MEETING ABSTRACTS

To appear in *Pigment Cell & Melanoma Research*, 2024; 37:713–740 https://doi.org/10.1111/pcmr.13192
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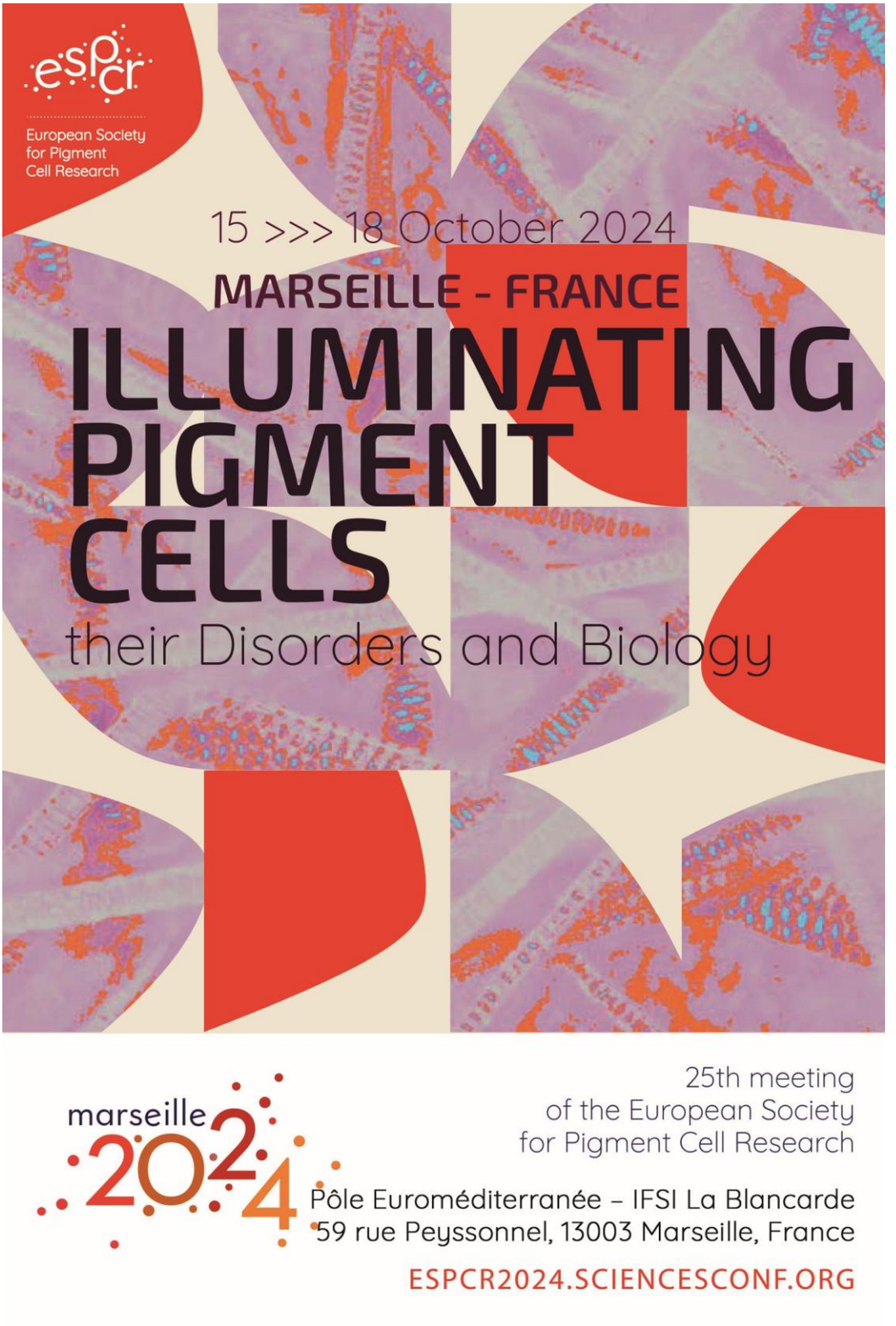

25th Annual Meeting of the European Society for Pigment Cell Research, Marseille, France

Tuesday 15 October 2024

14:15	**16:10**	**Chaired by Mauro Picardo**	**Session 1: Vitiligo, hair graying & other acquired pigmentary disorders**
14:15	14:40	Thierry Passeron	From translational data to clinical results: the crucial need for combination approaches to treat vitiligo
14:40	14:55	Barbara Bellei	IGF/Insulin defective signalling at cellular level correlates to metabolic syndrome markers in vitiligo patients
14:55	15:10	Joudi El Mir	XPC-deficient Xenopus embryos: an *in vivo* model system for studying the mechanisms underlying pigmentary abnormalities in XPC patients
15:10	15:30	Lionel Larue	UVB stress triggers hair greying: unveiling the Dicer-LEF/ B-catenin pathway's role in melanocyte dysfunction
15:30	15:45	Meri Tulic	Vitiligo auto-immune response upon oxidative stress-related mitochondrial DNA release opens up new therapeutic strategies
15:45	16:10	Katia Boniface	The skin immune memory response in vitiligo
16:40	**18:15**	**Chaired by Heather Etchevers**	**Session 2: Cutaneous mosaicism & cell-cell/-environmental interactions**
16:40	17:05	Lukas Sommer	Crosstalk between tumor cells and their microenvironment in melanoma disease progression and therapy response
17:05	17:20	Pierre Vabres	A new mosaic syndrome with linear skin hypopigmentation is associated with a GNA13 variant activating the RHOA/ROCK pathway and altering the cytoskeleton, cell morphology and melanosome transfer
17:20	17:35	Zackie Aktary	Targeting GRPR for sex-hormone dependent cancer after E-cadherin loss
17:35	17:50	Joanna Stefan	Anti-melanoma gene and protein expression in dermal sheath fibroblasts from hair follicles in Recessive Dystrophic Epidermolysis Bullosa (RDEB) scalp
17:50	18:15	Desmond Tobin	Epidermal-melanin units of haired human skin: remarkable life-affirming partnerships on our UVR-drenched planet
18:15	**19:15**	**Richard White**	**Keynote: What separates a melanocyte from a melanoma?**

Wednesday 16 October 2024

08:30	**10:20**	**Chaired by Graça Raposo**	**Session 3: Melanosome trafficking, cell biology & melanin biochemistry**
08:30	08:55	Michal Sarna	Physicochemical properties of melanin obtained from human induced pluripotent stem cell-derived melanocytes
08:55	09:10	Dvir Gur	Genetic and biochemical control over crystal formation in pigment cells
09:10	09:25	Shahram Mesdaghi	Structural insights into pink-eyed dilution protein (Oca2)
09:25	09:40	Sandra del Bino-Nokin	Unraveling UVA1-induced photo modifications of eumelanin and pheomelanin in human skin: insights into pigment darkening
09:40	10:05	Duarte Barral	Regulation of melanin secretion, transfer and processing in skin pigmentation
10:40	**13:00**	**Chaired by Lionel Larue**	**Session 4: Melanocyte development & extracutaneous pigmentation**
10:40	11:05	Igor Adameyko (ONLINE)	Nerve-dependent origin of melanocytes beyond skin: evolutionary and pathological implications
11:05	11:20	Yael Noy	Unraveling the ultrastructure and metabolome of unique pigment cells in the zebrafish fin
11:20	11:35	Beatriz Carazo Del Hoyo	Eye melanosomes: understanding activity patterns in extant and ancient mammals
11:35	11:50	Daniel Cirtina	A deep dive into the anatomical distribution and geometry of melanosomes in fish
11:50	12:05	Håvard Bjørgen	Melanin-synthesizing cells in muscle: inflammatory changes in the Atlantic salmon (*Salmo salar*)
12:05	12:30	Tatjana Sauka-Spengler (ONLINE)	Single-cell, spatial multiomics, and system probing decode complete gene regulatory network underlying cranial neural crest fate decisions
12:30	**13:00**		**Flash talks session 1: Poster teasers**
13:00	14:30		Posters/lunch
14:30	**16:05**	**Chaired by Stéphanie Mallet**	**Session 5: Melanocytic nevi: clinical and mechanistic facets**
14:30	14:55	E. Elizabeth Patton	Transcriptional and metabolic cellular heterogeneity in melanoma residual disease
14:55	15:10	Daniel Aldea	Deconvoluted methylation profiles discriminate between closely related melanocytic nevi
15:10	15:25	Dot Bennett	Telomeres in the senescence of human acquired naevi
15:25	15:40	Federica Papaccio	Hedgehog signalling inhibition by SMO inhibitors in melanoma cells reveals a potential value for a new therapeutic strategy
15:40	16:05	Veronica Kinsler	RNA therapy for congenital melanocytic naevi
16:35	**18:55**	**Chaired by Colin Goding**	**Session 6: Melanomagenesis & metastasis**
16:35	17:00	Chris Marine (ONLINE)	Unlocking the melanoma metastatic cascade at single-cell resolution
17:00	17:25	Julien Ablain	

			Investigating the mechanisms of melanoma dissemination using zebrafish
			(continued)
17:25	17:50	Daniela De Zio	Increasing evidence for the application of FAK1 inhibitors in melanoma therapy
17:50	18:15	Carmit Levy	The effect of melanosome systemic injection
18:15	18:30	Ahmed Najem	Microenvironment-driven adaptation mechanisms in Melanoma: 3D Bioprinting and Tyrosine-induced phenotype switching
18:30	18:55	Svenja Meierjohann	Stress adaptation in melanoma: the role of NRF2

Thursday 17 October 2024

08:30	**10:15**	**Chaired by Lionel Larribère**	**Session 7: Technological advances in melanoma detection & biomarkers**
08:30	08:55	Mitch Levesque	Single-cell molecular and functional landscapes of metastatic melanoma converge on clinically actionable features
08:55	09:10	Krishna Ravulapalli	e-DAM: An e-Delphi study to inform the clinical utility of AMBLor: a novel prognostic biomarker for early-stage cutaneous malignant melanoma
09:10	09:35	Luisa Lanfrancone	Optimizing drug delivery for more effective melanoma metastasis treatment
09:35	09:50	Andrew White	Melanoma macrophage and extracellular matrix transitions during the therapy-tolerant state
09:50	10:15	Anja Bosserhoff	Understanding molecular processes in tumor dormancy, metastasis and therapy
10:45	**12:30**	**Chaired by Eiríkur Steingrimsson**	**Session 8: (Epi+)genetics, post-transcriptional regulation**
10:45	11:10	Eiríkur Steingrimmson	MITF directly regulates the expression of CDH1 but indirectly regulates CDH2 through SETDB2 and chromatin modifications
11:10	11:35	Eleonora Leucci	Contribution of lncRNAs to the generation of drug-tolerant persister cells
11:35	11:50	Sebastien Corre	Role of circRNAs to control gene expression program and cell plasticity associated with BRAFi resistance in melanoma
11:50	12:05	Najla El Hachem	Valine aminoacyl-tRNA synthetase promotes therapy resistance in melanoma
12:05	12:30	Pierre Close	tRNA modifications: a new vulnerability in melanoma
12:30	**13:00**		**Flash talks session 2: Poster teasers**
13:00	14:30		Posters/lunch
14:30	**16:30**	**Chaired by Benoît Arveiler**	**Session 9: Albinism & other hereditary pigmentary disorders**
14:30	14:55	Lluis Montoliu	Making sense of the genetic heterogeneity of albinism
14:55	15:10	Brice Magne	Role of ciliopathy-associated protein TMEM138 in skin pigmentation
15:10	15:35	Fanny Morice-Picard	Phenotyping of albinism

15:35	15:50	João Charneca	Unraveling Griscelli's syndrome hypopigmentation using an *in vitro* model
15:50	16:05	Robert Aquaron	Oculocutaneous albinism type 2 (OCA2) in Cameroon and in sub-Saharan Africa: a 50-year-long-odyssey *(continued)*
16:05	16:30	Benoît Arveiler	Non-coding variants involved in the diagnosis of albinism
17:00	**18:00**		**ESPCR General Assembly** (auditorium)

Friday 18 October 2024

08:30	**11:40**	**Chaired by Nausicaa Malissen**	**Session 10: Innovations in therapy for pigment cell diseases**
08:30	08:55	Michael Hölzel	Modelling systemic immunity in adoptive T cell therapy of melanoma
08:55	09:20	Marie-Dominique Galibert	Investigating new therapeutic approaches to combat metastatic melanoma
09:20	09:35	Jacques Rouanet	A new dual-tumor murine model to study abscopal effect in metastatic melanoma targeted radionuclide therapy
09:35	09:50	Lingli Yang	Impact of EGFR-TKI on Skin Pigmentation and Its Therapeutic Potential for Hypopigmented Skin Disorders
09:50	10:05	Serena Sabbah	Targeting Prohibitins and CRAF: A Promising Therapeutic Strategy for Melanoma
10:50	11:05	Thomas Strub	LKB1-SIK2 loss drives uveal melanoma proliferation and hypersensitivity to SLC8A1 and ROS inhibition
11:05	11:30	Konrad Kleszczynski	Melatonin-boosted efficacy of targeted therapy of human melanoma
11:30	11:40		**Presentation of the ESPCR PRIZE**
11:40	**12:40**	**Miguel Seabra**	**Skin pigment: from birth in melanocytes to demise in keratinocytes**
12:40	12:45		Concluding remarks

The 25th annual meeting of the European Society for Pigment Cell Biology takes place in Marseille, France, on October 15–18, 2024. It was organized by a local and scientific committee presided by Heather Etchevers and composed of Corine Bertolotto, Meri Tulic, Stéphanie Mallet, Marie-Aleth Richard, and Thierry Passeron. The following are the abstracts of communications in either oral or poster form, arranged **in the order of the known presenting author's last name** and current as of going to press in early August, 2024. E-mail addresses will not appear in printed abstracts.

This preprint version has additional abstracts. For various reasons a few did not make it in to print. Sincere apologies for this to: Robert Aquaron, Mitch Levesque, Shahram Mesdaghi, Jilliana Monnier, Lluis Montoliu, Nina Séjourné, Tatjana Sauka-Spengler and Nina Tardif.

Poster abstracts are preceded by P and the assigned number of the board.

For more information and any updates on session times and orators, please consult the conference website, espcr2024.sciencesconf.org.

Investigating the mechanisms of melanoma dissemination using zebrafish

A.A. Mahi, S. Diazzi, S. Aires, J. Ablain

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Metastatic melanoma remains highly lethal despite the efficacy of targeted and immunotherapies. A better understanding of the mechanisms of tumor dissemination is needed to improve treatments. The tumor microenvironment is increasingly recognized as a critical regulator of cancer cell behaviors but the local signals that control melanoma cell spreading remain obscure. The zebrafish is uniquely positioned to probe the multiplicity of external cues influencing cancer cell invasion and dissemination *in vivo*. We developed genetic engineering tools to rapidly model any melanoma genotype in adult zebrafish, which has allowed us to study the function of candidate cancer genes in tumor initiation and progression. We uncovered a cooperation between the genetic loss of the adherens junction gene *NECTIN1* and a drop in the local concentration of the growth factor IGF1 in the regulation of melanoma spread. IGF1 inhibition induced the robust formation of adherens junctions between *NECTIN1*-wildtype melanoma cells, but not *NECTIN1*-knockout cells. Instead, NECTIN1-deficient cells activated a cell–matrix adhesion program resulting in FAK/SRC-mediated motility. In recent work, we have delineated a novel signaling cascade involving the kinase WNK1 and integrin β4 in the regulation of melanoma cell adhesion and migration downstream of the IGF1 receptor. Additionally, we have performed a large-scale genetic screen in zebrafish melanoma to identify cell-surface sensors promoting or hindering melanoma cell dissemination *in vivo*. These studies delineate new microenvironment-sensing mechanisms governing melanoma spread and suggest potential drug targets to interfere with cancer cell aggressiveness.

Targeting GRPR for sex hormone-dependent cancer after E-cadherin loss

Z. Aktary, J. Raymond, M. Pouteaux, V. Petit, V. Delmas, L. Larue

Inserm U1021, CNRS UMR 3347, Normal and Pathological Development of Melanocytes, Institut Curie, PSL Research University, Orsay, France

Sex inequalities in cancer are well documented, but limited understanding hinders advances in precision medicine and therapies. Consideration of ethnicity, age, sex, and gender is essential in the management of cancer patients, due to the significant differences in incidence and response to treatment. Age-related hormone production, a consistent divergence between the sexes, is underestimated in cancers that are not recognized as hormone-dependent. Here we show that premenopausal women present an increased vulnerability to cancers, and we identify the cell–cell adhesion molecule, ECAD, as a crucial component in the estrogen response in different cancers including melanoma. In a mouse melanoma model, we discovered an estrogen-sensitizing pathway connecting ECAD-βCAT-ERα-GRPR, promoting melanoma aggressiveness exclusively in females. Inhibition of this pathway by targeting GRPR reduces metastasis in mice, suggesting a therapeutic approach. Our study introduces a novel concept linking hormone sensitivity and tumor phenotype, where hormones significantly impact cell phenotype and aggressiveness. We have identified an integrated pro-tumor pathway in women, proposing that targeting a GPCR with drugs not commonly used for cancer treatment could be more effective in treating ECAD-dependent cancers in women. This study emphasizes the significance of sex-specific factors in cancer management and provides new hope for improving outcomes in various cancers.

P4 (withdrawn): Characterization of the UV-β-catenin-Dicer axis as a critical regulator of pigmentation

Z. Aktary, J.U. Bertrand, V. Petit, L. Larue

Inserm U1021, CNRS UMR 3347, Normal and Pathological Development of Melanocytes, Institut Curie, PSL Research University, Orsay, France

Deconvoluted methylation profiles discriminate between closely related melanocytic nevi

D. Aldea[1], N. Macagno[1,2], E. Marechal[1], M. Moreno[1], P. Romanet[1,3], M. Pertuit[3], J. Garcia[2], N. Degardin[4], S. Mallet[5], I. James[6], G. Captier[7], A. Barlier[1,3], H.C. Etchevers[1]

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Congenital melanocytic nevi (CMN) and common acquired melanocytic nevi (AMN) are melanoma-predisposing skin conditions presenting excessive numbers of melanocytes but arising at different times in life. Similar to early cutaneous melanoma, both types of nevi are associated with activating mutations in the RAS mitogen-activated protein kinase (MAPK) signaling pathway. Alterations in this pathway are linked to epigenetic changes that can affect gene expression. In this study, we investigated whether distinct genomic DNA methylation patterns exist between CMN and AMN patients. Through comparative epigenomic analysis, our study highlighted the wide cellular heterogeneity observed within the CMN samples analyzed, in terms of melanocytes, fibroblast and epidermal cells. Additionally, we identified differential DNA methylation patterns, with spectrin beta, non-erythrocytic 1 (*SPTBN1*) emerging as a potential molecular marker for CMN. Appropriately weighted whole-genome methylation analysis can be used as a basis for further research in dermatopathology and as applied here provides new insights into nevus biology. These

findings provide insights into early melanoma detection, as these skin lesions can undergo malignant transformation.

Oculocutaneous albinism type 2 (OCA2) in Cameroon and in sub-Saharan Africa: a 50-year-long-odyssey

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I had the pleasure of living in Cameroon from 1972 to 1977 as professor of biochemistry at the young faculty of medicine of Yaounde. This study on OCA began because this affection was easily recognizable and it was common to meet persons with albinism (PWAs) in the streets of Yaounde. With the help of my Cameroonian assistant (LK) I began to examine PWAs for visual impairment and skin abnormalities: keratosis, squamous and/or basal cell carcinoma. I referred some of them to colleagues in dermatology and ophthalmology departments for more investigations. As OCA is autosomal recessive, we noted the ethnic and the village of origin of parents. We examined in five years 230 PWAs and found a high percentage, nearly 60%, among the Bamileke tribe. Back to France, I pursued this study by regular travel to Cameroon. The molecular biology era of OCA began in 1989 with the description by Tomita of the first mutation in a Japanese patient of the tyrosinase gene (*OCA1*). In 1992, Muray Brillant and al. identified a non-enzymatic protein in the Pink-eyed albino mouse. The human equivalent gene was called" *P*" then *OCA2*. In 1994, Murray Brillant and al. identified a 2.7 kb intragenic deletion of the "*P*" gene in the homozygous or heterozygous state in 3 Africans (2 from Cameroon,1 from DRC) and 2 African-Americans. Moreover, this mutation was not found in Caucasian PWAs, pointing to an African origin for this specific mutation. We later showed that this mutation was specific to Bantu populations located in central, south, and east Africa.

Non-coding variants involved in the diagnosis of albinism

B. Arveiler[1,2], M. Diallo[1], C. Courdier[1,2], A. Defay-Sinat[1], A. Sequeira[1], E. Mercier[1], C. Plaisant[2], S. Javerzat[1], E. Lasseaux[2], V. Michaud[1,2]

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Albinism is characterized by generalized hypopigmentation and ocular features leading to poor visual acuity. Analysis of the 21 genes involved allows an about 70% diagnostic rate. Missing variants in the 30% of unsolved cases may reside in non-coding regions of the genes, which are not commonly analyzed in clinical laboratories. We screened both intronic and flanking regions of the genes to search for pathogenic variants. 121 patients heterozygous for a first pathogenic variant were analyzed by sequencing of either the whole genome or a panel comprising the exons of all albinism genes plus the introns and flanking sequences of *TYR*, *OCA2*, *SLC45A2*, *GPR143*, and *HPS1*. 14 variants in *trans* to a first variant had a splicing effect predicted by MaxEntScan, SpliceAI, SPiP, and RNASplicer. RT-PCR and/or minigene assays confirmed a splicing effect for 8 variants, leading to skipping of one or more exons or inclusion of pseudoexons. This allowed establishing the diagnosis in 11 patients. In addition to these splice variants, XX some variants fell in ENCODE-p redicted regulatory elements (visualized on the UCSC genome browser) including promoters, enhancers and CTCF binding regions. A large deletion upstream of *TYR* was found in 2 affected sibs. Single nucleotide variants are being tested using a dual luciferase assay in order to both characterize the regulatory nature of the region they lie in and their potential deleterious effect. These data suggest that the analysis of non-coding variants is warranted to increase the diagnostic rate of albinism.

Regulation of melanin secretion, transfer, and processing in skin pigmentation

D. Barral

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In the skin epidermis, melanin is produced by melanocytes and transferred to keratinocytes, where it is processed and forms supra-nuclear caps. Despite the crucial role of melanin secretion, transfer, and processing by keratinocytes for skin pigmentation, the pathways involved remain controversial and poorly characterized. We have been studying these processes by characterizing the pathways and regulators involved, and described that melanin is transferred through exo/phagocytosis of melanocores. Recently, we uncovered a new melanin secretion pathway, stimulated by keratinocyte-conditioned medium and regulated by Rab3a, Rab27a, and Rab3il1. We have also characterized the processing within keratinocytes and found that melanin resides in a weakly acidic and weakly degradative compartment, which we proposed to be named melanokerasome. We also gathered evidence that melanokerasomes represent a lysosomal-derived storage compartment that has exited the lysosome cycle, allowing melanin to persist for long periods and exert its photoprotective effects. These studies provide a better understanding of the skin pigmentary system and shed light on how skin phototype is determined, as well as how basal and facultative pigmentation are regulated.

P13: The transcriptome and ultrastructure of leucophores and their relation to other pigment cells

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Colors in nature are generated either through the absorption of light by pigments or by the interaction of light with nanometric crystalline materials, a phenomenon termed structural colors. Structural colors

are widespread in the animal kingdom and contribute to various functions, from vision to camouflage to the remarkable patterns seen in fish and chameleons. Fish exhibit a variety of pigment cells known as chromatophores, each producing a specific biomolecule (pigment) that contributes to their distinctive coloration. While most research has focused on melanophores, little is known about the uric acid (UA)-forming leucophores. Despite being recognized for years, their ultrastructure and the molecular dynamics of crystal formation have remained unexplored. Failure to control the crystal formation can be deleterious to organisms, obstructing fundamental aspects of their ecology and leading to human pathologies such as gout and kidney stones. To elucidate the molecular mechanisms involved in UA crystal formation, we investigated leucophores in the medaka (*Oryzias latipes*) model organism, which uses UA crystals in their skin for coloration, pattern formation, and signaling. We conducted the first single-cell RNA sequencing of pigment cells in medaka fish and discovered novel leucophore-specific genes, providing insights into their molecular mechanisms and cellular machinery. Additionally, we utilized advanced cryo-electron microscopy and spectroscopy to characterize the ultrastructure of leucophores, the crystals within them, and their molecular composition. This work not only sheds new light on the underexplored leucophore pigment cell but also holds promise for the development of innovative biomaterials and therapies to manage pathological UA crystallization.

IGF/Insulin defective signaling at cellular level correlates to metabolic syndrome markers in vitiligo patients

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Vitiligo (VTG) is characterized by white skin lesions due to melanocyte destruction by autoreactive CD8+ T cells. Recently, the emphasis has been on the final stages leading to clinical symptoms, overlooking the initial harmful process, which may be linked to an inherent metabolic deficiency. The current understanding lacks a detailed molecular explanation for the process that activates innate immune cells in the skin. Our research has identified various metabolic irregularities in cells taken from the pigmented skin of VTG patients, such as impaired intracellular IGF/INS signaling. IGF/insulin resistance is evidenced by the chronic hyperphosphorylated state of IRS1 at Ser612, which is correlated to defective mitochondrial dynamic network and functionality. This is reflected in low ATP, increased reactive oxygen species (ROS), and advanced glycation end products (AGEs), culminating in abundant production of immune system cell activators. Thus, considering the underlying vitiligo phenotype, we evaluated circulating markers of metabolic disorders in 50 patients diagnosed with non-segmental vitiligo to determine its systemic nature and similarities with metabolic syndrome. Biochemical approaches demonstrated abnormalities in the level of LDL cholesterol, folate, and vitamin D. We confirmed that vitiligo patients present an elevated inflammatory profile compared to controls. Particularly higher levels of IL-6, CXCL10, and IGFBP5, and an inappropriate activity of antioxidant enzymes, such as Superoxide Dismutase. Furthermore, we detected diminished Cysteine and augmented AGEs compared to a control group. Mass spectrometry evaluation revealed changes in lipid composition, indicating that vitiligo patients have higher levels of fatty acids closely associated with the inflammatory process. Collectively, our *in vitro* data and patient outcomes, support the hypothesis that vitiligo is a metabolic syndrome disorder.

Telomeres in the senescence of human-acquired nevi

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Acquired pigmented nevi or moles express many molecular markers of cell senescence, as reported by our group and others. They have been regarded as a valuable example of cell senescence *in vivo*, although this has been debated. Nearly all human benign nevi are known to express an activated oncogene, usually *BRAF(V600E)* or *NRAS(Q61K)*, so nevi have also been described as an example of oncogene-induced senescence (OIS) *in vivo*. However, "classical" OIS, as seen after oncogene overexpression in cell culture, occurs rapidly, with DNA replication stress and little cell division. Conversely, nevus cells have evidently divided many times before senescing, to form a visible lesion. This could therefore be described as either a different kind of OIS or a different kind of senescence. There is a wide range of published evidence—including from human familial melanoma genomics, precision tissue sequencing on nevi and associated melanomas, and immunohistochemical marker studies—that telomere length and telomere dysfunction are important in the senescence of human nevi as well as in the genetic changes seen during progression to melanoma. I will present an updated genetic model that may make sense of these findings.

Melanin-synthesizing cells in muscle inflammatory changes in the Atlantic salmon (*Salmo salar*)

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In ectothermic vertebrates, melanin-containing cells are present in secondary lymphoid organs and in the liver. In the literature, such cells are commonly referred to as melano-m acrophages, and we have previously shown that such organs express the genes for the tyrosinase gene family. In the industrialized production of Atlantic salmon, Salmo salar, focal melanized changes within the white musculature are a common problem affecting about 20% of all individuals. The economic consequences are substantial. These changes are proceeded by hemorrhages that may progress into a

chronic idiopathic inflammatory myopathy dominated by epithelioid cells, Langhan's giant cells and melano-macrophages. Virus infection may aggravate the inflammatory response but appears not to be the cause. Rather, lipid necrosis seems to be the driving force in the pathogenesis. Previously we have demonstrated the expression of the tyrosinase family genes in these changes as detected by RT-qPCR. Here we show by *in situ* hybridization that in early changes, amelanotic cells expressing these genes are present, but over time, the changes progress into a chronic inflammatory state with the presence of melano-macrophages. The pigmented cells are transcript-positive for tyrosinase and *Tyrp1*, indicating that they are producing melanin themselves and not simply phagocytosing the compound. Although commonly referred to in the piscine pathological nomenclature as melano-macrophages, the nature of these cells has not been resolved and is a matter of further investigation.

Understanding molecular processes in tumor dormancy, metastasis, and therapy

A. Bosserhoff

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Recent studies have unveiled significant plasticity and adaptability of cancer cells within tumor tissues. This is driven by several microenvironmental factors such as accumulation of metabolic byproducts, as well as conditions like hypoxia (oxygen deficiency) and acidification. Additionally, interactions to the extracellular matrix and with neighboring cells contribute to the diversity of tumor cell characteristics. Understanding these regulatory mechanisms at the molecular level is crucial, as they play a pivotal role in the tumor dormancy, progression of tumors, and their resistance to therapy. In the context of melanoma, our research has demonstrated various distinct effects of the microenvironment on the plasticity of melanoma cells supporting switching from proliferative to dormant/migratory and vice versa. In this presentation, an overview of recent findings from our research group is given, also highlighting the use of biofabricated 3D tumor models more closely mimicking the *in vivo* conditions. These findings can further aid the understanding of molecular alterations driven by the tumor microenvironment.

Eye melanosomes: Understanding activity patterns in extant and ancient mammals

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The early evolution of mammals is a hot topic in paleontology, with much attention focused on the impact of activity patterns on the evolutionary success of the clade. A previous hypothesis proposed that early mammals were adapted to nocturnal lifestyles, which is supported by anatomical, physiological and behavioral evidence from extant mammals. This hypothesis is, however, challenged by molecular evidence. Here we propose a new approach to distinguish between diurnal, nocturnal and crepuscular mammals using the geometry of eye melanosomes—melanin-rich organelles. Recent studies have reported that variation in the geometry of melanosomes among vertebrate taxa and tissues is linked to function. In the eyes of extant mammals, melanosomes play a crucial role in UV absorption and reduction of photo-oxidative stress. Given that the activity pattern of an animal controls the amount of ambient light available for vision, melanosomes in vertebrate eyes may vary in geometry according to the intensity of ambient light. To test this hypothesis, eyes were dissected from three replicates of each of six extant mammal species (two diurnal, two crepuscular, and two nocturnal). Melanosomes were extracted from the iris, retinal pigmented epithelium and choroid using a 12-day enzymatic digestion procedure and imaged using scanning electron microscopy. Multivariate analysis of a suite of melanosome attributes shows that melanosome geometry differs significantly between species with different activity periods. Our study provides a new approach to infer the active period of ancient mammals and thus may constrain evolutionary scenarios for the origins of diurnal and nocturnal lifestyles in mammals.

P24: $p21^{Waf1/Cip1}$ expression and cytoplasmic localization in low melanized pigment and melanoma cells: A potential mechanism to sensitize pigment cells to apoptosis

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Currently, 2.1% of the world's population will be diagnosed with cutaneous melanoma during their lifetime, with incidence rising especially in the fair skinned. Cutaneous pigment cells intrinsically resist apoptosis, which presents a significant barrier to melanoma treatment. While we know relatively little about apoptotic signaling pathways in pigment cells, our preliminary data suggest that the cyclin kinase inhibitor $p21^{Cip1/Waf1}$ is of interest. p21 expression is increased in response to several stimuli to arrest the cell cycle (to ensure genomic integrity). It is also involved in cell migration, apoptosis, differentiation, and DNA repair. Depending on subcellular localization, p21 can function as a tumor suppressor or oncogene. Here, we assess whether p21 subcellular localization and expression level is associated with melanin level/subtype in normal human (epidermal (HEM) and hair follicle (HFM) melanocytes) in human melanoma cells, as part of our search for melanomagenesis targets. Under basal culture conditions, low melanin HEM and amelanotic melanoma cells expressed high levels of p21 cytoplasmically. By contrast, p21 expression was downregulated and restricted to the nucleus in highly melanized pigment cells, concurring with a reduction in p21 expression after IBMX-stimulated melanogenesis. P21 was the most highly expressed marker in an apoptosis array of extracts of hair follicle, HFM,

followed by HEM and amelanotic melanoma cells. UVB-irradiation significantly increased p21 expression in cell extracts of whole human epidermis, HEM, and melanoma cells *in vitro*, suggesting that p21 may have a UVR-sensing and possibly UVR-protective function in pigment cells. Indeed, pigment cell apoptosis was more readily induced in low melanin HEM and amelanotic melanoma cells, suggesting that high p21 expression may "sensitize" normal pigment cells to apoptosis and inhibit melanomagenesis. Our Kaplan–Meier analysis showed that high p21 expression is associated with decreased overall survival (TCGA) in metastatic melanoma, concurring with published data showing that p21 overexpression is also associated with poor prognosis in many cancers (e.g., ovarian, prostate, and breast). Our data suggest that understanding how p21 subcellular localization and expression are regulated in non-malignant versus malignant pigment cells may reveal new targets for melanomagenesis.

Unraveling Griscelli's syndrome hypopigmentation using an *in vitro* model

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Griscelli syndrome (GS) is a rare disorder characterized by hypopigmentation of the hair and skin. GS leads to a greater susceptibility to ultraviolet radiation-induced DNA damage in the skin epidermis and a psychological burden, especially in dark skin patients. Skin pigmentation relies on melanin, which is produced and stored in specialized organelles termed melanosomes, within melanocytes. Melanin is then transferred to keratinocytes, where it accumulates in supranuclear caps and exerts a photoprotective effect. GS is caused by mutations in *MYO5A*, *RAB27A*, or *MLPH*, which encode for three proteins that form a tripartite complex required for the positioning of melanosomes in melanocyte dendrites. Strikingly, it is not understood why GS patients display hypopigmentation, as there is no significant difference in melanin transfer between co-cultures of keratinocytes and Rab27a-deficient melanocytes. This opens the need for an *in vitro* model that mimics GS patients' skin pigmentation defect. Moreover, we hypothesize that only such model can reproduce the deficient melanin transfer that occurs in the skin of GS patients, and the defects in melanin transfer caused by Rab27a depletion that are not apparent in co-cultures. We successfully established a GS *in vitro* model and confirmed that melanin is transferred by a different mechanism when Rab27a is depleted. Indeed, electron microscopy studies showed that Rab27a-silenced melanocytes secrete large globules from the dendrites and cell body, laden with melanosomes. Thus, the creation of a disease model that recapitulates GS etiology can shed light of the mechanisms of melanin transfer in pathological conditions.

A deep dive into the anatomical distribution and geometry of melanosomes in fish

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Melanosomes are cellular organelles associated with synthesizing, storing, and transporting melanin pigments in vertebrates. A lack of comparative studies of the anatomical distribution of melanosomes in contemporary species has limited our understanding of the origin(s) and functional evolution of melanin within the vertebrate clade. Fish melanosomes are of particular interest as they have putative functions in immunity and can be used as a biomarker for monitoring fish health and environmental conditions. The biology of fish melanosomes, however, is largely unknown. Here, we resolve these issues by sampling ten tissues in twelve species of extant fish, representing a wide phylogenetic spread of early-diverging vertebrates. Scanning electron microscopy reveals tissue-specific trends in the anatomical distribution and geometry of melanosomes. Bony fish species contain abundant melanosomes in the spleen and kidney, while melanosomes in cartilaginous fish are more frequently observed in the liver. Further, eye melanosomes differ significantly in geometry between the two groups, with large, spheroidal melanosomes present only in cartilaginous fish and long, rod-shaped melanosomes only in bony fish. In contrast, melanosomes geometry in internal organs appears to be highly conserved. These findings provide crucial insights into the ecological roles and evolutionary pathway of melanosomes, suggesting that the anatomical distribution and geometric diversity of melanosomes may have adapted in response to key physiological and environmental drivers.

tRNA modifications: A new vulnerability in melanoma

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Global regulation of mRNA translation has emerged as a central mechanism driving the adaptation of tumor cells during cancer progression and response to therapy. The emerging concept that translation reprogramming promotes the establishment of specific cancer phenotypes may position this important process as a key hallmark of cancer. Our previous work demonstrates that tRNA epitranscriptomics is a new regulatory module that contributes to the establishment of cancer phenotypes through the maintenance of specific proteomes. Therefore, modulation of tRNA epitranscriptomics and mRNA translation holds the potential to sustain tumor heterogeneity and shape tumor immune environment. Here, I will discuss how tRNA epitranscriptomic machineries may represent new regulatory modules that support cancer phenotypes and dictate interaction of cancer cells with their immune environment. We aim to discover new vulnerabilities in cancer and to uncover original therapeutic opportunities to be exploited for the development of future anti-cancer treatment.

Role of circRNAs to control gene expression program

and cell plasticity associated with BRAFi resistance in melanoma

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BRAF inhibitors are a first-line treatment for metastatic melanoma, significantly improving patient survival. However, resistance mechanisms systematically arise, limiting the treatment's long-term efficacy. Tumor phenotypic plasticity, governed by transcriptional expression programs, contributes to the development of this resistance. Noncoding RNAs (ncRNAs), due to their ability to control gene expression are critical components of cell plasticity and attractive targets for drug development. A recently discovered type of endogenous ncRNA is circular RNAs (circRNAs), which form closed loops and are highly represented in the eukaryotic transcriptome. circRNAs can function as microRNAs sponges, modulating gene expression during cancer development and thereby influencing cell fate. To explore the role of circRNAs in monitoring melanoma cell plasticity, we developed an innovative bioinformatics tool called Cirscan (https:// gitlab. com/ geobi oinfo/c irscan). This tool automates the identification of sponge mechanisms and maps circRNA-miRNA-mRNA networks between two conditions.
As a proof of concept, we validated *in vitro* noncoding RNAs subnetworks responsible for the upregulation of Ahr, AXL, and EGFR, three critical regulators of resistance. We identified several circRNAs (hsa_cir_0001610, _0002805, _0000442, _0005260) enriched in BRAFi-resistant cells that sponge specific miRNAs (miR-29a-3p, miR-151a-3p, miR-148a-3p, miR-221-3p) contributing to the overexpression of their target genes. Additionally, overexpression of these miRNAs or specific depletion of the identified circRNAs resulted in decreased AhR, AXL, and EGFR expression, increased BRAFi sensitivity, and reduced invasive capacity of melanoma cells. These findings underscore the importance of decoding the circRNA-miRNA-mRNA network in the context of melanoma resistance to targeted therapy. This approach could enhance our understanding of circRNA biology and aid in identifying circRNAs as new predictive markers of resistance and therapeutic targets for RNA-targeted therapies (ASOs).

Increasing evidence for the application of FAK1 inhibitors in melanoma therapy

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Mitogen-activated protein kinase inhibitors (MAPKi) face significant challenges in treating melanoma due to both intrinsic and acquired resistance mechanisms. Genetic alterations or tumor evolution processes have been described as mechanisms that coordinate this event. Prior data suggest that the absence of AMBRA1 (autophagy and beclin 1 regulator 1) enhances the growth and metastasis of melanoma. Our study reveals that resistance to MAPKi is intricately linked to decreased expression of the melanoma suppressor AMBRA1. We found that low AMBRA1 levels not only predict poor MAPKi response but also drive a phenotype switch, promoting an ERK-independent resistance pathway via focal adhesion kinase 1 (FAK1) activation. Through comprehensive *in vitro* and *in vivo* analyses, we demonstrate that melanomas with reduced AMBRA1 intrinsically resist MAPKi therapy yet remain highly sensitive to FAK1 inhibitors. Intriguingly, we observed that the rapid emergence of resistance in initially MAPKi-sensitive melanomas stems from pre-existing subclones with low AMBRA1 expression. However, a combination therapy targeting both MAPK and FAK1 pathways effectively inhibits the development of resistance. To sum up, our research highlights the significance of AMBRA1 in predicting the melanoma response to MAPKi and proves the therapeutic effectiveness of FAK1 inhibitors in overcoming MAPKi resistance.

Unraveling UVA1-induced photo modifications of eumelanin and pheomelanin in human skin: Insights into pigment darkening

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UVA exposure elicits immediate and persistent pigment darkening on the skin that are thought to result from oxidation, polymerization of existing melanin and/or precursors. Melanocytes produce eumelanin and pheomelanin. Eumelanin consists of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while pheomelanin consists of benzothiazine and benzothiazole units. Melanins can be analyzed through specific degradation products by HPLC. Eumelanin can be analyzed as pyrrole-2,3,5-tricarboxylic acid (PTCA) and pyrrole-2,3-dicarboxylic acid (PDCA), specific degradation products of DHICA

and DHI. Benzothiazole pheomelanin can be analyzed as thiazole-2,4,5-tricarboxylic acid (TTCA) and benzothiazine pheomelanin as 4-amino-3-hydroxyphenylalanine (4-AHP) and 3-amino-4-hydroxyphenylalanine (3-AHP). Melanins undergo structural modifications upon UVA exposure. Eumelanin undergoes oxidative cleavage to free pyrrole-2,3,5-tricarboxylic acid (free PTCA) and cross-linking to form pyrrole-2,3,4,5-tetracarboxylic acid (PTeCA). UVA exposure of pheomelanin induces oxidative conversion of the benzothiazine to the benzothiazole. Nevertheless, these structural modifications have never been characterized in human skin. In this study we exposed ex vivo skin to increasing UVA1 doses (60, 90 and 120 J/ cm^2) and characterized the induced pigment darkening before, immediately and two hours after exposure through colorimetry and HPLC. The results showed changes in the CIELAB colorimetric parameters, namely decrease in Luminance L*, yellow-blue component b* and Individual Typology Angle in UVA1-exposed samples, indicative of skin darkening. In parallel UVA1 exposure induced modifications of the levels of PTCA, TTCA, 4-AHP, and ratios of various markers, such as PTeCA/ PTCA, free/total PTCA, and TTCA/4-AHP, indicative of photooxidation/degradation of melanins. Our study shows first-time evidence of UVA-induced modifications of melanins associated with pigment darkening in human skin.

From pigment capture to intracellular positioning and photoprotection in keratinocytes

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The protection of human skin from harmful ultraviolet (UV) radiation relies on the production of melanin pigments by epidermal melanocytes and their storage in keratinocytes. While the role of melanocytes in pigmentation is well understood, the cellular and molecular mechanisms governing pigmentation in keratinocytes remain largely unexplored. Here, we introduce a cellular model using melanin particles secreted by melanoma cell lines and incubated with normal human keratinocytes that replicates the biology of pigmentation in keratinocytes and the protection of their genetic material from UV damage. Our model has unveiled initial events involved in the capture of melanin by keratinocytes and the role of cytoskeletal elements in the perinuclear positioning of pigment organelles for optimal genome photoprotection. When the molecular composition of secreted melanin particles is crucial for their effective capture and internalization, distinct cytoskeletons coordinate to strategically position pigment organelles on the sun-exposed side of the keratinocyte nucleus for minimizing DNA photodamage. Finally, the developed model of pigmented keratinocytes identified new fundamental molecular mechanisms underlying genome photoprotection. This provides opportunities for further elucidation of the strategic cellular adaptations that protect genetic material from UV-induced damage in health and disease.

P9: A chronic inflammation sustained by a molecular and cellular inflammatory loop contributes to the physiopathology of actinic lentigines

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Actinic lentigines (AL) or age spots, are hyperpigmented lesions associated with photoaging and characterized by a drastic disorganization of the cutaneous structure with deep epidermal invagination in the dermis, and melanin accumulation in basal keratinocytes. To better understand AL pathophysiology, an exploration of the inflammatory background was conducted in AL lesions versus adjacent non-lesional skin (NL) from 35 women (15 French, 20 Japanese; 50–70 y.o.) through genomic and histological analysis. A robust molecular signature associated with inflammation/immune response (31 modulated genes) was revealed in AL. Indeed, a proinflammatory environment was attested by the activation of proinflammatory pathways, the decrease of anti-inflammatory cytokines and the overexpression of genes regulating immune cells homeostasis. In line, the infiltration of CD4+ T cells and CD68+ macrophages was highlighted in the dermis of AL versus NL. Especially, a double immunostaining analysis revealed a significant increase of the number of CD80+/CD68+ macrophages, corresponding to proinflammatory M1 macrophages, in AL versus NL. To understand the cross talk between immune and cutaneous cells within AL lesions, *in vitro* approaches were developed to investigate (1) the role of keratinocyte dysregulations, particularly melanin retention, on the inflammatory environment, and (2) the impact of polarized M1 macrophages on fibroblasts/keratinocytes. The data showed a proinflammatory secretome of melanin-overloaded keratinocytes and a detrimental impact of M1 macrophages particularly on fibroblasts, by upregulating inflammation and ECM degradation–related genes and molecules. Altogether, these molecular and cellular signals may create a proinflammatory loop which should be targeted to normalize skin and improve age spot treatment.

XPC-deficient *Xenopus* embryos: An *in vivo* model system for studying the mechanisms underlying pigmentary abnormalities in XPC patients

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder characterized by defective DNA repair, leading to photosensitivity, skin damage, and an increased risk of skin cancers. Early signs include pigmentation changes and xerosis. Due to a lack of suitable models, research on XP's pigmentary abnormalities has been limited. This project seeks to model these abnormalities in XP-C patients using the amphibian *Xenopus laevis*. *Xenopus laevis* is an ideal *in vivo* model due to its evolutionary proximity to humans and its transparent skin, which facilitates studies related to melanocytes. We

first assessed the ability of *Xenopus laevis* to model UVB radiation effects on skin and melanocyte physiology. Our studies demonstrated that UVB exposure in *Xenopus* embryos activates the DNA damage response (DDR) network, inducing DNA repair systems, apoptosis, epidermal thickening, and increased melanocyte dendricity. These responses are similar to those seen in human skin following UVB exposure, validating *Xenopus* as a suitable model for studying UVB effects on skin physiology. Using antisense oligonucleotide morpholinos, we successfully downregulated xpc expression in *Xenopus* embryos, resulting in developmental abnormalities and pigmentary disturbances closely resembling XP-C clinical manifestations. Proteomic analysis of xpc morphant embryos provided insights into the underlying molecular mechanisms. Our findings establish *Xenopus* embryos as a sensitive and effective model for studying DNA damage response and pigmentary abnormalities in XP-C patients, offering a valuable tool for elucidating the cellular and molecular mechanisms of XP.

Valine aminoacyl-tRNA synthetase promotes therapy resistance in melanoma

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Transfer RNA dynamics contribute to cancer development through regulation of codon-specific messenger RNA translation. Specific aminoacyl-tRNA synthetases can either promote or suppress tumorigenesis. Here we show that valine aminoacyl-tRNA synthetase (VARS) is a key player in the codon-biased translation reprogramming induced by resistance to targeted (MAPK) therapy in melanoma. The proteome rewiring in patient-derived MAPK therapy-resistant melanoma is biased toward the usage of valine and coincides with the upregulation of valine cognate tRNAs and of VARS expression and activity. Strikingly, VARS knockdown re-sensitizes MAPK therapy-resistant patient-derived melanoma *in vitro* and *in vivo*. Mechanistically, VARS regulates the messenger RNA translation of valine-enriched transcripts, among which hydroxyacyl-CoA dehydrogenase mRNA encodes for a key enzyme in fatty acid oxidation. Resistant melanoma cultures rely on fatty acid oxidation and hydroxyacyl-CoA dehydrogenase for their survival upon MAPK treatment. Together, our data demonstrate that VARS may represent an attractive therapeutic target for the treatment of therapy-resistant melanoma.

P11: Study of the molecular etiology behind the pigmentary abnormalities caused by XPC protein deficiency: Insights from the epidermal melanin unit

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Skin disorders with abnormal pigmentation are challenging to treat due to their unclear etiology. Xeroderma pigmentosum (XP) is an autosomal recessive disorder characterized by extreme UVB sensitivity. XP-C, the most prevalent form globally and in France, primarily affects the skin, causing dyschromic macules within the first year of life without photoprotection. While many XP symptoms are linked to XPC protein's role in the global genome nucleotide excision repair (GG-NER) pathway, the mechanisms underlying pigmentary irregularities remain elusive. Our proteomic analysis comparing XPC knockout primary melanocytes to control cell counterparts revealed alterations in some proteins involved in skin pigmentation under basal conditions. Additionally, multiple pathways appear to be potentially altered, including the NRF2-mediated oxidative stress response. Nrf2's involvement in skin pigmentation may be linked to its modulation of autophagy-mediated melanosome degradation and melanogenesis, as indicated in existing literature. This study investigates the impact of XPC deficiency on the regulation of skin pigmentation, particularly at the dermo-epidermal melanin unit, using monoculture and coculture systems. It aims to elucidate the molecular connection between XPC protein deficiency, melanogenesis, and Nrf2-mediated melanosome degradation via autophagy under both basal and UV radiation conditions. Ongoing experiments appear to validate an upregulation of NRF2 and LC3B in XPC knockout cells and reveal distinct characteristics of XPC patients in cells with modulated NRF2. Research in this area may reveal potential therapeutic strategies for treating or managing skin pigmentation disorders, utilizing existing Nrf2-targeted pharmacological treatments.

P18: Generation and phenotyping of CRISPR-derived mouse models of ocular albinism

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Albinism is a rare and complex genetic condition caused by mutations in at least 21 known genes. It is classified into two main groups: syndromic albinism, which affects multiple cell types including melanocytes and retinal pigment epithelium cells, and non-syndromic albinism, which affects only these two cell types. Non-syndromic albinism is further divided into oculocutaneous albinism, affecting the skin, hair, and eyes, and ocular albinism, affecting only the eyes. The latter includes conditions like ocular type 1 albinism (OA1) and foveal hypoplasia, optic nerve decussation defects, and anterior segment dysgenesis (FHONDA). All types of albinism are characterized by severe and variable visual deficits, such as reduced visual acuity due to foveal hypoplasia, photophobia, nystagmus, and anomalies in the axonal chiasm connections between the retina and the brain. The primary goal of this study is to improve understanding of the relationship between genetic mutations and their phenotypic

expressions in patients. Focusing on OA1 and FHONDA syndromes, which exclusively affect the visual system, could be key to uncovering the link between vision and pigmentation in albinism. Mouse models for OA1 and FHONDA, generated using CRISPR-Cas9 to carry specific patient mutations, were analyzed through various tests including melanin quantification, histology, chiasm connection studies, and optomotor tests for visual acuity. These models successfully replicate many of the clinical signs of OA1 and FHONDA seen in humans. Understanding these mouse models is crucial for comprehending the pathogenesis of ocular albinism, improving diagnostic methods, and validating potential new therapies.

Genetic and biochemical control over crystal formation in pigment cells

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To enable capabilities such as camouflage, communication, thermal regulation, pattern formation, and vision, organisms have developed strategies to regulate the functionality of pigment cells. While much is known about the cellular regulation of melanophores, the mechanisms behind the formation of diverse intracellular crystals in iridophores and leucophores remain largely unknown. Here, we have unraveled both genetic and biochemical controls over crystal morphogenesis in zebrafish iridophores. Our study shows that the chemical composition of the crystals determines their shape. These variations are genetically controlled through tissue-specific expression of specialized paralogues, which exhibit remarkable substrate selectivity. This orchestrated combination enables organisms to generate a broad spectrum of crystal morphologies. In addition, utilizing a combination of advanced spectroscopy and microscopy techniques and pharmacological perturbations, we reveal that organelle pH plays a crucial role in this process. We discovered that amorphous guanine accumulates in early-stage iridosomes and is initially protonated. Live imaging with a pH sensor revealed that early-stage iridosomes are acidic, with their pH gradually approaching neutrality during maturation. Overall, our findings suggest a novel mechanism for the morphological and functional diversity of biogenic crystals, integrating genetic regulation and pH-dependent biochemical processes. This ability of cells to manipulate the microenvironment and composition of intracellular organelles to regulate the crystallization of molecular crystals is novel and sheds new light on this underexplored avenue of cell biology—the biochemical regulation of crystal-forming cells.

P6: Regulation of ITGA5 and ITGB1 levels by MGRN1 led to enhanced adhesion and decreased migration of human melanoma cells

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Mahogunin Ring Finger-1 is a E3-ubiquitin ligase encoded by *MGRN1*, a color gene involved in the regulation of eumelanogenesis in mice. Kaplan–Meier analysis of the cohort of skin melanoma patients from the TCGA database showed that low MGRN1 expression in the tumor was significantly associated with better survival of the patients. Permanent MGRN1 downregulation in human melanoma cells led to bigger cellular clusters, increased E-cadherin levels, higher cell–cell and cell–matrix adhesion, and lower migration rate. Moreover, enhanced intercellular adhesion was mostly mediated by induction of E-cadherin and activation of CDC42, suggesting a new MGRN1-dependent pathway regulating melanoma cell shape, motility, and invasion potential. Bioinformatics analysis of the RNA-Seq data from control and MGRN1-null melanoma cells revealed that MGRN1 regulated several sets of genes involved in cell–matrix adhesion. In this study, we investigated MGRN1-mediated regulation of integrins and their effects on the phenotype of MGRN1-silenced cells. We found that loss of MGRN1 resulted in higher protein expression of ITGA5 and ITGB1 measured by Western blot and immunofluorescence. We also found higher levels of membrane-associated ITGB1 in MGRN1-KO clones than control cells by flow cytometry in non-permeabilized cells. Furthermore, double silencing of ITGA5/ITGB1 decreased the size of cellular clusters formed on collagen I in MGRN1-null cells and reverted the increased adhesion and decreased migration observed upon permanent repression of MGRN1. In summary, these findings point at MGRN1 as an important regulator of melanoma dissemination at least in part by contributing to the regulation of the integrin pool of melanoma cells.

Modeling systemic immunity in adoptive T-cell therapy of melanoma

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Immunotherapy has revolutionized the treatment landscape of melanoma. Adoptive T-cell therapy is an emerging treatment option, but the dynamics of the systemic immune response is poorly understood. Here we used a syngeneic mouse model of melanoma and employed our CRISPR-Cas9–based platform termed CRISPitope (CRISPR-assisted insertion of epitopes) to study the spatiotemporal dynamics of adoptively transferred T cells directed against an endogenously encoded oncogene. CRISPitope is a flexible approach for generating tumor cells expressing model CD8+ T-cell epitopes fused to endogenously encoded gene products of choice. CRISPitope-engineered tumor cells can be recognized by T-cell receptor-transgenic (TCRtg) CD8+ T cells that are widely used in immunology research. We dissected the expansion and contraction of adoptive transferred T cells and the changes in other key immune cell populations including reactively recruited neutrophils. Importantly, we compare tumor-draining versus non-draining lymph nodes and reveal unexpected differences regarding neutrophil recruitment. We also compared treatment protocols with or without targeted Type I interferon activation in the tumor and studied the consequences on T-cell expansion and reactive neutrophil recruitment. In summary, we performed a comprehensive

assessment how adoptively T cell expand and contract in secondary lymphoid organs in a well-defined mouse model of melanoma and the interrelationships with other key immune cell populations. We believe that our results provide important novel mechanistic insights and will help improve current regiments of adoptive T-cell therapy.

P22: Minimally invasive cryosurgery for the aesthetic removal of congenital melanocytic nevi

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Introduction: Congenital melanocytic nevi require excellent cosmetic treatment, but the treatment of cases for which surgical excision or laser ablation is not suitable remains controversial. The author created an ex vivo experimental skin model using human melanocytic nevus tissue and devised a minimally invasive cryosurgery method by film dressings and dry ice (Film dry ice treatment). Here we report the results of histological validation and several clinical cases. **Method:** A pseudo-skin model was created by placing human nevus tissue on a heat block heated to around 36°C. Freezing experiments were conducted under various conditions to investigate safe and effective treatment methods. Several patients with congenital melanocytic nevi that were difficult to treat with surgical excision or laser therapy were treated with film dry ice and followed up for 6 months after the last treatment. **Result:** It was shown that nevus cells can be destroyed deep into the dermis by applying dry ice for 7~10 seconds. The application of a single film significantly suppressed the rate of epidermal damage, but did not affect the effectiveness of the treatment. In all cases, excellent cosmetic results were obtained without scar formation after 10–15 treatments. **Conclusion:** Film dry ice treatment is a low-cost and simple procedure that can be accomplished at any facility. Although it requires a number of treatments, it is useful because it is a minimally invasive treatment that can destroy deep lesion cells while leaving no ugly scars.

P20 (withdrawn): Compared analysis of mouse models of oculocutaneous albinism: is incomplete melanin better than nothing?

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RNA therapy for congenital melanocytic nevi

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RAS proteins regulate cell division, differentiation and apoptosis via multiple downstream effector pathways. Oncogenic RAS variants are the commonest drivers in the context of cancer; however, they also drive many benign lesions with a presdispositioin to malignancy, including melanocytic nevi, thyroid nodules, and colonic polyps. Reversal of such benign lesions could reduce cancer incidence; however, the effects of oncogenic RAS have been extremely difficult to target with downstream pathway inhibitors. Here we show successful suppression of oncogenic and undruggable *NRAS*^{Q61K} in primary cells from congenital melanocytic nevi using siRNA targeted to the recurrent causal variant. This results in reduction in expression of *ARL6IP1*, a known inhibitor of endoplasmic reticulum stress-induced apoptosis but which has not previously been linked to NRAS. We go on to show that a single dose of siRNA in primary cultures triggers apoptosis, which is not seen with MEK inhibition. Packaging of the siRNA into lipid nanoparticles is protective and permits successful delivery into a humanized mouse model of melanocytic nevi ((*Tg*(*Tyr-NRAS*Q61K*)*1Bee*; MGI:3768645 (Ackermann et al., 2005)), which results in variant *NRAS* knockdown *in vivo*. These data show that RAS-induced protection from apoptosis is involved in persistence of *NRAS*-driven melanocytic nevi and support the potential use of targeted siRNA in clinical trials for RAS-driven benign tumors.

Melatonin-boosted efficacy of targeted therapy of human melanoma

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Melanoma is a leading cause of cancer deaths worldwide. Although targeted therapy and immunotherapy have improved the outcome of patients with metastatic disease, unwanted side effects remain a problem. Herein, by using human melanoma cell lines *in vitro*, we explore that melatonin enhances anti-tumor activity of commonly used BRAF/MEK inhibitors, vemurafenib (VF), and cobimetinib (CB), respectively. Our results have demonstrated that compared to VF/CB alone, melatonin significantly reduced proliferation (scratch

or drop assay) and induced apoptosis (cleaved Casp-3, PARP) in melanoma cells. Concurrently, VF/CB+melatonin decreased melanoma invasiveness related protein (E-cadherin), inducible nitric oxide synthase (iNOS), epithelial cell adhesion molecule (EpCAM), and proliferating cell nuclear antigen (PCNA); important players in melanoma tumorgenesis, tumor growth, and metastasis. In addition, we also found that the combined treatment caused significant mechanistic changes in cellular bioenergetics by (i) uncoupling of oxidative phosphorylation (OXPHOS), (ii) attenuation of glycolysis (Seahorse assessment), (iii) dissipation of mitochondrial transmembrane potential (mtΔΨ) (FACS), (iv) changes in mitochondrial morphology (TEM), and (v) supported by a xenograft model *in vivo* using zebrafish embryos. Our results extend previously published data and they provide new perspectives and evidence for introduction of melatonin as an add-on complementary therapy in future treatment of melanoma-affected patients.

P27: Active and stable non-segmental vitiligo: Role of T-cell membrane bound PD-1 and PD-L1

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Introduction: Immune-mediated depigmentation resembling vitiligo has been reported as an adverse effect of immune checkpoint inhibitor (ICI) therapy, sparking discussions on the similarities and distinctions between this ICI-induced depigmentation and classical vitiligo. **Aim:** To investigate the expression of membrane-bound programmed death 1 (mPD1) and programmed death ligand 1 (mPDL1) on different subsets of circulating T cells, including cytotoxic T cells (Tc), T-helper 1 cells (Th1), and T-regulatory cells (Tregs). **Methods:** In this prospective, cross-sectional, observational study, a total of 40 patients with NSV (20 in the stable phase and 20 in the active phase) were enrolled. 40 healthy controls, matched for age and gender, were included. Blood samples were collected and examine the change in frequency of T cells expressing mPDL1 and mPD1 using flow cytometry analysis of various T-cell subsets. **Results:** Among active vitiligo patients, the median percentage of Tc cells expressing mPDL1 was significantly lower compared to HC (1.165% vs. 4.25%, p = 0.006). In stable vitiligo patients, there was no difference in mPDL1 distribution (4.1% vs. 4.25%, p =0.507). The Th1 cells, in active vitiligo showed significanlty lower mPDL1 expression than controls (1.73% vs. 6.89%, p = 0.046). Interestingly, Tregs of patients who had achieved stability displayed a significantly high mPDL1 expression compared to controls (10.5% vs. 0.755%, p = 0.004). Furthermore, the Tregs expressing mPD1 was observed to be higher in patients with active vitiligo compared to controls (27.9% vs. 19.4%, p = 0.09). **Discussion:** The findings highlight the differential expression of PD-1/PDL-1 axis among different stages of vitiligo. Notably, the increased expression of mPDL-1 on Treg-cells in stable vitiligo demonstrates a compensatory mechanism. Conversely, active vitiligo patients exhibited reduced mPDL-1 expression on cytotoxic and Th1-cells, signifying a disrupted PD-1 axis in de-novo vitiligo. **Conclusion:** Considering localized pathological involvement, a comprehensive tissue-based immune cell profiling need to fully understand this immunological shift.

P3: Elevated inflammatory chemokines in segmental vitiligo

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In vitiligo pathogenesis, chemokines play a crucial role in promoting the infiltration of immune cells into the skin, leading to melanocyte destruction and subsequent depigmentation. Th1-type chemokines, notably CXCL9, CXCL10, and CXCL11, have been implicated as key mediators in vitiligo development. While most studies have focused on non-segmental generalized vitiligo (NSV), the role of chemokines in segmental vitiligo (SV), a distinct clinical subtype with potentially distinct pathogenic mechanisms, remains underexplored. This study investigated chemokine profiles in suction blister fluid from lesional (NSV: N=1, SV: N=2) and non-lesional (NSV: N=3, SV: N=2) skin of vitiligo patients, and healthy controls (N=3), using the Bio-P lex system. Elevated expression of inflammatory chemokines, including CCL2, CCL13, CCL20, CXCL1, CXCL11, CXCL9, and CXCL10, was observed in vitiligo lesions. Notably, inflammatory cytokine levels of CCL7, CCL26, CXCL2, CXCL5, and CXCL6 were specifically increased in SV lesions. These findings indicate that a broad range of inflammatory chemokines are involved in the pathogenesis of both NSV and SV, highlighting their significant role in vitiligo. Elucidating chemokine expression patterns in various vitiligo subtypes may provide insights into pathogenic mechanisms, facilitate disease activity monitoring, and identify potential targets for therapeutic interventions.

UVB stress triggers hair graying: Unveiling the Dicer-LEF/β-Catenin pathway's role in melanocyte dysfunction

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Stress, including ultraviolet (UV) exposure, affects pigmentation by influencing various cellular mechanisms. A key factor is the enzyme Dicer, essential for the maturation of miRNAs that regulate mRNA stability and translation. While stress is known to reduce Dicer level, the consequences of UVB on Dicer have not been explored. Here, we show that UVB irradiation suppresses Dicer expression, linked to the activation of PI3K, RSK, and WNT/β-catenin signaling pathways, and associated with transcriptional repression by β-catenin (bcat). Notably, we identified specific binding sites for the LEF/bcat complex in the Dicer promoter. These findings highlight the UV-induced LEF/ bcat pathway's role in regulating Dicer expression and melanocyte physiology. Given that bcat can influence pigmentation and melanocyte renewal, we investigated whether the lack of Dicer affects pigmentation, mature melanocytes, and their renewal *in vivo* in mice. Using several mouse genetic models and human and mouse cell lines, we found that Dicer

inactivation in melanocytes leads to their misplacement within the hair follicle and cell death. This misplacement results in a lack of melanin transfer to keratinocytes in growing hair and the exhaustion of the melanocyte stem cell (McSC) pool. Additionally, miR-92b, which regulates ItgaV mRNA and protein levels, plays a critical role in altering melanocyte migration. Overall, our findings suggest that the UVB-bcat-Dicer-miR92b-ItgaV pathway is a major signaling mechanism linking stress to premature hair graying. This integrated view underscores Dicer's crucial role in maintaining melanocyte function under environmental stress, offering insights into potential therapeutic targets for skin protection and preventing premature hair graying.

Contribution of lncRNAs to the generation of drug-tolerant persister cells

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Long non-coding RNAs (lncRNAs) generated from remote areas of the human genome constitute a major source of innovation for the adaptation to the numerous stresses encountered by melanoma cells during progression and therapy resistance. As such, research in this field offers a unique opportunity to study melanoma evolution and unveil potentially novel biology. Over the years we have characterized several novel transcripts interacting with the translational machinery, either in the mitochondria or in the cytosol, and affecting the generation of drug-tolerant cells. These transcripts represent previously unexplored cancer-specific vulnerabilities and thus extend the palette of therapeutic opportunities to include RNA-based medicines.

Single-cell molecular and functional landscapes of metastatic melanoma converge on clinically actionable features

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We performed multi-modal, single-cell, and functional profiling of 126 melanoma tumors from 116 patients (with 10 patients providing longitudinal samples) in an observational, technology-demonstration clinical study (BASEC: 2018-02050). The exploratory analysis of the melanoma datasets identified 6 novel melanoma cell subtypes based on single-cell CyToF data generated from 1.7 million cells, corresponding to 7 melanoma tumor types. These tumor types had distinct biomarker profiles and ex vivo drug responses, suggesting future biomarker-driven interventional clinical trials that better stratify advanced melanoma patients for more efficacious therapies.

P7: Modeling epidermal melanocyte behavior during plaque psoriasis and the impact of pro-inflammatory cytokines on melanogenesis

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Melanocytes reside in the basal layer of human epidermis in a 1:10 ratio to neighboring keratinocytes. They do not proliferate, even after exposure to UVR. In psoriasis, this ratio is maintained despite significant keratinocyte hyperplasia, suggesting a proportional increase in melanocyte numbers. Here we aim to investigate whether and how melanocyte numbers may change both in active plaques and after treatment with biologic therapy. We also explored the impact of psoriasis-related cytokines on human epidermal melanocytes *in vitro*. Immunohistochemistry (IHC) was conducted on lesional and non-lesional samples (n=11) at baseline and after 12 weeks of treatment using antibodies to MITF (melanocyte marker) and Ki67 (proliferation marker). Melanocyte number/mm of basal layer and melanocyte/keratinocyte ratio were estimated (ImageJ analysis). Primary human melanocytes were treated with proinflammatory cytokines (IL-17, TNF-α, IL-22, IL-23) to assess their effect on melanogenesis via immunocytochemistry (ICC), western blotting (WB), and Warthin–Starry melanin staining. Proliferating melanocytes were detected in both non-lesional and lesional skin and in resolving plaques (12 weeks post-treatment) but never in healthy epidermis. Melanocyte number/mm of basal layer was significantly higher in non-lesional than lesional sections suggesting melanocyte expansion already occurs prior to clinically-appreciable lesions are established. ICC and WB analysis revealed TNF-α reduces tyrosinase and TRP1 protein expression alone and in combination with other test cytokines. Overall, this study shows psoriasis cytokines directly impact melanogenesis, and that melanocyte proliferation occurs in lesional and non-lesional (clinically normal) psoriasis, which suggests early melanocyte deregulation in psoriasis supporting a role for these cells in psoriasis pathogenesis.

P17: How does the anatomic position and differentiation state of the melanocyte influence its transformation to melanoma?

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Oncogenic competence is a significant concept in understanding whether melanocytes become melanoma. Oncogenic competence can be defined as the factors beyond genetic mutations that enable a cell to be transformed into cancer. By leveraging melanoma modeling in zebrafish, our lab has shown that anatomic positioning and differentiation state are key competence factors and contribute to a melanocyte's transformation into melanoma. For example, our work in acral melanoma found that melanocytes in posterior locations have a distinct *HOX* code in comparison to body

melanocytes, and this determines which mutations are transforming. Moreover, a less differentiated/melanoblast more readily gives rise to melanoma than a differentiated melanocyte. Although our understanding of oncogenic competence in melanoma has greatly advanced, we are still lacking an *in vitro* system that is able to: (1) manipulate the differentiation state of the melanocyte (melanoblast vs. melanocyte) and (2) generate anatomically distinct melanocytes (body vs. limb). To this end, we developed an iPS-derived melanocyte system to differentiate melanocytes from the cranial (anterior) and sacral (posterior) neural crests, thereby representing discrete anatomic locations. Moreover, cAMP depletion of our melanocytes has dedifferentiatied cells to a more melanoblast-like state. These cAMP depletion experiments have also suggested the important role TFAP2B may play in controlling melanocyte differentiation state. Our *in vitro* studies and RNA sequencing data show promise in uncovering the intrinsic differences between melanocytes that are not only anatomically different, but of distinct differentiation states. Altogether, we hope this system enables the study of diverse melanoma subtypes (cutaneous vs. acral melanoma).

P19: Human choroidal melanocytes, Opsin 3 and the effects of blue light exposure

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Background: The choroid between inner and outer eye layers, is a dynamic vascular pigmented tissue including abundant melanocytes, critical for homeostasis including melanin-light absorption. Whether the choroid may be light-responsive remains unclear, including how and which cells. Opsins (OPN) are light-sensing molecules, including nonvisual opsins such as OPN3 found in skin melanocytes. Could OPN3 be involved in choroid responses to light? We investigated for OPN3 in adult human choroid and whether human choroid melanocytes (HCMs) respond to blue light (BL) including transcriptome pathway analysis. **Methods:** Human *postmortem* eyes (n=5), and HCMs (n=4) were studied for OPN3, and melanosome/melanogenesis and fibroblast markers (immunolabeling and confocal). HCMs (n=4) were exposed to BL (λ=460nm, 75 to 370J/cm^2), and cell death, cell metabolism, cell cycle and intra-and extracellular melanin assessed. RNA-Seq was used to compare control and BL-exposed HCMs (n=4) for genes and possible pathways involved. **Results:** Human choroid showed OPN3+ cells including melanocytes, fibroblasts and endothelium. BL up to 80hrs (374 J/ cm^2) decreased HCM metabolism without significant toxicity. HCMs also increased intracellular, but decreased extracellular, melanin post-80 h BL, including increased perinuclear melanin. Preliminary transcriptomics indicated pathways that protect against oxidative stress and 'dampen' immunity/inflammation with BL. **Conclusion:** Human choroid contains heterogeneous OPN3+ cells (melanocytes, fibroblasts and vascular endothelium). Accumulated HCM perinuclear melanin post-BL may be photoprotective as in skin melanocytes and keratinocytes with UV. Overall, responses of HCMs to blue light are complex but likely include a role in choroid defense against oxidative stress. The function(s) of OPN3 in choroid cells remains to be established. Supported by Australia Vision Research (Ophthalmic Research Institute of Australia) (MCM, AVC, and RMC).

Role of ciliopathy-associated protein TMEM138 in skin pigmentation

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The primary cilium is an essential organelle for cell polarity and signaling in most mammalian cells. Defects in its assembly or function underlie the pathogenesis of developmental disorders known as ciliopathies. Interestingly, expression of ciliopathy-associated protein TMEM138 is regulated by non-pathogenic alleles that are found in African populations with diverse skin phototypes. TMEM138 supports primary cilia biogenesis in photoreceptor cells, but whether it regulates pigmentation via signaling from the primary cilium, melanogenic cargo trafficking, and/ or pigment dissemination in the epidermis is unknown. To test for a role in melanogenesis, we assessed the impact of altered TMEM138 expression in pigment cells. Thus, we generated immortalized C57BL/6-derived mouse melanocytes stably overexpressing TMEM138, or depleted of TMEM138 by shRNA expression. Knockdown of TMEM138 caused a marked loss of pigmentation (n=5 to 10), which was restored by re-expression of epitope-tagged human TMEM138. Expression of melanogenesis genes TYRP1 and PMEL was reduced (n=3) and residual TYRP1 was mislocalized to lysosomes in TMEM138-depleted cells (n=3), suggesting that TMEM138 may function in both signaling and trafficking during melanogenesis. Ongoing experiments are testing the underlying mechanism by which TMEM138 impacts both processes in melanocytes, whether other ciliopathy gene products play a similar role, and whether TMEM138 functions in melanin transfer to keratinocytes.

P5: Investigating the cross talk between melanoma and keratinocytes

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Background: The tumor microenvironment (TME) plays a key role in melanoma development and progression, as well as contributes to the therapeutic resistance and disease spreading. The TME is heterogeneous and consists of multiple cell types encompassing both dermal and epidermal compartments. However, the influence of keratinocytes on melanoma still remains to be elucidated.

Methods: Primary human keratinocytes and patient-derived melanoma cells were isolated from fresh biopsies. Keratinocytes were cultured in melanoma conditioned medium to assess gene and protein expression signatures among growth factors, cytokines, chemokines, pro-angiogenic factors, and proteins involved in extracellular matrix remodeling. **Results:** Data showed that among the categories of mitogens, inflammation and cell–cell adhesion, bFGF, CXCL16, and E-cadherin are the highest factors induced in keratinocyte by melanoma conditioned-medium. Of interest, autocrine and paracrine bFGF is considered the most important growth factor in melanoma. CXCL16 that is implicated in the recruitment of activated T cells also might cause the activation of MAPK and Akt/PKB pathways leading to increased proliferation, migration and formation of apoptosis-resistant tumor cells. By contrast, up-modulation of E-cadherin was unexpected since loss of this adhesion protein is considered the most critical marker for melanoma invasion. Farther, among factors involved in tumor invasion, metastasis and wound healing the multifunctional cytokine TGF-β and the gelatinase B MMP-9 are less expressed. **Conclusions:** Our study provide evidences that melanoma cells can influence surrounding keratinocytes driving the production of molecule that support tumor growth. Taken together, keratinocytes could have a great impact on cancer onset and early phases of progression.

P23: Agent-based modeling of the two major patterns of human congenital melanocytic diseases

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Two recurrent patterns of human congenital melanocytic disease have been described. The first, the "segmental" pattern, is compatible with animal data on neural crest melanocytic development and migration. The second, "non-segmental" pattern, is not compatible with what is known of neural crest melanocytes and was hypothesized to be of mesodermal origin (cf. https://doi.org/10.1111/pcmr.1264). Whether both contribute to human pigmentation in healthy conditions is not yet known, however evidence of the patterns of pigment loss in segmental and non-segmental vitiligo, which closely mimic the patterns of the two populations, would suggest that they do. This study used a form of computational modeling, known as agent-based modeling, to understand how cell migration and proliferation result in these two recurrent patterns seen in humans. Cells are represented as autonomous individuals on a grid, following simple, yet accurate rules of local interaction with each other and nearby surroundings, such that experiments on the scale of tens of thousands of cells are possible. By varying model parameters: precursor cell number and positioning, cell cycle length, migratory cell speed, the number of migratory cells, and chemoattractant strength, we can recreate various real-world pigmentation patterns, different in shape, size, perceived density, and number of foci. Thus, the models provide a way to associate cell-level behaviors with human congenital macroscopic patterns, suggesting underlying causes of real-world pattern variation. Furthermore, the model supports the different origin hypothesis, as the two categories of patterns cannot be recreated under similar conditions, requiring the restriction of parameter values, or the introduction of additional mechanisms.

New light on the evolution of melanin by integrating data from extant vertebrates, experiments and the fossil record

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* Unfortunately, Dr McNamara could not present this work in person, but other lab members are in Marseille to discuss it.

Melanin is among the most common pigments in animals and has a fossil record stretching back over 300 million years. Despite a rich tradition of analytical research, and despite increasing interest in fossil melanin, the evolutionary history of melanin and its functions in animals remains poorly resolved. This is due, in part, to the fact that aspects of melanin biology that can preserve in fossils are incompletely characterized. In vertebrates, key knowledge gaps relate to the biology and biochemistry of melanin-rich organelles termed melanosomes. Our research aims to map the anatomical distribution of melanosomes in modern and fossil vertebrates, including fish, amphibians, reptiles, birds and mammals, and to integrate these data with new information on melanosome geometry, melanin monomer chemistry and the melanosomal metallome. Comparative analysis of variation in these characters among internal and integumentary tissues within individual species has revealed important tissue-level controls that likely relate to melanin function, especially metal homeostasis. Laboratory experiments reveal how melanin characters alter during fossilization, constraining interpretations of preserved melanosomes in fossils. Our current research reveals shifts in melanin characters for certain body tissues at high taxonomic levels, implicating a role for melanin in major transitions in vertebrate ecology. These new data are the basis of an emerging new model for melanin evolution in vertebrates, which allows more targeted analysis of fossils from particular geological time periods and phylogenetic groups, to better understand the ultimate biological origins and functional roles of melanin in vertebrates.

Stress adaptation in melanoma: the role of NRF2

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The transcription factor NRF2 is activated upon oxidative and electrophilic stress and orchestrates a cellular response associated with redox regulation and metabolic reprogramming. In contrast to non-small cell lung cancer, where the NRF2 pathway is often activated by mutations and its contribution to poor disease outcome is well established, cancer types such as melanoma do not harbor mutations in this pathway. Despite this, accumulating evidence shows that NRF2 plays a major role in melanoma by deploying several mechanisms that lead to cellular reprogramming and tumor resistance.

Structural insights into pink-eyed dilution protein (Oca2)

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Recent innovations in computational structural biology have opened an opportunity to revise our current understanding of the structure and function of clinically important proteins. This study centres on human Oca2 which is located on mature melanosomal membranes. Mutations of Oca2 can result in a form of oculocutanous albinism, which is the most prevalent and visually identifiable form of albinism. Sequence analysis predicts Oca2 to be a member of the SLC13 transporter family, but it has not been classified into any existing SLC families. The modelling of Oca2 with AlphaFold2 and other advanced methods show that, like SLC13 members, it consists of a scaffold and transport domain and displays a pseudo inverted repeat topology that includes re-entrant loops. This finding contradicts the prevailing consensus view of its topology. In addition to the scaffold and transport domains, the presence of a cryptic GOLD domain is revealed that is likely responsible for its trafficking from the endoplasmic reticulum to the Golgi prior to localisation at the melanosomes. The GOLD domain harbours some known glycosylation sites. Analysis of the putative ligand binding site of the model shows the presence of highly conserved key asparagine residues that suggest Oca2 may be a Na+/dicarboxylate symporter. Known critical pathogenic mutations map to structural features present in the repeat regions that form the transport domain. Exploiting the AlphaFold2 multimeric modelling protocol in combination with conventional homology modelling allowed the building of plausible homodimers in both inward-and outward-facing conformations, supporting an elevator-type transport mechanism.

P12: Automated melanoma detection. An algorithm inspired from human intelligence characterizing disordered pattern of melanocytic lesions improving a convolutional neural network

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Introduction: Interpretation of Convolutionnal Neural Network (CNN) results for melanoma detection remains a limitation. Most melanomas share disorder color distribution, in contrast, most nevi share an organized color distribution. Such an approach based on ordered/disordered aspect of melanocytic lesions (MLs) has never been integrated into a handcrafted model. Our objective was to compare a model based on features characterizing color disorder of ML (entropy characterizing disorder in image processing) named the "disorder model" with a CNN model and to test if the fusion of these two models could increase CNN performances. *Methods*: We developed two binary models to differentiate melanoma from nevi on dermoscopic images on the same data set including 6296 nevi and 1361 melanomas from the ISIC 2019 public dataset: a handcrafted algorithm characterizing disorder color in ML (the disorder model), based on 48 features characterizing disorder (entropy, skewness, SD, and kurtosis from 4 color spaces) associated with a classifier (K-nearest neighbors), and a CNN based on ResNet-50. *Results*: The disorder model reached high performances, (AUC 0.91, sensitivity 91%, specificity 74%, balanced accuracy (BAC) 83%). The CNN model displayed equivalent performances, (AUC 0.89, sensitivity 86%, specificity 75%, BAC 81%). The fusion of the two algorithms yielded higher performances, (AUC 0.921, sensitivity 86%, specificity 82%, BAC 84%). Classification errors made by disorder model were more interpretable than those by the CNN model. *Conclusion*: The disorder model inspired from human intelligence reached the same performances as the CNN model. The fusion increased performances, notably specificity and reduced obvious errors.

Making sense of the genetic heterogeneity of albinism

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Albinism is one of the first congenital rare diseases investigated due to the obvious phenotype associated with the first cases reported. The absence or partial reduction of pigmentation has been diagnostic and used to identify this genetic condition over the years. However, the number of genes whose mutations are associated with different types of albinism has been increasing over time. The latest count in the field corresponds to 21 genes identified and 22 types of albinism reported (one albinism type has not yet been associated with a given gene). In addition, the diagnostic phenotype is no longer the alterations in pigmentation but the abnormal vision, present in all types of albinism, irrespective of their absence or reduction in

pigmentation. It is therefore intriguing what are the relationships between all these genes whose mutations cause the same visual phenotype. Recently, a group of researchers involved in investigating albinism have proposed a linear correlation between the different types of albinism and the genes mutated in each case. In one end, there will be the syndromic albinisms, HPS and CHS, and their 12 subtypes, followed by the 8 known types of oculocutaneous albinism and by ocular albinism. At the other end we have a new syndrome, called FHONDA, where the same visual abnormalities are present without affecting pigmentation. Through various animal models (in mice) we and others have been investigating the role of all these genes in albinism. The current view of the genetic heterogeneity of albinism will be summarized in this talk.

Microenvironment-driven adaptation mechanisms in Melanoma: 3D bioprinting and tyrosine-induced phenotype switching

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Melanoma cells exhibit remarkable plasticity, enabling adaptation to diverse microenvironmental cues a process known as phenotype switching, which is linked to epithelial-mesenchymal transition (EMT). Tyrosine, the precursor of melanin biosynthesis, plays a critical role in this dynamic cell state transition. In our first study, we identified that high tyrosine concentrations in the microenvironment induce oxidative stress, promoting a phenotypic switch in melanoma primary cultures toward either a mesenchymal-like invasive state or a senescence-like state, highlighting mechanisms of cellular adaptation. Utilizing advanced 3D bioprinting technology, which mimics the tumor microenvironment, we investigated the interplay among microenvironmental cues, cell pigmentation, and phenotypic diversity. Our findings demonstrate that tyrosine-dependent phenotype switching is associated with increased invasive activity and heightened vulnerability to tyrosine kinase inhibitors (TKIs) in 3D culture models. Additionally, primary cultures maintained in different extracellular matrix (ECM) such as skin and brain hydrogels exhibited distinct phenotypes, proliferation rates, and gene expression profiles. Notably, melanoma cells in a brain-like microenvironment displayed neuronal like morphology and elevated expression of neural and stem cell markers, such as SOX2 and NGFR, compared to cells in 2D or skin-like conditions. To further explore the interactions between skin and brain microenvironments and the significance of phenotype switching, we injected primary cultures, established under varying tyrosine concentrations, both subcutaneously and intracranially into mice. These tumor cells exhibited different growth rates and phenotypes, confirming the *in vivo* relevance of phenotype switching. In conclusion, our study provides a comprehensive context for understanding melanoma plasticity, offering critical insights into the relationship among tumor microenvironment, pigmentation, cell phenotype, invasion, and drug vulnerability.

P16: DNA methylation after UVB exposure may be associated with facultative pigmentation in human skin

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Ultraviolet-B (UVB) radiation is a significant environmental stress on human skin, triggering facultative (tanning) pigmentation above the skin's constitutive melanin levels. While epigenetic changes (DNA methylation/ histone modification) are involved in skin photoaging, we know little about how these are differentially regulated in constitutive versus facultative skin pigmentation. Here we investigate how single or multiple (4 sequential) UVB exposures alter DNA methylation in *ex vivo* human skin cultures. Biopsies were collected from four SPT-II donors and irradiated (4 J/cm^2) with UVB (peak 311 nm). Melanin content and melanin distribution (within epidermis) were assayed. Cyclobutane pyrimidine dimer (CPD) detection, DNA methyltransferase (DNMT1/3a/3b) activity, DNMT1/DNMT3a protein levels, and global DNA methylation of 5-methylcytosine (5mC) were investigated. Facultative pigmentation was detected after single and multiple UVB exposures, with melanin level increasing significantly already 24 h after first dose. CPD formation, which was detected within 0–24h of first UVB dose, persisted for at least 96 h in both single and multiple-dosed tissue. While single and multiple UVB irradiations decreased DNMT activity and DNMT1/DNMT3A protein level, only multiple irradiations significantly reduced *global* DNA methylation compared to non-irradiated controls. In summary, while facultative pigmentation-associated changes (melanin level/distribution, CPD formation, DMNT activity/protein expression) all occurred within 24h of a single UVR dose, detectable reduction in *global* DNA methylation appeared to require multiple UVB doses. Investigating the interplay between DNA methylation after initial UVR exposure at a target gene level (via bisulfite sequencing of MITF for example) will be important, that these gene-specific epigenetic events may presage subsequent pigmentation phenotypic changes.

Unraveling the ultrastructure and metabolome of unique pigment cells in the zebrafish fin

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Pigments are ubiquitous in nature, and responsible for the diverse colors and patterns of our world, serving essential roles in camouflage, mating, thermoregulation, and social interactions across species. Zebrafish exhibit a variety of pigment cells known as chromatophores, which include melanophores, xanthophores, iridophores, and leucophores. Each type of chromatophore produces a specific biomolecule (pigment) that contributes to their distinctive coloration. Recently, a new type of pigment cell was discovered in zebrafish (*Danio rerio*), termed melanoleucophores (ML). These cells, located at the distal margin of the dorsal fin, undergo a

remarkable transformation: they shift from synthesizing melanin to producing guanine crystals. The elusive process behind this cellular metamorphosis, termed fate plasticity, and the precise reprogramming events that occur remain poorly understood. Here, we employed state-of-the-art advanced electron microscopy coupled with molecular biology techniques to intricately map the ultrastructure of these cells and the organelles within them and elucidate the compositional changes that take place during transdifferentiation. Additionally, we have investigated the biosynthesis of these organelles and mapped the initial stages of pigment degradation and crystal formation, pinpointing the intracellular origin of the new organelles. Our findings shed new light on intracellular processes occurring during transdifferentiation and provide new insights into the pathways of melanin breakdown and guanine crystal formation. This research enhances our understanding of pigment cell biology and the intricate mechanisms of cellular biology and transdifferentiation.

P25: Usefulness of film-dry ice treatment as a second treatment for congenital melanocytic nevi

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Background: Congenital melanocytic nevi (CMN) are often difficult to treat, depending on the location and size of the lesion. Especially in cases where surgical resection is difficult and lesions are deep, complete treatment with laser and curettage alone is difficult. Here, we report on the usefulness of cryosurgry using a combination of film dressing material and dry ice (hereafter referred to as film-dry ice treatment), including histological observations.
Methods: Patients with CMN who developed recurrence after curettage or laser irradiation underwent film-dry ice treatment. Treatment was performed every 1–3 months with postoperative follow-u p, and the effect of blanching and scarring were evaluated by Dermocamera and Antera 3D. In addition, pain during the procedure was evaluated by VAS.
Results: In all cases, prominent bleaching of the lesions without scar was observed, and there was no recurrence of lesions for six months after the last treatment. The post-treatment satisfaction rate was also high, and pain was relatively low at 3-4 on the VAS pain scale at the time of outpatient treatment, indicating that pain control had been achieved.
Discussion: In this study, film-dry ice treatment was performed on residual lesions that were difficult to resect surgically or that had failed to respond to laser irradiation, and good results were obtained without leaving disfiguring scars. The film-dry ice treatment can be performed without leaving the skin intact, minimizing the risk of scarring. Furthermore, it can also treat deeper lesions and is useful as a second treatment for recurrent lesions.

P21: The mitochondria-shaping protein Opa1 is required for melanocyte stem cell maintenance

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Changes in mitochondrial function are linked to melanin production, pointing to a role for these organelles in melanocyte function. How mitochondrial function and morphology however impact melanocyte biology is unclear. Here we show that the master regulator of mitochondrial inner membrane fusion optic atrophy 1 (Opa1) is required to sustain differentiated melanocytes during the hair follicle cycle. Conditional Opa1 ablation *in vivo* in mouse melanocytes reduced melanocyte stem cells as well as differentiated melanocytes during hair cycles, ultimately resulting in early hair graying and indicating a critical role for Opa1 in melanocyte stem cell survival. Activation of ligand stem cell factor (SCF)-receptor tyrosine kinase KIT pathway is crucial for melanoblast survival, migration, and the hair follicle melanogenesis. Mutants or inhibition of SCF-KIT receptor are reported giving mice white coat. Mechanistically, SCF-induced Ser473 phosphorylation of AKT was impaired in melanoblasts lacking Opa1. Our data indicate that Opa1 is a key player for melanocyte stem cells homeostasis by allowing SCF-induced downstream signaling.

Hedgehog signaling inhibition by SMO inhibitors in melanoma cells reveals a potential value for a new therapeutic strategy

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Background: Melanoma is one of the most aggressive cancers due to its cell plasticity which results in high metastatic potential and chemoresistance. Further, melanomas frequently develop resistance to targeted therapy against MAPK; therefore, in this context, the testing of new small molecules to target other signaling pathways is required. **Methods:** Patient-derived melanoma cells isolated from fresh biopsies were characterized for the level of hedgehog (Hh) pathway activation using a gene array card system encompassing 93 genes of interest. Primary human melanocytes were used for control. Then, melanoma cells were treated with different doses of SMO inhibitors (Vismodegib, Sonidegib, and Taladegib) to assess the effect on cell cycle regulation. After selecting Sonidegib as the most effective compound in mitigating cell growth and inducing cell death, this molecule was used to study melanoma cell migration and gene expression profile. **Results:** Melanoma cells displayed markers of Hh signaling activation mostly due to significant down-

modulation of the repressors PTCH1 and SUFU and the up-modulation of the downstream effector Gli2. Inhibition of SMO by Sonidegib failed to recover the normal level of expression of these proteins except for a mild decrease of Gli2. At the same time, treatment with Sonidegib significantly reduced the migratory potential of primary and metastatic melanoma cells compared to untreated conditions. This effect was also accompanied by the modulation of the E to N-cadherin transition, which is crucial in melanoma progression and invasiveness Dose-dependent reduction of the proliferation was characterized by arrest in the G2/M phase associated with apoptotic cell death at higher doses. **Conclusions**: The analysis of Hh signaling in melanoma revealed a clear signature of activation, indicating a possible pathogenic role in melanomagenesis and clinical relevance in therapy.

Transcriptional and metabolic cellular heterogeneity in melanoma residual disease

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Cancer cellular heterogeneity and therapy resistance arise substantially from metabolic and transcriptional adaptations, but how these are interconnected is poorly understood. Our laboratory is using zebrafish models of melanoma residual disease to understand the transcriptional and cellular states that emerge in persister cells, and how these cells contribute to disease recurrence. We find that the cancer stem cell marker aldehyde dehydrogenase 1A3 (ALDH1A3) forms an enzymatic partnership with acetyl-coenzyme A (CoA) synthetase 2 (ACSS2) in the nucleus to couple high glucose metabolic flux with acetyl-histone H3 modification of neural crest (NC) lineage and glucose metabolism genes. Importantly, we show that acetaldehyde is a metabolite source for acetyl-histone H3 modification in an ALDH1A3-dependent manner, providing a physiologic function for this highly volatile and toxic metabolite. In our zebrafish melanoma residual disease models, we identify an ALDH1-high subpopulation that emerges following BRAF inhibitor treatment, and targeting these with an ALDH1 suicide inhibitor, nifuroxazide, delays or prevents BRAF inhibitor drug-resistant relapse. Our work reveals that the ALDH1A3-ACSS2 couple directly coordinates nuclear acetaldehyde-acetyl-CoA metabolism with specific chromatin-based gene regulation and represents a potential therapeutic vulnerability in melanoma.

P15: Non-integumentary melanosome geometry suggests a highly conserved role of melanin between amphibia and reptilia

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Melanosomes are melanin-rich organelles that, in extant vertebrates, occur in the integument, eyes and internal tissues. Although melanin underpins critical physiological functions, certain fundamental aspects of melanin biology remain poorly understood.

Previous studies reported tissue-specific melanosome geometries in a limited number of extant vertebrates. Whether that dataset is representative of vertebrates more broadly is unclear. To test this, our new expanded dataset incorporates 18 taxa and 10 tissue types. We used an enzymatic digestion process to extract melanosomes from 143 tissues from four reptile and two amphibian taxa. Scanning electron microscopy, morphospace and statistical analyses validate existing models of tissue-specific melanosome geometry in Reptilia and Amphibia. Intriguingly, we found that melanosome geometries are highly conserved in the skin, eyes and liver but not in the gonads—in the latter, melanosomes diverge strongly in geometry in amphibians and reptiles—the meaning behind this is unexplored. The distinct melanosome geometries in the gonads of amphibians and reptiles suggests divergence of gonad melanosome functions in these two groups. Future work combining data on melanosome chemistry and geometry may provide deeper insight into the biological controls underlying these trends and on the functional evolution of melanin in vertebrates.

P8: ARHGAP29 in malignant melanoma

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ARHGAP29 is an intracellular GTPase-activating protein (GAP) that has a high affinity for Ras Homolog Family Member A (RhoA). The small GTPases of the Rho family regulate a variety of cellular mechanisms, e.g. the organization of the cytoskeleton and the cell cycle. GAPs like ARHGAP29 cause the Rho proteins to hydrolyze GTP and thus switch to their inactive state. By stimulating the intrinsic GTPase activity of RhoA through ARHGAP29, the RhoA/Rho-associated protein kinase (ROCK) signaling pathway is inactivated. As a result, ARHGAP29 could influence tumor progression, e.g. by altering the structure of the actin cytoskeleton. The function and significance of ARHGAP29 in malignant melanoma has not yet been described. Using expression analyses, we demonstrate that ARHGAP29 is strongly expressed in various melanoma cell lines compared to human epidermal melanocytes (NHEMs). Therefore, it seems that ARHGAP29 may play an important role in melanoma progression. To further investigate the role of ARHGAP29 in malignant melanoma, siRNA- mediated knockdown experiments were performed. These showed that ARHGAP29 influences the actin cytoskeleton, cell morphology and cell-cell contacts. It was observed that ARHGAP29 promotes cell spreading by inhibiting ROCK and stabilizes 3D cell aggregates. Furthermore, functional assays demonstrated a stimulating effect of ARHGAP29 on cell migration and invasion, which was confirmed by an altered expression of cell adhesion molecules (CAMs) and matrix metalloproteases (MMPs) through ARHGAP29. Overall, our data indicate that ARHGAP29 plays a crucial role in melanoma progression, in particular influencing the invasive behavior of melanoma cells, and thus could promote the formation of metastases.

e-DAM: An e-Delphi study to inform the clinical utility of AMBLor—a novel prognostic biomarker for early-stage cutaneous malignant melanoma

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Aims: AMBLor—a novel IHC-based prognostic biomarker for non-u lcerated Stages I and II primary cutaneous malignant melanoma—identifies patients at lower risk of disease progression. We aim to develop expert consensus on the optimal utility of the AMBLor test in clinical practice. **Methods:** An e-Delphi consensus survey of 25 UK skin specialist MDT (SSMDT) consultants was performed. Rounds 1 and 2 identified and built consensus on clinical-decision making and service provision challenges. The challenges identified were used to build clinical scenarios in Round 3, asking participants how an AMBLor result may change their clinical decision making. Moderate consensus was defined as 51–74% and strong consensus as ≥75% of participants selecting positive answers (likely/ agree, very likely/strongly agree). **Results:** Round 1. Most commonly reported themes: "Estimating risk of disease progression" (10/40) and "Decision to perform sentinel lymph node biopsy (SLNB)" (10/40). Round 2. Only "Estimating risk of disease progression" surpassed strong consensus levels as a clinical decision-making challenge (86.5% agreement). Potential use-case scenarios identified for AMBLor in clinical practice that reached a consensus agreement included: 1. Targeting radiological surveillance (100%) 2. Refining clinical follow-up (100%) and 3. Improving surgical decision-making when considering SLNB (90.9%). Round 3. Consensus was reached on participants reconsidering the offer of SLNB to patients with T1b non-ulcerated melanoma who are AMBLor low-risk and using a low-risk result to prioritize the provision of SLNB. **Conclusion:** Strong SSMDT consensus suggests that the AMBLor prognostic biomarker could help clinicians estimate risk of disease progression, and improve stratification for SLNB.

Targeting prohibitins and CRAF: A promising therapeutic strategy for melanoma

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Given the significant challenge posed by acquired resistance in BRAF mutant melanoma and the lack of effective therapies for BRAF wild-type melanoma, there are unmet clinical needs for new efficient targeted therapies. One of the emerging targets in cancer is the Mitochondrion. In melanoma, mitochondria play a central role in disease progression. Prohibitins (PHBs) largely found in the mitochondrial membrane are overexpressed in several cancer types, and control mitochondrial integrity, metabolism, and apoptosis. In our previous study, we showed that PHBs are highly expressed in melanoma and such expression is associated with a worse patient survival and resistance to MAPKi. Moreover, we designed novel specific PHB inhibitors that can affect cell growth by acting on the mitochondrion, promoting p53 expression and significantly inhibiting two main melanoma signaling pathways MAPK (CRAF-ERK axis) and PI3K/AKT. Otherwise, CRAF whose activation is PHB-dependent is key effector of MAPK and control mitochondrial metabolism and has a critical role anti-apoptotic role through mitochondrial translocation. Novel CRAF/pan-RAFi are evaluated in solid tumors (including melanoma). In our study, we pursued our deep investigation of the mechanisms of action of PHBi as new class of MTAs alone and in combination with CRAF/pan-RAFi as novel therapeutic strategy. We demonstrated that the combination of PHBi and CRAF/pan-RAFi synergistically inhibited cell growth, induce apoptosis and overcomes acquired resistance to BRAF/MEK inhibitors. In conclusion, targeting PHBs and CRAFi represent a very promising therapeutic strategy in melanoma, regardless of mutational status and constitute a new approach in cancer treatment.

P10: A MGRN1-based combination of biomarkers accurately predicting melanoma patient survival

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The tumor–node–metastasis (TNM) classification of the American Joint Committee on Cancer, employed for staging melanoma patients, predicts the overall survival (OS) with medium/ low accuracy. Approximately 1/3 low-grade TNM (0-IIIb) patients of *a priori* good prognostic undergo an aggressive disease, with OS comparable to high TNM (IIIc-IV) patients. Therefore, reliable prognostic markers are still lacking. The MGRN1 ubiquitin ligase modulates pigmentation, shape and motility of melanoma cells, with low MGRN1 expression associated with less aggressive phenotypes. We analyzed the effects of MGRN1 deficiency on the transcriptional landscape of melanomas and OS of patients, using the available information in the TCGA and other melanoma datasets. *MGRN1* was overexpressed in melanoma *versus* normal skin or nevi and was mutated in a subset of melanomas with a mutually exclusive pattern with *TP53* or *CDKN1A* mutations. Kaplan-Meier analysis showed longer survival for carriers of melanoma with low *MGRN1* expression. Gene set enrichment analysis of melanomas with low or high *MGRN1* expression revealed differential expression of gene sets associated with cell cycle, DNA damage and repair processes. Moreover, the combination of MGRN1 with melanoma-specific biomarkers reliably complemented the prognostic information of TNM staging, as it accurately identified the melanoma patients with TNM 0-IIIb that underwent an aggressive disease with low OS, despite their *a priori* good prognostic. Therefore, MGRN1

expression provides a prognostic biomarker that might complement the current TNM staging of melanomas. Supported by grant PI22/00404 from ISCIII, cofinanced by EC.

Physicochemical properties of melanin obtained from human-induced pluripotent stem cell-derived melanocytes

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Although melanin is viewed as a natural sunscreen that protects pigmented cells against adverse effects of solar radiation, recent studies have demonstrated that under certain conditions the pigment can actually contribute to light-induced oxidative damage of the cells. However, the main issue with such studies is finding natural pigments without photooxidative modifications. Recently, melanin obtained from melanocytes, generated from human induced pluripotent stem cells (hiPSC-Mel), was suggested as a promising source of the pigment without significant photooxidation. Although different studies have demonstrated the feasibility of the technique to generate melanin-producing cells, no thorough analysis of physicochemical properties of the pigment was performed. To address this issue, we examined key physicochemical parameters, including aerobic photoreactivity of melanin isolated from hiPSC-Mel and compared them with melanin from other known sources of the pigment, such as bovine retinal pigment epithelium (bRPE) and phototype V (PT-V) hair. Electron paramagnetic resonance (EPR) spectroscopy, dynamic light scattering, UV-Vis absorption and chemical analysis of melanin degradation products were used. The ability of the examined melanins to photogenerate and quench reactive oxygen species was determined employing EPR oximetry, EPR spin-trapping and time-resolved singlet oxygen phosphorescence. The obtained results demonstrated that melanin obtained from hiPSC-Mel exhibited physicochemical properties typical for eumelanin. Moreover, the pigment had significantly higher photoprotective properties when compared to bRPE melanin and PT-V hair melanin. Our findings indicate that hiPSC-Mel could be an excellent source of high-quality pigment for photoprotection studies.

Single-cell, spatial multiomics, and system probing decode complete gene regulatory network underlying cranial neural crest fate decisions

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In vivo regulatory blueprints behind cell fate decisions (CFDs) are essential to understanding development and disease but remain unclear. Multipotent neural crest (NC) cells, which migrate throughout the embryo adopting multiple fates, are an excellent model for studying CFDs. Using time-resolved single-cell multiomics, spatial transcriptomics, and systematic genetic perturbations combined with newly developed *in silico* latent-time-embedded simulations, we fully deciphered the cranial NC programs in zebrafish. Identifying 23 NC cell states and three spatial trajectories, we reconstructed and tested the complete underlying gene regulatory network (GRN), which, unlike previous qualitative models, demonstrated high predictivity, with a 3 to 6.3-fold increase in correlation between *in vivo* and *in silico* perturbations. Using a new computational framework for regulatory synchronization, we discovered a post-epithelial-mesenchymal-transition endothelial-like program crucial for migration, identified motif coordinators for dual-fate priming, and quantified lineage-specific cooperative transcription factor functions. This study provides a comprehensive, quantitative, and validated NC Waddington regulatory landscape, offering general regulatory models for CFDs in vertebrates.

The 2024 ESPCR Award Lecture:
Skin pigment: From birth in melanocytes to demise in keratinocytes

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Here, I will review our contributions to pigment cell research over 25 years. Our interest in melanocytes and melanosomes started in 1999 with the discovery by Jenkins and Copeland that the *ashen* mutation in mice was due to mutations in the *Rab27a* gene. We had identified this gene as highly expressed in the retinal pigment epithelium (RPE) and a candidate for retinal degeneration in a rare disease called Choroideremia. Studying the role of Rab27a in melanocytes, we (with Alistair Hume) and others discovered that a tripartite complex composed of Rab27a, melanophilin and myosin Va was responsible for the peripheral distribution of melanosomes. Studying the role of Rab27a in the RPE, we (with Clare Futter) discovered that a similar tripartite complex composed of Rab27a, MyRIP and MyosinVIIa was responsible for the apical distribution

of melanosomes. We then became interested in the controversial transfer process of melanin between melanocytes and keratinocytes. We (with Duarte Barral) proposed that the main transfer process is through exocytosis of the melanosome core (melanocore) followed by phagocytosis. We then demonstrated that keratinocyte phagocytosis of melanocores is specifically stimulated by PAR-2 and that 3D reconstructed human pigmented epidermis/skin models recapitulate this mode of transfer. More recently, we described the melanokerosome, a terminal melanin-containing organelle in keratinocytes that forms the parasol, essential in photoprotection.

Long-lasting and safe photoprotection using a skin-bioadhesive technology: a proof of concept with a novel M10 skin-bioadhesive UVA filter

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Protection against solar UV radiation is a global public health need due to the increasing incidence of skin cancer including melanoma. Innovating in sunscreen is a challenging research and technology effort to reduce melanoma incidence. Health authorities and consumers support the use of safe sunscreens that are highly protective, without causing adverse environmental effects. Organic UV filters, while providing cosmetic advantages, require frequent re-application, may penetrate the skin, and may induce ecotoxicity. Based on skin-bioadhesive technology, we developed new organic UV filters that bind to the stratum corneum. Our first filter, M10, was built on diethylamino-hydroxybenzoyl-hexyl-benzoate (DHHB), a UVA filter widely used in European, Asian and South American sunscreens. The efficacy and safety of M10 were evaluated *in vitro* and *ex vivo* using UV spectrum analysis, skin autofluorescence, diffusion cell permeation and Raman confocal spectroscopy. The persistence of photoprotection was analyzed *in vivo* in a clinical study using UVA imaging on human volunteers' arms. *Ex vivo*, comparison of formulated M10 and DHHB demonstrated superiority of skin-bioadhesive M10 regarding its efficacy and safety. *In vivo*, M10 was 47% more protective than DHHB, with 84% persistence 6 hours after application. As a human proof of concept of the technology, M10 showed resistance to rubbing, reduced diffusion, a good tolerance and persistence of photoprotection substantially longer than the recommendation to reapply sunscreen every two hours. The development of safe and long- lasting skin-bioadhesive UV filters could be a major advance in photoprotection research that the industry has been seeking over the past 20 years.

P1: Sebum promotes wound healing and suppresses pigmentation

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Wound healing is often followed by post-inflammatory hyperpigmentation (PIH), which commonly develops in sebum-poor areas. Here we investigated the effects of sebum on wound healing, especially relative to basement membrane recovery and PIH. Using 100% trichloroacetic acid, we created deep dermal wounds on both sides of male HRM-2 hairless melanin-containing mice. The left side served as the control group, while the right side received daily sebum application for 21 days. The wound healing status and degree of pigmentation were evaluated by visual wound assessments and image analyses. Histological analysis and electron microscopy (EM) examinations were performed on Days 7, 14, and 21, to compare basement membrane recovery and pigmentation. The sebum group exhibited faster wound healing, including quicker crust removal and wound contraction, and reduced pigmentation, although this difference was not statistically significant. Histological and EM analyses revealed accelerated basement membrane recovery and delayed onset of melanosome accumulation with sebum treatment. We also performed cell viability and melanin contents assays using B16F10 melanoma cells, revealing that sebum decreased melanogenesis by inhibiting tyrosinase activity, without cytotoxicity. Overall, our results suggest that sebum promotes wound healing, with faster basement membrane recovery and reduced PIH.

P2: A clinical study on the recurrence of non-segmental vitiligo

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Background: Vitiligo, characterized by depigmented patches on the skin, poses challenges in achieving lasting repigmentation after treatment. Identifying factors contributing to vitiligo recurrence is vital for effective management, yet there's a significant knowledge gap on post-treatment recurrence factors for vitiligo patients. **Objectives:** We aimed to investigate factors linked to the recurrence of non-segmental vitiligo patients. **Methods:** We retrospectively analyzed patients' medical records from 2000 to 2023, defining a "cure group" with cosmetically satisfactorily repigmented and maintained for at least 6 months. This group was split into recurrence and non-recurrence groups. We analyzed their clinical characteristics and various factors about treatments. We explored cure and recurrence rates based on vitiligo location. We performed survival analysis for recurrence with or without maintenance therapy. **Results:** Among the 70 cured patients, 19 relapsed. With or without maintenance treatment was the most important factor on the recurrence of vitiligo ($p < .0001$, OR = 0.029). There were no statistically significant differences in initial age, sex, disease duration, sites, subtype, treatment duration, treatment modality, and comorbidity. After 100 months, 87.3% in the maintenance therapy group stayed recurrence-free, while only 20% did in the non-maintenance therapy group. Head and neck lesions had the highest cure rates, whereas the lower limb region showed higher recurrence rates. In the recurrence group, about 63.2% occurred at the original site, higher than the 31.6% outside the original site. **Conclusion:** The absence of maintenance therapy is the most important factor involved in the recurrence of non-segmental vitiligo.

Crosstalk between tumor cells and their microenvironment in melanoma disease progression and therapy response

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Despite impressive progress in treatment, metastatic melanoma is still a fatal disease for many patients. Melanoma cells can become metastatic and therapy resistant due to their capacity to reversibly switch between different cellular states. In addition, pro-tumorigenic remodeling of the tumor microenvironment contributes to disease progression and therapy response. To study these processes in human patients, we established a method by which tissues from patients before and on treatment can be obtained by means of fine needle aspiration (FNA). Repeated sampling from BRAFi/MEKi treatment-resistant and responding lesions, followed by single cell RNA sequencing analyses, revealed significant changes in the cellular landscapes between resistant versus responder patients. Notably, unlike in the therapy responders, resistant patients exhibited a strong increase in a particular macrophage population associated with targeted therapy resistance (TTR). We show that the TTR phenotype can be induced by extracellular signals derived from tumor cells and its microenvironment and that TTR macrophages protect melanoma cells from targeted therapy-mediated killing. Furthermore, we demonstrate that signaling pathways, such as TGFβ, which are normally associated with melanoma disease progression, can be redirected toward anti-tumorigenic activities in the context of targeted therapy. Thus, integration of signaling cues derived from tumor cells and their microenvironment dictates the responsiveness of melanoma cells to targeted therapy.

Anti-melanoma gene and protein expression in dermal sheath fibroblasts from hair follicles in Recessive Dystrophic Epidermolysis Bullosa (RDEB) scalp

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Cutaneous melanoma is rare in patients with the skin blistering disease Recessive dystropic epidermolysis bullosa (RDEB), in contrast to aggressive squamous cell carcinoma. However, RDEB patients may exhibit unusual nevi with striking melanoma-like clinical features but which are benign. Here, we leverage RNA-Seq/proteomics analysis to explore whether mesenchyme associated with hair follicles (HF) in RDEB scalp may protect from melanoma and other cancers. Fibroblasts from HF dermal sheath (DS) were cultured from the scalp of a generalized intermediate type RDEB patient and from two healthy donors. Ingenuity Pathway Analysis (IPA) (z-score) of differential RNA-Seq expression data from RDEB DS fibroblasts (vs. healthy DS) showed downregulation for tumor-associated pathways: *Tumor tissue* (z-score=-2.33, p-value=3.59e-03); *Occurrence of tumor* (z-score= -2.65, p-value=2.07e-02); *Advanced melanoma* (z-score=-1.51, p-value=2.07e-02); *Progressive solid tumor* (z-score=-1.73, p-value=7.19e-05) and *Progressive malignant solid tumor* (z-score=-1.39, p-value=1.6e-04). Other inhibited pathways included: *Skin hyper-pigmentation* (z-score=-1.51, p-value=1.41e-03); *Aryl hydrocarbon receptor* (z-score =-2.18, p-value= 5.72e-03); *WNT/beta-catenin* (z-score=-1.48, p-value=4.37e-06); IL1 (z-score=-1.51, p-value=2.52e-03); *Acute phase response* (z-score=-1.71, p-value=5.73e-03) ; *IL17* (z-score=-1.18, p-value=6.8e-03); *Sonic Hedgehog* (z-score=-1.41, p-value=2.89e-03), and *IL-6* (z-score=-1.52, p-value=1.31e-02). IPA Upstream Regulator analysis revealed inhibition of UVA-inducible cytokines: *TNF* (p=3.64e-73) and *IL1B* (p=2.18e-53). Meanwhile, proteomics analysis showed statistically significant downregulation of proteins expression: MMP2 (fold change=-5.24 vs. control), S100A2 (fold change=-4.47) and TGFBI (fold change=-5.01). In summary, DS fibroblasts in RDEB scalp exhibited unexpected tumor and pigmentation biology gene/protein differences compared to similar cells isolated from healthy donors. This may explain apparent protection of RDEB patients and their scalp skin from melanoma and from skin cancer in general.

MITF directly regulates the expression of CDH1 but indirectly regulates CDH2 through SETDB2 and chromatin modifications

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The MITF transcription factor is a key regulator of melanocyte development and is also important in melanoma where it regulates phenotype plasticity by switching the cells from a proliferative, non-invasive state to a non-proliferative, invasive state. How MITF mediates the switch is still an open question. One of the changes that take place during the switch is that the cells change from expressing E-cadherin (CDH1) to expressing N-cadherin (CDH2). In order to investigate if MITF regulates the expression of these two plasticity markers we have performed CUT-n-RUN, RNA-Seq and co-t ransfection studies. We show that MITF can directly regulate the expression of CDH1 through regulatory elements in intron 2 of the CDH1 gene; we identify four MITF-binding sites as most important for this regulation. We also show that MITF does not directly regulate CDH2 expression but instead positively regulates the expression of the epigenetic regulator SETDB2 which in turn represses CDH1 expression by chromatin modifications. Our results suggest that MITF mediates melanoma plasticity through direct and indirect mechanisms and identify SETDB2 as a partial plasticity mediator.

LKB1-SIK2 loss drives uveal melanoma proliferation and hypersensitivity to SLC8A1 and ROS inhibition

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Metastatic uveal melanomas are highly resistant to all existing treatments. To address this critical issue, we performed a kinome-wide CRISPR-Cas9 knockout screen, which revealed the LKB1-SIK2 module in restraining uveal melanoma tumorigenesis. Functionally, LKB1 loss enhances proliferation and survival through SIK2 inhibition and upregulation of the sodium/calcium (Na+/Ca2+) exchanger SLC8A1. This signaling cascade promotes increased level of intracellular calcium and mitochondrial reactive oxygen species, two hallmarks of cancer. We further demonstrate that combination of an SLC8A1 inhibitor and a mitochondrion-targeted antioxidant promotes enhanced cell death efficacy in LKB1-and SIK2-negative uveal melanoma cells. Our study also identified an LKB1-loss gene signature for the survival prognostic of patient with uveal melanoma that may be also predictive of response to the therapy combination. Our data thus identify not only metabolic vulnerabilities, but also new prognostic markers, thereby providing a therapeutic strategy for particular subtypes of metastatic uveal melanoma.

P26: RNA-based therapy to reach unmet needs in melanoma: ASOs against undruggable targets

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In the last decade, significant advances in the understanding of mechanisms driving neoplastic diseases have led to the development of highly efficient therapies. Targeted therapy and immunotherapy are now largely being used to propose adapted medicine. Despite their success the emergence of resistance remains a major obstacle to complete cure and some cancer remained refractory, highlighting the need to develop new treatments. Recently, RNA-based therapies, especially antisense oligonucleotides (ASO), have emerged as a new class of promising therapies. This project aims to introduce ASOs as a treatment for metastatic melanoma by targeting driver genes. ASOs are single-stranded synthetic nucleic acids, with a mean length of 12 to 25 nucleotides, specifically binding to their RNA- target. They endorse versatile mode of action according to their chemical structure, giving important opportunity in targeting any amenable genes. Here, we aim to propose splice switch ASOs (SSOs) as an original treatment for metastatic melanoma by targeting genes that remain undruggable to this day. SSOs were designed to force the splice of given exons, leading to a shift in the reading frame and the appearance of a premature stop codon, thereby reducing the expression of the target gene. Amongst the ASOs we designed, three showed strong efficacy and specificity, impacting cell proliferation and survival. Remarkably, these ASOs are equally effective in BRAF and NRAS mutated melanoma cell lines, opening new hopes for these patients.

Epidermal-melanin units of haired human skin: Remarkable life-affirming partnerships on our UVR-drenched planet

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Skin and hair pigmentation is the product of a remarkably complex communication between two histologically distinct cell types; the pigment-producing melanocyte of neural crest origin and the

pigment-accepting keratinocyte of epithelial origin. Perhaps the most dramatic feature of this communication is the routine exchange of one partner's organelle (melanosome) to the other. This example of uni-directional organelle transfer, between the near-permanent melanocyte and the labile keratinocyte, is essentially unique in mammalian biology. It represents an intercellular communication that is distinct from bi-directional exchanges that occur via extracellular vesicles, cellular fusion, and tunneling nanotubes/filopodia. Indeed, the interaction of each melanocyte with a remarkably stable number of viable keratinocytes generates a range of functionally distinct melanin units. These contribute considerably to the homeodynamic balance of both epidermis and the uniquely mammalian pilo-sebaceous unit. Apart from the melanocyte's "primary" role of melanin synthesis, they also function as contributors to our skin's immune response, stress-sensing or neuroendocrinology. Our melanocytic "sentinels" operate within and beyond UVA/UVB's reach. Thus, differences in cutaneous melanocyte post-coding (i.e., epidermis vs. pilosebaceous/follicular) can have dramatic consequences for their stability throughout our life-span. As essentially post-mitotic cells, the melanocyte of the human interfollicular epidermis is much more likely to become a feral melanoma cell than their cousins in the continually cycling hair follicle. This is despite the latter's enormous plasticity and proliferative habit. In this talk, I hope to tease out some key aspects of the remarkable double lives of cutaneous melanocytes, and what this may mean for our human health and well-being.

Vitiligo auto-immune response upon oxidative stress– related mitochondrial DNA release opens up new therapeutic strategies

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Background: Vitiligo is a complex autoimmune skin disease characterized by melanocyte loss and skin depigmentation. Despite the efficacy of JAK inhibitors, repigmentation is long and response often incomplete. We have shown innate immunity to play critical role in melanocyte destruction and have identified presence of mitochondrial DNA (mtDNA) in skin of some vitiligo patients. We questioned whether instead of being byproduct of melanocyte destruction, mtDNA may be directly involved in vitiligo pathogenesis. **Methods:** MtDNA was sequenced from non-lesional melanocytes isolated from skin biopsies of healthy and vitiligo patients. Melanocyte mitochondrial immune and metabolic function, their morphology, release of mtDNA and antioxidant activity was measured. To identify germline predictive biomarkers, we looked for changes in redox balance genes in PBMCs using whole-exome-sequencing (WES). **Findings:** Vitiligo melanocytes have increased number of mtDNA variants compared to healthy melanocytes and can be classified as having low-(LV) or high-variant (HV) load. Vitiligo HV melanocytes have increased mitochondrial mass and function, increased ROS production, and reduced catalase activity compared to LV or healthy melanocytes. Sensing of mtDNA by the cGAS-STING pathway results in pro-inflammatory response promoting the recruitment of CD8+ T cells. These events can be blocked with mitochondrial-specific SOD2, NRF2 activators (DMF, NK-252) and TBK1 inhibitor. **Interpretation:** We demonstrate two previously undescribed sub-groups of vitiligo patients based on mitochondrial variant load in their melanocytes. These findings have clinical implications as HV melanocytes are more likely to respond to treatments that specifically target their mitochondria.

A new mosaic syndrome with linear skin hypopigmentation is associated with a GNA13 variant activating the RHOA/ROCK pathway and altering cell morphology and melanosome transfer

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The genetic basis of pigmentary mosaicism in humans has increasingly been identified. Here, we found a recurrent postzygotic *GNA13* variant in a recognizable syndrome with hypomelanosis of Ito (HI). Through deep exome sequencing on affected skin in a cohort of patients with HI, we identified a postzygotic *GNA13* R200K variant in one patient, also found through direct *GNA13* sequencing in three additional patients with HI. Acral anomalies, delayed wound healing, digestive tract atresia, and ocular involvement (microphthalmia, coloboma) were present in two or more patients. Expression of the *GNA13* R200K variant in B16-F0, SK-MEL-28 or NHEM melanocytes resulted in increased actin polymerization, reduced perimeter, increased circularity and solidity, reduced focal adhesions and upregulation of the RHOA/ROCK pathway. We also found a defect in cell migration, but no changes in cell proliferation. The amount of melanin was unaltered, but dendrites and melanosome transfer were reduced. Inhibition of RHOA or ROCK blocked actin polymerization and restored the initial cell shape. These findings highlight the interaction between G-proteins and the RHOA/ROCK pathway, and their role in melanocyte function. Clinical manifestations also support their involvement in wound healing and gastrointestinal tract

development. *GNA13* expands the list of Gα protein genes (*GNAQ*, *GNA11*, or *GNAS*) involved in pigmentary mosaic skin disorders. They also add new evidence for involvement of the RHOA/ROCK pathway in syndromes with linear hypopigmentation, in keeping with our previous findings in a similar neuroectodermal mosaic disorder caused by dominant-negative *RHOA* variants.

Melanoma macrophage and extracellular matrix transitions during the therapy-tolerant state

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Cancer therapeutics cause changes in the tumor ecosystem beyond the eradication of targeted cells, but our understanding of these environmental alterations is often limited. However, defining this evolution through specific stages in cancer treatment can provide new targets that may lead to more effective therapy combinations. Using pre-clinical models, we identify that during the transition from Braf/Mek inhibitor therapy-induced tumor regression to a drug-tolerant, minimal residual "persister" state, both Ccr2 monocyte-derived macrophage infiltration influx and extracellular matrix (ECM)–related gene expression rises. These data suggest that macrophage influx and alterations in ECM at this time point could facilitate the trajectory of drug resistance mechanisms, which almost always occurs with continued treatment. To test the role of macrophage influx, this process was inhibited through the loss of the Ccr2 receptor. Surprisingly, though resistance still evolved, the tumor cells from Ccr2 loss of function recipients remained targeted therapy-sensitive when removed from their native environment, indicating that the nature of resistance is directed by macrophage presence. To test the role of increased ECM deposition at the transition to a drug-t olerant tumor, ECM deposition and organization were inhibited. This resulted in a delay in drug resistance, likely due to changes in immune cell infiltration in this context. Overall, these studies suggest that altering the tumor microenvironment at specific time points during targeted therapy could change the route to resistance to predictable and potentially more manageable paths.

What separates a melanocyte from a melanoma?

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Oncogenes are not able to initiate tumors in all cellular contexts, a phenomenon referred to as oncogenic competence. Such competence depends on both cell-intrinsic programs (i.e., develop-mental and epigenetic state) as well as cell-extrinsic influences from the TME. To study this, we have developed zebrafish and human pluripotent stem cell models of melanoma. In the talk, I will discuss how convergence of intrinsic and extrinsic programs determines the likelihood of oncogenic transformation.

Impact of EGFR-TKI on skin pigmentation and its therapeutic potential for hypopigmented skin disorders

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Epidermal melanin unit (EMU) integrity is vital for skin homeostasis and pigmentation. The epidermal growth factor receptor (EGFR) plays a significant role in cell growth and wound healing, but its influence on skin pigmentation remains underexplored. This study investigated the impact of EGFR tyrosine kinase inhibitors (EGFR-TKIs), specifically, gefitinib and PD153035, on skin pigmentation and their underlying mechanisms. We performed quantitative real-time PCR, western blot, and immunofluorescence for evaluating the expression of EGF and EGFR, found in epidermal keratinocytes. Treatment with EGFR-TKIs significantly increased the expression of stem cell factor (SCF) and endothelin-1 (ET-1) in cultured keratinocytes, resulting in enhanced melanocyte migration and proliferation in co-culture systems. Time-lapse live imaging and single-cell tracking assays confirmed these findings. Moreover, topical application of gefitinib on guinea pig dorsal skin induced increased pigmentation and mitigated rhododendrol-induced leukoderma. Our results indicate that suppression of EGF signaling indirectly enhances skin pigmentation by upregulating SCF and ET-1 in keratinocytes. This novel mechanism highlights the crucial role of EGF signaling in regulating skin pigmentation and suggests that topical EGFR-TKI therapy, at appropriate doses, could be a promising strategy for managing depigmentation disorders.