Year of publication 2019


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

*Scientific reports*: 1055 : [DOI: 10.1038/s41598-018-37522-6](https://doi.org/10.1038/s41598-018-37522-6)

**Summary**

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.


**Thymine DNA glycosylase as a novel target for melanoma.**

*Oncogene*: [DOI: 10.1038/s41388-018-0640-2](https://doi.org/10.1038/s41388-018-0640-2)

**Summary**

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.

Year of 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]

**Summary**

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.

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

Summary

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.


Simulation of melanoblast displacements reveals new features of developmental migration.

Development (Cambridge, England) : DOI : dev160200

Summary

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](http://dx.doi.org/10.1007/s12035-018-0996-x)

**Summary**

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](http://dx.doi.org/10.1111/pcmr.12645)

**Summary**

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.

**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

Summary

The individual molecular pathways downstream of Cdc42, Rac, and Rho GTPases are well documented, but we know surprisingly little about how these pathways are coordinated when cells move in a complex environment in vivo. In the developing embryo, melanoblasts originating from the neural crest must traverse the dermis to reach the epidermis of the skin and hair follicles. We previously established that Rac1 signals via Scar/WAVE and Arp2/3 to effect pseudopod extension and migration of melanoblasts in skin. Here we show that RhoA is redundant in the melanocyte lineage but that Cdc42 coordinates multiple motility systems independent of Rac1. Similar to Rac1 knockouts, Cdc42 null mice displayed a severe loss of pigmentation, and melanoblasts showed cell-cycle progression, migration, and cytokinesis defects. However, unlike Rac1 knockouts, Cdc42 null melanoblasts were elongated and displayed large, bulky pseudopods with dynamic actin bursts. Despite assuming an elongated shape usually associated with fast mesenchymal motility, Cdc42 knockout melanoblasts migrated slowly and inefficiently in the epidermis, with nearly static pseudopods. Although much of the basic actin machinery was intact, Cdc42 null cells lacked the ability to polarize their Golgi and coordinate motility systems for efficient movement. Loss of Cdc42 de-coupled three main systems: actin assembly via the formin FMNL2 and Arp2/3, active myosin-II localization, and integrin-based adhesion dynamics.
UVB represses melanocyte cell migration and acts through β-catenin.

Summary

The exposure of skin to ultraviolet (UV) radiation can have both beneficial and deleterious effects: it can lead, for instance, to increased pigmentation and vitamin D synthesis but also to inflammation and skin cancer. UVB may induce genetic and epigenetic alterations, and have reversible effects associated with post-translational and gene regulation modifications. β-catenin is a main driver in melanocyte development; although infrequently mutated in melanoma, its cellular localization and activity is frequently altered. Here, we evaluate the consequence of UVB on β-catenin in the melanocyte lineage. We report that in vivo, UVB induces cytoplasmic/nuclear relocalization of β-catenin in melanocytes of newborn mice and adult human skin. In mouse melanocyte and human melanoma cell lines in vitro, UVB increases β-catenin stability, accumulation in the nucleus, and co-transcriptional activity, leading to the repression of cell motility and velocity. The activation of the β-catenin signaling pathway and its effect on migration by UVB are increased by an inhibitor of GSK3β, and decreased by an inhibitor of β-catenin. In conclusion, UVB represses melanocyte migration and does so by acting through the GSK3-β-catenin axis. This article is protected by copyright. All rights reserved.

Autophagy deficient melanocytes display a senescence associated secretory phenotype that includes oxidized lipid mediators.

Summary

Autophagy is a recycling program which allows cells to adapt to metabolic needs and to stress. Defects in autophagy can affect metabolism, aging, proteostasis and inflammation. Autophagy pathway genes, including autophagy related 7 (Atg7), have been associated with the regulation of skin pigmentation, and autophagy defects disturb the biogenesis and transport of melanosomes in melanocytes as well as transfer and processing of melanin into keratinocytes. We have previously shown that mice whose melanocytes or keratinocytes lack Atg7 (and thus autophagy) as a result of specific gene knockout still retained functioning melanosome synthesis and transfer, and displayed only moderate reduction of pigmentation. In cell culture the Atg7 deficient melanocytes were prone to premature senescence and dysregulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling. To elucidate the biochemical basis of this phenotype, we performed a study on global gene expression, protein secretion and phospholipid composition in Atg7 deficient versus Atg7 expressing
melanocytes. In cell culture Atg7 deficient melanocytes showed a pro-inflammatory gene expression signature and secreted higher levels of C-X-C motif chemokine ligand -1,-2,-10 and -12 (Cxc1, Cxc12, Cxc10, Cxc12), which are implicated in the pathogenesis of pigmented disorders and expressed higher amounts of matrix metalloproteinases -3 and -13 (Mmp3, Mmp13). The analysis of membrane phospholipid composition identified an increase in the arachidonic- to linoleic acid ratio in the autophagy deficient cells, as well as an increase in oxidized phospholipid species that act as danger associated molecular patterns (DAMPs). The secretion of inflammation related factors suggests that autophagy deficient melanocytes display a senescence associated secretory phenotype (SASP), and we propose oxidized lipid mediators as novel components of this SASP.

Christine Grill, Lionel Larue (2016 Sep 25)
**NRAS, NRAS, Which Mutation Is Fairest of Them All?**

**Summary**

In 28% of melanomas, NRAS is mutated in one of two hotspots: G12 or Q61. Phosphoproteomic analysis of primary human melanocytes transduced with G12 and Q61 showed different phosphorylation events in the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. Surprisingly, NRAS(G12) modulates the PI3K pathway and overexpresses the kinase PIM2, whereas NRAS(Q61) is associated with the MAPK pathway and overexpression of CK2α.

Supawadee Sukseree, Ying-Ting Chen, Maria Laggner, Florian Gruber, Valérie Petit, Ionela-Mariana Nagelreiter, Veronika Mlitz, Heidemarie Rossiter, Andreas Pollreisz, Ursula Schmidt-Erfurth, Lionel Larue, Erwin Tschachler, Leopold Eckhart (2016 Aug 19)
**Tyrosinase-Cre-Mediated Deletion of the Autophagy Gene Atg7 Leads to Accumulation of the RPE65 Variant M450 in the Retinal Pigment Epithelium of C57BL/6 Mice.**
*PloS one* : e0161640 : [DOI : 10.1371/journal.pone.0161640]

**Summary**

Targeted gene knockout mouse models have helped to identify roles of autophagy in many tissues. Here, we investigated the retinal pigment epithelium (RPE) of Atg7f/f Tyr-Cre mice (on a C57BL/6 background), in which Cre recombinase isexpressed under the control of the tyrosinase promoter to delete the autophagy gene Atg7. In line with pigment cell-directed blockade of autophagy, the RPE and the melanocytes of the choroid showed strong accumulation of the autophagy adaptor and substrate, sequestosome 1 (Sqstm1)/p62, relative to the levels in control mice. Immunofluorescence and Western blot analysis demonstrated that the RPE, but not the choroid melanocytes, of Atg7f/f Tyr-Cre mice also had strongly increased levels of retinoid isomerase RPE65, a pivotal enzyme for the maintenance of visual perception. In contrast to Sqstm1, genes involved in retinal
regeneration, i.e. Lrat, Rdh5, Rgr, and Rpe65, were expressed at higher mRNA levels. Sequencing of the Rpe65 gene showed that Atg7f/f and Atg7f/f Tyr-Cre mice carry a point mutation (L450M) that is characteristic for the C57BL/6 mouse strain and reportedly causes enhanced degradation of the RPE65 protein by an as-yet unknown mechanism. These results suggest that the increased abundance of RPE65 M450 in the RPE of Atg7f/f Tyr-Cre mice is, at least partly, mediated by upregulation of Rpe65 transcription; however, our data are also compatible with the hypothesis that the RPE65 M450 protein is degraded by Atg7-dependent autophagy in Atg7f/f mice. Further studies in mice of different genetic backgrounds are necessary to determine the relative contributions of these mechanisms.

Valérie Petit, Lionel Larue (2016 Apr 28)
Any route for melanoblasts to colonize the skin!
Experimental dermatology: DOI: 10.1111/exd.13061

Summary
Melanocytes arise from the fourth embryonic layer, the neural crest. They emerge from the roof plate of the neural tube and migrate throughout the body. In mammals, these cells have the capacity to migrate in any type of environment and use various pathways and mechanisms to colonize the skin and hair, and for their maintenance throughout the life of the animal. This article is protected by copyright. All rights reserved.

Year of publication 2015

Regulation of melanoma progression through the TCF4/miR-125b/NEDD9 cascade.
The Journal of investigative dermatology: DOI: 10.1016/j.jid.2016.02.803

Summary
Melanoma progression from a primary lesion to a distant metastasis is a complex process associated with genetic alterations, epigenetic modifications and phenotypic switches. Elucidation of these phenomena may indicate how to interfere with this fatal disease. The role of microRNAs (miRNAs) as key negative regulators of gene expression, controlling all cellular processes including cell migration and invasion, is now being recognized. Here we show from in silico analysis of miRNA expression profiles of primary and metastatic melanomas, and from functional experiments that miR-125b is a determinant candidate of melanoma progression: (i) miR-125b is more strongly expressed in aggressive metastatic than primary melanomas, (ii) there is an inverse correlation between the amount of miR-125b and overall patient survival, (iii) invasion/migration potentials in vitro are inversely correlated with the amount of miR-125b in a series of human melanoma cell lines and (iv) inhibition of miR-125b reduces migratory and invasive potentials without affecting cell
proliferation in vitro. Furthermore, we show that NEDD9 is a direct target of miR-125b and is involved in modulating melanoma cell migration and invasion. Also, TCF4, associated with EMT, and invasion, induces the transcription of mir-125b-1. In conclusion, the TCF4/miR-125b/NEDD9 cascade promotes melanoma cell migration/invasion.