sensitive to the combination of Mek and Akt inhibitors in vitro and in vivo.
Pigment cell & melanoma research : 829-841 : DOI : 10.1111/pcmr.12807

Résumé

RAS is frequently mutated in various tumors and known to be difficult to target. NRAS are the second most frequent mutations found in human skin melanoma after BRAF . Aside from surgery, various approaches, including targeted therapies, immunotherapies, and combination therapies, are used to treat patients carrying NRAS mutations, but they are inefficient. Here, we established mouse NRAS melanoma cell lines and genetically derived isografts (GDIs) from Tyr::NRAS mouse melanoma that can be used in vitro and in vivo in an immune-competent environment (C57BL/6) to test and discover novel therapies. We characterized these cell lines at the cellular, molecular, and oncogenic levels and show that NRAS melanoma is highly sensitive to the combination of Mek and Akt inhibitors. This preclinical model shows much potential for the screening of novel therapeutic strategies for patients harboring NRAS mutations that have limited therapeutic options and resulted in poor prognoses.

Patrick Laurette, Sébastien Coassolo, Guillaume Davidson, Isabelle Michel, Giovanni Gambi, Wenjin Yao, Pierre Sohier, Mei Li, Gabrielle Mengus, Lionel Larue, Irwin Davidson (2019 May 9)
Chromatin remodellers Brg1 and Bptf are required for normal gene expression and progression of oncogenic Braf-driven mouse melanoma.
Cell death and differentiation : 29-43 : DOI : 10.1038/s41418-019-0333-6

Résumé

Somatic oncogenic mutation of BRAF coupled with inactivation of PTEN constitute a frequent combination of genomic alterations driving the development of human melanoma. Mice genetically engineered to conditionally express oncogenic Braf and inactivate Pten in melanocytes following tamoxifen treatment rapidly develop melanoma. While early-stage melanomas comprised melanin-pigmented Mitf and Dct-expressing cells, expression of these and other melanocyte identity genes was lost in later stage tumours that showed histological and molecular characteristics of de-differentiated neural crest type cells. Melanocyte identity genes displayed loss of active chromatin marks and RNA polymerase II and gain of heterochromatin marks, indicating epigenetic reprogramming during tumour progression. Nevertheless, late-stage tumour cells grown in culture re-expressed Mitf, and melanocyte markers and Mitf together with Sox10 coregulated a large number of genes essential for their growth. In this melanoma model, somatic inactivation that the catalytic Brg1 (Smarca4) subunit of the SWI/SNF complex and the scaffolding Bptf subunit of the NuRF

complex delayed tumour formation and deregulated large and overlapping gene expression programs essential for normal tumour cell growth. Moreover, we show that Brg1 and Bptf coregulated many genes together with Mitf and Sox10. Together these transcription factors and chromatin remodelling complexes orchestrate essential gene expression programs in mouse melanoma cells.

Katrin Möller, Sara Sigurbjornsdottir, Asgeir O Arnthorsson, Vivian Pogenberg, Ramile Dilshat, Valerie Fock, Solveig H Brynjolfsdottir, Christian Bindesboll, Margret Bessadottir, Helga M Ogmundsdottir, Anne Simonsen, Lionel Larue, Matthias Wilmanns, Vesteinn Thorsson, Eirikur Steingrimsson, Margret H Ogmundsdottir (2019 Feb 2)

MITF has a central role in regulating starvation-induced autophagy in melanoma.

Scientific reports : 1055 : DOI : 10.1038/s41598-018-37522-6

Résumé

The MITF transcription factor is a master regulator of melanocyte development and a critical factor in melanomagenesis. The related transcription factors TFEB and TFE3 regulate lysosomal activity and autophagy processes known to be important in melanoma. Here we show that MITF binds the CLEAR-box element in the promoters of lysosomal and autophagosomal genes in melanocytes and melanoma cells. The crystal structure of MITF bound to the CLEAR-box reveals how the palindromic nature of this motif induces symmetric MITF homodimer binding. In metastatic melanoma tumors and cell lines, MITF positively correlates with the expression of lysosomal and autophagosomal genes, which, interestingly, are different from the lysosomal and autophagosomal genes correlated with TFEB and TFE3. Depletion of MITF in melanoma cells and melanocytes attenuates the response to starvation-induced autophagy, whereas the overexpression of MITF in melanoma cells increases the number of autophagosomes but is not sufficient to induce autophagic flux. Our results suggest that MITF and the related factors TFEB and TFE3 have separate roles in regulating a starvation-induced autophagy response in melanoma. Understanding the normal and pathophysiological roles of MITF and related transcription factors may provide important clinical insights into melanoma therapy.

Pietro Mancuso, Rossella Tricarico, Vikram Bhattacharjee, Laura Cosentino, Yuwaraj Kadariya, Jaroslav Jelinek, Emmanuelle Nicolas, Margret Einarson, Neil Beeharry, Karthik Devarajan, Richard A Katz, Dorjbal G Dorjsuren, Hongmao Sun, Anton Simeonov, Antonio Giordano, Joseph R Testa, Guillaume Davidson, Irwin Davidson, Lionel Larue, Robert W Sobol, Timothy J Yen, Alfonso Bellacosa (2019 Jan 25)

Thymine DNA glycosylase as a novel target for melanoma.

Oncogene : DOI : 10.1038/s41388-018-0640-2

Résumé

Melanoma is an aggressive neoplasm with increasing incidence that is classified by the NCI as a recalcitrant cancer, i.e., a cancer with poor prognosis, lacking progress in diagnosis and treatment. In addition to conventional therapy, melanoma treatment is currently based on targeting the BRAF/MEK/ERK signaling pathway and immune checkpoints. As drug resistance remains a major obstacle to treatment success, advanced therapeutic approaches based on novel targets are still urgently needed. We reasoned that the base excision repair enzyme thymine DNA glycosylase (TDG) could be such a target for its dual role in safeguarding the genome and the epigenome, by performing the last of the multiple steps in DNA demethylation. Here we show that TDG knockdown in melanoma cell lines causes cell cycle arrest, senescence, and death by mitotic alterations; alters the transcriptome and methylome; and impairs xenograft tumor formation. Importantly, untransformed melanocytes are minimally affected by TDG knockdown, and adult mice with conditional knockout of Tdg are viable. Candidate TDG inhibitors, identified through a high-throughput fluorescence-based screen, reduced viability and clonogenic capacity of melanoma cell lines and increased cellular levels of 5-carboxylcytosine, the last intermediate in DNA demethylation, indicating successful on-target activity. These findings suggest that TDG may provide critical functions specific to cancer cells that make it a highly suitable anti-melanoma drug target. By potentially disrupting both DNA repair and the epigenetic state, targeting TDG may represent a completely new approach to melanoma therapy.

Année de publication : 2018

Elise Bonvin, Enrico Radaelli, Martin Bizet, Flavie Luciani, Emilie Calonne, Pascale Putmans, David Nittner, Nitesh Kumar Singh, Sara Francesca Santagostino, Valérie Petit, Lionel Larue, Jean Christophe Marine, François Fuks (2018 Dec 13)

TET2-Dependent Hydroxymethylome Plasticity Reduces Melanoma Initiation and Progression.

Cancer research : 482-494 : DOI : 10.1158/0008-5472.CAN-18-1214

Résumé

: Although numerous epigenetic aberrancies accumulate in melanoma, their contribution to initiation and progression remain unclear. The epigenetic mark 5-hydroxymethylcytosine (5hmC), generated through TET-mediated DNA modification, is now referred to as the sixth base of DNA and has recently been reported as a potential biomarker for multiple types of cancer. Loss of 5hmC is an epigenetic hallmark of melanoma, but whether a decrease in 5hmc levels contributes directly to pathogenesis or whether it merely results from disease progression-associated epigenetic remodeling remains to be established. Here, we show that NRAS-driven melanomagenesis in mice is accompanied by an overall decrease in 5hmC and specific 5hmC gains in selected gene bodies. Strikingly, genetic ablation of in mice cooperated with oncogenic NRAS to promote melanoma initiation while suppressing specific gains in 5hmC. We conclude that TET2 acts as a barrier to melanoma initiation and progression, partly by promoting 5hmC gains in specific gene bodies. SIGNIFICANCE: This work emphasizes the importance of epigenome plasticity in cancer development and highlights the involvement of druggable epigenetic factors in cancer.

Véronique Delmas, Lionel Larue (2018 Dec 12)
Molecular and cellular basis of depigmentation in vitiligo patients.
Experimental dermatology : 662-666 : DOI : 10.1111/exd.13858

Résumé

Vitiligo is a chronic skin disease characterized by the appearance of zones of depigmentation. It is mostly described as an autoimmune disease in which the immune system destroys the melanocytes. Consistent with this origin, genetic studies have implicated genes encoding proteins mediating the immune response targeting melanocytes in the aetiology of this disease, together with proteins specific to these cells. However, the destruction of melanocytes by the immune system is neither global nor complete, because the patients do not display total depigmentation. The etiopathology of vitiligo is clearly complex and cannot be simply reduced to an autoimmune reaction directed against pigmented cells. Intrinsic changes have been observed in the melanocytes, keratinocytes and dermal cells of vitiligo patients. Identification of the molecular and cellular changes occurring in normally pigmented skin in vitiligo patients, and an understanding of these changes, is essential to improve the definition of trigger events for this disease, with a view to developing treatments with long-term efficacy. This review focuses on the early events identified to date in the non-lesional regions of the skin in vitiligo patients and discusses the process of repigmentation from melanocyte stem cells.

Pierre Sohier, Léa Legrand, Zackie Aktary, Christine Grill, Véronique Delmas, Florence Bernex, Edouard Reyes-Gomez, Lionel Larue, Béatrice Vergier (2018 May 31)
A histopathological classification system of Tyr::NRAS murine melanocytic lesions: A reproducible simplified classification.
Pigment cell & melanoma research : 423-431 : DOI : 10.1111/pcmr.12677

Résumé

Genetically engineered mouse models offer essential opportunities to investigate the mechanisms of initiation and progression in melanoma. Here, we report a new simplified histopathology classification of mouse melanocytic lesions in Tyr::NRAS derived models, using an interactive decision tree that produces homogeneous categories. Reproducibility for this classification system was evaluated on a panel of representative cases of murine melanocytic lesions by pathologists and basic scientists. Reproducibility, measured as inter-rater agreement between evaluators using a modified Fleiss' kappa statistic, revealed a very good agreement between observers. Should this new simplified classification be adopted, it would create a robust system of communication between researchers in the field of mouse melanoma models.

Pascal Laurent-Gengoux, Valérie Petit, Zackie Aktary, Stuart Gallagher, Luke Tweedy, Laura Machesky, Lionel Larue (2018 May 18)

Simulation of melanoblast displacements reveals new features of developmental migration.
Development (Cambridge, England) : DOI : dev160200

Résumé

To distribute and establish the melanocyte lineage throughout the skin and other developing organs, melanoblasts undergo several rounds of proliferation, accompanied by migration through complex environments and differentiation. Melanoblast migration requires interaction with extracellular matrix of the epidermal basement membrane and with surrounding keratinocytes in the developing skin. Migration has been characterized by measuring speed, trajectory and directionality of movement, but there are many unanswered questions about what motivates and defines melanoblast migration. Here, we have established a general mathematical model to simulate the movement of melanoblasts in the epidermis based on biological data, assumptions and hypotheses. Comparisons between experimental data and computer simulations reinforce some biological assumptions, and suggest new ideas for how melanoblasts and keratinocytes might influence each other during development. For example, it appears that melanoblasts instruct each other to allow a homogeneous distribution in the tissue and that keratinocytes may attract melanoblasts until one is stably attached to them. Our model reveals new features of how melanoblasts move and, in particular, suggest that melanoblasts leave a repulsive trail behind them as they move through the skin.

Supawadee Sukseree, Lajos László, Florian Gruber, Sophie Bergmann, Marie Sophie Narzt, Ionela Mariana Nagelreiter, Romana Höftberger, Kinga Molnár, Günther Rauter, Thomas Birngruber, Lionel Larue, Gabor G Kovacs, Erwin Tschachler, Leopold Eckhart (2018 Mar 19)
Filamentous Aggregation of Sequestosome-1/p62 in Brain Neurons and Neuroepithelial Cells upon Tyr-Cre-Mediated Deletion of the Autophagy Gene Atg7.
Molecular neurobiology : 8425-8437 : DOI : 10.1007/s12035-018-0996-x

Résumé

Defects in autophagy and the resulting deposition of protein aggregates have been implicated in aging and neurodegenerative diseases. While gene targeting in the mouse has facilitated the characterization of these processes in different types of neurons, potential roles of autophagy and accumulation of protein substrates in neuroepithelial cells have remained elusive. Here we report that Atg7 Tyr-Cre mice, in which autophagy-related 7 (Atg7) is conditionally deleted under the control of the tyrosinase promoter, are a model for accumulations of the autophagy adapter and substrate sequestosome-1/p62 in both neuronal and neuroepithelial cells. In the brain of Atg7 Tyr-Cre but not of fully autophagy competent control mice, p62 aggregates were present in sporadic neurons in the cortex and other brain regions as well in epithelial cells of the choroid plexus and the ependyma. Western blot analysis confirmed a dramatic increase of p62 abundance and formation of high-molecular weight species of p62 in the brain of Atg7 Tyr-Cre mice relative to Atg7

controls. Immuno-electron microscopy showed that p62 formed filamentous aggregates in neurons and ependymal cells. p62 aggregates were also highly abundant in the ciliary body in the eye. Atg7 Tyr-Cre mice reached an age of more than 2 years although neurological defects manifesting in abnormal hindlimb clasping reflexes were evident in old mice. These results show that p62 filaments form in response to impaired autophagy in vivo and suggest that Atg7 Tyr-Cre mice are a model useful to study the long-term effects of autophagy deficiency on the homeostasis of different neuroectoderm-derived cells.

Veronica A Kinsler, Lionel Larue (2018 Jan 31)

The patterns of birthmarks suggest a novel population of melanocyte precursors arising around the time of gastrulation.

Pigment cell & melanoma research : 95-109 : DOI : 10.1111/pcmr.12645

Résumé

Systematic work in the mouse and chicken has mapped out two neural crest-derived pathways of melanocyte precursor migration. With these in mind, this study reappraises the patterns of congenital pigmentary disorders in humans and identifies three recurrent patterns consistent across genetically different diseases. Only two of these are seen in diseases known to be melanocyte cell-autonomous. The segmental pattern correlates well with the classical dorsolateral population from animal studies, demonstrating respect of the midline, cranio-caudal axial mixing, unilateral migration and involvement of key epidermally derived structures. Importantly however, the melanocyte precursors responsible for the non-segmental pattern, which demonstrates circular, bilateral migration centred on the midline, and not involving key epidermally derived structures, have not been identified previously. We propose that this population originates around the time of gastrulation, most likely within the mesoderm, and ultimately resides within the dermis. Whether it contributes to mature melanocytes in non-disease states is not known; however, parallels with the patterns of acquired vitiligo would suggest that it does. The third pattern, hypo- or hyperpigmented fine and whorled Blaschko's lines, is proposed to be non-cell-autonomous.

C Grill, L Benzekri, A Rubod, Z Aktary, K Ezzedine, A Taïeb, Y Gauthier, L Larue, V Delmas (2018 Jan 18)

Epidermal melanocytes in segmental vitiligo show altered expression of E-cadherin, but not P-cadherin.

The British journal of dermatology : 1204-1206 : DOI : 10.1111/bjd.16352

Résumé