20TH ANNUAL MEETING OF THE PANAMERICAN SOCIETY FOR PIGMENT CELL RESEARCH PROGRAM AND ABSTRACTS

Seeing the Light: Pigment Cells, Their Responses, and the Generation of Diversity

ROYAL SONESTA HARBOR COURT
BALTIMORE, MARYLAND
OCTOBER 5-8, 2016
ORGANIZED BY PASPCR

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2016 PASPCR MEETING PROGRAM

Seeing the Light: Pigment Cells, Their Responses, and the Generation of Diversity

Wednesday, October 5, 2016

Royal Sonesta Harbor Court, Baltimore, MD

6:00 – 8:00 p.m.: PASPCR Council Meeting, Royal Sonesta Conference Room

Thursday, October 6, 2016

8:00 a.m.: Continental Breakfast at Royal Sonesta

MORNING SESSION

8:30 a.m.: Welcome and Opening Remarks, Thomas J. Hornyak, M.D., Ph.D., VA Maryland Health Care System and University of Maryland School of Medicine; Lead Organizer, PASPCR 2016; President-Elect, PASPCR

Plenary Session 1 – Melanin: Structure, Function, and Modification

Invited Speakers:
8:40 – 9:05 a.m.: David R. Adams, M.D., Ph.D., Senior Staff Clinician, National Human Genome Research Institute and NIH Undiagnosed Diseases Program, “Emerging Models of Melanin Biosynthesis and the Spectrum of Oculocutaneous Albinism”

9:05 – 9:30 a.m.: Brian Brooks, M.D., Ph.D., Senior Investigator, National Eye Institute and National Human Genome Research Institute, “Albinism: Can It Become a Treatable Disease?”

Oral presentations from submitted abstracts
9:30 – 9:42 a.m.: Albinism genetics and its pathophysiological correlation with melanogenesis (Abstract #19) Sairah Yousaf1,2, Mohsin Shahzad1, Yar M. Waryah3, Hadia Gul4, Tasleem Kausar2, Nabeela Tariq2, Umair Mehmood1, Muhammad Ali5, Muzammil A. Khan4, Ali M. Waryah3, Rehan S. Shaikh2, Saima Riazuddin1, Zubair M. Ahmed1

1Department of Otorhinolaryngology Head and Neck Surgery, School of Medicine, University of Maryland, Baltimore, MD, USA. 2Institute of Molecular Biology & Biotechnology, Bahauddin Zakariya University, Multan, Pakistan. 3Molecular Biology & Genetics Department, Medical Research Center, Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan. 4Gomal Centre of Biochemistry and Biotechnology, Gomal University, DI Khan, Pakistan. 5Government College University, Faisalabad, Pakistan. 6University of Washington Center for Mendelian Genomics.
9:42 – 9:56 a.m.: Bioorganic chemistry of eumelanin (Abstract #16)  Jason M. Belitsky
Department of Chemistry and Biochemistry, Oberlin College, Oberlin, Ohio

9:56 – 10:08 a.m.: Aromatic residues drive melanosomal formation of PMEL core amyloid (Abstract #1)  Ralf M. Leonhardt(1), Jiashee Hee(1), Xinran Liu(2), Peter Cresswell(1,2).
(1)Department of Immunobiology and (2) Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut, USA

COFFEE BREAK

10:30 a.m.: Remarks from Anthony F. Lehman, M.D., M.S.P.H., Senior Associate Dean for Clinical Affairs and Professor and former Chairman of the Department of Psychiatry, University of Maryland School of Medicine

Invited Speaker:
10:35 – 11:05 a.m.: Valerie Harvey, M.D., M.P.H. Eastern Virginia Medical School; Co-Director, Skin of Color Research Institute at Hampton University, “Pigmentary Disorders and Health Disparities in Skin of Color”

Oral presentations from submitted abstracts
11:05 – 11:17 a.m.: Deregulation of chemotactic signals, leukocyte recruitment, and immunity in segmental and non-segmental vitiligo (Abstract #21)  Rezk A Rezk(1,2), Marley Kemp D(1), Uitto J(1), Igoucheva O(1) and Alexeev V(1).
(1)Department of Dermatology and Cutaneous Biology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia Pennsylvania, (2)Department of Dermatology Faculty of Medicine, Al-Minia University, Al-Minia, Egypt.

11:17 – 11:29 a.m.: Adoptive transfer of TCR-transgenic, tyrosinase reactive T cells can induce human vitiligo (Abstract #31)  Emilia Dellaceca(1), Tamson Moore(1), Gina Scurti(1), Kelli Hutchens(2), Joseph I Clark(3), Courtney Regan(5), Elizabeth Garrett-Mayer(6), Michael Nishimura(1), I. Caroline Le Poole(1,2).
(1)Oncology Research Institute, Loyola University Chicago, Chicago, IL. (2)Department of Pathology, Loyola University Chicago, Chicago, IL. (3)Department of Hematology/Oncology, Loyola University Chicago, Chicago, IL. (4)Department of Biostatistics, Hollings Cancer Center, Medical University of South Carolina (Charleston, SC).

11:29 – 11:41 a.m.: A role for the PERK arm of the unfolded protein response in determining melanocyte viability and the pathogenesis of vitiligo (Abstract #42)  Omotayo A. Arowojolu, Seth J. Orlow and Prashiela Manga. The Ronald O. Perelman Department of Dermatology, New York University School of Medicine, New York, NY.

11:41 – 11:53 a.m.: Morphological and transcriptomic analyses reveal alterations of dermal extracellular matrix in actinic lentigines (Abstract #2)  Emilie Warrick(1), Christine Duval(1), Stéphanie Nouveau(1), Philippe Bastien(1), Virginie Piffaut(1), Jean-Paul Ortonne(2), Olivier de Lacharrière(1), Françoise Bernerd(1).
(1)L’Oréal Research and Innovation, Aulnay-sous-Bois, France. (2)Department of Dermatology, Hôpital l’Archet–2, Nice, France.
11:53 a.m. – 12:05 p.m.: Decreased level of cathepsin V/L2 in seborrheic keratosis: evidence that ineffective degradation of melanosomes in the keratinocytes play a role in this disorder (Abstract #5) Tie-Chi Lei, Men-Yun Su, Wen-Juan Yi, Shi Yin, Long-Fei Luo
Department of Dermatology, Renmin Hospital, Wuhan University, Wuhan, CHINA.

BREAK / LUNCH BOXES AVAILABLE

12:30 – 1:20 p.m.: Panel and Discussion Session: “Women in Science: Addressing the Gender Gap in Pigment Cell Research and Beyond”, Caroline Le Poole, Ph.D., President, PASPCR.

AFTERNOON SESSION

Plenary Session 2 – Pigment Cells Across the Phylogenetic Tree

Keynote Address:
1:30 – 2:30 p.m.: Michel Milinkovich, Ph.D., University of Geneva, “The EvoDevo and Physics of Skin Appendage and Skin Colour Patterning in Vertebrates”

Oral presentations from submitted abstracts
2:30 – 2:42 p.m.: Do morphological secondary sexual characteristics correlate with excited body coloration in Angel’s chameleons? (Abstract #25) Randall L. Morrison (1) and Lorna Cudmore (2)
(1) Department of Biology, McDaniel College, Westminster, Maryland and (2) School of Biological and Environmental Science, University College Dublin, Dublin, Ireland.

2:42 – 2:56 p.m.: The MITF family member tfec functions in zebrafish neural crest pigment cell fate diversification (Abstract #27) Samantha A. Spencer (1) and James A. Lister (1,2)
(1) Department of Human and Molecular Genetics and, (2) Massey Cancer Center, Virginia Commonwealth University School of Medicine, Richmond, Virginia.

2:56 – 3:08 p.m.: Neural tube-derived factors are required for the initial expression of MITF in the induction of melanocyte precursors (Abstract #9) Jing Wang, Juan Yang, Yu Chen, Zhongyuan Su, Huirong Li, Xiaoyin Ma, Ling Hou
Laboratory of Developmental Cell Biology and Disease, Center for Vision Science Research and Eye Hospital, Wenzhou Medical University, Wenzhou, 325003, China.

3:15 – 4:30 p.m.: Poster Session (All poster presenters should be present at their posters)

Oral presentations from submitted abstracts
4:30 – 4:42 p.m.: Repair of UV photodamage in melanocytes by the MSH-MC1R signaling axis: a new role for AKAP12 (Abstract #3) Stuart G. Jarrett and John A. D’Orazio
Markey Cancer Center, University of Kentucky, Lexington, KY.

4:42 – 4:54 p.m.: MC1R and endothelin B receptor signaling activates common targets that modulate the DNA damage response of melanocytes (Abstract #29) Viki B. Swope (1), Renny Starner (1), Ranjan Perera (2), and Zalfa A. Abdel-Malek (1)
4:54 – 5:06 p.m.: Impact of heterozygosity of germline p16 and MC1R mutations on human melanocytes in vitro (Abstract #28) Zalfa A. Abdel-Malek (1), Viki Swope (1), Kevin Choi (1), Steven Guard (1), Ayesha Anwar (1), Pamela Cassidy (2), and Sancy Leachman (2)  
(1) Department of Dermatology, University of Cincinnati and (2) Department of Dermatology, Oregon Health and Sciences University.

5:06 – 5:18 p.m.: In vivo function of Vitamin D Receptor (VDR) signaling in UVB-induced DNA damage and melanocyte homeostasis (Abstract #34) Sharmeen Chagani1,2, Sergiy Kryyachenko2, Yoko Yamamoto3, Shigeaki Kato4, Gitali Ganguli-Indra1,2 and Arup K. Indra1,2,5,6,7,8  
1Molecular and Cellular Biology Program, OSU, Corvallis, OR, 97331, USA; 2Department of Pharmaceutical Sciences, College of Pharmacy, OSU, Corvallis, OR, 97331, USA; 3Department of Surgical Oncology, The University of Tokyo, Tokyo, Japan; 4Research Institute of Innovative Medicine, Jyoban Hospital, Tokiwa Foundation, Fukushima, Japan; 5Department of Biochemistry & Biophysics, OSU, Corvallis, OR, 97331, USA; 6Linus Pauling Institute, OSU, Corvallis, OR, USA; 7Department of Dermatology, Oregon Health & Science University (OHSU), Portland, OR, 97239, USA; 8Knight Cancer Institute, OHSU, Portland, OR, 97239, USA.

5:18 – 5:30 p.m.: Regulation of melanocortin-1 receptor via heterodimerization with opsin3 in human epidermal melanocytes (Abstract #14) Rana N. Ozdeslik, Lauren E. Olinski, and Elena Oancea (1)  
(1) Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, RI  
Oral presentations from submitted abstracts
8:30 – 8:55 a.m.: Genomic analysis of human hair color variation (Abstract #23) Michael Morgan (1), Albert Tenesa (2), Jonathan Rees (3), Margaret Keighren (4) and Ian J. Jackson (2,4)  
(1) Department of Physiology, Anatomy and Genetics, University of Oxford, (2) Roslin Institute, University of Edinburgh, (3) Department of Dermatology, University of Edinburgh, (4) MRC Human Genetics Unit, University of Edinburgh.

Invited Speaker:  
8:55 – 9:20 a.m.: Andy McCallion, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, “Lessons from Freckles: Functional Genetic Dissection of Disease-Implicated Variation”
9:20 – 9:40 a.m.: Identification of hypoxia-induced HIF1A targets in melanocytes reveals a gene profile associated with poor prognosis for melanoma (Abstract #24) Stacie K. Loftus (1), Laura L. Baxter (1), Julia C. Cronin (1), Temesgen D. Fufa (2) and William J. Pavan (1) (1) National Human Genome Research Institute, (2) NISC Comparative Sequencing Program National Institutes of Health, Bethesda, Maryland.

9:40 – 9:52 a.m.: SASH1 is a novel gene involved in human skin pigmentation (Abstract #38) Karoline A. Lambert (1), Adam Alm(eida) (1), Donald S. Backos (2), David Norris (1), Kristin Artinger (3), Theresa Pacheco (1) and Yiqun G. Shellman (1) (1) Department of Dermatology; (2) Computational Chemistry and Biology Core Facility; and (3) Department of Craniofacial Biology, University of Colorado AMC.

9:52 – 10:04 a.m.: A structural variant in the 5'-flanking region of the TWIST2 gene affects melanocyte development in belted cattle (Abstract #4) Nivedita Awasthi Mishra (1,2,3), Cord Drögemüller (1,2,3), Vidhya Jagannathan (1,2,3), Rémy Bruggmann (4), Jule Beck (5), Ekkehard Schütz (5), Bertram Brenig (5), Steffi Demmel (1,2,3), Heidi Signer-Hasler (6), Aldona Pieńkowska-Schelling (7), Claude Schelling (7), Robert Kelsh (8), Nadia Mercader (9), Tosso Leeb (1,2,3) (1) Institute of Genetics, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland, (2) DermFocus, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland, (3) Swiss Competence Center of Animal Breeding and Genetics, University of Bern, Bern University of Applied Sciences HAFL & Agroscope, 3001 Bern, Switzerland, (4) Interfaculty Bioinformatics Unit, University of Bern, 3012 Bern, Switzerland, (5) Department of Molecular Biology of Livestock, Georg August University, 37077 Göttingen, Germany, (6) Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences, 3052 Zollikofen, Switzerland, (7) Clinic for Reproductive Medicine, University of Zurich, Zurich, Switzerland, (8) Department of Biology and Biochemistry, University of Bath, Claverton Down, United Kingdom, (9) Institute of Anatomy, University of Bern, 3012 Bern, Switzerland.

COFFEE BREAK

Invited Speaker:
10:30 – 11:00 a.m.: Craig Ceol, Ph.D., University of Massachusetts Medical School, “Using the Zebrafish to Understand Regeneration and Tumorigenesis of Melanocytes”

Oral presentations from submitted abstracts
11:00 – 11:12 a.m.: The role of BRD9 (Bromodomain Containing Protein 9) in melanogenesis and melanoma proliferation (Abstract #8) Tupa Basu Roy and Ivana de la Serna Department of Biochemistry and Cancer Biology, University of Toledo College of Medicine and Life Sciences, Toledo, Ohio, United States.

11:12 – 11:32 a.m.: Cooperation between EZH2 and transcription factors regulates control of genes involved in tumor suppression and immune responses in melanoma (Abstract #15) Stephen Wilson*, Stuart J. Gallagher#, Jessamy Tiffen$, Peter Hersey#, Fabian V. Filipp* #Melanoma Immunology and Oncology Group, The Centenary Institute, University of Sydney, Camperdown, New South Wales, Australia; Systems Biology and Cancer Metabolism, Program for Quantitative Systems Biology, University of California Merced, Merced, CA.
11:32 – 11:45 a.m.: Defining the epigenome response to targeted MEK inhibition in melanoma (Abstract #13) Temesgen D. Fufa, Julia C. Cronin, Stacie K. Loftus and William J. Pavan
Genetic Disease Research Branch, National Human Genome Research Institute, Bethesda, MD.

Lunch on your own

AFTERNOON SESSION

1:00 – 1:40 p.m.: Career Mentoring Session – Emphasis upon Scientific Careers Outside of Academics. Led by William J. Pavan, Ph.D. Senior Investigator and Director, Intramural Training Office, National Human Genome Research Institute

Participants:
Laura Brockway-Lunardi, Ph.D., Center for Research Strategy, National Cancer Institute
Sergio Coelho, Ph.D., Interdisciplinary Scientist, Division of Nonprescription Drug Products, FDA
Connie Lin, Ph.D., Director, Skin Health Research, GlaxoSmithKline-Stiefel

1:40 - 2:00 p.m.: Short oral presentations from selected posters

Plenary Session 4 – Melanocyte Development, Differentiation, and Transformation

PASPCR Lerner Award Lecture:
2:00 – 3:00:– Eirikur Steingrímsson, Faculty of Medicine, School of Health Sciences, University of Iceland, “MITF and Transcription Factor Networks”

Oral presentations from submitted abstracts
3:00 – 3:12 p.m.: Characteristics of quiescent hair follicle melanocytes during anagen (Abstract #46) Bishal Tandukar (1), Sandeep Joshi (1), Jennifer Huang (1), Thomas J. Hornyak (1, 2)
(1)Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland (2)Research & Development Service, VA Maryland Health Care System, Baltimore, Maryland.

3:12 – 3:24 p.m.: Genetic modifiers of the age-related phenotype of melanocyte stem cell differentiation (Abstract #12) Melissa L. Harris (1), William J. Pavan (2)
(1)Department of Biology, University of Alabama, Birmingham, Birmingham, Alabama; (2)National Human Genome Research Institute, NIH, Bethesda, Maryland.

COFFEE BREAK

Invited Speaker:
4:00 – 4:30 p.m.: Todd Ridky, M.D., Ph.D., University of Pennsylvania Perelman School of Medicine, “New insights into mechanisms regulating melanocyte homeostasis”
**Oral presentations from submitted abstracts**

4:30 – 4:45 p.m.: A mutation in the Cdon gene potentiates congenital nevus development mediated by NRAS Q61K (Abstract #18)  
Arash Chitsazan (1,2), Blake Ferguson (1), Ramesh Ram (3), Pamela Mukhopadhyay (1), Herlina Y. Handoko (1), Brian Gabrielli (2), Peter H. Soyer (4), Grant Morahan (3) and Graeme J. Walker (1)  
(1)QIMR Berghofer Medical Research Institute, Herston, QLD, Australia  (2)The University of Queensland Diamantina Institute, Translational Research Institute, The University of Queensland (UQ), Brisbane, QLD, Australia  (3)Centre for Diabetes Research, Harry Perkins Institute of Medical Research, Perth, WA, Australia, Australia, Dermatology Research Centre, UQ School of Medicine, Translational Research Institute, Brisbane, QLD, Australia.

4:45 – 5:00 p.m.: In vivo E2F reporting on efficacious dosing schedules of MEK plus CDK inhibition in melanoma (Abstract #6)  
Jessica L.F. Teh (1), Neda Nikbakht (1), Timothy J. Purwin (1), Inna Chervoneva (1), Michael A. Davies (2), Andrew E. Aplin (1)  
(1)Thomas Jefferson University, Philadelphia, Pennsylvania, (2)University of Texas MD Anderson Cancer Center, Houston, Texas.

5:00 – 5:15 p.m.: An in vitro model for late relapse of metastatic melanoma and a system analysis of the molecular characteristics of the quiescent/dormancy (QD) state (Abstract #7)  
Feng Liu-Smith (1,2,7), Parvital Paresh Panchal (7), Zi Wang (1,3,7), Ahmed Farhat (1,7), Angela Garcia (1,7), T Charles Fagundes (7), Fabian Fellip (9), Frank L. Meyskens, Jr (1,2,5,6,7)  

**7:30 p.m.: Gala Dinner/Banquet at Royal Sonesta**

**Saturday, October 8, 2016**

**7:15 a.m.: Continental breakfast at Royal Sonesta**

**7:30 – 8:00 a.m.: PASPCR Business Meeting**

**MORNING SESSION**

**Plenary Session 5 - Melanoma: Animal Models, Metastasis, and Therapeutics**

**Invited Speaker:**  
8:00 – 8:30 a.m.: Sheri Holmen, Ph.D., Huntsman Cancer Institute, University of Utah. “Determinants of Melanoma Metastasis: the RCAS System as a Discovery Tool in Melanoma”

**Oral presentations from submitted abstracts**

8:30 – 8:42 a.m.: NME1 mediates a switch in beta integrin subunits that correlates with prolonged patient survival (Abstract #10)  
M. Katie Leonard (1), Marián Novak (1), Joseph R. McCorkle (2), Xiuwei H. Yang (2), Alexey Belkin (1,3) and David M. Kaetzzel (1)  
Departments of 1Biochemistry and Molecular Biology, and Greenebaum Cancer Center, University of Maryland–Baltimore, Baltimore, MD; 2Department of Molecular and Biomedical Pharmacology,
8:42 – 8:54 a.m.: Deletion of chromosome 2q37.3 as a driver of metastasis in melanoma (Abstract #37) Archana Gopalan,1 Kasey L Couts,1 Ichiro Nakachi,2 Yuchun Luo,1 Hieu Van,1 Akihiro Fujisawa,1 Steven E Robinson,2 William A Robinson,2 Mark W Geraci,2 and Mayumi Fujita1,3
1Department of Dermatology and 2Medicine, University of Colorado AMC, Aurora, Colorado 3Denver VA Medical Center, Denver, Colorado.

8:54 – 9:06 a.m.: Vacuolar protein sorting 11 gene promotes chemoresistance in zebrafish melanocytes (Abstract #33) Kersten A. Peterson(1), Miranda E. Bean(2), Jallanie V. Negussie(2), Lauren F. Clancey(2), Allison B. Coffin(3) and Cynthia D. Cooper (2, 4)
(1)School of Biological Sciences, Washington State University, (2)College of Arts and Sciences, Washington State University,(3)Integrative Physiology and Neuroscience, Washington State University, (4)School of Molecular Biosciences, Washington State University, Vancouver WA 98686.

9:06 – 9:18 a.m.: Regulation of glutaminase in GRM1-expressing melanoma cells (Abstract #17) Raj Shah (1), Andrew Boreland (1) and Suzie Chen (1,2)
(1)Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, School of Pharmacy, Rutgers, the State University of New Jersey, Piscataway, NJ (2)Rutgers Cancer Institute of New Jersey, New Brunswick, NJ.

9:18 – 9:30 a.m.: YAP1 and TAZ as therapeutic targets in BRAF inhibitor-resistant melanoma (Abstract #44) Matthew L. Fisher, Daniel Grun, Gautam Adhikary, Richard L. Eckert
Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland.

9:30 – 9:42 a.m.: NLRP1 plays a positive role in melanoma chemoresistance (Abstract #40) Zili Zhai (1), Archana Gopalan (1), David A. Norris (1,2), Richard A. Spritz (1) and Mayumi Fujita (1,2)
(1)University of Colorado Denver SOM, Aurora, Colorado; (2) Denver Veterans Affairs Medical Center, Denver, Colorado.

COFFEE BREAK

10:15 – 10:30 a.m.: Acknowledgements, Closing Remarks, and IPCC 2017

Invited Speaker:
10:30 – 11:00 a.m.: Ashani Weeraratna, Ph.D., Wistar Institute, Philadelphia, “In the Wnt-er of life: How the aged microenvironment promotes melanoma metastasis and therapy resistance”

Oral presentations from submitted abstracts
11:00 – 11:12 a.m.: Targeted deactivation of cancer-associated fibroblasts by beta-catenin ablation suppresses melanoma growth (Abstract #35) Linli Zhou, Kun Yang, Randall R. Wickett and Yuhang Zhang
Division of Pharmaceutical Sciences, College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267, USA.
11:12 – 11:24 a.m.: Regulation of glutathione- and thioredoxin-based antioxidant systems in melanocytes and melanoma (Abstract #30) Pamela Cassidy(1), Matthew Honnegar(1), Madeleine Laws(1), Chelsey Kline(1), Zalfa Abdel-Malek(2) and Sancy Leachman(1)
(1) Department of Dermatology and Knight Cancer Institute, Oregon Health & Science University, Portland, OR. (2) and Department of Dermatology, University of Cincinnati, Cincinnati, OH.

11:24 – 11:36 a.m.: Driving NKT cell-mediated immune response toward ganglioside D3 (Abstract #32) Steven W. Henning (1), Levi W. Barse (1), Manuel F. Fernandez (1), Jonathan M. Eby (1), Adam M. Hammer (1), Edward R. Kessler (1,2), Emily R. Gilbert (1,2), Daniel F. Dilling (1,2) and I. Caroline Le Poole (1,2)
(1) Oncology Research Institute, Loyola University Chicago, Chicago, IL. (2) Departments of Pathology and Medicine, Loyola University Chicago, Chicago, IL.

Lunch – on your own and departure
POSTER PRESENTATIONS
Thursday, October 06, 2016, 3:15 – 4:30 p.m.

Ab. 11. NRF2 is an Important Target for the Protective Action of Melatonin and Its Metabolites Against UVB-induced Damage in Human Melanocytes
Zorica Janjetovic; University of Alabama at Birmingham

Ab. 20. Identifying Key Effectors in NME1-Mediated Metastasis Suppression
Nidhi Pamidimukkala; Univ. of Maryland School of Medicine, Baltimore, Maryland

Ab. 22. Keeping Tabs on Your Lab: Recognition and Detection of Data Manipulation
Helene Z. Hill; Rutgers NJ Medical School, Newark, NJ

Ab. 26. Galactomyces Ferment Filtrate in Reducing Pigmentation and Oxidative Stress
JàNay K. Woolridge Cooper; University of Cincinnati, Cincinnati, Ohio

Ab. 36. Novel mutations in LYST, a gene mutated in Chediak-Higashi disease
Elena-Raluca Nicoli; National Human Genome Research Institute, Bethesda, MD

Ab. 39. The Role of Endothelin 3 during Melanoma Lung Premetastatic Niche Formation
Juliano Freitas; University of Colorado AMC

Ab. 41. Identification and Characterization of the Metastatic Cell Populations in a Mouse Model of Melanoma
Xiaoshuang Li; Florida International University

Ab. 43. The Ezh2 Polycomb Group Protein Drives an Aggressive Phenotype in Melanoma Cancer Stem Cells and is a Target of Diet Derived Sulforaphane
Matthew L. Fisher; University of Maryland School of Medicine, Baltimore, Maryland

Ab. 45. Functional Roles and Molecular Mechanisms of Nm23 in Skin Melanocytes
Li Pan; University of Maryland School of Medicine, Baltimore, Maryland

Ab. 47. Transcriptional Coactivators YAP1 and TAZ Drive Melanoma Progression and Survival through Differential Pathways
Jason W. Lui; University of Chicago, Chicago, Illinois

Ab. 48. Outcomes of Melanoma in situ Treated with Mohs Micrographic Surgery Compared with Wide Local Excision
Adi Nosrati; University of California, San Francisco, California

Ab. 49. Using CRISPR Cas9 to Generate Thioredoxin Reductase 1 (TR1) Knock-outs in the Human Melanoma Cell Line, M-14
Chelsey D Kline; Oregon Health and Science University, Portland, Oregon

Ab. 50. Inhibition of Vemurafenib-Associated MAP Kinase (MAPK) Activation and Induction of Melanization in BRAFWT Melanoma Cells by 17-AAG
Sandeep S. Joshi; University of Maryland School of Medicine, Baltimore, Maryland
Ab. 51. **Determination of the Epigenetic Status of Melanocyte Lineage-associated Genes in throughout the Melanocyte Life Cycle**  
Sandeep S. Joshi; University of Maryland School of Medicine, Baltimore, Maryland

Ab. 52. **NBUVB treatment of human vitiligo significantly activates integrin-linked kinase signaling in the hair follicle bulge**  
Stance A. Birlea; University of Colorado School of Medicine

Ab. 53. **NLRP1 and IL-1β Play a Positive Role in Melanoma Chemoresistance**  
Zili Zhai; University of Colorado Denver SOM, Aurora, Colorado

Ab. 54. **TFAP2 Paralogs Drive Melanocyte Differentiation in Parallel with MITF**  
Robert A. Cornell; University of Iowa
### ORAL AND POSTER PRESENTATIONS AT A GLANCE

<table>
<thead>
<tr>
<th>Invited Speakers</th>
<th>Date &amp; Time</th>
<th>Oral Presentation</th>
</tr>
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<tbody>
<tr>
<td><strong>Invited Speakers</strong></td>
<td><strong>Date &amp; Time</strong></td>
<td><strong>Oral Presentation</strong></td>
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<tr>
<td>David R. Adams</td>
<td>Oct. 6, 8:40 a.m.</td>
<td>Abdel-Malek, Zafra A.</td>
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<td>Brian Brooks</td>
<td>Oct. 6, 9:06 a.m.</td>
<td>Alexeeev, Vitaliy</td>
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<td>Valerie Harvey</td>
<td>Oct. 6, 10:35 a.m.</td>
<td>Almeida, Adam</td>
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<td>Michel Millinkovich</td>
<td>Oct. 6, 1:30 p.m.</td>
<td>Belitsky, Jason M.</td>
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<td>Andy McCallion</td>
<td>Oct. 7, 8:58 a.m.</td>
<td>Cassidy, Pamela</td>
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<td>Craig Celis</td>
<td>Oct. 7, 10:00 a.m.</td>
<td>Chitsazan, Arash</td>
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<td>Errikur Stenngrensson</td>
<td>Oct. 7, 2:00 p.m.</td>
<td>Cooper, Cynthia V.</td>
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<td>Todd Risdy</td>
<td>Oct. 7, 4:00 p.m.</td>
<td>DellaCeca, Emilia</td>
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<td>Shani Holmen</td>
<td>Oct. 8, 8:00 a.m.</td>
<td>D’Orazio, John A.</td>
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<td>Ashani Weeraratna</td>
<td>Oct. 8, 10:30 a.m.</td>
<td>Filipp, Fabian V.</td>
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<td><strong>Poster Presentation</strong></td>
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<td>Oct. 6, 3:15 - 4:30 p.m.</td>
<td>Umeda, Arup K.</td>
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<td>Jackson, Ian J</td>
<td>Oct. 7</td>
<td>8:30 a.m.</td>
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<td>Lei, Tie-Chi</td>
<td>Oct. 6</td>
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<td>Birela, Stanca A.</td>
<td>52</td>
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</tr>
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<td>Cooper, Janay K.W.</td>
<td>26</td>
<td>Leonhardt, Ralf M.</td>
</tr>
<tr>
<td>Cornell, Robert A.</td>
<td>54</td>
<td>Lister, James A.</td>
</tr>
<tr>
<td>Fisher, Matthew L.</td>
<td>43</td>
<td>Loftus, Stacie K.</td>
</tr>
<tr>
<td>Freitas, Juliano</td>
<td>39</td>
<td>Manga, Prashiela</td>
</tr>
<tr>
<td>Hill, Helene Z.</td>
<td>22</td>
<td>Meyskens, Frank L. Jr</td>
</tr>
<tr>
<td>Janjetovic, Zorica</td>
<td>11</td>
<td>Mishra, N. Awasthi</td>
</tr>
<tr>
<td>Joshi, Sandeep S.</td>
<td>51</td>
<td>Morrison, Randall L.</td>
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<tr>
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<td>50</td>
<td>Ozdeslik, Rana N.</td>
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<tr>
<td>Kline, Chelsey D.</td>
<td>49</td>
<td>Roy, Tupa Basu</td>
</tr>
<tr>
<td>Li, Xiaoshuang</td>
<td>41</td>
<td>Shah, Raj</td>
</tr>
<tr>
<td>Lui, Jason W.</td>
<td>47</td>
<td>Swope, Viki B.</td>
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<td>Nicoll, Elena-Raluca</td>
<td>36</td>
<td>Tendukar, Bishal</td>
</tr>
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<td>Nosrat, Adi</td>
<td>48</td>
<td>Teh, Jessica L.F.</td>
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<td>Zhai, Zili</td>
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<td>Zhou, Linli</td>
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INVITED SPEAKERS
Keynote Address
The EvoDevo and Physics of Skin Appendage and Skin Colour Patterning in Vertebrates

Michel C. Milinkovitch, Ph.D.
Laboratory of Artificial & Natural Evolution
Dept. of Genetics & Evolution
Swiss Institute of Bioinformatics
University of Geneva, Switzerland

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Combining evolutionary developmental biology, physics and computer science, my research group investigates the emergence of complexity and diversity of integumentary traits in vertebrates. More specifically, we perform descriptive and mechanistic analyses of morphogenesis and patterning of skin colour and skin appendages in reptiles and mammals. Using as showcases some of our recent results in snakes and lizards, I will argue that it becomes possible to understand, in non-model species, the genetic and physical determinisms of developmental processes that generate both intra- and inter-specific variation of skin traits. First, I will show that that rapid skin colour changes in chameleons are not caused by dispersion/aggregation of pigment-containing organelles but by the active tuning of an intracellular 3D photonic structure. Second, I will discuss our analyses of skin patterning in snakes and lizards, with special emphasis on a gene mapping program in corn snakes for the identification of mutations affecting colour traits.
Emerging Models of Melanin Biosynthesis and the Spectrum of Oculocutaneous Albinism

David R Adams, M.D., Ph.D.
National Human Genome Research Institute,
National Eye Institute, and NIH Clinical Center, NIH, Bethesda, MD

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Oculocutaneous albinism (OCA) is an important cause of inherited visual impairment. The care of persons with OCA, and the development of rational therapies, are complicated by the presence of outstanding questions surrounding the genetics and cellular mechanisms of melanin production and regulation. This talk will: 1. Highlight current challenges in diagnosing and designing therapies for OCA, and, 2. Review current models that may shed light on the diagnostic uncertainty associated with many cases of OCA.
Albinism: Can It Become a Treatable Disease?

Brian Brooks, M.D., Ph.D.
National Eye Institute and
National Human Genome
Research Institute, Bethesda, MD

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Patients with oculocutaneous albinism (OCA) have decreased best-corrected visual acuity due, at least in part, to hypoplasia of the fovea—a highly-specialized region of the neurosensory retina. Because foveal development continues after birth, we posit that children identified as having albinism at birth could benefit from interventions that promote foveal development. At present, we do not understand how mutations in various melanin-pigment-related genes expressed in the retinal pigment epithelium and the choroid of the eye lead to non-cell-autonomous effects on retinal development. However, we hypothesize that improving the process of melanin deposition in melanosomes during development may aid in the formation of the fovea.

We have previously shown that oral administration of an FDA-approved drug, nitisinone, is capable of improving the melanin pigment in the fur and eyes of a mouse model of one form of albinism, OCA1b, caused by hypomorphic alleles of the tyrosinase gene. We are currently conducting a one year pilot clinical trial of 2mg/day oral nitisinone in five adults (ages, 24-52; 3 female, 2 males) with molecularly-proven OCA1b. Visual acuity, contrast sensitivity, iris transillumination, fundus pigmentation, electroretinograms, and foveal morphology have been documented at baseline and are being documented at pre-specified intervals (3, 6, 9, and 12 months). Skin melanin content is similarly quantified using skin reflectometry, hair is collected for biochemical analysis of melanin, and qualitative changes in hair and skin are photographed. The last patient completed 12 months of treatment recently and data are being analyzed; preliminary data from the trial will be discussed.

In parallel, we have ascertained if nitisinone improves the melanin pigment in a mouse model of OCA3 (Tyrp1 mutation) and OCA4 (Slc45a2 mutation). Preliminary data indicate that nitisinone does not have a clinically-significant effect in our model of OCA3, but does improve melanin content in melanosomes of our OCA4 mouse model.

Lastly, in an effort to find compounds that may stabilize tyrosinase (and therefore improve its folding) and/or activate its enzymatic activity, we have purified the intramelanosomal portion of human wild-type and mutant forms of tyrosinase and performed enzymology. In collaboration with the National Center for Advancing Therapeutics, we have screened 10,770 compounds at a single dose (115µM) wild-type enzyme. Nine compounds have potential activator activity, while 115 are potential inhibitors. These compounds are currently undergoing more detailed enzymatic screening and confirmation in tertiary model systems.
Lessons From Freckles: Functional Genetic Dissection of Disease-Implicated Variation

Andy McCallion
McKusick-Nathans Institute of Genetic Medicine,
Johns Hopkins University School of Medicine,
Baltimore, Maryland

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Pigmentary Disorders and Health Disparities in Skin of Color

Valerie M. Harvey, M.D., M.P.H.
Hampton University Skin of Color Research Institute, Eastern Virginia Medical School

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Patients with skin of color represent a growing segment of the US population. This presentation will provide clinical perspective on select benign and malignant pigmentary processes, such as post inflammatory hyperpigmentation and cutaneous melanoma, which disproportionately burden minority patients. It will also highlight current knowledge gaps and provide recommendations for the future areas of research needed to advance the understanding and treatment of these clinically important disorders.
Using the Zebrafish to Understand Regeneration and Tumorigenesis of Melanocytes

Craig J. Ceol, Ph.D.
University of Massachusetts Medical School

Craig.Ceol@umassmed.edu

Through the lens of the pigmentary system we use the zebrafish to address fundamental questions relating to regenerative and cancer biology. In studying regeneration of adult zebrafish melanocytes, we have used a combination of targeted cell ablation and single cell lineage tracing to define the process by which melanocytes recover following injury. This process couples direct differentiation of stem cells with divisions of other stem cells. This is a new mode of regeneration that serves to reconstitute differentiated cells while simultaneously maintaining the capacity for additional regenerative cycles. Melanocytes in adult zebrafish exist in an anatomically uniform epithelium without a defined niche, and this mode of regeneration may be common in similar epithelia such as interfollicular regions of human skin.

For studies of melanoma biology, we have performed comparative genomic analyses to uncover new genes involved in melanomagenesis. Through this work we have identified a BMP ligand that can promote melanoma progression and is required for melanoma cell survival. BMP pathway signaling is specifically activated in melanoma cells, where it serves to prevent differentiation and promote survival. Ligand expression correlates with melanoma patient outcome, and current efforts are aimed at targeting BMP signaling as an anti-melanoma therapy.
The MITF transcription factor is a master regulator of melanocyte development and a critical factor in melanoma progression. MITF has been shown to regulate most aspects of melanocyte function including proliferation, survival, differentiation and genome stability. In order to mediate these different effects MITF participates in different transcription factor networks. One such network involves the MITF, TFAP2A and IRF4 transcription factors which together play an important role in human pigmentation by regulating expression of pigment genes. Another network involves the related transcription factors TFEB and TFE3 which play a key role in autophagy regulation in various cell types. Our work shows that, like TFEB and TFE3, MITF is also involved in regulating autophagy. In melanoma cells and tumors, the expression of MITF correlates with the expression of genes involved in autophagy in melanoma cell lines and in melanoma tumors. Similarly, in melanoma cells MITF binds to the promoters and activates expression of autophagy genes. Surprisingly, however, CRISPR-mediated MITF knockout cells also exhibit increased expression of autophagy genes. This is due to increased expression of TFE3 in the absence of MITF. This suggests that MITF, TFEB and TFE3 are all involved in regulating autophagy in melanoma and that they form a regulatory feedback loop. Regulation of autophagy is therefore maintained, even in the absence of one member of this family of transcription factors. Thus, TFE3 and TFEB also need to be considered when analyzing MITF function in melanocytes and melanoma.
New Insights into Mechanisms Regulating Melanocyte Homeostasis

Todd Ridky, M.D., Ph.D.
University of Pennsylvania
Perelman School of Medicine

ridky@mail.med.upenn.edu

Common melanocytic nevi usually result from acquisition of a BRaf\(^{V600E}\) mutation that drives a transient period of melanocyte proliferation that halts when the nevus reaches 3-6 mm in size. Mechanisms mediating this growth arrest have been elusive. Using primary melanocytes isolated directly from over 30 benign human nevi, we have recently demonstrated that growth arrest downstream of BRaf activation results largely from TGFβ mediated induction of CDKN2B/p15. We have also recently discovered that neoplastic BRaf\(^{V600E}\) melanocytes, as well as normal primary melanocytes respond reciprocally to the sex steroids estrogen and progesterone, likely explaining some of the morphologic changes in nevi and skin pigmentation commonly observed during pregnancy. We demonstrated that melanocytes lack classic nuclear estrogen and progesterone receptors, and that steroid effects on melanocyte differentiation are mediated through the recently-discovered membrane bound G protein-coupled receptors PAQR7 and GPER. Selective agonists of these receptors may be useful for a variety of human pigmentation disorders.
Determinants of Melanoma Metastasis: The RCAS System as a Discovery Tool in Melanoma

Sheri L. Holmen, Ph.D.
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Salt Lake City and Huntsman Cancer Institute, Salt Lake City, UT

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A wealth of genetic data has been generated from tumor samples and compared with other tumor types, melanoma harbors a large number of somatic mutations. The gold standard for accurately classifying these alterations as driver or passenger mutations is biological analysis in model organisms. However, these methods have traditionally not been well-suited for high throughput analysis. To overcome this challenge, we developed a somatic cell gene delivery mouse model of melanoma that allows for the rapid validation of these alterations. This method is based on the RCAS/TVA retroviral vector system that allows for tissue- and cell-specific targeted infection of mammalian cells through ectopic expression of the viral receptor. This system utilizes a viral vector, RCAS, derived from the avian leukemia virus (ALV). The receptor for RCAS is encoded by the TVA gene and is normally expressed in avian cells; infection with ALV results in stable integration of the virus into the genome of replicating cells. In mammalian cells that express TVA, the viral vector is capable of stably integrating into the DNA and expressing the inserted experimental gene, but the virus is replication-defective, which allows for multiple rounds of infection. A major advantage of this system is the ability to model the multi-step process of carcinogenesis in immune-competent mice without the generation of multiple new mouse strains. We have successfully modeled melanoma by delivery of mutant NRAS and BRAF and identified genes that promote metastasis. We have further modified this model system to allow for the regulation of gene expression post-delivery using the tetracycline (tet)-regulated system and have used CRISPR/Cas9 technology to knock out tumor suppressor function in vivo. This mouse model enables high throughput analysis of genes that drive melanoma initiation, progression and metastasis and is highly useful for furthering our understanding of the biology of this disease as well as identifying viable targets for therapy.
In the Wnt-er of life: How the aged microenvironment promotes melanoma metastasis and therapy resistance

Ashani Weeraratna, Ph.D.
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While the clinical landscape of melanoma is rapidly evolving, malignant melanoma remains an aggressive disease. Individuals over the age of 55 have a much poorer prognosis for melanomas of equal grade and stage than younger individuals (1). This may be due to multiple factors, from accumulated environmental damage to increases in chronic inflammation, and changes in adaptive immunity. Recently, we have developed an interest in how changes in normal cells such as fibroblasts affect tumor progression. We have shown that factors secreted by fibroblasts in the aged microenvironment drive the local aggressiveness and systemic dissemination of melanoma cells. These age-related factors are involved in multiple processes leading to tumor progression including the acquisition of genomic instability, a breakdown of matrix surrounding the tumor cells, and the acquisition of a pro-metastatic phenotype. We will discuss the contribution of various age-related secreted factors and how they contribute to the increased aggression we see in melanoma in older patients.
ABSTRACTS
Aromatic residues drive melanosomal formation of PMEL core amyloid
Ralf M. Leonhardt (1), Jiashee Hee (1), Xinran Liu (2), Peter Cresswell (1,2)

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(1) Department of Immunobiology and (2) Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut, USA

Abstract:
PMEL is a melanocyte protein that forms physiological amyloid in melanosomes. Many amyloids and/or their oligomeric precursors are toxic, causing or contributing to severe, incurable diseases including type 2 diabetes, Alzheimer’s, Parkinson’s, prion diseases, and cancer. Striking similarities between the intracellular formation pathways of PMEL and various pathological amyloids including Aβ and PrPSc suggest PMEL may be an excellent model system to better understand endocytic amyloid. Learning how PMEL fibrils accumulate in melanosomes without apparent toxicity, may help developing therapeutic strategies that disarm pathological fibrils. Here, using mass spectrometry, we identify the critical ~76 amino acid PMEL subunit that forms the melanosomal amyloid core (CAF) and map PMEL domains on fibril-associated fragments. An unbiased alanine-scanning screen covering the entire CAF region combined with quantitative electron microscopy analysis of the full set of mutants identifies numerous essential residues, many of which rely on aromaticity for function. Some mutants appear to be defective in amyloid nucleation. This extensive data set informs the first structural model of the PMEL core amyloid.

ORAL PRESENTATION  Oct. 6, 9:56 a.m.
Abstract No: 2

Morphological and transcriptomic analyses reveal alterations of dermal extracellular matrix in actinic lentigines
Emilie Warrick (1), Christine Duval (1), Stéphanie Nouveau (1), Philippe Bastien (1), Virginie Piffaut (1), Jean-Paul Ortonne (2), Olivier de Lacharrière (1), Françoise Bernerd (1)

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(1)L’Oréal Research and Innovation, Aulnay-sous-Bois, France ; (2)Department of Dermatology, Hôpital l’Archet-2, Nice, France

Abstract:
Although actinic lentigines (AL) are common skin hyperpigmented lesions associated with age and chronic sun exposure, the biological mechanisms underlying their development remain unclear. This study aimed at better describing the alterations associated with AL displaying a high deformation of the dermo-epidermal junction (DEJ). AL from the dorsal side of hands of 15 Caucasian women were carefully selected using dermatoscopy. Lesional and adjacent non-lesional (NL) skin biopsies were processed for transcriptomic and histologic analyses. Histological staining confirmed a severe deformation of the DEJ in AL, with deep epidermal protrusions into the dermal compartment. Melanin content was significantly higher in AL as compared to NL skin, with a preferential localization in the epidermal basal layer. However, the number and distribution of melanocytes along the DEJ were not altered in AL. Analysis of gene expression profiles revealed a signature of 529 genes that clearly discriminated AL from adjacent skin. In accordance with morphological observations, alteration of epidermal homeostasis was confirmed by the up-regulation of several markers of basal keratinocytes and the down-regulation of markers of terminal differentiation. Surprisingly, the canonical genes involved in melanogenesis regulation were not differentially expressed in AL as compared to NL samples. A striking finding was the overexpression in AL of many genes encoding proteins involved in extracellular matrix (ECM) organization, matrix remodeling, DEJ structure and cell-matrix interactions. Increased expression of several ECM-associated proteins in the dermis of AL was confirmed in situ using immunohistochemistry, suggesting that a local disorganization of the dermal ECM could contribute to the formation of AL. Altogether, these results emphasize the interest of developing multi-targeted treatment for AL, in order to sustainably reduce pigmentation by restoring both dermal and epidermal homeostasis.

ORAL PRESENTATION  Oct. 6, 11:41 a.m.
Repair of UV photodamage in melanocytes by the MSH-MC1R signaling axis: a new role for AKAP12
Stuart G. Jarrett and John A. D’Orazio

Abstract:
By promoting development of covalent and mutagenic photoproducts in DNA, UV radiation is a major causative risk factor for melanoma. Melanocytes repair UV damage by the nucleotide excision repair (NER) genomic maintenance pathway, which is regulated in part by the melanocortin signaling axis. Besides the amount of UV that a cell is exposed to, NER efficiency regulates how susceptible a melanocyte will be to UV mutagenesis and, therefore, malignant degeneration. Loss-of-signaling melanocortin 1 (MC1R) polymorphisms that lead to defective cAMP second messenger generation are major inherited risk factors for melanoma development in part because of suboptimal NER and higher rates of UV mutagenesis. We have determined that the MC1R-cAMP signaling axis enhances NER through activation of cAMP-dependent protein kinase (PKA) and phosphorylation of ataxia telangiectasia and Rad3-related protein (ATR) on the S435 residue. This post-translational event recruits the key NER factor XPA to ATR and together, XPA and p-S435 ATR efficiently localize to sites of UV photodamage in chromatin to facilitate NER. We now identify A kinase anchoring protein 12 (AKAP12) as a critical molecular scaffold needed for PKA-mediated phosphorylation of ATR and nuclear translocation of the repair complex. Without AKAP12, levels of p-S435 ATR remain at basal levels and melanocytes lose the MC1R NER “boost” to help them recover from UV damage, accruing higher rates of UV mutagenesis as a result. Further, we have determined that the MC1R signaling pathway accelerates the 5’ strand incision step of NER, providing critical insight into the molecular mechanisms by which cAMP signaling impacts melanocyte genomic stability.

ORAL PRESENTATION  Oct. 6, 4:30 p.m.
A structural variant in the 5’-flanking region of the TWIST2 gene affects melanocyte development in belted cattle

Nivedita Awasthi Mishra¹,²,³, Cord Drögemüller¹,²,³, Vidhya Jagannathan¹,²,³, Rémy Bruggmann⁴, Jule Beck⁵, Ekkehard Schütz⁵, Bertram Brenig⁵, Steffi Demmel¹,²,³, Heidi Signer-Hasler⁶, Aldona Pierkowska-Schelling¹,⁷, Claude Schelling⁷, Robert Kelsh⁸, Nadia Mercader⁹, Tosso Leeb¹,²,³

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Abstract:
Belted cattle have a circular belt of unpigmented hair and skin around their midsection. The belt is inherited as a monogenic autosomal dominant trait. We mapped the causative variant to a 54 kb segment on bovine chromosome 3. Whole genome sequence data of 2 belted and 130 control cattle yielded only a single genetic variant in the critical interval, which was private to the two belted animals. The belt-associated variant was a copy number variant (CNV) involving the quadruplication of a 6 kb non-coding sequence located approximately 16 kb upstream of the TWIST2 gene. Increased copy numbers at this CNV were strongly associated with the belt phenotype in a cohort of 239 cases and 1303 controls (p = 1 exp-265). We hypothesized that the CNV causes aberrant expression of TWIST2 during neural crest development, which might negatively affect melanoblasts. Functional studies showed that ectopic expression of bovine TWIST2 in neural crest in transgenic zebrafish led to a decrease in melanocyte numbers. Our results thus implicate an unsuspected involvement of TWIST2 in regulating pigmentation and reveal a non-coding CNV underlying a captivating Mendelian character.
Decreased level of cathepsin V/L2 in seborrheic keratosis: evidence that ineffective degradation of melanosomes in the keratinocytes plays a role in this disorder
Tie-Chi Lei, Men-Yun Su, Wen-Juan Yi, Shi Yin, Long-Fei Luo

Abstract:
Background and Aim: Seborrheic keratosis (SK) is the most common benign disorder, which usually appears as a brown, black or light-tan skin growth on the face, chest, shoulders, especially the sun-exposed area. The pathogenesis of SK is not known, however, many observations strongly suggested that sun exposure and skin aging are the primary causes. Based on the histological findings that much more prominent melanin granules are accumulated in the affected skin lesions, we propose whether the interrupted degradation of melanosomes occurs in the keratinocytes, thereby stimulates epidermal hyperplasia. Method: 1) 15 seborrheic keratosis patients were recruited in this study, the biopsies were taken from lesional and clinically normal perilesional sites; 2) HaCaT human keratinocyte was routinely cultured in our lab and photoaging status was induced by repeated exposures of UVB; 3) Melanosomes were purified from MNT1 melanoma cells or from retinal pigment epithelium (RPE) in human eyeball; 4) Melanin granule distribution profile in tissue sections was visualized by Fontana-Masson staining; 5) Ultrastructural changes of melanosomes in tissues and melanosome specimens were examined using a transmission electron microscopy (TEM); 6) cathepsin V/L2 (CTSV/L2) mRNA was analyzed by semiquantitative reverse transcription-polymerase and its protein was assessed by Western blotting; CTSV/L2 enzyme activity was estimated with the fluorogenic substrate Z-Leu-Arg-AMC; and 7) In vitro proteolytic attack assay was carried out by melanosome incubated with the keratinocyte derivatives at an acidic pH. Results: 1) RT-PCR analyses showed that CTSV/L2 mRNA levels were significantly downregulated in SK lesional tissues to compare with those in normal perilesional skin tissues; 2) TEM data indicated that some broken melanosomes were seen in perilesional skin samples, barely seen in SK lesional tissue samples; 3) β-galactosidase-positive keratinocytes induced by UVB radiation suppressed CTSV.
In vivo E2F reporting on efficacious dosing schedules of MEK plus CDK inhibition in melanoma
Jessica L.F. Teh (1), Neda Nikbakht (1), Timothy J. Purwin (1), Inna Chervoneva (1), Michael A. Davies (2), Andrew E. Aplin (1)

Abstract:
Pharmacological targeting of cyclin dependent kinases 4 and 6 (CDK4/6) could represent a viable therapeutic option in combination with FDA-approved targeted therapies such as BRAF and/or MEK inhibitors (MEKi). Indeed, continuous and concurrent dosing of MEKi plus a CDK4/6 inhibitor (CDK4/6i) leads to melanoma regressions in in vivo models and delays the onset of MEKi resistance. Current scheduling in the clinic for the CDK4/6i inhibitor, palbociclib in breast cancer patients is 3 weeks on/1 week off (3/1) or 2 weeks on/1 week off (2/1). However, it is unclear what schedule of CDK4/6i would be most effective and safe in combination with a MEKi. Utilizing an E2F reporter system, we sought to analyze the efficacy of different CDK4/6i plus MEKi schedules in a quantitative and temporal manner. The schedules tested were intermittent dosing scheduling of the combination (3 weeks on/1 week off), continuous MEKi with intermittent CDK4/6i (3/1) and continuous CDK4/6i with intermittent MEKi (3/1). Intermittent dosing scheduling of the combination resulted in tolerant tumors and rapid reactivation of the pathway during drug holiday. Overall, continuous MEKi with intermittent CDK4/6i (3/1) inhibition led to more complete responses as compared to continuous CDK4/6i with intermittent MEKi (3/1). Furthermore, weight loss was evident in the continuous CDK4/6i arm suggesting adverse events related to continuous CDK inhibition. Taken together, in vivo reporting allows for quantitative measurement of pathway activity associated with inhibitor resistance and can be utilized to optimize combination schedules to improve the therapeutic index in patients.

ORAL PRESENTATION  Oct. 7, 4:45 p.m.
An in vitro model for late relapse of metastatic melanoma and a system analysis of the molecular characteristics of the quiescent/dormancy (QD) state

Feng Liu-Smith (1,2,7), Parvital Paresh Panchal (7), Zi Wang (1,3,7), Ahmed Farhat (1,7), Angela Garcia (1,7), T Charles Fagundes (7), Fabian Fellip (4), Frank L. Meyskens, Jr (1,2,5,6,7)

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Abstract:
It has been known clinically for eons that revitalized dormant cells lead frequently to late tumor relapse and eventual death. Our unique in vitro cell model that evolved in response to three common clinical dosage approaches demonstrates that there are multiple cellular strategies leading to drug resistance that correspond to low but increasing doses, medium dose and high dose treatment and include respectively the induction of the phenomena of adaptive response, genetic selection and dormancy. How these individual cell fates occurs is not clear. Publications of acquired drug resistance has shown that a few studies of genetic mutations and many of non-genetic kinase network rewiring underlie the basis for the first two phenomena. To our knowledge, the biological basis of dormancy and its role in drug resistance has not been characterized. Through RNA-Seq profiling and system biology analysis, we compared the gene expression in parental SK-Mel28 cells and adapted SK-Mel28A2-1b, and discovered a number of altered signal cascades: the TGF-β-SMAD9-FGF1, PDGF-MAPK-DUSP-HIF2α and EDNRB-GNAI2-PLCB4-PKA-MiTF pathways. Induction of HIF2α was confirmed by qRT-PCR in adapted Mel28A2-1b and in A375C2 cells as well. This was accompanied by up-regulation of HIF2α targets and the negative regulator EGLN1 and EGLN3. Induction of MiTF was not shown at the transcript level in the adapted cells but we observed melanin accumulation in the dormant cells and hypothesize that both HIF2α and MiTF are up-regulated in dormancy. The details of these novel findings will be presented and suggest that Plx4032-induced dormant cells may be dependent on HIF2α/MiTF-mediated p21WAF1 up-regulation for maintaining the dormancy status. Our findings suggest that the study of dormancy using an appropriate in vitro model has the potential to begin to understand clinical dormancy and late relapse mechanistically and offering a novel translational runway to developing new therapies.
Abstract:
Melanocytes are cells that produce melanin pigment and protect skin against damage from UV radiation. Malignant melanoma develops from the transformation of melanocytes. SWI/SNF chromatin remodeling complexes interact with master regulators of melanocyte differentiation and melanoma oncogenes to regulate the expression of genes important for melanogenesis and melanoma proliferation. Heterogeneous SWI/SNF complexes that contain either BRG1 or BRM as the catalytic subunit and an assortment of associated factors (BAFs) have been identified. BRG1 and BRM as well as some BAFs have bromodomains (BrDs) which bind to acetylated lysine residues in histone tails. Little is known about the role of BrD-proteins in regulating SWI/SNF function. Small molecules that specifically inhibit the association of BrD-containing proteins with chromatin can be used as tools to interrogate BrD function and may have therapeutic potential. I-BRD9 is a chemical inhibitor specific for BRD9, a newly identified BrD-containing component of SWI/SNF complexes that have BRG1 as catalytic subunit. We found that BRD9 is highly expressed in melanocytes and melanoma cell lines. Co-immunoprecipitation studies indicated that BRD9 and BRG1 physically interact in melanoma cells. To test the hypothesis that BRD9 has function in melanogenesis and melanoma proliferation, we treated melanocyte precursors with I-BRD9. I-BRD9 inhibited melanin synthesis and expression of genes that regulate melanocyte function. Decreased expression of genes that regulate melanin synthesis was associated with altered chromatin structure at regulatory sites. Depletion of BRD9 by siRNA had similar effects on gene expression as treatment with I-BRD9. I-BRD9 treated melanoma cells compromised proliferation and colony survival. Our data indicate that Brd9 has important roles in regulating melanogenesis and melanoma proliferation; also, its chemical inhibition may be useful for treating melasma and melanoma.
Neural tube-derived factors are required for the initial expression of MITF in the induction of melanocyte precursors
Jing Wang, Juan Yang, Yu Chen, Zhongyuan Su, Huirong Li, Xiaoyin Ma, Ling Hou

Presenting Author
Ling Hou

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Abstract:
During early vertebrate development, the melanocyte lineage arises with remarkable spatial and temporal precision from neural crest stem cells. Although the development of melanoblasts to melanocytes is known to depend on many distinct factors, it is still poorly understood which factors lead to the induction of melanocyte precursors. MITF, a basic-helix-loop-helix leucine zipper transcription factor, is critical regulator in melanoblast development and is involved in cell cycle regulation, cell survival, cell differentiation, and cell migration. Given the critical role that MITF plays early in melanocyte lineage development, it becomes crucial to determine the mechanisms that lead to its initial expression in the induction of melanocyte precursors. Here we approach the question of the extrinsic control of the initial expression of MITF by using an embryonic NT explant culture system. Our results show in the primary neural tube explant system that the MITF-positive cells in primary neural crest cells only occurs when neural tubes remain in the dish, but not when they are removed, suggesting that embryonic neural tubes provide one or more signals capable of inducing MITF expression that leads to the induction of melanoblasts. To determine which factors might be involved, we used gene expression profiling of neural tube explants, followed by whole mount in situ hybridization screening of candidate genes in early embryos and finally testing a set of candidate factors for their ability to induce the initial expression of MITF in neural crest stem cell-based melanocyte differentiation system. We further discuss whether candidate factors play any potential roles in inducing the initial expression of MITF in vivo from multipotential neural crest stem cells.
NME1 Mediates a Switch in Beta Integrin Subunits that Correlates with Prolonged Patient Survival

M. Katie Leonard (1), Marián Novak (1), Joseph R. McCorkle (2), Xiuwei H. Yang (2), Alexey Belkin (1,3) and David M. Kaetzel (1)

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Abstract:

Expression of the metastasis suppressor NME1 in melanoma is associated with reduced cellular motility, invasion, and metastasis, but the molecular mechanisms underlying this activity are not completely understood. Herein we report a novel mechanism through which NME1 modulates focal adhesion dynamics via regulation of integrins ß1 and ß3. Stable expression of NME1 significantly altered focal adhesion turnover at the cell periphery. Interestingly, over-expression of NME1 resulted in a switch from predominantly fast recycling α4ß1 integrins to slower recycling αvß3 integrins. Contrary to its regulation of other cell surface receptors, the inhibition of integrin ß1 and induction of integrin ß3 by NME1 was found to occur at the transcriptional level rather than through dynamin mediated endocytosis. Induction of integrin ß3 required both the 3-5’ exonuclease and nucleoside diphosphate kinase (NDPK) activities of NME1, which are also required for its metastasis suppressor activities in vivo. Further suggesting that the induction of integrin ß3 is involved in the metastasis suppression function of NME1, knockdown of integrin ß3 significantly increased the invasion capability of cells expressing NME1 in vitro compared to control cells. Analysis of metastatic melanoma patients in the TCGA showed individuals with a 1.5x or greater increase in integrin ß3 mRNA had a significantly longer overall survival times. Additionally, an inverse correlation was observed between NME1 and integrin ß1 mRNA in an independent microarray of primary melanomas. The inverse correlation of NME1 and integrin ß1 RNA was also a strong predictor of prolonged distant disease free and overall survival in patients with the basal-like subtype of breast carcinoma. Together, these data strongly suggest NME1 prevents metastasis of human melanoma and some types of breast cancers by altering beta integrin expression to reduce recycling of focal adhesions and, ultimately suppress of cell motility.
NRF2 is an important target for the protective action of melatonin and its metabolites against UVB-induced damage in human melanocytes

Zorica Janjetovic, Elisabeth F. Lee, Corey Duprey, Taban Ghaffari, Adrzej T. Slominski

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Abstract:
UV light is an inducer of radical oxygen species (ROS) as well as 6-4-photoproducots and cyclobutane pyrimidine dimers (CPD) in the skin, which further cause damage to the skin cells. Irradiation of cultured human melanocytes with ultraviolet B (UVB) (25, 50, or 75 mJ/cm2) stimulated ROS production which was reduced in cells treated with melatonin or its metabolites: 6-hydroxymelatonin (6-OHM), N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), N-acetylsertotonin (NAS), and 5-methoxytryptamine (5-MT). Melatonin and its derivatives also stimulate the expression of NRF2 (nuclear factor erythroid 2 [NF-E2]-related factor 2) and its target HO-1 (heme oxygenase), that play an important role in cell protection from different damaging factors, such as radiation. PCR, blotting and immunofluorescent (IF) analysis have been used to confirm the above statement. Silencing NRF2 gene, using siRNA, in human melanocytes diminished the protective effects of melatonin and its derivatives, while silencing of melatonin receptors, either MT1 or MT2, did not affect the protective role of melatonin or its derivatives from ROS, most probably due to very low detectable levels of MT1 or MT2 in melanocytes (confirmed by immunoprecipitation experiments). Using comet assay and IF staining for CPDs we observed that both melatonin and its metabolites enhanced the DNA repair in cells exposed to UVB. In addition, melatonin and its metabolites further enhanced expression of p53 phosphorylated at Ser-15. In conclusion, melatonin, its precursor NAS, and its metabolites 6-OHM, AFMK, 5-MT, protect melanocytes from UVB-induced oxidative stress and DNA damage, through the action on NRF2 receptor.
Abstract No: 12

Genetic modifiers of the age-related phenotype of melanocyte stem cell differentiation
Melissa L. Harris (1), William J. Pavan (2)

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Abstract:
A useful model to assess how genetic variation affects age-related phenotypes is through the study of adult stem cell populations in highly regenerative tissues; disruption of these cell populations often lead to acute and measurable phenotypic outcomes that are useful for genetic mapping. In particular, we have focused on assessing how genetic variation contributes to changes in melanocyte stem cell (McSC) maintenance in the hair follicle. One critical aspect of maintaining McSC identity is to suppress terminal differentiation, and we know that the ability of McSCs to prevent this differentiation program decreases with age. Using a genetic approach to predispose mice to McSC differentiation (the transgenic mouse line Tg(Dct-Sox10)) we ask here whether we can identify additional genetic variants that modify this phenotype in attempt to discover novel mechanisms involved stem cell maintenance. In one approach, by mating Tg(Dct-Sox10) mice to other mice carrying specific genetic mutations, we find that haploinsufficiency for the transcription factor, Mitf (Mitfmi-vga9/+) severely exacerbates McSC differentiation. Transcriptome analysis of McSCs isolated from wild type and Mitfmi-vga9/+ mice reveals the differential expression of immune-related genes between these animals and suggests an interesting relationship between McSC maintenance and immune-modulation. In a second approach, by mating Tg(Dct-Sox10) mice to inbred mouse lines, we found that the extent of McSC differentiation varies widely based on genetic background. Using genetic mapping approaches we are now identifying the genetic basis of these strain-specific contributions to McSC maintenance. These studies underscore the relevance of evaluating genetic modifiers in age-related phenotypes, and suggest future investigations into whether these modifiers contribute more broadly to aging phenotypes in other tissues and whether this approach can predict genetic predisposition for aging phenotypes in senile aging.

ORAL PRESENTATION  Oct. 7, 3:12 p.m.
Defining the Epigenome Response to Targeted MEK Inhibition in Melanoma
Temesgen D. Fufa, Julia C. Cronin, Stacie K. Loftus and William J. Pavan

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Abstract:
Activating mutations in the MAPK signaling cascade are key drivers of melanoma initiation and progression. Although gene targeted therapies have revolutionized the treatment of melanoma, adaptive resistance linked to molecular and phenotypic switching of tumor cells remains problematic. We used the MEK-1/2 inhibitor selumetinib (AZD6244) to disrupt MAPK signaling in melanoma cells, and analyzed the dynamics of the epigenome. We show that chemical loss of MEK function induces major changes in global gene expression, mechanistically involving extensive reprogramming of gene super-enhancers (SE) and motif signatures of a number of transcriptional regulators. We identified 963 SE-associated genes, of which 422 showed significant expression changes following AZD6244 treatment. Interestingly, AZD6244 treatment upregulated SE-associated gene categories involved in the regulation of melanocyte lineage and epithelial-mesenchymal transition (EMT) programs suggesting that SE driven transcription is a key epigenomic determinant of melanoma response to MEK inhibition. Furthermore, we demonstrate that MEK inhibition provokes the redistribution of SOX10 binding to novel genomic loci, including regulatory regions near genes involved in EMT/cell invasion. Thus, combined targeting of SOX10, SE and MEK inhibition may offer potential alternative therapeutic avenues for melanoma.

ORAL PRESENTATION  Oct. 7, 11:32 a.m.
Regulation of melanocortin-1 receptor via heterodimerization with opsin3 in human epidermal melanocytes
Rana N. Ozdeslik, Lauren E. Olinski, and Elena Oancea

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Abstract:
Melanin, the main pigment in mammals, is synthesized by a complex mechanism in melanocytes. A key component of the melanogenic pathway is the melanocortin-1 receptor (MC1R), a G-protein coupled receptor (GPCR) expressed in uveal, cutaneous and hair follicle melanocytes. The activity of MC1R determines skin and hair pigment and variants of MC1R are linked to skin cancer susceptibility. MC1R is Gas-coupled and upon stimulation by a-melanocortin stimulating hormone (aMSH), increases cAMP levels, activating a signaling cascade that leads to expression of melanocyte master regulator, MITF, and pigment production. While the regulation of MC1R gene expression has been extensively studied, the regulatory mechanisms that govern MC1R-mediated melanogenesis are not fully understood. We show that MC1R forms heterodimers with opsin3 (OPN3), a light-activated GPCR highly expressed in primary human epidermal melanocytes. Intriguingly, the interaction between MC1R and OPN3 is potentiated by aMSH stimulation. In addition, OPN3 and MC1R are present and co-localize both at the plasma membrane, but primarily in intracellular vesicles. Interestingly, reducing the expression of OPN3 in human epidermal melanocytes leads to increased cellular melanin, suggesting that OPN3 negatively regulates the activity of MC1R. We hypothesize that OPN3, a Gai-coupled receptor, modulates the amount of cAMP produced in response to MC1R activation, thus regulating melanin synthesis. Our results suggest that heterodimerization of MC1R and OPN3 in melanocytes is a novel regulatory mechanism for MC1R and, because OPN3 is a photosensitive protein, it postulates that light could regulate melanin production via MC1R dimerization with OPN3.

ORAL PRESENTATION  Oct. 6, 5:18 p.m.
Cooperation between EZH2 and transcription factors regulates control of genes involved in tumor suppression and immune responses in melanoma

Stephen Wilson*, Stuart J. Gallagher#, Jessamy Tiffen$, Peter Hersey#, Fabian V. Filipp*

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Abstract:
EZH2 is a promising anticancer drug target in melanoma due to somatic hyperactivation by amplification or mutation. However, the genomic foundation determining its oncogenic function are still poorly understood. In view of this we have used an integrated systems biology approach to analyze ChIP-Seq, MethylSeq, and RNASeq data of skin cutaneous melanoma specimen for transcriptional targets. H3K27me3 ChIP-Seq events were used to enrich for EZH2 activity. As a control we introduced inhibitor GSK126 to observe specific loss of binding of events mediated by the histone methyltransferase EZH2. We conducted a motif search of corresponding promoter and transcription start sites to identify transcriptional cooperation of the polycomb repressive complex 2. The identified target genes share a purine-rich motifs at their promoter sites pointing towards interaction with distinct transcription factors. Mass spectrometry in combination with reversible cross-link immunoprecipitation captured cooperation of the polycomb repressive complex 2. Target genes were validated by ChIP-PCR at genomic locations of enriched transcription factor motifs, DNA hypermethylation, and transcriptional silencing. Hypermethylation of DNA co-localizes with H3K27 trimethylation, and modulates gene expression that promotes melanoma progression. Prior in silico systems biology predictions of EZH2 targets in patients of The Cancer Genome Atlas (Tiffen J, Wilson S, Gallagher SJ, Hersey P, Filipp FV. Neoplasia. 2016 Feb; 18(2):121-32. doi: 10.1016/j.neo.2016.01.003) were validated in vitro using ChIP-Seq of cellular models of melanoma at a 28.0% success rate. These results provide mechanistic insights into the malignant role of EZH2 upon transformation and facilitate therapeutic targeting of an epigenetic modifier.

ORAL PRESENTATION   Oct. 7, 11:12 a.m.
Abstract No: 16

Bioorganic Chemistry of Eumelanin
Jason M. Belitsky

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Abstract:
A challenge to pigment cell research from a biochemical perspective is that many questions remain about the fundamental chemistry of pigments such as eumelanin, the brown-to-black form of melanin in humans. We are using the tools of bioorganic chemistry to investigate the formation and structure of eumelanin, as well as to develop melanin-inspired materials for environmental applications. Initiated by the oxidative transformation of tyrosine/L-dopa to dihydroxyindoles, eumelanin is thought to form from the self-assembly of heterogeneous dihydroxyindole oligomers. We are investigating this process by two complimentary routes: synthesis of well-defined dihydroxyindole oligomers and small molecule screening for modulators of biomimetic oxidative polymerizations. Eumelanin is known to bind a wide variety of metal ions and organic compounds; in addition to using these interactions as probes to interrogate eumelanin formation and structure, these molecular recognition interactions can be leveraged for water purification and heavy metal sensing applications. In particular, we are developing synthetic eumelanin-based water purification agents and catechol-based colorimetric sensors for lead. The latter project has assumed particular importance given the recent incidences of lead contamination in Flint, Michigan and other US cities. This presentation will give an overview of the melanin-related projects in our lab, including the involvement of approximately 60 undergraduates per year through a course-based research experience, “Bioorganic Chemistry of Eumelanin,” in our second-semester organic chemistry class.

ORAL PRESENTATION  Oct. 6, 9:42 a.m.
Regulation of Glutaminase in GRM1-expressing Melanoma Cells

Raj Shah (1), Andrew Boreland (1) and Suzie Chen (1,2)

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Abstract:
Aberrant glutamatergic signaling has been implicated in many cancer types, and is associated with dysregulated growth leading to cellular transformation and tumorigenesis. Our laboratory has previously illustrated the oncogenic properties of a neuronal receptor, metabotropic glutamate receptor 1 (GRM1) in melanocytes. Glutamate is the major excitatory neurotransmitter in the central nervous system and is the natural ligand of GRM1. Our group has demonstrated that glutamate production/release is upregulated in melanoma cells, which leads to constitutive activation of GRM1 and GRM1-associated downstream signaling pathways. We hypothesize that this activation of GRM1 in melanoma cells is associated with higher expression and/or increased enzymatic activity of glutaminase (GLS). We utilized CB-839, a potent, selective, and orally bioavailable GLS inhibitor, to downregulate GLS in GRM1 positive human melanoma cell lines. Various in vitro assays were performed to assess the consequences of inhibiting GLS with respect to cell proliferation, viability and alteration of glutamate levels in treated cells. We found that the suppression of GLS significantly inhibits cell proliferation in GRM1 positive cell lines in comparison to GRM1 negative cell lines. Additionally, our cell proliferation analysis found cells harboring wild-type BRAF to be more sensitive than those with mutated BRAF. Furthermore, our data indicate that CB-839 acts by reducing the function, not the expression, of GLS. Currently, we are investigating the relationship between GRM1, c-Myc and glutaminase by genetic and pharmacological approaches, and if this cascade of events is linked with altered glutamine metabolisms described for many human cancers including melanoma.
A mutation in the Cdon gene potentiates congenital nevus development mediated by NRAS Q61K

Arash Chitsazan (1,2), Blake Ferguson (1), Ramesh Ram (3), Pamela Mukhopadhyay (1), Herlina Y. Handoko (1), Brian Gabrielli (2), Peter H. Soyer (4), Grant Morahan (3) and Graeme J. Walker (1)

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Abstract:
Congenital nevi develop before birth, and sometimes cover large areas of the body. They are presumed to arise from the acquisition of a gene mutation in an embryonic melanocyte that becomes trapped in the dermis during development. To better understand the development and biology of congenital nevi we have used a mouse model carrying an NRASQ61K mutation in all melanocytes. These transgenics develop melanocytic lesions ostensibly identical histologically to many human congenital nevi. The murine lesions start to form at post-natal day 10, and by day 40 are fully formed. The lesions emanate from melanocytes escaping hair follicles. We combined the nevus-prone transgenics with the Collaborative Cross (CC), a resource of ~100’s of genetically diverse mouse strains that enable rapid mapping of quantitative trait loci and is specifically designed to permit the discovery of genes for complex diseases. We examined variation in nevus cell density in 70 CC strains and mapped a large effect quantitative trait locus controlling nevus cell density to murine chromosome 9. We stratified candidate genes within the interval by cataloguing DNA variants that vary between the susceptible and resistant strains. This revealed a very strong candidate gene (Cdon) carrying a missense mutation. Cdon is a positive regulator of sonic hedgehog (Shh). No genome wide association studies have been performed to help us understand the susceptibility to the development of congenital nevi. For the first time, we utilized a systems genetics approach to define innate variation that dramatically influences the density of congenital nevi. Understanding genes that control the development of these lesions will be a first step in the development of new treatments and control measures for these lesions.
**Albinism genetics and its pathophysiological correlation with melanogenesis**

Sairah Yousaf¹,², Mohsin Shahzad³, Yar M. Waryah¹, Hadia Gul⁴, Tasleem Kausar², Nabeela Tariq², Umair Mehmood¹, Muhammad Ali¹, Muzammil A. Khan⁴, Ali M. Waryah¹, Rehan S. Shaikh², Saima Riazuddin¹, Zubair M. Ahmed¹

**Abstract**: In our society, visual impressions of body form and color have certain importance to interact within and between the human communities. Oculocutaneous albinism (OCA) is a heterogeneous genetic disorder characterized by partial or complete loss of pigmentation in the skin, hair and iris, due to a decrease or absence of melanin production. Our goal here is to identify common and/or novel OCA alleles by cost-efficient genetic diagnostic assays in large families segregating OCA and evaluate their functional consequences in heterologous expression system. Through the combination of Sanger and whole exome sequencing (WES) techniques, we have identified 40 mutations, including 24 novel variants, segregating with OCA phenotype in 81 families. The 24 novel mutations include 10 missense, 4 splice site, 4 truncating and 6 gross deletions in four known albinism genes (TYR, OCA2, TYRP1 and SLC24A5). All of the ten novel missense variants were predicted pathogenic by various web-based programs. To decipher the effect of temperature on the synthesis and stability of protein encoded by identified mutated genes we are conducting immunostaining and Cycloheximide Chase assay at 37 °C and 31 °C. Some of the identified variants showed a kind of temperature-sensitive phenotype by being present on melanosomes in the cytoplasm at 31 °C but not at 37 °C. We adopted “Humanized Zebrafish Orthologous Rescue” (HuZOR) approach to evaluate the effect of human mutations on slc24a5 gene function. Constructs containing morpholino resistant wild-type slc24a5 mRNA rescued the pigmentation phenotype (up to 60 %) by 72 hpf in morphants. Whereas the identified mutations were unable to rescue the pigmentation phenotype, which support their pathogenicity. Furthermore, to understand the impact of these alleles on human SLC24A5 protein, we employed the CRISPR/Cas9 gene editing technology in primary melanocytes and introduce these mutations in the endogenous genes. Currently, studies are underway to evaluate the effect on the pigmentation of primary melanocytes. **Conclusion**: Our studies provide further support to comprehend the role of different pigmentation genes. Overall, our study contributes to the development of genetic testing protocols and genetic counseling for OCA in Pakistani families.
Abstract No: 20

Identifying Key Effectors in NME1-Mediated Metastasis Suppression

Nidhi Pamidimukkala(1), M. Katie Leonard (1), Joseph R. McCorkle (2), Qingbei Zhang (2), Anup Mahurkar(3), Amol Shetty (3), David M. Kaetzel (1,2)

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Abstract:
NME 1 was the first described metastasis suppressor, discovered through its reduced expression in metastatic melanoma cells compared to non-metastatic counterparts. Despite extensive research over the years, the mechanism with which NME 1 exhibits its metastasis suppressing activity is not well understood. NME 1 has two known enzymatic activities which may contribute to its metastasis suppressor function: a nucleoside diphosphate kinase (NDPK) activity mediating the reciprocal transfer of a phosphate from a nucleoside triphosphate to a nucleoside diphosphate, and a 3’ to 5’ exonuclease activity which removes 3’ overhanging bases on DNA. Our lab discovered the 3’ to 5’ exonuclease activity, and likely the NDPK activity, to be necessary for metastasis suppression. NME 1 has further been shown to regulate transcription, which may also contribute to its metastasis suppressor function. NME 1 has been shown to bind to the promoter region and suppresses transcription of the platelet derived growth factor A (PDGF-A) oncogene (Ma,D. et al, 2002). In breast cancer, NME 1 was shown to regulate expression of several genes, with suppression of LPA receptor, EDG2, largely contributing to its metastasis suppressor function (Horak, C.E. et al, 2007). These results strongly suggest that NME 1 regulates expression of target genes that modulate the metastatic phenotype of malignant cells. To identify genes whose expression is modified by NME 1 and also tracks with its metastasis suppressor function in melanoma cells, NME 1 mutants abrogating the metastasis suppressor function were generated to compare RNA expression to NME 1 wild-type expressing cells. Expression analysis revealed thirteen genes that were significantly modulated in the NME 1 wild-type cells, but not the mutants, and these genes were denoted by our lab as the metastasis suppressor signature (MSS). Taken together, the data supports the hypothesis that NME 1 exerts its metastasis suppressing activity by regulating expression of key effector genes within the MSS.
Deregulation of chemotactic signals, leukocyte recruitment, and immunity in segmental and non-segmental vitiligo

Rezk A Rezk (1,2), Marley Kemp D (1), Uitto J (1), Igoucheva O (1) and Alexeev V (1)

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Abstract:
Autoimmunity was suggested as a mechanism involved in progression of non-segmental (NSV) and, to certain extent, segmental vitiligo (SV). As cutaneous autoimmunity coincides with deregulated recruitment of leukocytes to the skin, vitiligo-associated chemotactic signals were investigated. Initial analysis of chemokines demonstrated up-regulation of several chemokines, including CCL5 and CXCL12. Analysis of vitiliginous skin demonstrated that CXCL12 is primarily expressed by epidermal melanocytes and recruited CD11c+ dendritic cells (DC). Furthermore, chemokine-focused antibody array revealed only minor changes in the serum levels of the chemotactic molecules. Deregulation of chemotactic signals within lesional and perilesional skin of the SV and NSV samples was more pronounced with substantially higher levels of CCL27, CCL11, CCL24, CXCL1, CXCL10, CCL22, CCL5, CCL17 and CXCL12. These lesions were also characterized by the prominent infiltration with leukocytes. Immuno-phenotyping showed a predominant infiltration of skin with CD11c+ DC often extended to the epidermis. In the perilesional skin of early NSV and SV majority of skin-infiltrating DC express CD86 maturation marker. Analysis of the chemokine receptor repertoire demonstrated that CD11c+CD86+ DC express CCR3 receptor for CCL11, CCL24 and CCL5 chemokines. CD3+ T cells co-localized with these CD11c+CD86+ DC were also positive for CCR3. Among CD8+ T cells, CCR7 and CCR4-positive populations were detected. These observations suggested that in early SV and NSV DC and T cells could be recruited to the cutaneous tissue by several epidermis and melanocyte-derived chemokines following by acquisition of melanocytic antigens by DC, intracutaneous priming/activation of the T cells and melanocytes-specific CTL activity. These findings suggest autoimmunity as a common initiation mechanism for both SV and NSV where SV-associated local immunity extends to acquisition of melanocyte-specific immunologic memory and progression.

ORAL PRESENTATION  Oct. 6, 11:05 a.m.
Abstract No: 22

Keeping Tabs on Your Lab: Recognition and Detection of Data Manipulation
Helene Z. Hill

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Abstract:
Over the last 10 years, there has been a marked increase in retractions of articles in the scientific literature. Even more unsettling is the fact that well over half of these retractions are due to scientific misconduct. Scientists are increasingly becoming aware that not all data generated in their laboratories is reflective of the truth. In order to make certain that results have not been influenced by the experimenter(s), the wise chief can employ certain tests to ascertain that no tampering of results has occurred. Data alteration can fall into at least 3 categories: plagiarism, image manipulation and numerical fabrication. Commercial products are available to test for plagiarism and image alteration. An excel spreadsheet has been developed that allows for the easy testing of three types of number manipulation that might be encountered when counting cells in suspension or colonies. Examples will be shown to demonstrate image rearrangements and suspicious numbers. Ways to avoid these pitfalls will be discussed.

POSTER PRESENTATION  Oct. 6, 3:15 – 4:30 p.m.
Genomic Analysis of Human Hair Colour Variation
Michael Morgan (1), Albert Tenesa (2), Jonathan Rees (3), Margaret Keighren (4) and Ian J. Jackson (2,4)

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Abstract:
The genetics of human hair colour is a complex interaction between numerous genes. The knowledge we have of the biology of melanocytes and their interactions presents an excellent opportunity to functionally dissect this genetic system. UK Biobank is a project in which 500,000 individuals have been recruited and data collected for many clinical and physiological parameters, including hair colour. We have performed a genome wide association study of UK Biobank for red and blonde hair colour. The red hair analysis reveals the well-established association with multiple alleles of MC1R, including novel variants outside the coding region. We show that the poorly penetrant (“r”) missense alleles of MC1R show epistatic interactions with variants at the ASIP locus, encoding the agouti protein. There are only 3 further loci which contribute to red hair colour. By contrast we find over 25 separate loci associated with blonde hair colour, including many genes known to be involved in melanocyte biology but not previously shown to contribute to human hair colour variation. We also identify novel, melanocyte specific genes of previously unknown function. We are functionally analysing these genes, including making mouse mutants, as candidates for new pathways in pigment cell biology.

ORAL PRESENTATION  Oct. 7, 8:30 a.m.
Identification of hypoxia-induced HIF1A targets in melanocytes reveals a gene profile associated with poor prognosis for melanoma

Stacie K. Loftus (1), Laura L. Baxter (1), Julia C. Cronin (1), Temesgen D. Fufa (2) and William J. Pavan (1)

Abstract:
HIF1A is a critical regulator of cellular response to changes in oxygen concentration within the surrounding tissue microenvironment. HIF1A signaling broadly regulates multiple cellular processes critical to tumor progression, including metabolism, cellular proliferation, chromatin remodeling, vascularization and invasion. However, gene-specific responses to both hypoxia and HIF1A signaling are highly tissue dependent. Given the complexity found with HIF1A gene regulation across tissues, we utilized an integrated genomics approach to identify a melanocyte-specific response to hypoxia, and investigate the role of HIF1A-responsive genes in melanoma tumor progression. We identified a cohort of 81 HIF1A direct target genes regulated by HIF1A signaling under hypoxia exposure, 19 of which are novel HIF1A targets. Analysis of individual gene expression levels for this cohort within the cutaneous melanoma primary tumor dataset generated by TCGA Research Network (http://cancergenome.nih.gov/) found that individual expression levels for 12 HIF1A direct target genes were significantly correlated with reduced time of Disease Free Status (DFS). The cumulative expression profile of the 12 targets also finds significant correlation with DFS by logistic regression (P-value =0.0013) and ROC curve analysis (AUC= 0.849, P-value <0.0001). This panel of HIF1A-responsive genes identifies a microenvironment-driven expression profile correlated with primary melanoma tumor progression to metastasis, and defines a melanocyte-specific gene response to regulation of metabolism, cellular proliferation, chromatin remodeling, and vascularization reflected in this gene set. Furthermore, this 12 gene panel provides targets for evaluation and refinement of diagnostic markers associated with primary melanoma tumor metastatic potential, and also provides gene targets for therapeutic strategies targeting HIF1A and hypoxia-driven metastatic disease progression.
Do morphological secondary sexual characteristics correlate with excited body coloration in Angel’s chameleons?
Randall L. Morrison (1) and Lorna Cudmore (2)

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Abstract:
Chameleons have a variety of secondary sexual characteristics that are used for intraspecific communication between individuals. Angel’s chameleons (Furcifer angeli) occur in the dry deciduous forests of northwest Madagascar. Morphological secondary sexual characteristics include a casque on the back of the head and a rostral appendage that extends beyond the mouth. These features are particularly prominent in large males. Angel’s chameleons also can change color from a subdued color pattern to a brighter excited color pattern. In this study we wished to determine if a more intense excited color pattern correlated with larger casques and rostral appendages. Various measurements of morphological features were made using digital calipers. We also measured the excited color state of three color pattern elements, the light vertical stripe, the dark vertical stripe and the horizontal white stripe using reflectance spectrometry. The large number of data points in each spectrum was reduced by averaging reflectance across the entire spectrum within 38 evenly distributed bins. This binned data was then used in a Principal Components Analysis (PCA) to generate principle components that can be analyzed statistically to determine if there is any statistically significant correlation with morphological characteristics. Several principle components of the dark, light and white stripes correlated significantly with rostral appendage measurements. Having two independent correlated signaling systems (morphological characteristics and range of color variability) may convey information at different stages of intraspecific interaction between individuals. Color may be used early in the display interaction and morphological features of the rostral appendage and casque during actual fights.

ORAL PRESENTATION  Oct. 6, 2:30 p.m.
Abstract:

**Background/Objective:** Galactomyces ferment filtrate (GFF, Pitera™) is a yeast derived extract currently used as a moisturizing agent in cosmetics. GFF demonstrates anti-aging and hypopigmenting effects on skin within 14 days. The mechanisms of action underlying GFF are relatively unknown and therefore are the focus of our study. **Methods:** In vitro human pigment cell models, including foreskin derived normal human epidermal melanocytes (NHEM) and human melanoma (SK MEL), were treated with or without GFF 0-10%. Melanin content was quantified by spectrophotometric assay at 490 nm. Tyrosine hydroxylase activity was determined with tritiated tyrosine and assayed by liquid scintillation. RNA-seq transcriptome profiling was completed with RNA extracted from NHEM. Reactive oxygen species (ROS) were visualized through fluorescent microscopy. Protein expression was quantitated by Western Blot and densitometric analyses. **Results:** GFF suppressed constitutive pigmentation in NHEM and SK MEL and reduced tyrosine hydroxylase activity in NHEM and protein lysate. However, GFF did not significantly alter the RNA levels of melanogenic proteins required for melanin synthesis (MITF, PMEL, TYR, TYRP1, TYRP2/DCT). GFF stifled the induction of ROS in NHEM exposed to 4-tertiary-butyphenol (4TBP). Expression levels of proteins from the antioxidant response elements (ARE) pathway were increased after GFF treatment, including transcription factor nuclear factor, erythroid 2-like 2 (Nrf2) and phase II enzymes heme oxygenase 1 (HO1) and NAD(P)H dehydrogenase, quinone 1 (NQO1). **Conclusions:** GFF inhibits melanin synthesis by deterring tyrosine hydroxylase activity. GFF also nullifies oxidative stress by priming the intracellular environment with increased antioxidant phase II enzymes via the Nrf2-ARE pathway.
The MITF family member tfec functions in zebrafish neural crest pigment cell fate diversification

Samantha A. Spencer (1) and James A. Lister (1,2)

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Abstract:
In organisms such as fish and reptiles where the neural crest produces more than one type of pigment cell, the existence of a lineage-restricted precursor of all pigment cell types has been speculated upon but not conclusively demonstrated. In zebrafish, null mutations in the microphthalmia-associated transcription factor (MITF) ortholog mitfa lack all neural crest-derived melanocytes but retain the other two neural crest pigment cell types, xanthophores and iridophores. In fact, mitfa mutants display an increased number of iridophores compared to wild-type, suggestive of a possible cell fate switch, and cell lineage experiments indicate the existence of a bipotent iridophore/melanocyte precursor. We have begun to examine another member of the zebrafish MITF family, tfec, which is expressed in premigratory and migrating neural crest cells and later in differentiating iridophores. Knockdown of tfec with antisense morpholino oligonucleotides, or by a CRISPR/Cas9 approach indicates that tfec is necessary for the development of iridophores. The leukocyte tyrosine kinase (ltk) gene has also been shown to be required for iridophore development, although its expression persists in neural crest cells lacking the Sox10 transcription factor, required for all three pigment cell lineages. Whereas mitfa expression in the melanophore lineage requires sox10, we observe that tfec, like ltk, is still expressed in sox10 mutant neural crest. However, expression of ltk appears to require tfec. Intriguingly, loss of tfec and mitfa together additionally eliminates the third pigment cell type, xanthophores. These data indicate that all three pigment cell lineages in zebrafish are dependent upon one or a combination of MITF family proteins. Moreover, they provide genetic evidence for a distinct pan-pigment precursor in the hierarchy of zebrafish neural crest cell fate diversification.

ORAL PRESENTATION  Oct. 6, 2:42 p.m.
Impact of heterozygosity of germline p16 and MC1R mutations on human melanocytes in vitro
Zalfa A. Abdel-Malek (1), Viki Swope (1), Kevin Choi (1), Steven Guard (1), Ayesha Anwar (1), Pamela Cassidy (2), and Sancy Leachman (2)

Abstract:
Carriage of germline mutations in both p16 and the melanocortin 1 receptor (MC1R) genes results in a greater increase in melanoma risk than expression of a mutation in either gene. To elucidate the possible interaction of p16 and MC1R mutations, we compared their effects on proliferation, senescence, and response to UV, of primary human melanocyte cultures derived from biopsies of carriers of germline V126D or 5'UTR-34G>T/+mutation in p16 with or without one MC1R red hair (RHC) variant, R160W or D294H. Melanocytes with these genotypes had comparable proliferation rates and doubling times to melanocytes with wild type p16 and MC1R. With increase in passage number, melanocytes heterozygous for p16 with or without MC1R mutation, similar to wild type melanocytes, exhibited reduction in proliferation rate and increase in senescence. The response of the formal melanocytes to single or multiple irradiations with UV was similar to that of wild type melanocytes in that they underwent cell cycle arrest and increase in doubling time, and repair of DNA photoproducts. Whether carriage of a p16 mutation would disrupt the function of MC1R in melanocytes heterozygous for MC1R RHC variant or wild type for MC1R was investigated. No effect of p16 mutation on stimulation of cAMP formation or enhanced repair of DNA photoproducts by a-MSH treatment was observed. Although epidemiological studies have linked heterozygosity for both p16 and MC1R mutations to exaggerated melanoma risk, our results suggest that melanocytes with this genotype do not behave differently in culture and in their response to UV from melanocytes with mutation in either gene, or wild type for p16 and MC1R. We postulate that alterations in the microenvironment and/or an additional hit, such as epigenetic silencing of p16I, promotes the malignant transformation of melanocytes heterozygous for p16 and MC1R mutations to melanoma.
MC1R and endothelin B receptor signaling activates common targets that modulate the DNA damage response of melanocytes

Viki B. Swope (1), Renny Starner (1), Ranjan Perera (2), and Zalfa A. Abdel-Malek (1)

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Abstract:
We had reported that activation of the MC1R by a-MSH and the endothelin-1 B receptor (ENDBR) by endothelin-1 (End-1) results in synergistic increase in human melanocyte (hMC) survival following UV irradiation, and that this is mediated by activating Mitf, resulting in increased Bcl2 levels, and Akt that inhibits Bad. We and others established that the MC1R signaling pathway enhances repair of UV-induced DNA photoproducts and reduces oxidative DNA damage. Recently we reported that ENDBR signaling reduces induction of DNA photoproducts, as well as enhances their repair in UV-irradiated hMC. We hereby report that although MC1R and ENDBR activate different signaling pathways, they affect common downstream targets that modulate the DNA damage response of hMC to UV. Activation of either receptor in UV-irradiated hMC resulted in the rapid phosphorylation of ATM and the translocation of phospho ATM and its downstream target BRCA1 from the cytoplasm to chromatin. Additionally, the nucleotide excision repair enzymes XPC and XPA localization on chromatin was markedly increased. Activation of MC1R or ENDBR induced the phosphorylation of the stress-induced MAP kinases JNK and p38, and resulted in accumulation of their downstream target p53. MicroRNA seq experiments revealed that in UV-irradiated hMC, activation of MC1R or ENDBR modulated the expression of common miRNAs, and reversed the effect of UV on a number of miRNAs. Expression of 2 red hair allelic variants of the MC1R results in reduced repair capacity due to lack of response to a-MSH. However, treatment of melanocytes expressing loss-of-function with End-1 enhanced their DNA repair capacity. Given that loss of function of MC1R increases melanoma risk, and the significance of DNA repair in prevention of photocarcinogenesis, we propose that downstream effector(s) of ENDBR might be druggable and targeted for melanoma prevention in high risk individuals, including those with loss-of-function MC1R.

ORAL PRESENTATION  Oct. 6, 4:42 p.m.
Regulation of Glutathione- and Thioredoxin-based Antioxidant Systems in Melanocytes and Melanoma

Pamela Cassidy (1), Matthew Honnegar (1), Madeleine Laws (1), Chelsey Kline (1), Zalfa Abdel-Malek (2) and Sancy Leachman (1)

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Abstract:
UV-induced reactive oxygen species are known to damage DNA in UV irradiated melanocytes. This damage, if left unrepaired, can result in mutations and ultimately to melanoma tumor formation. The thiol antioxidants glutathione (GSH) and thioredoxin (Trx) utilize the reducing power of NADPH to maintain redox homeostasis in the melanocyte, and to combat the deleterious effects of UV. Our labs have worked for some time to understand the regulation of these systems in melanocytes with the goal of designing new melanoma prevention strategies. These studies have led naturally to a focus on the transcription factor Nrf2, which regulates key elements of both GSH and Trx networks. We have examined these networks in normal melanocytes using a number of genetic and pharmacologic tools in immortalized cell lines and primary melanocyte cultures, including cultures from donors who have germline mutations in melanoma susceptibility loci MC1R and CDKN2A. We have also performed immunohistochemical analyses of nevus and tumor arrays for the Nrf2-regulated protein thioredoxin reductase 1, and we have interrogated TCGA to characterize the relationship between Nrf2-driven genes and their products with tumor progression and patient survival. We have found that although Nrf2 regulated proteins are beneficial in the setting of melanoma prevention, some of these proteins are also differentially expressed in melanomas relative to normal melanocytes. This raises concerns for the safety of chemoprevention agents that modulate the transcriptional activity of Nrf2 because such agents might be harmful to patients who have undiagnosed or early stage tumors that could progress. We are modeling this scenario in pre-clinical cell and organ culture models.

ORAL PRESENTATION Oct. 8, 11:12 a.m.
Adoptive transfer of TCR-transgenic, tyrosinase reactive T cells can induce human vitiligo

Emilia Dellacecca (1), Tamson Moore (1), Gina Scurti (1), Kelli Hutchens (2), Joseph I Clark (3), Courtney Regan (3), Elizabeth Garrett-Mayer (4), Michael Nishimura (1), I. Caroline Le Poole (1,2)

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Abstract:
Vitiligo patients present with progressive depigmentation in response to melanocyte-reactive T cells, while melanoma is caused by proliferation of malignant melanocytes. Tyrosinase, involved in melanin production, is expressed by both melanocytes and melanoma cells. A phase 1 dose escalation trial was developed wherein melanoma patients undergo adoptive T cell transfer. Patient T cells are transduced with a lentivirus encoding the 1383i T cell receptor reactive to tyrosinase 368-377, and truncated CD34. We postulated that successfully treated patients would develop vitiligo. In cohort 1, 3 eligible patients with intratumoral HLA-A2 and tyrosinase expression were lymphodepleted before infusing 2.5 X10^6/kg transgenic T cells and treatment with low dose IL-2 for a week. Transgenic T cells were followed in the blood over time and punch biopsies were taken from depigmenting skin to determine if vitiligo was associated with transgenic T cell infiltration. Patient lymphocytes were stained for transgene expression by FACS analysis and biopsies were cryosectioned and stained with fluorescent antibodies to CD3, CD34, and Vß12 (to the 1383i TCR) and a DAPI nuclear stain. Staining was evaluated by confocal microscopy. Circulating transgenic T cells spiked between 2 and 3 weeks after adoptive transfer. Patient 1 expired within 3 months of treatment. Patient 2 exhibited initial tumor remission and was treated by Pembrolizumab and high-dose IL-2 upon relapse, resulting in a second transgenic T cell spike and total vitiligo 14 months after treatment; this patient is now tumor free. Patient 3 developed vitiligo three weeks after treatment and has since expired. Transgenic T cells were observed at 2-fold and 12-fold increased abundance compared to blood samples, at 4% and 13% of infiltrating T cells, respectively. These therapeutic procedures presented an opportunity to induce vitiligo in humans and show that vitiligo is a powerful measure of T cell responses and anti-tumor efficacy.

ORAL PRESENTATION Oct. 6, 11:17 a.m.
Driving NKT cell-mediated immune response toward ganglioside D3

Steven W. Henning (1), Levi W. Barse (1), Manuel F. Fernandez (1), Jonathan M. Eby (1), Adam M. Hammer (1), Edward R. Kessler (1,2), Emily R. Gilbert (1,2), Daniel F. Dilling (1,2) and I. Caroline Le Poole (1,2)

Abstract:
GD3 is expressed in tumors of neuroendocrine origin, such as melanoma, with limited expression in normal tissue. We questioned whether vaccinating with the gene encoding for the enzyme GD3 synthase (SIAT8) can drive an NKT cell-mediated immune response toward GD3. An expression plasmid and recombinant human adenovirus type 5 encoding for mouse SIAT8 was generated. Mouse vascular smooth muscle cells were transfected or transduced with the respective construct to assess resulting GD3 expression. C57BL/6 and CD1d knockout (KO) mice were gene gun vaccinated weekly for 5 weeks with adjuvant HSP70i and either empty vector (EV), SIAT8 or OptTRP-1 plasmid DNA and tumor challenged bilaterally with or without low or high GD3 expressing B16.F10 melanoma cells. NK/T/NKT cells were quantified by fluorocytometry. C57BL/6 mice were injected with a single dose of EV or SIAT8 adenovirus and tumor challenged bilaterally with low GD3 expressing B16.F10s. Immune monitoring was performed. All SIAT8 transfected or transduced cells expressed significantly increased levels of GD3. GD3 overexpressing tumor cells exhibited accelerated tumor growth. Gene gun vaccinated SIAT8 and OptTRP-1 C57BL/6 mice developed significantly smaller tumors than DV vaccinated mice, and OptTRP-1 vaccinated mice depigmented, but SIAT8 vaccinated mice did not. SIAT8 vaccinated C57BL/6 mice developed significantly smaller tumors than CD1d KO mice, which lack NKT cells, implicating NKT cell dependent responses. High GD3 expressing tumors from SIAT8 vaccinated C57BL/6 mice contained 9 NKT cells per mm2 versus 5 from EV vaccinated C57BL/6 tumors, a significant increase. C57BL/6 mice injected with SIAT8 adenovirus saw 42% reduced tumor growth 17 days post-tumor challenge, and qRT-PCR trended toward increased levels of IL-17 in these tumors. We conclude that GD3 contributes to tumor growth and that vaccination with the underlying enzyme can support anti-tumor responses in mice.
Vacuolar protein sorting 11 gene promotes chemoresistance in zebrafish melanocytes

Kersten A. Peterson¹, Miranda E. Bean², Jallanie V. Negussie², Lauren F. Clancey², Allison B. Coffin³ and Cynthia D. Cooper², ⁴

Abstract:
Melanoma, cancer of black pigment cells or melanocytes, is the deadliest form of skin cancer and its treatment is inefficient due to its inherent resistance to chemotherapy. Using model organism zebrafish, our long term goal is to improve the treatment of melanocyte-related disorders, such as melanoma, by elucidating the cell biological mechanisms that promote chemoresistance. Here, we test the hypothesis that vacuolar protein sorting (Vps) 11 gene promotes drug sequestration within melanocyte specific organelles, melanosomes. The approach for testing this hypothesis involves using a zebrafish melanocyte mutant harboring a loss of function mutation in Vps11, whose protein product belongs to the vesicle tethering complex, HOPS. Due to the ease of adding chemotherapeutic drugs to fish water, conserved roles for Vps proteins to mammals and structurally similar skin architecture, zebrafish larvae offer a biologically relevant model for understanding chemoresistance in humans. Following treatment with chemotherapeutic drug, cisplatin, we note a drop in the number of mutant and wildtype melanocytes as compared to untreated controls. However, the percentage of melanocytes lost in mutant larvae is significantly greater. Additionally, this increase in drug sensitivity in Vps11 mutants was melanocyte specific as confirmed by similar analysis of cisplatin treated hair cells. Thus, Vps11 promotes resistance to cisplatin in melanocytes. Future research goals include further elucidation of the mechanism of chemoresistance as well as testing of other melanoma relevant drugs in zebrafish and in melanoma models.
Abstract No: 34

**In vivo function of Vitamin D Receptor (VDR) signaling in UVB-induced DNA damage and melanocyte homeostasis**

Sharmeen Chagani¹,², Sergiy Kyryachenko², Yoko Yamamoto³, Shigeaki Kato⁴, Gitali Ganguli-Indra¹,² and Arup K. Indra¹,²,⁵,⁶,⁷,⁸

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Abstract:
**Background:** Loss of VDR expression is inversely correlated to melanoma progression in humans. Certain VDR polymorphisms are associated with malignant melanoma risk and may be of importance for melanoma pathogenesis. **Methods:** VDR was selectively ablated in the melanocytes in vivo using the Cre-loxP strategy and effects of acute UVB exposure on neonatal mice was evaluated by histopathological and immuno-histochemical (IHC) analyses. Vitamin D3 was topically applied on the mutant and control mice skin and the protective effects against UVB induced DNA damage was evaluated by IHC analyses. **Results:** In vivo ablation of VDR in melanocytes (VDRmel⁻/⁻ mice) reduced percentage of melanocyte precursors and mature differentiated melanocytes, as well as melanocyte proliferation, both pre- and post-UVB irradiation. VDR ablation significantly increased the proportion of UVB induced Cyclopyridine dimer (CPD) positive melanocytes in the VDRmel⁻/⁻ mice. Topical application of VitD3, inhibited UVB induced DNA damage of melanocytes in the presence of functional VDR and this inhibition was abrogated in the absence of melanocytic VDR. VDRmel⁻/⁻ mice also exhibited a reduction in melanocyte apoptosis after UV exposure. **Conclusions:** Our results identify a photo-protective role of VDR in murine melanocytes against UVB induced DNA damage in vivo and establish its critical cell-autonomous role in regulating responses of melanocytes to neonatal UVB exposure by controlling their proliferation, differentiation and overall survival (1). The in vivo protective effects of Vitamin D3 against the UV-induced DNA damages is mediated by functional VDR. **Reference:** Chagani S, Kyryachenko S, Yamamoto Y, Kato S, Ganguli-Indra G, Indra AK. In vivo role of Vitamin D Receptor (VDR) signaling in UVB induced DNA damage and melanocyte homeostasis. J Invest Dermatol. 2016 Jun 17. pii: S0022-202X(16)31352-5. doi: 10.1016/j.jid.2016.06.004.

ORAL PRESENTATION  Oct. 6, 5:06 p.m.
Targeted deactivation of cancer-associated fibroblasts by beta-catenin ablation suppresses melanoma growth
Linli Zhou, Kun Yang, Randall R. Wickett and Yuhang Zhang

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Abstract:
Background: v-Raf murine sarcoma viral oncogene homolog (BRAF) mutations occur in over 50% of cutaneous melanoma. Melanoma cells actively interact with a heterogeneous mix of non-cancer cells in the surrounding microenvironments. Genetically stable fibroblasts in the melanoma stroma, termed as cancer-associated fibroblasts (CAFs), remodel the extracellular matrix (ECM) and secrete chemical factors, which altogether support tumor cells to grow, migrate and evade cell death induced by therapeutic agents. We recently discovered loss of beta-catenin signaling deactivated fibroblasts and reduced the production of growth factors and ECM proteins.

Methods: To determine the in vivo effects of CAF deactivation on melanoma growth, we ablated β-catenin expression in melanoma-associated CAFs after Braf-driven melanoma tumor was formed. Tumor growth was carefully monitored and compared between deactivated CAF and control groups.

Results: Our results showed that melanoma growth was significant suppressed after CAFs were deactivated. Apace with this finding, melanoma cell growth was significantly inhibited while cell death was increased. Moreover, reduced ECM protein collagen and fibronectin production were found in Braf-driven melanoma tumors with deactivated CAFs. Expression of various cell cycle proteins, including cyclin D, and cyclin-dependent kinases (CDKs), was down-regulated while production of CDK inhibitors p16, p18, p21 and p27 was enhanced. Further analysis revealed that expression of phospho-Erk1/2 and phospho-Akt was greatly reduced in Braf-driven melanoma tumor upon CAF deactivation, accounting for the likely cause of inhibited melanoma growth. Conclusion: Overall, our data suggested that CAFs reciprocally interact with melanoma cells to promote their co-evolution. Melanoma growth could be suppressed by targeted CAF deactivation via tumor-stroma interactions. Thus, targeting CAFs has the potential to be a novel therapeutic approach for improved melanoma treatment.
Novel mutations in LYST, a gene mutated in Chediak-Higashi disease
Elena-Raluca Nicoli, Chulaluck Kuptanon, Camilo Toro, May Christine Malicdan, William A. Gahl, Wendy J. Introne

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Abstract:
Chediak-Higashi disease (CHD; OMIM #214500) is a rare autosomal recessive disease with congenital immunodeficiency resulting from mutations in the lysosomal trafficking regulator gene (LYST OMIM #606897). CHD is characterized by hematologic, immunologic, pigmentary, and neurologic manifestations with a predilection for hemophagocytic lymphohistiocytosis (HLH), also known as the accelerated phase. The LYST gene has 53 exons with only 51 of them encoding a 429-kDa protein made of 3801 amino acids. More than 65 mutations have been reported so far, scattered throughout the gene. Through our NIH clinical protocol (NCT00005917, Study of Chediak-Higashi Syndrome, clinicaltrials.gov), we have investigated patients with CHD and performed extensive clinical evaluations and molecular genotyping. In our cohort, we have 6 patients with novel mutations in LYST. All of the patients presented with characteristic features and were confirmed to have CHD by identifying giant inclusions with the leucocytes. Analysis of dermal fibroblasts from forearm skin biopsies revealed perinuclear and enlarged lysosomes and reduced LYST mRNA levels, features that are typically observed in CHD. Our results are valuable for understanding the spectrum of disease in CHD, establishing diagnosis, and genotype-phenotype correlation.
Deletion of chromosome 2q37.3 as a driver of metastasis in melanoma
Archana Gopalan¹, Kasey L Couts,¹ Ichiro Nakachi,² Yuchun Luo,¹ Hieu Van,¹ Akihiro Fujisawa,¹ Steven E Robinson,² William A Robinson,² Mark W Geraci,² and Mayumi Fujita¹,³

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Abstract:
Metastasis is responsible for most cancer-related deaths in melanoma. Recent studies have elucidated that genetic events responsible for metastasis are pre-programmed into primary tumors. To test this, we used SNP arrays to analyze the genomics of 35 melanoma samples; 10 patient tumors (F0) and their passage in a patient-derived xenograft (PDX) model (F1, F2, F3). Our data show primary melanoma tumors develop more genomic changes than metastatic melanoma tumors when passaged through generations of the PDX model. Interestingly, in patient-matched primary/metastatic tumors, the primary tumor developed nearly identical genomic alterations to those in the metastatic tumor (correlation between primary F0 and metastatic F0, R=0.24; primary F3 and metastatic F0, R=0.77), implying that metastatic genetic changes are pre-programmed into the primary tumor. Most changes that evolved were deletions, including regions in chromosomes 2, 7, 10 (PTEN region), 15 and 16. We identified a 1.27Mb homozygous deletion in chromosome 2q37.3 that becomes apparent in primary melanoma PDX tumors (F1-F3) when compared with the F0 tumor and that was maintained in metastatic melanoma PDX tumors. This deletion was confirmed by FISH and copy number PCR. SNP array data analysis of melanoma tumors in cBioPortal tumor database (n=336) showed that Chr2q37.3 deletion occurs in ~ 17% of tumors. CRISPR/Cas9 system was used to create chromosomal deletion in primary and metastatic melanoma cell lines, and the successful deletion of the 1.27Mb region that contains 16 genes was confirmed using copy number PCR in A375 cells. Chr2q37.3-deleted A375 clones displayed higher sphere formation capacity in a sphere assay compared to control clones. Other metastatic phenotypes are to be examined. In summary, we have identified a novel deletion (Chr2q37.3) that contains potential metastasis suppressor genes in melanoma and demonstrate that metastatic melanoma genetic changes are pre-programmed into primary melanoma.

ORAL PRESENTATION  Oct. 8, 8:42 a.m.
Abstract No: 38

**SASH1 is a novel gene involved in human skin pigmentation**

Karoline A. Lambert(1), Adam Almeida(1), Donald S. Backos(2), David Norris(1), Kristin Artinger(3), Theresa Pacheco(1) and Yiqun G. Shellman(1)

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**Abstract:**
Dysregulation of melanocyte functions leads to disease, including pigmentation abnormalities. Lentigines are a common form of hyperpigmentation in human skin; however, the etiology of lentigines is not well defined. Here we studied a unique autosomal dominant hyper-pigmented lentiginous phenotype associated with a SNP in the SASH1 gene in a family of Hispanic descent. The variant is a heterozygous missense substitution in exon 13 of SASH1, c.1556 G>A, p. S519N, and is the only SNP that co-segregated perfectly with the disease in this family. The function of SASH1 is largely unknown. Histological examination of skin biopsies from affected individuals showed increased numbers of melanocytes and increased epidermal cell proliferation. Many known familial lentigines are classified as RASopathies, a group of rare genetic conditions caused by mutations in genes of the RAS/RAF/MAPK pathway. Transfection experiments with human melanocytes in vitro showed that SASH1 over-expression did not alter MAPK signaling, but instead activation of RAS/RAF/MAPK signaling upregulated SASH1 expression, suggesting SASH1 is downstream of this pathway. Molecular computational modeling indicates that the S519N mutation alters the confirmation of a putative binding interface within the SASH1 protein, suggesting that the mutation affects SASH1’s interaction with its partners. We are performing yeast two-hybrid screens to identify potential binding partners, and are developing both CRISPR-Cas9 mediated SASH1 knockout zebrafish and a patient-derived iPSC model for further investigation of this disease. Considering the recent reports of three additional inherited SASH1 mutations in patients with lentiginous phenotypes, SASH1 is emerging as a novel gene involved in human pigmentation. Our preliminary studies suggest the SASH1-S519N disorder could be a RASopathy, and a better understanding of SASH1’s functions may provide new insights into the development of hyper-pigmentation in human skin.

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ORAL PRESENTATION Oct. 7, 9:40 a.m.
The Role of Endothelin 3 during Melanoma Lung Premetastatic Niche Formation

Juliano Freitas, Jospeh Palmer, Martina Cavallini and Lidia Kos

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Abstract:
A number of studies have shown that there is an intricate crosstalk between primary tumors and future sites of metastases in order to create a suitable microenvironment in the distant organs for the tumorigenic cells. This microenvironment is called the premetastatic niche, and is set to receive disseminating cancer cells, and support the growth of the metastatic colony. Melanoma is a highly metastatic cancer which preferentially establishes secondary lesions in the lungs. Human melanoma initiation is mainly driven by activation of oncogenic protein BRaf and deletion of PTEN gene and has recently been modeled in mouse cell lines. The cytokine Endothelin 3 (Edn3) and its receptor Endothelin receptor b (Ednrb) have been implicated in melanoma metastasis. Our laboratory has created a mouse model (K5-Edn3) that overexpresses Edn3. In order to establish whether Edn3 drives melanoma lung premetastatic niche formation we injected 3 different BRaf^{V600E/+};PTEN^{-/-} murine melanoma cell lines (D4M, YUMM1.1 and YUMM1.7) into K5-Edn3 and control mice. The appearance of bone marrow derived cells (BMDCs) clusters and metastatic cells in the lungs was monitored by immunofluorescence and flow cytometry at different time points of tumor progression. Analysis of the earlier stages of tumor development has demonstrated there are no differences in the numbers of BMDCs clusters and metastatic cells between K5-Edn3 and control mice. Although none of the three cell lines express Ednrb in vitro, upon injection into K5-Edn3 mice YUMM1.1-derived tumors expressed Ednrb. Interestingly, YUMM1.1 tumors produced into K5-Edn3 mice were statically larger than the control mice and these tumors were able to metastasize to the lungs. These data suggest that Edn3/Ednrb signaling may be an important player in melanoma progression and the metastatic potential of a BRaf^{V600E/+};PTEN^{-/-} murine melanoma cell line.

POSTER PRESENTATION  Oct. 6, 3:15 – 4:30 p.m.
Abstract

NLRP1 plays a positive role in melanoma chemoresistance
Zili Zhai (1), Archana Gopalan (1), David A. Norris (1,2), Richard A. Spritz (1) and Mayumi Fujita (1,2)

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Abstract:
NLRP1 inflammasome is a NF-kB activator and a tumor promotor in metastatic melanoma. Since activated NF-kB has been shown to confer melanoma resistance to temozolomide (TMZ), we investigated the involvement of NLRP1 in TMZ resistance in metastatic melanoma cells. Pharmacological relevant doses of TMZ not only enhanced NLRP1 expression and NF-kB activity, but increased the expression of Notch1 and ALDH1A3, two key stem cell regulators, in 1205Lu and HS294T cells. To understand the role of NLRP1 in TMZ resistance, TMZ-resistant 1205Lu and HS294T cells were generated, which showed increased NLRP1 inflammasome activation, IL-1ß secretion, NF-kB activity, and expression of Notch1 and its downstream molecules, including XIAP, BCL-XL, cyclin D1, and PI3K/AKT, all contributing to cell survival. Whereas Notch1 inhibitor DAPT inhibited TMZ-induced NLRP1 and ALDH1A3 expression in parental 1205Lu and HS294T cells, IL-1 receptor antagonist (IL-1Ra) had no influence on TMZ-induced Notch1 expression in parental cells and silencing NLRP1 did not alter Notch1 expression in both parental and resistant cells. However, IL-1Ra and NLRP1 knockdown decreased ALDH1A3 expression. Moreover, we found that either NLRP1 silencing or DAPT treatment partially increased the sensitivity of resistant cells to TMZ. These data suggest that upregulated expression of NLRP1 and Notch1 confers melanoma resistance to TMZ and that NLRP1 is an effector downstream of Notch1 signaling but a positive regulator of ALDH1A3. We report a new role of NLRP1, known as a danger signaling sensor, in tumor chemoresistance.
Identification and Characterization of the Metastatic Cell Populations in a Mouse Model of Melanoma
Xiaoshuang Li and Lidia Kos

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Abstract:
Melanoma is the deadliest form of skin cancer due to its high propensity to metastasize and resistance to current therapies. We have created an inducible mouse model of metastatic melanoma (Dct-Grm1/K5-Edn3) where metastasis to the lungs is 80% penetrant. The primary tumors of these mice present cellular heterogeneity with cells at varying levels of differentiation. The main goal of this study is to determine if the primary tumor resident Tyrosinase positive cells are the major contributor to lung metastases and evaluate the dynamic pattern of gene expression as those cells move from the primary tumors to the sites of metastasis. To accomplish this aim we crossed the Dct-Grm1/K5-Edn3 mice to CreERT2/ ROSAmT/mG mice to indelibly label Tyrosinase cell populations within the primary tumor and perform lineage tracing in the metastatic lesions. We found that Tyrosinase positive cells enter the circulation at the very early stages of tumor progression and establish close interactions with blood vessels. Metastatic cells in close association with the inner wall of the blood vessels loose pigmentation and do not express melanocytic markers. In the lung tissue they are capable of establishing successful metastases. The results of this study will increase our understanding of the etiology and pathogenesis of melanoma metastasis. Further characterization of those more aggressive cells in melanoma will allow for the development of new prognostic tests and novel therapeutic strategies to eliminate metastasis.
Abstract No: 42

A role for the PERK arm of the unfolded protein response in determining melanocyte viability and the pathogenesis of vitiligo
Omotayo A. Arowojolu, Seth J. Orlow and Prashiela Manga

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Abstract:
Vitiligo, characterized by progressive skin depigmentation, results from autoimmune-mediated melanocyte loss. The mechanisms underlying disease onset are poorly delineated. Triggers, including exposure to phenols such as monobenzone (MB), are thought to disrupt melanocyte homeostasis and ultimately instigate an autoimmune reaction. We have shown that MB disrupts cellular homeostasis and induces endoplasmic reticulum (ER) stress that leads to activation of the unfolded protein response (UPR). Three proteins, including PERK, each activate UPR arms that orchestrate the restoration of homeostasis. When activated, PERK phosphorylates eIF2α, a translation initiation factor, thus reducing protein synthesis and ER stress. In this study, we investigated the impact of the PERK-eIF2α cascade on melanocyte viability and sensitivity to MB.

Results: Basal levels of phospho-eIF2α are higher in melanocytes compared to cutaneous fibroblasts or keratinocytes. When PERK expression is downregulated by RNAi, there is a significant reduction in melanocyte viability (88% decrease, p<0.05 shPERK versus non-target/shNT; n=3). Some melanocytes (shPERKLT) can however survive despite prolonged PERK downregulation. Survival correlated with a paradoxical increase in phospho-eIF2α levels and reduced sensitivity to MB (Cleaved/c-PARP levels lower in shPERKLT cells treated with 400μM MB compared to shNT cells). Chemical inhibition of PERK kinase activity, using GSK2606414, prevented eIF2α phosphorylation and sensitized melanocytes to MB (c-PARP observed with 250μM MB + GSK2606414, compared to 400μM MB + vehicle). Conclusion: PERK-eIF2α axis activity contributes to melanocyte viability and determines sensitivity to MB. Pathways, such the UPR, which has also been implicated in autoimmune diabetes, may link exposure to vitiligo-inducing triggers and onset of autoimmunity. These pathways represent novel therapeutic targets to prevent vitiligo progression or improve efficacy of repigmentation protocols.
The Ezh2 polycomb group protein drives an aggressive phenotype in melanoma cancer stem cells and is a target of diet derived sulforaphane


Abstract:
Melanoma is a metastatic cancer associated with poor survival. Here, we study a subpopulation of melanoma cancer cells displaying melanoma cancer stem cell (MCS cells) properties including elevated expression of stem cell markers, increased ability to survive as spheroids, and enhanced cell migration and invasion. We show that the Ezh2 stem cell survival protein is enriched in MCS cells and that Ezh2 knockdown or treatment with small molecule Ezh2 inhibitors, GSK126 or EPZ-6438, reduces Ezh2 activity. This reduction is associated with a reduced MCS cell spheroid formation, migration, and invasion. Moreover, the diet-derived cancer prevention agent, sulforaphane (SFN), suppresses MCS cell survival and this is associated with loss of Ezh2. Forced expression of Ezh2 partially reverses SFN suppression of MCS cell spheroid formation, migration, and invasion. A375 melanoma cell-derived MCS cells form rapidly growing tumors in immune-compromised mice and SFN treatment of these tumors reduces tumor growth and this is associated with reduced Ezh2 level and H3K27me3 formation, reduced matrix metalloproteinase expression, increased TIMP3 expression and increased apoptosis. These studies identify Ezh2 as a MCS cell marker and cancer stem cell prevention target, and suggest that SFN acts to reduce melanoma tumor formation via a mechanism that includes suppression of Ezh2 function.
YAP1 and TAZ as therapeutic targets in BRAF inhibitor-resistant melanoma
Matthew L. Fisher, Daniel Grun, Gautam Adhikary, Richard L. Eckert

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Abstract:
Treating BRAF-inhibitor-resistant melanoma is an important therapeutic goal. A variety of approaches have been tried, but as yet a reliable treatment has not been developed. YAP1 and TAZ are adaptor proteins that interact with TEAD transcription factors in the Hippo signaling pathway, and are important drivers of cancer cell survival. In the present manuscript we explore whether YAP1/TAZ are therapy targets in PLX4032-resistant melanoma. We show that PLX4032-resistant cells display elevated YAP1/TAZ levels and that YAP1/TAZ serve as PLX4032-resistance factors. We further show that Verteporfin, an agent that inhibits YAP1 interaction with TEAD transcription factors, reduces survival of PLX4032-resistant cells via a mechanism that involves dissociation of the YAP1/TEAD complex and loss of YAP1/TAZ and TEAD transcription factors. Verteporfin also inhibits tumor formation via a mechanism that also involves loss of YAP1, TAZ, and TEAD factors. Based on these studies, we propose that Verteporfin is a candidate agent for the treatment of PLX4032-resistant melanoma.
Abstract:

Melanoma is an aggressive skin cancer, which arises from the transformation of normal melanocytes. The five-year survival rate of early stage melanoma can be high after adequate treatment. However, metastatic disease is difficult to treat. Hence, understanding the determinants of melanoma metastasis will provide new strategies for treatment therapies. Nm23-m1 is the first described metastasis suppressor gene. Previous studies have shown that it is involved in regulating melanoma metastasis, repair of UV-induced DNA damage, cell morphology, self-adhesion, and motility. However, the functional roles and molecular mechanisms of Nm23-m1 in melanocytes in vivo are not well understood. Here, we crossed Nm23-m1m2 double knockout heterozygous mice to Dct-H2BGFP mice, whose GFP expression pattern matches with endogenous Dct. Fluorescence-activated cell sorting was used to isolate GFP expressing melanocytes from P11 Nm23-m1m2 heterozygous deficient pups and wild-type littermates. To determine genes regulated by Nm23-m1 and Nm23-m2 in vivo, expression microarray analysis was done. Compared with wild-type melanocytes, several cell adhesion related genes were upregulated in Nm23-m1m2 deficient melanocytes. Interestingly, the most significant change was in Ccl27, encoding the chemokine Ccl27 which plays important roles in skin lymphocyte trafficking and inflammation through interaction with its cognate receptor Ccr10. Quantitative RT-PCR results confirmed that Ccl27 was upregulated in Nm23-m1m2 deficient melanocytes. Flow cytometry analysis showed that expression of the pan-T cell marker CD3e was increased in Nm23-m1m2 deficient skin, although the total number of CD3e-expressing T cells remained unchanged. These results suggest that Nm23 deficiency correlates with cutaneous immune activation. Further analysis will be performed to determine the basis for increased CD3e expression in Nm23-deficient skin.
Characteristics of Quiescent Hair Follicle Melanocytes during Anagen

Bishal Tandukar (1), Sandeep Joshi (1), Jennifer Huang (1), Thomas J. Hornyak (1, 2)

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Abstract:
Melanocyte stem cells (MeSCs) are key components of the hair follicle (HF) stem cell system that are derived from neural crest during embryogenesis and are responsible for regeneration of differentiated melanocytes during successive HF cycles. Our analysis of MeSC quiescent and proliferative properties throughout the HF cycle using BrdU, surprisingly revealed quiescent melanocytes maintained outside of the HF bulge region throughout the anagen. This observation has implications for maintenance of MeSCs throughout successive follicular cycles. Here, we wanted to characterize more fully these quiescent anagen melanocytes and compare them to their proliferative counterparts. Our Dct-H2BGFP bitransgenic doxycycline-regulated mouse model permits accurate identification of the MeSCs and melanocytes in murine HFs through GFP expression. To confirm the existence of quiescent GFP expressing cells during anagen, we treated the animals with doxycycline from P19 to P30 to extinguish the expression of GFP. The results show a subpopulation of Ki67- and GFP-retaining cells predominately in the HF outer root sheath (ORS), confirming their quiescence compared to proliferating and differentiated melanocytes located at the HF bulb with low GFP retention and higher Ki67 expression. Further investigation shows existence of Kit+ Nestin+ quiescent melanocytes at the HF bulge and Kit+ Nestin- quiescent melanocytes below the bulge area. Both subpopulations of quiescent melanocytes express lower levels of melanocyte differentiation markers Mitf, Dct, Tyrp1 and Tyr compared to proliferating Kit- Nestin- melanocytes located at HF bulb. These results suggest that quiescent melanocytes, localized in the ORS above the HF bulb in anagen, retain the stem cell phenotype observed in quiescent MeSCs during telogen.

ORAL PRESENTATION  Oct. 7, 3:00 p.m.
Transcriptional coactivators YAP1 and TAZ drive melanoma progression and survival through differential pathways
Jason W. Luï (1), Kelsey Ogomori (1), and Deborah Lang (1,2)

Abstract:
Melanoma is a disease with an aggressive nature, resistance to apoptosis, and a high degree of metastasis. YAP1 (Yes associated protein 1) and TAZ (WW domain-containing transcription regulator protein 1) are transcriptional coactivators that have both recently been implicated to be active drivers of many cancers, including melanoma. Traditionally YAP1 and TAZ have been shown to bind transcription factors and subsequently drive expression of antiapoptotic and proliferative genes. They have similar structures, and are often cited as paralogs with identical function but differing levels of expression. We find that both YAP1 and TAZ are expressed in a panel of melanoma cell lines. To determine the consequences of loss of YAP1 and/or TAZ in melanoma progression and survival, expression of YAP1, TAZ, or both was inhibited via siRNA mediated knockdowns in A375 and mel537 melanoma cells. While YAP and TAZ are often thought of as redundant in function, we found that the YAP and TAZ knockdown groups had major differences in cellular morphology, with YAP knockdowns leading to higher levels of dendritic extensions as compared to both control and TAZ knockdown groups. We next performed RNA-sequencing on the three knockdown groups (siYAP1, siTAZ, siYAP1/TAZ) and compared to siScrambled control to generate differentially expressed gene profiles. Comparisons of three groups showed vastly different profiles between each one. Although YAP1 and TAZ are traditionally thought to have similar structure and overlapping function, our data suggests this might be not the case in melanoma as there is evidence for both YAP1 and TAZ specific targets.
Abstract No: 48

Outcomes of melanoma in situ treated with Mohs micrographic surgery compared with wide local excision
Adi Nosrati (1,2), Jacqueline G. Berliner (1,2), Joseph McGuire (1), Shilpa Goel (2), Vera Morhenn (2), Juliana R. de Souza (1), Yildiray Yeniay (1), Ann Griffin (1), Barbara Grimes (1), Eleni Linos (1), Mary M. Chren (1,2), Roy Grekin (1), Maria Wei

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Abstract:
Melanoma in situ (MIS) is increasing in incidence; expert consensus opinion recommends surgical excision for treatment and wide local excision (WLE) is standard of care. A growing subset of individuals with MIS is treated with Mohs micrographic surgery (MMS), in which the entire surgical margin is evaluated intraoperatively. We assessed the outcomes of MIS treated with MMS compared with those treated with WLE at an academic tertiary care referral center, using a prospective, single-institution, multidisciplinary database from 1983 to 2013, with 385 WLE and 277 MMS treated patients. The median follow-up interval was 8.6 years [range 0.2-37 years]. Unadjusted recurrence rate did not differ between the MMS and WLE treatments (HR=0.41, 95% CI 0.15, 1.09); no difference in recurrence was seen after adjustment for risk factors (HR=0.55, 95% CI 0.18, 1.70). There was no difference in overall survival for patients treated with MMS compared with WLE in the unadjusted (HR=1.25, 95% CI 0.83, 1.88) or adjusted (HR=0.87, 95% CI 0.53, 1.42) model. Melanoma specific survival did not differ between the two groups (HR=0.80, 95% CI 0.17, 3.80). MMS can be utilized for accommodating anatomic considerations, to achieve clear clinical margins and can provide an effective alternative therapeutic technique to WLE.
Using CRISPR Cas9 to generate thioredoxin reductase 1 (TR1) knock-outs in the human melanoma cell line, M-14
Chelsey D Kline, Pam Cassidy, Sancy Leachman, and Madeleine Laws

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Abstract:
Antioxidant networks are involved in cell signaling and redox sensitive pathways which play an important role in maintaining the health of a melanocyte. Detoxification of ROS produced by exposure of the skin to the sun’s UV radiation is controlled in part by the antioxidant networks glutathione (GSH) and thioredoxin reductase 1 (TR1) (Denat et al., 2014). Recently our lab found that TR1 expression levels varied in human melanoma tissues, with increased expression being associated with advanced stages of melanoma and increased metastasis (Cassidy et al., 2015). Based on these observations we believe that a melanocyte’s ability to respond to UV-induced oxidative stress might be the basis of increased risk for melanoma, while hyper activation of the TR1 antioxidant system facilitates progression of disease in melanomas. We have started to investigate the importance of the antioxidant network(s) in the human melanoma cell line M-14, which has the highest level of TR1 in all of the melanoma lines in the NCI 60 panel (Gholami et al., 2013). In order to directly evaluate the role of TR1 in M-14 cell lines we are using the genome editing tool CRISPR Cas9 to generate TR1 knockouts and assess the effects on the antioxidant response to simulated solar radiation. In this study, we hope to elucidate the mechanisms by which TR1 levels effect response of the complex antioxidant networks of the melanocytes to UV-induced oxidative stress and oncogenic transformation. This information is vital for developing effective chemoprevention agents in addition to understanding the mechanism of melanoma progression.
Inhibition of Vemurafenib-associated MAP Kinase (MAPK) activation and induction of melanization in BRAF\textsuperscript{WT} melanoma cells by 17-AAG

Sandeep S. Joshi\textsuperscript{(1)}, Shunlin Jiang\textsuperscript{(4)}, Emmanuel Kalapurakal\textsuperscript{(1)} and Thomas J. Hornyak\textsuperscript{(1,2,3,4)}

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Departments of \textsuperscript{(1)}Biochemistry and Molecular Biology, \textsuperscript{(2)}Dermatology, University of Maryland School of Medicine, \textsuperscript{(3)}VA Maryland Health Care System, Baltimore, Maryland, USA, \textsuperscript{(4)}Dermatology Branch, CCR, NCI, NIH, Bethesda, Maryland, USA.

Abstract:
Heat shock protein 90 (HSP90) is a molecular chaperone which stabilizes client proteins with important roles in tumor growth. 17 allylamino-17-demethoxygeldanamycin (17-AAG), an inhibitor of HSP90 ATPase activity, occupies the ATP binding site of HSP90 causing a conformational change which destabilizes client proteins and directs them towards proteosomal degradation. Melanomas have active MAPK signaling due to a BRAF\textsuperscript{V600E} mutation or activation of BRAF\textsuperscript{wt} and CRAF through mutations in NRAS or KIT. 17-AAG inhibits cell growth in both types of melanomas (mutant BRAF and NRAS) and selectively induces degradation of BRAF\textsuperscript{V600E} which is a HSP90 client protein but does not affect the stability of BRAF\textsuperscript{wt}. Here, we demonstrate that BRAF\textsuperscript{wt} and CRAF are bound by HSP90 in BRAF\textsuperscript{wt} melanoma cells. HSP90 inhibition by 17-AAG inhibits ERK signaling and cell growth by destabilizing CRAF but not BRAF\textsuperscript{wt} in the majority of NRAS mutant melanoma cells studied. The highly-selective BRAF\textsuperscript{V600E} inhibitor, PLX4032 (vemurafenib), inhibits ERK signaling and cell growth in mutant BRAF melanoma cells, but enhances signaling in cells with wild-type BRAF. We further investigated the effect of 17-AAG on PLX4032-enhanced ERK signaling in BRAF\textsuperscript{wt} melanoma cells. PLX4032 alone enhanced ERK signaling in the BRAF\textsuperscript{wt} melanoma cell lines Mel-Juso, SK-Mel-2, and SK-Mel-30, and inhibited signaling and cell growth in BRAF\textsuperscript{V600E} A375 cells. However, 17-AAG inhibited PLX4032-enhanced ERK signaling and inhibited cell growth by destabilizing CRAF. Surprisingly, 17-AAG also stimulated melanin production in SK-Mel-30 cells and enhanced TYRP1 and DCT expression without stimulating TYR production in all 3 BRAF\textsuperscript{WT} cell lines studied. These results suggest that HSP90 inhibitors may be useful adjuncts to highly-selective BRAF inhibitors by inhibiting the effects of secondary mutations and restoring expression of melanogenic proteins that function as immune targets.
Determination of the epigenetic status of melanocyte lineage-associated genes in throughout the melanocyte life cycle
Sandeep S. Joshi \(^{(1)}\), Jennifer Huang \(^{(1)}\), Bishal Tandukar \(^{(1)}\), and Thomas J. Hornyak \(^{(1,2,3)}\)

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Abstract:
Melanocytes are neural crest-derived cells responsible for pigmentation of skin, eyes and hair. During embryonic development, neural crest cells generate melanoblasts which migrate along the dorsolateral region of the neural tube. After migration, melanoblasts invade the overlying epidermis and enter the developing hair follicle (HF) contributing to the differentiated HF melanocytes and melanocyte stem cells (MeSCs). Recently, we found that MeSCs from the bulge (CD34+) and secondary hair germ (SHG; CD34-) telogen hair follicle possess distinct functional properties. CD34- MeSCs primed for melanocyte differentiation and CD34+ MeSCs exhibiting a broader, neural crest-like phenotype. Here we determined how the expression of select genes changes throughout the melanocyte life cycle beginning with the neural crest (E9.5), and melanoblasts (E12.5), and including differentiated melanocytes (P8), and CD34+ and CD34- MeSCs (P56). Our goal was to determine whether gene expression changes were correlated with key changes in histone modification. We found that the melanogenic markers Dct, Tyrp1, and Tyr are highly expressed in melanoblasts, melanocytes, and CD34+ and CD34- MeSCs in comparison to neural crest cells. Apart from undifferentiated neural crest cells, bulge CD34+ MeSCs showed lower expression for all melanogenic markers. Next, we determined the changes in the histone modifications H3K27me3 and H3K4me3, which are indicators of repressed and active gene transcription respectively, of the Dct, Tyrp1, and Tyr genes. We found that ratio of H3K27me3 to H3K4me3 was higher for Tyrp1 and Tyr genes in CD34+ MeSCs relative to other cell types and was significantly lower in differentiated melanocytes. Thus, these findings suggest that Lysine 27 and Lysine 4 methylation of histone 3 regulated by the Polycomb and Trithorax systems respectively are key regulators of differentiation gene expression in MeSCs.

POSTER PRESENTATION  Oct. 6, 3:15 – 4:30 p.m.
Abstract No: 52

NBUVB treatment of human vitiligo significantly activates integrin-linked kinase signaling in the hair follicle bulge
Nathaniel B. Goldstein(1), Michael J. Wright(1), Maranke I. Koster(1), Laura G. Hoaglin(1), Steven E. Robinson(2), William A. Robinson(2), Bifeng Gao(3), Kenneth L. Jones(4), Dennis R. Roop(1), David A. Norris(1), Stanca A. Birlea(1)

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Abstract:
We developed a model to study epidermal repigmentation in human vitiligo treated with narrow band UVB (NBUVB), by isolating genetic material from melanocytes and keratinocytes from specific anatomic locations using laser capture microdissection. We isolated RNA from 100 melanocytes and separately from 100 keratinocytes from the hair follicle (HF) bulge outer root sheath (ORS) of vitiligo patients (6 untreated and 6 NBUVB-treated for 4 months), and performed whole transcriptome RNA sequencing. Using the Ingenuity Pathway Analysis tool, we identified the integrin-linked kinase (ILK) pathway as top activated in response to NBUVB (P=5.2E-06; z-score=3.3), connecting genes of intercellular adhesion (CDH3, CDH11, ITGB4, ITGB5) with genes involved in signal transduction of cytoskeleton dynamics (PI3K, CTNNB1, ACTN4, RHO, SLUG, DSP, VIM, FN1), all differentially expressed in the sequencing data. Key signals of ILK pathway were subjected to immunostaining and qRT-PCR confirmation. Immunostaining revealed a main upstream component of this pathway, PI3K p110ß, to be significantly upregulated by NBUVB in the bulge ORS (2.1-fold, P=2.0E-03). We also confirmed by qRT-PCR increased expression of CTNNB1 in the bulge melanocyte samples in response to NBUVB (5.8-fold, P=2.3E-02) (with attributed proliferation and differentiation roles in melanocyte stem cells (MSCs)). This was associated with activation of VIM (2.2-fold increase, P=1.2E-02), a transcriptional target of CTNNB1 and a downstream ILK pathway effector, that was associated with motile phenotypes. Using qRT-PCR, we confirmed RHOJ as being significantly upregulated (2.8-fold, P=3.0E-03) by NBUVB in the bulge melanocyte samples, with attributed pro-migratory roles in melanoma. Our results identify key-components of a biochemical pathway that governs proliferation, migration, and differentiation of MSCs emerging from the bulge during NBUVB. This is the key-pathway in activating MSCs to initiate vitiligo repigmentation.

POSTER PRESENTATION  Oct. 6, 3:15 – 4:30 p.m.
Abstract:
Inflammation in tumor microenvironment promotes tumorigenesis and chemoresistance. We have demonstrated that metastatic melanoma cells are of autoinflammation property, characterized by constitutive NLRP inflammasome activation and IL-1ß secretion. We have also demonstrated that aldehyde dehydrogemase (ALDH)1A isozymes are markers of human melanoma stem cells and ALDH-high melanoma cells are more resistant to chemotherapeutic agents. Here, we investigated the involvement of NLRP1 and ALDH in TMZ resistance in metastatic melanoma cells. We found that ALDH-high melanoma cells exhibited higher levels of IL-1ß production and secretion. Pharmacological relevant doses of TMZ not only enhanced NLRP1 expression and NF-kB activity, but increased the expression of Notch1 and ALDH1A3, two core stem cell regulators, in 1205Lu and HS294T cells. To understand the role of NLRP1 in chemoresistance, TMZ-resistant 1205Lu and HS294T cells were generated, which showed increased NLRP1 inflammasome activation, IL-1ß secretion, NF-kB activity, and expression of Notch1 and its downstream molecules, including XIAP, BCL-XL, cyclin D1, and PI3K/AKT, all contributing to cell survival. Whereas Notch1 inhibitor DAPT inhibited TMZ-induced NLRP1 and ALDH1A3 expression in parental 1205Lu and HS294T cells, IL-1 receptor antagonist (IL-1Ra) had no influence on TMZ-induced Notch1 expression in parental cells, and silencing NLRP1 did not alter Notch1 expression in both parental and resistant cells. However, IL-1Ra significantly decreased ALDH1A3 expression. Moreover, we found that either NLRP1 silencing or DAPT treatment partially increased the sensitivity of resistant cells to TMZ. These data suggest that upregulated NLRP1 and Notch1 expression confers melanoma resistance to TMZ and that NLRP1 is an effector downstream of Notch1 signaling but a positive regulator of ALDH1A3. We report a new role of NLRP1, known as a danger signaling sensor, in tumor chemoresistance.
**Abstract:**

Mutations in the gene encoding Transcription factor activator protein 2 alpha (TFAP2A), one of four closely-related proteins (i.e., TFAP2A, TFAP2B, TFAP2C, TFAP2E) result in reduced pigmentation in humans, mice, and zebrafish. Moreover, TFAP2A expression levels are consistently reduced in advanced melanoma in comparison to benign nevi. However, it has been unclear whether the genes regulated by TFAP2A and its paralogs are cell-lineage specific or generic, and the extent to which the target genes are shared with MITF, a master regulator of melanocyte biology and melanoma progression. To determine the position of TFAP2A in the melanocyte gene regulatory network, we first conducted anti-TFAP2A ChIP-seq to create profiles of TFAP2A-bound loci in mouse immortalized melanocytes (melan-a cells) and human primary melanocytes. Comparison of our TFAP2A ChIP-seq profile with a published H3K27Ac ChIP-seq profiles showed that TFAP2A peaks more than 70% of promoters and enhancers active in these cells. We then used microarray analysis in mouse melan-a cells depleted of tfap2a, and zebrafish tfap2a mutants. Genes at the intersection of the microarray and ChIP-seq profiles are likely direct targets of TFAP2A. These include genes encoding growth factor receptors implicated in melanocyte development (e.g., mc1r), melanin synthesis enzymes, and melanosome structural proteins. In reporter assays, deletion of TFAP2A binding sites decreased activity of a minimal TRPM1 promoter, similar to published results for deletion of MITF binding sites from this element. However, the reduction of pigmentation in mitf loss-of-function mutants is much more severe than in tfap2a mutants, suggesting redundant activity of a Tfap2 paralog. Consistent with this prediction, we find that mice with simultaneous Wnt1:CRE mediated depletion of Tfap2a and Tfap2b in the neural crest lack melanocytes, while retaining other trunk neural crest derivatives like sensory neurons and sympathetic neurons. In vivo experiments in zebrafish showed a genetic interaction between tfap2a and mitfa, but overexpression of tfap2a was not sufficient to rescue pigmentation in mitfa mutants. These findings indicate that TFAP2A and its paralogs and MITF work in parallel to promote gene expression in melanocytes. In addition, they show that a widely-expressed transcription factor, TFAP2A, cooperates with a more tissue-restricted transcription factor, MITF, to directly regulate expression of lineage-specific melanocyte genes.

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**Abstract:**

Mutations in the gene encoding Transcription factor activator protein 2 alpha (TFAP2A), one of four closely-related proteins (i.e., TFAP2A, TFAP2B, TFAP2C, TFAP2E) result in reduced pigmentation in humans, mice, and zebrafish. Moreover, TFAP2A expression levels are consistently reduced in advanced melanoma in comparison to benign nevi. However, it has been unclear whether the genes regulated by TFAP2A and its paralogs are cell-lineage specific or generic, and the extent to which the target genes are shared with MITF, a master regulator of melanocyte biology and melanoma progression. To determine the position of TFAP2A in the melanocyte gene regulatory network, we first conducted anti-TFAP2A ChIP-seq to create profiles of TFAP2A-bound loci in mouse immortalized melanocytes (melan-a cells) and human primary melanocytes. Comparison of our TFAP2A ChIP-seq profile with a published H3K27Ac ChIP-seq profiles showed that TFAP2A peaks more than 70% of promoters and enhancers active in these cells. We then used microarray analysis in mouse melan-a cells depleted of tfap2a, and zebrafish tfap2a mutants. Genes at the intersection of the microarray and ChIP-seq profiles are likely direct targets of TFAP2A. These include genes encoding growth factor receptors implicated in melanocyte development (e.g., mc1r), melanin synthesis enzymes, and melanosome structural proteins. In reporter assays, deletion of TFAP2A binding sites decreased activity of a minimal TRPM1 promoter, similar to published results for deletion of MITF binding sites from this element. However, the reduction of pigmentation in mitf loss-of-function mutants is much more severe than in tfap2a mutants, suggesting redundant activity of a Tfap2 paralog. Consistent with this prediction, we find that mice with simultaneous Wnt1:CRE mediated depletion of Tfap2a and Tfap2b in the neural crest lack melanocytes, while retaining other trunk neural crest derivatives like sensory neurons and sympathetic neurons. In vivo experiments in zebrafish showed a genetic interaction between tfap2a and mitfa, but overexpression of tfap2a was not sufficient to rescue pigmentation in mitfa mutants. These findings indicate that TFAP2A and its paralogs and MITF work in parallel to promote gene expression in melanocytes. In addition, they show that a widely-expressed transcription factor, TFAP2A, cooperates with a more tissue-restricted transcription factor, MITF, to directly regulate expression of lineage-specific melanocyte genes.

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1. Interdisciplinary Graduate Program in Genetics, University of Iowa, 2. Department of Anatomy and Cell Biology, University of Iowa, 3. Genetic Disease Research Branch, National Human Genome Research Institute, Bethesda, MD, 4. Bioinformatics Division, Iowa Institute of Human Genetics, University of Iowa

**Abstract:**

Mutations in the gene encoding Transcription factor activator protein 2 alpha (TFAP2A), one of four closely-related proteins (i.e., TFAP2A, TFAP2B, TFAP2C, TFAP2E) result in reduced pigmentation in humans, mice, and zebrafish. Moreover, TFAP2A expression levels are consistently reduced in advanced melanoma in comparison to benign nevi. However, it has been unclear whether the genes regulated by TFAP2A and its paralogs are cell-lineage specific or generic, and the extent to which the target genes are shared with MITF, a master regulator of melanocyte biology and melanoma progression. To determine the position of TFAP2A in the melanocyte gene regulatory network, we first conducted anti-TFAP2A ChIP-seq to create profiles of TFAP2A-bound loci in mouse immortalized melanocytes (melan-a cells) and human primary melanocytes. Comparison of our TFAP2A ChIP-seq profile with a published H3K27Ac ChIP-seq profiles showed that TFAP2A peaks more than 70% of promoters and enhancers active in these cells. We then used microarray analysis in mouse melan-a cells depleted of tfap2a, and zebrafish tfap2a mutants. Genes at the intersection of the microarray and ChIP-seq profiles are likely direct targets of TFAP2A. These include genes encoding growth factor receptors implicated in melanocyte development (e.g., mc1r), melanin synthesis enzymes, and melanosome structural proteins. In reporter assays, deletion of TFAP2A binding sites decreased activity of a minimal TRPM1 promoter, similar to published results for deletion of MITF binding sites from this element. However, the reduction of pigmentation in mitf loss-of-function mutants is much more severe than in tfap2a mutants, suggesting redundant activity of a Tfap2 paralog. Consistent with this prediction, we find that mice with simultaneous Wnt1:CRE mediated depletion of Tfap2a and Tfap2b in the neural crest lack melanocytes, while retaining other trunk neural crest derivatives like sensory neurons and sympathetic neurons. In vivo experiments in zebrafish showed a genetic interaction between tfap2a and mitfa, but overexpression of tfap2a was not sufficient to rescue pigmentation in mitfa mutants. These findings indicate that TFAP2A and its paralogs and MITF work in parallel to promote gene expression in melanocytes. In addition, they show that a widely-expressed transcription factor, TFAP2A, cooperates with a more tissue-restricted transcription factor, MITF, to directly regulate expression of lineage-specific melanocyte genes.

**POSTER PRESENTATION**

Oct. 6, 3:15 – 4:30 p.m.
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