Department of Genetics
2019-2020

GENETICS FACULTY

2019-2020

	Room	Building	Phone
Gil Atzmon, Associate Professor (primary appointment, Medicine/Endocrinology)	502C	Golding	430-3628
Nicholas E. Baker, Professor (secondary appointment, Developmental and Molecular Biology) (tertiary appointment, Ophthalmology and Visual Sciences)	805	Ullmann	430-2854
Nir Barzilai, Professor (primary appointment, Medicine/Endocrinology)	701A	Belfer	430-3144
Hannes Buelow, Associate Professor (secondary appointment, Neuroscience)	709	Ullmann	430-3621
Roy S. Chuck, Professor (primary appointment, Chair, Ophthalmology and Visual Sciences)	3332 Rochambeau Ave., MMC		920-6665
Ales Cvekl, Professor (primary appointment, Ophthalmology and Visual Sciences)	123	Ullmann	430-3217
Meelad Dawlaty, Assistant Professor	419	Price	678-1224
Winfried Edelmann, Professor (primary appointment, Cell Biology)	277	Price	678-1086
Scott Emmons, Professor (secondary appointment, Neuroscience)	703	Ullmann	430-3130
John Greally, Professor (secondary appointment, Medicine/Hematology) (tertiary appointment, Pediatrics)	322	Price	678-1234
Jean Hébert, Professor (primary appointment, Neuroscience)	237	Kennedy	430-3494
William R. Jacobs, Jr., Professor (primary appointment, Microbiology & Immunology)	577	Price	678-1075
Andreas Jenny, Professor (primary appointment, Developmental and Molecular Biology)	503	Chanin	430-4183
Ganjam V. Kalpana, Professor (secondary appointment, Microbiology & Immunology)	823	Ullmann	430-2354
Herb Lachman, Professor (primary appointment, Psychiatry and Behavioral Sciences) (secondary appointment, Medicine/Hematology) (tertiary appointment, Genetics) (tertiary appointment, Neuroscience)	103	Forchheimer	430-2428
Jack Lenz, Professor (secondary appointment, Microbiology & Immunology)	717	Ullmann	430-3715

Wei Liu, Assistant Professor (primary appointment, Ophthalmology and Visual Sciences)	117A	Ullmann	839-7926
Sridhar Mani, Professor (primary appointment, Medicine/Oncology)	302D-1	Chanin	430-2871
Cristina Montagna, Associate Professor (secondary appointment, Pathology)	401	Price	678-1158
Bernice Morrow, Professor (secondary appointment, Obstetrics & Gynecology and Women's Health) (tertiary appointment, Pediatrics)	402	Price	678-1121
Jayanta Roy-Chowdhury, Professor (primary appointment, Medicine/Liver Diseases)	523	Ullmann	430-2265
Namita Roy-Chowdhury, Professor (primary appointment, Medicine/Liver Diseases)	523	Ullmann	430-2254
Julie Secombe, Associate Professor (secondary appointment, Neuroscience)	809	Ullmann	430-2698
Frank Soldner, Assistant Professor (primary appointment, Neuroscience) (seconday appointment, Genetics)	235	Kennedy	839-7770
Simon D. Spivack, Professor (primary appointment, Medicine/Pulmonary Medicine) (secondary appointment, Epidemiology & Population Health) (tertiary appointment, Genetics)	301	Price	678-1040
Yousin Suh, Professor (secondary appointment, Medicine/Endocrinology)	475	Price	678-1111
Jan Vijg, Professor and Chair (secondary appointment, Ophthalmology & Visual Sciences)	450	Price	678-1151
David Q.-H. Wang, Professor (primary, Medicine/Gastroenterology & Liver Diseases) (secondary, Genetics)	611	Ullmann	430-8865
Tao Wang, Associate Professor (primary, Epidemiology & Population Health) (secondary, Genetics)	1303A	Belfer	430-4007
Melissa Wasserstein, Professor (primary appointment, Pediatrics) (secondary appointment, Genetics)	3411 Wayne Avenue, MMC		741-2318
Daniel Weiser, Assistant Professor (primary appointment, Pediatrics)	813	Ullmann	430-2181
Zhengdong Zhang, Associate Professor	353A	Price	678-1139
Deyou Zheng, Professor (primary appointment, Genetics and Neurology) (tertiary appointment, Neuroscience)	320	Price	678-1217

Bin Zhou, Professor (secondary appointment, Pediatrics) (tertiary appointment, Medicine/Cardiology)	420	Price	678-1067

RESEARCH FACULTY

Department of Genetics

2019-2020

Name (Mentor)	Title	Room	Building	Phone
Xiao Dong (Vijg)	Associate	455	Price	678-1194
Jhih-Rong Lin (Zhang)	Associate	353	Price	678-1147
Shahina Maqbool (Greally)	Research Assoc. Prof.	157	Price	678-1163
Alexander Maslov (Vijg)	Research Asst. Prof.	468	Price	678-1135
Kateryna Morozova (Montagna)	Associate	407	Price	678-1159
Silvia Racedo (Morrow)	Research Asst. Prof.	402	Price	678-1122
David Reynolds (Morrow)	Associate	1203	Ullmann	929-246-6735
Jidong Shan (Montagna)	Research Asst. Prof.	413	Price	678-1155
Masako Suzuki (Greally)	Research Asst. Prof.	319	Price	678-1571
Bingruo Wu (Zhou)	Research Asst. Prof.	420	Price	678-1551
Lei Zhang (Vijg)	Associate	468	Price	678-1135

POSTDOCTORAL FELLOWS

Department of Genetics

2019-2020

Name (Mentor)	Telephone	Lab Location
Kristina Brazhnik (Vijg)	678-1135	468 Price
Wei Cheng (Zhou)	678-1551	414 Price
Stephanie Chrysanthou (Dawlaty)	678-1210	413 Price
Christopher DeBono (Morrow)	678-1122	408 Price
Updesh Kumar Dixit (Kalpana)	430-2404	823 Ullmann
Xuhui Feng (Zhou)	678-1551	414 Price
Reza Jabal Ameli Forooshani (Zhang)	678-1147	353 Price
Erica Hasten (Morrow)	678-1122	408 Price
Johanna Heid (Vijg)	678-1135	468 Price
Zhenqui Huang (Vijg)	678-1135	468 Price
Antonie Abou Jaoude (Dawlaty)	678-1210	413 Price
Chen Jin (Suh)	430-1112	475 Price
Chaitali Khan (Baker)	430-2855	805 Ullmann
Seungsoo Kim (Suh)	678-1112	475 Price
Marianthi Kiparaki (Baker)	430-2855	805 Ullmann
Amit Kumar (Baker)	430-2855	805 Ullmann
Hequn Liu (Zheng)	678-1147	353 Price
Yang Liu (Zheng)	678-1166	353 Price
Pengfei Lu (Zhou)	430-1551	414 Price
Liyang Ma (Dawlaty)	678-1210	413 Price
Elaine Maggi (Montagna)	678-1159	407 Price
Joydeep Mitra (Zhang)	678-1147	353 Price
Sudershana Nair (Baker)	430-2855	805 Ullmann
Hiroko Nomaru (Morrow)	678-1122	408 Price
Venkateswara Reddy (Baker)	430-2855	805 Ullmann
Rajiv Pathik (Kalpana)	430-2404	823 Ullmann
Mirunalini Ravichandran (Dawlaty)	678-1210	413 Price
Naoko Sakai (Emmons)	430-2249	703 Ullmann
Shixiang Sun (Vijg)	678-1135	478 Price
Leo Tsz-Ho Tang (Buelow)	430-3622	709 Ullmann
Qin Tang (Dawlaty)	678-1210	413 Price
Jiping Yang (Suh)	678-1112	475 Price
Zhen Wang (Zhang)	678-1147	353 Price
Quanwei Zhang (Zhang)	678-1147	353 Price
Yingjie Zhao (Morrow)	678-1122	408 Price
Yizhou Zhu (Suh)	678-1112	475 Price

GRADUATE STUDENTS

Department of Genetics

2019-2020

Name (Mentor)	Telephone	Lab Location
Jesse Barnes (Lachman)	430-2491	103 Forchheimer
Helen Belalcazar (Secombe)	430-4463	809 Ullmann
Dora (Zhongxuan) Chi (Morrow)	678-1122	408 Price
Reanna Doňa* (Greally)	678-1570	319 Price
Philip Galbo (Zheng)	678-1166	Price 353
Brenda Gonzalez (Suh)	678-1112	469 Price
Hayden Hatch* (Secombe)	430-4463	809 Ullmann
Adam Hudgins (Suh)	430-1112	475 Price
Ameya Kulkarni (Barzilai)	430-3620	705 Belfer
Nicolas Le Texier (Suh)	678-1112	475 Price
Garrett Alexander Lee (Buelow)	430-3622	709 Ullmann
Albert Lowe (Liu)	839-7926	117 Ullmann
Kassidy Lundy (Greally)	678-1570	319 Price
Xiaoping Luo (Mani)	430-2871	302D-Chanin
Virginia Folgado Marco (Baker)	430-2855	805 Ullmann
Maisha Rahman (Buelow)	430-3622	709 Ullmann
Michael Rogers* (Secombe)	430-4463	809 Ullmann
Archana Tare (Suh)	678-1112	469 Price
Taylor Thompson (Greally)	678-1570	319 Price
Meera Trivedi (Buelow)	430-3622	709 Ullmann
Christopher Salazar (Buelow)	430-3622	709 Ullmann
Blair Schneider (Secombe)	430-4463	809 Ullmann
Lijie Shi (Morrow)	678-1122	408 Price
Hansoo Song* (Morrow)	678-1122	408 Price
Jacob Stauber* (Greally)	678-1570	319 Price
Taylor Thompson (Greally)	678-1570	319 Price
Xizhe Wang (Suh)	678-1112	469 Price

*M.D./Ph.D. Students

GIL ATZMON, Ph.D.

Epigenetic Profiling in Healthy Aging and Exceptional Longevity

Examples of epigenetic regulation of genome architecture and gene expression are paved across the evolutionary lineage. Even if only a small proportion of human genes are subject to similar effects, they may still play a major role in the phenotypic variation and susceptibility to diseases. My long-term research goal is to investigate changes in the epigenetic control of gene expression which may be one of the central mechanisms by which aging predisposes to many age-related diseases and therefore lifespan.

Despite some early work, the role of epigenetics in human life span and age related diseases has remained unexplored. Discovering that methylated loci are involved in the genetic control of cellular existence, modify the risk for age-related disease and influence mortality is a novel and extremely important concept that would significantly enhance our understanding of the biology of aging. In addition, accumulating evidence supports the notion that major age-associated diseases (such as diabetes, metabolic syndrome and cancer) are regulated by epigenetic alterations. Epigenetic changes may provide information on the pivotal points between healthy and sick stages in the lifespan of an individual. Hence, epigenetic changes could serve as landmarks of events such as onset of disease and these events can be tracked long after its occurrence (forms of epimutation and the Barker theory).

To test our hypothesis, we propose to employ a novel high-throughput genome-wide methylation assay, HELP-tag. Additionally, we will utilize a combination of large-scale epigenomic analysis (EWAS) to identify the most distinctive epigenetic loci that show greatest differential methylation. We will then perform Multi-locus validation for methylation status using MassARRAY. We will test expression of candidate loci to explore possible mechanisms of methylation regulation. We will combine these results with whole genome sequences to assess the interaction between the genetic blueprint and the environment as it manifested through epigenetic changes.

This research will further our understanding of the complexity of healthy lifespan process by identifying loci that when altered epigenetically have important ramifications for age-related diseases and lifespan. Validating the genes whose function is modulated epigenetically could lead to interventions to delay or even prevent the development of age-associated diseases.

Recent Publications:

1. **Gielen M, et.al..** BMI is negatively associated with telomere length: a cross-sectional meta-analysis of 67 observational studies. American Journal of Clinical Nutrition. 2018 108(3):453-475.
2. **Udler MS, et.al.**. Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: A soft clustering analysis. PLoS Med. 2018 15(9):e1002654.
3. **Gurinovich A, et.al..** PopCluster: an algorithm to identify genetic variants with ethnicity-dependent effects. Bioinformatics. 2019.
4. **Yu D et.al..** Interrogating the Genetic Determinants of Tourette's Syndrome and Other Tic Disorders Through Genome-Wide Association Studies. Am J Psychiatry. 2019 176(3):217-227.
5. **Flannick J, et.al**.. Exome sequencing of 20,791 cases of type 2 diabetes and 24,440 controls. Nature. 2019 570(7759):71-76.

NICHOLAS BAKER, Ph.D.

Regulatory genes that control translation, cell competition, and neuronal development

One of the unresolved questions in biology is that of how organs grow. It is thought that cancer and neurodegenerative diseases involve defects in the regulation of cell growth and survival that are fundamental to organ growth.

Genetic studies can be used to uncover new genes controlling tissue growth and maintenance, and to characterize their roles in vivo. Our current research uses *Drosophila* and mice to address new mechanisms of growth regulation. We have uncovered novel steps in the regulation of ribosome biogenesis and translation, the mechanisms and functions of 'cell competition' in development and pathology, and the role of caspase enzymes in non-apoptotic process that control neuronal development and function.

Regulation of ribosome biogenesis

Ribosomes are essential for growth. There is increasing interest in how their biogenesis and function are regulated, both during growth and in neurodegenerative disease, and recent discoveries about these mechanisms. Our laboratory has discovered novel signaling pathways that are activated when defects occur in ribosome assembly. We are studying the molecular signaling mechanisms involved, and their conservation in mammals. Ribosomal proteins are affected in several human diseases and also appear to act as tumor suppressors for multiple cancers, but the mechanism of tumor suppression by ribosomal proteins is not yet clear. There is also evidence that defects in translation underlie some neurological disease.

Cell competition When organs contain mixed populations of cells, for example because of somatic mutation during aging, 'cell competition' can eliminate less fit cells through selective apoptosis of only these cells. Our studies show that ribosomal protein genes play a key role in targeting cells for elimination by cell competition. Because ribosomal protein genes are distributed across almost all chromosomes, they serve as markers for large-scale genetic changes. Our studies show that cell competition is a mechanism that can remove cells that have become aneuploid, or acquired other large-scale genetic changes, and may be important in preventing birth defects and cancer.

Non-apoptotic caspase function

Proneural bHLH proteins are the master regulators for most neuronal differentiation. We have found that defects in bHLH expression activate the Hippo pathway of tumor suppressors, which in turn activate caspase enzymes. Although caspases are well known as the executors of apoptosis, when regulated by bHLH proteins they function instead in non-apoptotic processes that appear to control neuronal cell fate specification. Homologs of many of these genes are implicated in schizophrenia, axon and dendrite patterning, suggesting that transcriptional control of non-apoptotic caspase signaling may be relevant to brain diseases.

Selected recent publications

Lee, C.H., Kiparaki, M., Blanco, J., Folgado, V., Ji, Z., Kumar, A., Rimesso, G., and Baker, N.E. (2018) A regulatory response to ribosomal protein mutations controls translation, growth, and cell competition. *Dev Cell*, **46**, 456-469.

Baker, N.E., and Brown, N.L. (2018) All in the family: neuronal diversity and proneural bHLH genes. *Development*, **145:** dev159426.

Li, K., and Baker, N.E. (2018) Regulation of the *Drosophila* ID protein Extra Macrochaetae by proneural dimerization partners. *Elife* **7**: e33967.

Kale, A., Ji, Z., Kiparaki, M., Rimesso, G., Flibotte, S., and Baker, N.E. (2018) Ribosomal protein S12e has a distinct function in cell competition. *Dev Cell* **44**, 42-55.

Baker, N.E. (2017) Mechanisms of cell competition emerging from *Drosophila* studies. *Curr Opin Cell Biol* **48**, 40-46.

Wang, L.-H. and Baker, N.E. (2015) E-proteins and Id-proteins: helix-loop-helix partners in development and disease. *Developmental Cell* **35**: 269-280

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Wang, L.-H. and Baker, N.E. (2015) Salvador-Warts-Hippo pathway in a developmental checkpoint monitoring Helix-Loop-Helix proteins. *Developmental Cell* **32:**.191-202.

Bhattacharya, A., and Baker, N.E. (2011). A network of broadly-expressed HLH genes regulates tissue-specific cell fates. *Cell*, **147**: 881-892.

NIR BARZILAI, M.D.

Searching for Longevity Genes in Humans

Why do some people live much longer than others? What allows these individuals to escape age-associated diseases that contribute to mortality in the elderly? Is this a result of favorable genes or merely a healthy life style? If the genome does play a role, what are the mechanisms?

To address these questions, we recruited over 1500 Ashkenazi Jews. The Ashkenazi Jewish population is unique as it is derived from a small number (several thousands) of founders and therefore it is genetically homogeneous. This population has been utilized for identification of several genes, a prominent example being the breast cancer gene. The subjects fall into three groups; probands, subjects with exceptional longevity (1:10000 in the general population); their offspring; and a control group consisting of spouses of the offspring and other Ashkenazi Jewish people recruited from the Einstein Aging Study.

Studying the clinical and metabolic phenotype, revealed certain physiological characteristics in the centenarians and their offspring such as high levels of high-density lipoprotein (HDL), high adiponectin levels, and high IGF-1 levels. In collaboration with Dr. Atzmon and Suh, we showed that each of those phenotype is now associated with a genotype that has a functional meaning, and each of those genotypes have been validated independently in at least one other population of centenarians. One of the genotypes is also specifically protective from cognitive decline, and this was also validated as an Alzheimer's protective gene. We studied telomere length demonstrating longer telomeres in our longest living subjects and their offspring compared to control. These findings may indicate longer telomeres at birth or slower attrition rate in their length, and this was associated with a specific haplotype of the telomere gene. Most important, since the trait of longer telomeres is associated with protective lipoprotein profile and less age-related disease, this test may be used as a predictor for longevity.

Using an un-biased approach we have employed an Affimetrix 6.0 platform with almost 2MM markers and across the genome. Comparing the centenarian genotype to a younger un-related control, we established 35 genotypes that increase monotincally with aging (from age 60 to 112)) and were linked significantly ($p<10-6$) to genes that have not been previously linked to aging. In collaboration with Drs. Greally and Atzmon, we have used high throughput methylation assay (HELP) to demonstrate that centenarians methylation pattern across the genome is significantly different than in younger un-related subjects.

Our lab has trained many graduates and post-docs, and the latest graduate, Reid Thompson, MD/Ph.D. student, can be a reference. We offer a clinical platform for variety of genomic studies in collaboration with many of the Einstein faculty.

Recent Publications:

Kato K, Zweig R, Schechter CB, Verghese J, Barzilai N, Atzmon G. Personality, self-rated health, and cognition in centenarians: Do personality and self-rated health relate to cognitive function in advanced age? Aging (Albany NY). 2013 Mar 23. PMID: 23524310

Han J, Ryu S, Moskowitz DM, Rothenberg D, Leahy DJ, Atzmon G, Barzilai N, Suh Y. Discovery of novel nonsynonymous SNP variants in 988 candidate genes from 6 centenarians by target capture and next-generation sequencing. Mech Ageing Dev. 2013 Jan 31. doi:pii: S0047-6374(13)00020-1. PMID: 23376243

Barzilai N and Ferrucci L. Insulin Resistance and Aging: A Cause or a Protective Response? J Gerontol A Biol Sci Med Sci. August 2012; PMID: 22859390

Gombar S, Jung HJ, Dong F, Calder B, Atzmon G, Barzilai N, Tian XL, Pothof J, Hoeijmakers JH, Campisi J, Vijg J, Suh Y. Comprehensive microRNA profiling in B-cells of human centenarians by massively parallel sequencing. BMC Genomics. 2012 Jul 31;13(1):353 PMID: 22846614

Barzilai N, **Guarente L, Kirkwood TB, Partridge L, Rando TA, Slagboom PE**. The place of genetics in ageing research. Nat Rev Genet. 2012 Jul 10;13(8):589-94. doi: 10.1038/nrg3290. PMID: 22777128

Huffman DM, Deelen J, Ye K, Bergman A, Slagboom EP, Barzilai N, Atzmon G. Distinguishing Between Longevity and Buffered-Deleterious Genotypes for Exceptional Human Longevity: The Case of the MTP Gene. J Gerontol A Biol Sci Med Sci. 2012 Apr 10. PMID: 22496539

Conneely KN, Capell BC, Erdos MR, Sebastiani P, Solovieff N, Swift AJ, Baldwin CT, Budagov T, Barzilai N, Atzmon G, Puca AA, Perls TT, Geesaman BJ, Boehnke M, Collins FS. Human longevity and common variations in the LMNA gene: a meta-analysis. Aging Cell. 2012 Feb 16. PMID: 22340368

Rajpathak SN, Liu Y, Ben-David O, Reddy S, Atzmon G, Crandall J, **Barzilai N**. Lifestyle Factors of People with Exceptional Longevity. J Am Geriatr Soc. 2011 Aug 3. PMID: 2181276

HANNES E. BUELOW, Ph.D.

Genetics of Nervous System Development and Function

My lab uses the small nematode *C. elegans* with its simple and well characterized nervous system as a genetic model. We are trying to understand how growing axons and dendrites navigate the extracellular space to connect to their partners and be appropriately patterned and, how they function within a neural circuit.
The extracellular space is filled with a complex mixture of proteins and proteoglycans e.g. heparan sulfate (HS) proteoglycans, which are a particular focus of the lab. We are asking how specific modification patterns of HS sugars determine the navigation of developing axons and neurons. For instance, we have shown that distinct modification patterns in HS serve specific functions during neural development leading us to formulate the 'HS code' hypothesis. Our goal is to decipher the information contained in HS, determine the factors that create and modulate it and describe the genes that respond to it. In a second project we are studying the development of dendrites in polymodal multidendritic neurons of *C. elegans*. We are aiming to understand how the complex dendritic arbors that resemble menorah-like candelabras are patterned. In a third project we study how synaptic connections are shaped by experience and how these plastic changes correlate with behavior. In summary, we are using genetic approaches coupled with biochemical, behavioral and advanced imaging approaches to understand genes involved in development and function of the nervous system.

Selected Recent Publications:

Townley R.A., and Bülow H.E. (2011) Genetic Analysis of the Heparan modification network in *Caenorhabditis elegans*. ***J. Biol. Chem***, *286:16824–16831, published March 24, 2011 as doi:10.1074/jbc.M111.227926.*

Tornberg J., Sykiotis G.P., Keefe K., Plummer L., Hoang X, Hall J.E., Quinton R., Seminara S.B., Hughes V., Van Vliet G., Van Uum S., Crowley, Jr W.F., Habuchi H., Kimata K., Pitteloud N.*, Bülow H.E.* (2011) *Heparan sulfate 6-O-sulfotransferase 1*, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotrophic hypogonadism. ***Proc Natl Acad Sci USA***, *108(28):11524-11529*, *published online June 23, 2011 as doi:10.1073/pnas.1102284108*, * contributed equally.

Attreed M., Desbois M., van Kuppevelt T.H., and Bülow H.E. (2012) Direct visualization of specifically modified extracellular glycans in living animals. ***Nat. Methods***, *9(5):477-479*, *published online April 1, 2012 as doi:10.1038/nmeth.1945*.

Salzberg Y., Diaz-Balzac C.A., Ramirez-Suarez N.J., Attreed M., Tecle E., Desbois M., Kaprielian Z., and Bülow H.E. (2013) Skin-derived cues control arborization of sensory dendrites in *Caenorhabditis elegans.* ***Cell***, *155(2): 308–320*, *published online on October 10 as http://dx.doi.org/10.1016/j.cell.2013.08.058.*

Díaz-Balzac C.A., Lázaro-Peña M.I., Ramos-Ortiz G.O., Bülow H.E. (2015) The Adhesion molecule KAL-1/anosmin-1 regulates Neurite Branching through a SAX-7/L1CAM–EGL-15/FGFR Receptor Complex. ***Cell Reports***, *11:1–8, published online on May 21 as http://dx.doi.org/10.1016/j.celrep.2015.04.057.*

Díaz-Balzac C.A., Rahman M., Lázaro-Peña M.I., Martin Hernandez L.A., Salzberg Y., Aguirre-Chen C., Kaprielian Z., and Bülow H.E. (2016) Muscle- and skin-derived cues jointly orchestrate patterning of somatosensory dendrites. ***Current Biology***, *26:1-9*, *published online on July 21 as http://dx.doi.org/10.1016/j.cub.2016.07.008.*

Celestrin K., Díaz-Balzac C.A., Tang L.TH., Ackley B.D., and Bülow H.E. (2018) Four specific Ig domains in UNC-52/Perlecan function with NID-1/Nidogen during dendrite morphogenesis in *Caenorhabditis elegans.* ***Development***, *published online on 20 April 2018 as https://doi.org/10.1242/dev.158881.*

Ramirez-Suarez N.J., Belalcazar H.M., Salazar C.J., Beyaz B., Raja B., Nguyen K.C.Q., Celestrin K., Fredens J., Færgeman N.J., Hall D.H., and Bülow H.E. (2019) Axon-dependent patterning and maintenance of somatosensory dendritic arbors, ***Developmental Cell****, 48:229-244, published online on January 17, 2019 as https://doi.org/10.1016/j.devcel.2018.12.015.*

Tang L.TH., Díaz-Balzac C.A.*, Rahman M., Ramirez-Suarez N.J., Salzberg Y., Lázaro-Peña M.I., and Bülow H.E. (2019) TIAM-1/GEF can shape somatosensory dendrites independently of its GEF activity by regulating F-actin localization.* ***eLife****; 8:e38949 DOI: 10.7554/eLife.38949.*

ALES CVEKL, Ph.D.

Genetic and Epigenetic Regulatory Mechanisms in Mammalian Eye Development and Ocular Diseases

We are studying mouse eye as a model system to elucidate basic molecular mechanisms of embryonic development, transcriptional regulation, signal transduction, cellular differentiation, nuclear organization, and aging. We are particularly interested in the genes that control embryonic lens induction and differentiation. We seek to identify and characterize the complete set of genetic and epigenetic instructions that control lens development. We are studying DNA-binding transcription factors (e.g. Pax6, c-Maf, Hsf4, Gata3, and Prox1), BMP and FGF signaling, and chromatin remodeling (e.g. CBP, p300, Brg1, Snf2h, and Ncoa6) and their role in lens induction and differentiation. Aberrant function of these genes causes not only lens abnormalities in mouse embryos, but also other human congenital eye diseases affecting the cornea, lens, iris, and retina. Mutations in PAX6 cause aniridia, characterized by the absence of iris, as well as early onset cataract, foveal hypoplasia and glaucoma. Mutations in p300, HSF4, and MAF cause distinct types of lens opacities resulting in cataracts.

Transcriptional regulatory mechanisms of Pax6, c-Maf, Prox1, and Gata3 are investigated through the identification and functional characterization of lens-specific enhancers of these genes. Gene regulatory networks (GRNs) of crystallin gene expression are comprised of multiple feed-forward loops and represent excellent models to study principles of tissue morphogenesis.

We are also studying the biology of lens fiber cell nuclei as transcriptional factories for crystallin gene synthesis. The crystallin encode highly abundant lens structural proteins that accumulate in elongating lens fiber cells and are required for lens transparency and its refraction. We are interested in nascent transcription/ transcriptional bursting, mRNA splicing and transport, crystallin mRNA stability control, and their translational regulation. We use RNA FISH and MS2 system to visualize mRNAs at single molecule levels. Multiple RNA-binding proteins were recently identified in different lens compartments and ongoing experiments are aimed to probe functions of carhsp1 and Rbm38 RNA-binding proteins in the eye.

Pax6 also plays important roles in the formation of other organs, including brain and pancreas. Several novel roles of PAX6 are used to explain differences between primate and rodent brains. Our studies also have impact on understanding of eye evolution and formation of new genes through gene duplication.

Our interest in age-related ocular diseases is focused on age-related macular degeneration, cataract, and glaucoma. Using eye, retinal, retinal pigmented epithelium, and lens organoids differentiated from human ES cells we develop human models to understand disease mechanisms through CRISPR-based genome engineering and use these systems for discovery of novel therapeutic interventions.

Recent Publications:

Ninkovic, J., L. Pinto, S. Petricca, J. Sun, M.A. Rieger, T. Schroeder, A. Cvekl, J. Favor and M. Gotz. 2010. The transcription factor Pax6 regulates survival of dopaminergic olfactory bulb neurons via crystallin αA. Neuron **68:**682-694.

Xie, Q. and A. Cvekl. 2011. The orchestration of mammalian tissue morphogenesis through a series of coherent feed-forward loops. J. Biol. Chem. **286:**43259-43271.

Wolf, L., W. Harrison, J. Huang, Q. Xie, N. Xiao, J. Sun, L. Kong, S.A. Lachke, M.R. Kuracha, V. Govindarajan, P.K. Brindle, R. Ashery-Padan, D.C. Beebe, P.A. Overbeek, and A. Cvekl. 2013. Histone posttranslational modifications and cell fate determination: lens induction requires the lysine acetyltransferases CBP and p300. Nucleic Acids Res. **41:**101989-10214.

Sun, J., S. Rockowitz, Q. Xie, D. Zheng, and A. Cvekl. 2015. Identification of *in vivo* DNA-binding mechanisms of Pax6 and reconstruction of Pax6-dependent gene regulatory networks during forebrain and lens development. 2015. Nucleic Acids Res. **43:**6827-6846.

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Esteban-Martinez, L., E. Sierra-Filardi, R.S. McGreal, M. Salazar-Roa, G. Marino, E. Seco, S. Durand, D. Enot, O. Grana, M. Malumbres, A. Cvekl, A.M. Cuervo, G. Kroemer, and P. Boya. 2017. Programmed mitophagy is essential for the glycolytic switch during cell differentiation. EMBO J. **36:**1688-1706.

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MEELAD DAWLATY, Ph.D.

Epigenetics of embryonic and pluripotent stem cells, development and cancer

Our research focuses on understanding the epigenetic mechanisms governing the biology of stem cells, development and cancer, with an emphasis on the role of DNA modifying enzymes (Tet1/2/3). We utilize embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and mice as model systems to study how these enzymes reshape the epigenome and regulate stem cell biology. The lab specializes in advanced technologies in genome editing and generating complex mouse strains. We integrate mouse genetics with cellular, molecular, biochemical and bioinformatics approaches to dissect epigenetic pathways and mechanisms in stem cells.

The Tet family of enzymes (Tet1/2/3) convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and promote DNA demethylation. They are abundant in various cell types including the zygote, ESCs, germ cells, hematopoietic stem cells (HSCs) and neurons. Over the years, our work in the field has defined key functions of Tet enzymes in ESC differentiation, germ cell reprogramming and development *(Cell Stem Cell 2011, Developmental Cell 2013, Developmental Cell 2014, Cell Reports 2019)* as well as in hematopoietic stem cells (HSCs) and malignancies *(Nature Immunology 2015, Cell Reports 2015, Cell Reports 2019),* and in memory extinction *(Neuron 2013)*. Recently in my lab:

- We have shown that the biological roles of Tet enzymes go beyond their enzymatic activity in DNA demethylation. Such non-canonical functions present a novel layer of epigenetic regulation. We study their role in ESC pluripotency and development, as well as in HSCs and hematologic malignancies.
- We investigate how Tet enzymes regulate lineage specification and organogenesis, and research the biologically critical functions of Tet proteins in post gastrulation development.
- We have identified two DNA binding proteins, Rinf and Idax, as partners of Tet enzymes and pluripotency factors. We study how they target Tet enzymes and pluripotency factors to gene regulatory regions and control their transcription in ESCs and during differentiation.
- We probe the role of 5hmC and its derivatives as independent epigenetic marks. We study their involvement in regulation of gene expression during development and in onset of diseases.

Our research investigates novel biological roles of DNA modifying enzymes in regulation of stem cell biology, development and cancer. This line of research will unveil new mechanisms of epigenetic regulation by Tets/5hmC, and can lead to identification of new markers and targets in stem cell applications and for treatment of diseases.

For more details on our research please visit our lab website: https://www.dawlatylaboratory.com

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WINFRIED EDELMANN, Ph.D.

Genomic Instability and Cancer in Murine Models

The maintenance of genomic integrity in all organisms requires multiple DNA repair pathways that are involved in the processes of DNA replication, repair and recombination. Perturbations in these pathways can lead to increased mutation rates or chromosomal rearrangements that ultimately result in cancer. MMR is one of the repair systems that mammalian cells employ to maintain the integrity of its genetic information by correcting mutations that occur during erroneous replication. Mutations in MMR genes are linked to one of the most prevalent human cancer syndromes, Lynch syndrome and a significant number of sporadic colorectal cancers. At the molecular level tumors that develop in these patients display increased genomic mutation rates as indicated by increased instability at microsatellite repeat sequences (termed microsatellite instability, MSI). MMR in eukaryotes is complex and involves several homologs of the bacterial MutS and MutL proteins. In mammals, the initiation of the repair process requires two complexes formed by three different MutS homologs (MSH): A complex between MSH2-MSH6 for the recognition of single base mismatches and a complex between MSH2-MSH3 for the recognition of insertion/deletions. The repair reaction also requires a complex between the two MutL homologs MLH1 and PMS2 that interacts with the MSH complexes to activate subsequent repair events which include the excision of the mismatch carrying DNA strand and its re-synthesis. In addition to correcting DNA mismatches, the MMR system mediates an apoptotic response to DNA damage and both of these functions are thought to be important for genome maintenance and tumor suppression. We have generated gene targeted mouse lines with inactivating mutations in all the different MutS and MutL homologs, and also in genes that function in the later MMR steps to study their roles in genome maintenance and tumor suppression. In addition, we have generated knock-in mouse lines with missense mutations and conditional knockout mouse lines that inactivate specific MMR functions and/or model mutations found in humans. Our studies indicate that specific MMR functions play distinct roles in maintaining genome stability and that defects in these functions have important consequences for tumorigenesis. These studies have also revealed that MMR proteins play essential roles in class switch recombination and somatic hypermutation during antibody maturation and the control of meiotic recombination in mammals. We are currently studying the functions of MMR in intestinal stem cells (ISCs) and cancer stem cells (CSCs) in preclinical mouse models and how loss of MMR in stem cells affects tumorigenesis and the response of tumors to anticancer treatment.

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SCOTT W. EMMONS, Ph.D.

Genetic Encoding of Neural Circuits, Connectomics

How complex neural circuits form and how they function are major unsolved problems in neurobiology. We use the nematode *Caenorhabditis elegans* to study these questions at the cellular and molecular levels. Connectivity in the *C. elegans* nervous system is assayed by serial section electron microscopy. *C. elegans* is the only animal species for which the complete nervous system wiring diagram, now available for both male and hermaphrodite adults, is known, providing an unprecedented foundation for *C. elegans* neuroscience research.

The *C. elegans* nervous system is a complex neural network that is genetically specified. To investigate how the patterns of connectivity are encoded in the genome, we make use of transgenes that express fluorescent proteins targeted to specific classes of synapses. We use these synapse-specific labels to identify mutants and genes that affect formation of particular cellular synaptic contacts. We are determining the expression patterns of genes that encode neural cell adhesion proteins in the neural network that governs the mating behavior of the adult male. This class of transmembrane proteins is thought to include the molecular cell labels by which appropriate pre- and post-synaptic cells recognize each other. By correlating the expression of these molecules with connectivity, we hope to uncover the molecular code that determines the wiring diagram of the nervous system.

Visit our websites: http://worms.aecom.yu.edu. http://wormwiring.org

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JOHN M. GREALLY, D.Med., Ph.D.

Epigenomics in Human Disease

The Greally lab has interests in both basic science and clinical research, focusing on transcriptional regulatory processes and mechanisms mediating long-term cellular memory. We have a long-standing interest in developing and using functional genomic assays to reveal insights into human phenotypes.

During the last several years, we have changed our definition of epigenetics and our approach to phenotypic associations, as summarized in the 2017 ***Nature Reviews in Genetics*** paper below. Our group's work is now focused on what we call somatic cellular genomics, an extension of our prior focus on what have been called epigenetics studies of transcriptional regulation.

We are funded to perform the most detailed somatic cellular genomic study ever performed of human ageing, and we also work as part of a multi-institutional consortium working on myalgic encephalopathy/chronic fatigue syndrome. We are testing how adverse environmental exposures cause disease by studying priming of stem cells by endocrine disrupting chemicals, vitamins and physical microenvironmental conditions. Non-alcoholic fatty liver disease and steatosis represent primary human disease foci in applying these new insights. To understand inter-individual variability in responses to the same environmental challenges, we are developing a discipline that we refer to as environmental epigenetics, testing how functional genetic variants mediate a cell's response to stresses.

Our clinical application of somatic cellular genomics is through our involvement in the NYCKidSeq project, part of the CSER initiative of the NHGRI. We are developing the GenomeDiver tool to allow improved diagnostic capabilities of whole genome sequencing in our diverse paediatric population. As part of the development of novel analytical approaches to identify unusual somatic cellular genomic mechanisms of human disease, we are developing MADSEQ to identify mosaic chromosomal aneuploidy in this cohort.

Recent Publications:

Wijetunga NA, Delahaye F, Zhao YM, Golden A, Mar JC, Einstein FH, **Greally JM.** The meta-epigenomic structure of purified human stem cell populations is defined at cis-regulatory sequences. ***Nature Communications*** 2014 Oct 20;5:5195. doi:10.1038/ncomms6195. PubMed PMID: 25327398; PubMed Central PMCID: PMC4300104.

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JEAN HÉBERT, Ph.D.

Regenerating the Neocortex

The Hébert lab has traditionally studied how the forebrain develops using conditional genetic methods in mice. Recently, the focus of the lab has transitioned to a new area of interest. Based on what has been learned about the molecular and cellular mechanism regulating neocortex development, we are devising novel methods to regenerate the damaged neocortex, the part of our brains that we use for our highest cognitive and perceptual functions. Neocortical damage can be local, due for example to stroke or trauma, or widespread, due for example to neurodegeneration or aging. Among the approaches we are taking, we are developing ways of replacing the principle neurons of the adult neocortex without significantly disrupting the function of existing neural circuits. These experiments include testing mixed human and mouse cell populations that resemble the normal cell composition of the neocortex to establish tissue replacement paradigms for local damage. In addition, for widespread degenerative damage, we are testing the ability of highly migratory cells that disperse throughout the neocortex to repopulate and bolster existing neural circuits with new neocortical neurons. These projects are highly collaborative as they require multidisciplinary methods including molecular genetic, cellular, single cell transcriptomic, surgical, and electrophysiological techniques, among others.

Selected Recent Publications

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WILLIAM R. JACOBS JR., Ph.D.

Sterilizing Chemotherapies and Immunotherapies against Tuberculosis, Herpes, and Influenza

Tuberculosis:
Tuberculosis (TB) remains the single leading infectious disease in the world, causing over 10 million new cases per year and accounting for 1.6 million deaths annually. The onset of the HIV epidemic worsened the TB global health burden leading to increases in incidences, reactivated disease, and the emergence of drug resistance. The worsening problem of TB is surprising because both a vaccine and sterilizing chemotherapy exist to treat this disease. A major reason for the ineffectiveness of these therapies, is TB's ability to persist; persistence is the capacity of *Mycobacterium tuberculosis* (*Mtb*) to survive sterilization in animals and humans. Persistence is also an epigenetic process found in all bacteria and cancer cells. Recently, we demonstrated populations of *Mtb* have a subpopulation of *Mtb* cells that are phenotypically resistant to bactericidal antibiotics. We have identified specific transcriptional patterns that regulate phenotypic resistance and developed dual reporter mycobacteriophages to rapidly identify this subpopulation of cells. Moreover, we discovered the addition N-acetylcysteine or Vitamin C to cultures of *Mtb* prevent the formation of persisters and allow for rapid sterilization in the presence of bactericidal drugs. Current efforts are focused on characterizing the mechanisms by which persisters are formed and identifying relevant targets to eliminate these persisters.

Herpes and Influenza:
In collaboration with Dr. Betsy Herold, we have generated a precise deletion of the gene encoding *gD* of Herpes Simplex Virus (HSV) 2, termed ΔgD-2, that upon immunization in mice elicits sterilizing immunity against challenge with HSV-1 and HSV-2. This unprecedented protection results from the induction of a special type of antibodies that mediate antibody dependent cell mediated killing (ADCK) of herpes infected cells. We have subsequently found that many pathogens do not elicit ADCK antibodies but we hypothesized that by cloning genes encoding important antigens into our herpes viral vector, we could elicit protection against other pathogens such as influenza. Recently, our lab generated recombinant ΔgD-2 herpes virus expressing genes encoding flu antigens and demonstrated that we can confer complete protection against the homologous influenza challenge. This proof of principle suggests that by cloning antigens from other pathogens, such as *Mtb*, it is possible to make novel vaccines and elicit ADCK antibodies. Thus other efforts in Jacobs lab focus on characterizing the mechanisms by which the ADCK antibodies facilitate the collaboration of innate immunity with adaptive immune responses.

Lab Website: http://williamrjacobs.org/

Select Publications:

- **Tiwari S, vanTonder AJ, Vilchèze C, Mendes V, Thomas SE, Malek A, Chen B, Chen M, Kim J, Blundell TL, Parkhill J, Weinrick B, Berney M, Jacobs WR Jr.** (2018). "Arginine-deprived-induced ozidative damage sterilizes Mycobacterium tuberculosis." *PNAS*. 115(39):9779-9784. PMCID: PMC6166831
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ANDREAS JENNY, Ph.D.

Canonical and non-canonical Wnt signaling: patterning and cell polarization

Wnt/Wingless (Wg) growth factors commonly signal through either the canonical Wnt (Wg)-Frizzled (Fz)/β-catenin pathway or through non-canonical Wnt pathways such as the Wnt/Fz-planar cellular polarity (PCP) pathway, resulting the polarization of cells within the plane of the epithelium. These two pathways are highly conserved between humans, mice, fish, and flies. Canonical Wnt/β-catenin signaling is essential for many aspects of development. For example in vertebrates, it controls the specification of the dorsal-ventral (D-V) embryonic axis, cell proliferation in many tissues, and the maintenance of stem cells and during vascularization. In addition, aberrant canonical Wnt signaling in humans causes cancer. Our lab studies the function of Wnk kinases, for which we have identified a novel role in Wnt signaling in addition to their well-known role in the regulation of ion homeostasis in the kidney, where their misregulation causes hypertension (Gordon syndrome).

Non-canonical Wnt signaling established polarity within the plane o an epithelium, commonly referred to as epithelial planar cell polarity (PCP) and allows a cell to form structures that require not only positional, but also vectorial information. Examples of PCP in vertebrates can be very obvious, as in the ordered arrangement of scales on fish or hairs of mammalian skin. Less visible examples are the cilia of the respiratory tract and oviduct as well as the stereocilia of the sensory epithelium of the organ of Corti in the vertebrate inner ear. Aberrant PCP can lead to left/right asymmetry defects, open neural tubes, deafness and kidney disease. PCP signaling is, however, best studied in *Drosophila melanogaster*, mainly because of the versatility of the fly as model system. Our lab is particularly interested in how Rho kinase (Rock) is required for the migration aspect of PCP establishment which will help to understand tumor cell migration.

A genetic model for Endosomal Microautophagy

Autophagy delivers cytosolic components to lysosomes for degradation and is thus essential for cellular homeostasis and to cope with different stressors. As such, autophagy counteracts various human diseases and its reduction leads to aging like phenotypes. Macroautophagy (MA) can selectively degrade organelles or aggregated proteins, but selective degradation of single proteins has only been described for Chaperone-mediated autophagy (CMA) and endosomal Microautophagy (eMI). These two autophagic pathways, described to date only in mammals, are specific for proteins containing KFERQ-related targeting motifs. In collaboration with the Cuervo lab, we have developed a fluorescent reporter to characterize an eMI or CMA-like process in *Drosophila in vivo*. Our data provide evidence for a novel, starvation inducible catabolic process resembling endosomal microautophagy in a non-mammalian species. We are thus for the first time able to perform genetic screens for regulatory components of eMI, this only recently identified form of autophagy about which barely anything is known.

It is our goal to use *Drosophila* as model system to address fundamental questions that are relevant for development and disease in general.

Lab homepage: http://jennylab.aecom.yu.edu/

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GANJAM V. KALPANA, Ph.D.

Molecular Genetic Analysis of INI1/hSNF5 in HIV-1 Replication, HIV-1 latency and Cancer: Single molecule analysis to Organismal studies

INI1/hSNF5/SMARCB1/BAF47 is a component of the chromatin remodeling SWI/SNF complex. We first discovered it as a binding partner for HIV-1 integrase (IN). *INI1/SMARCB1* gene is also a tumor suppressor biallelically mutated/deleted in many human cancers. The goal of our laboratory is to determine how INI1 affects HIV-1 replication, tumor suppression, and in general cellular function.

(i) RNA mimicry of IN-binding domain of INI1/SMARCB1, its role in HIV-1 replication and its potential as a drug target for intervention of AIDS: HIV-1 is the retrovirus that causes AIDS. We have established that INI1/hSNF5 plays multiple roles during HIV-1 replication including viral integration, transcription and post-transcriptional events. Perturbing INI1 have potent inhibitory effects. Structural information often provide functional clues. In collaboration with Drs. Girvin, Cowburn and Almo, we have solved an NMR structure of IN-binding INI1 Rpt1 domain and have discovered that this domain structurally mimics TAR RNA. RNA mimicry of INI1 sheds novel insights about the cellular and viral function of INI1. We are in the process of determining these mechanisms and use it novel srug targets to inhibit HIV-1 replication.

(ii) Study of HIV-1 latency- Application of a novel single cell and single molecule RNA-FISH and IF method: A final hurdle to eradicate HIV-1 is the persistence of the virus in latent reservoirs, which are transcriptionally suppressed and low in number. While the current drugs control viremia, they are unable to eliminate the virus from the infected cells. To detect these rare reactivated latent cells in patient samples, we have developed novel Single Cell Single Molecule Immuno-fluorescence and RNA-FISH assay (SMIRA) in collaboration with Dr. Robert Singer. This novel assay will be applied to characterize latent reservoirs in various anatomical locations and the effect of various Latency Reversing Agents (LRA, that are in clinical trials) and various drugs of abuse will be studied.

(iii) Mechanism of tumor suppression by *INI1/hSNF5* and developing novel and effective therapeutic strategies to combat INI1-deficient tumors: By using a series of genetic systems developed in our laboratory (knock-out, knock-in mouse models, cell culture models), we are dissecting the mechanism of INI1-medaited tumor suppression and developing molecularly targeted therapies. Previously we demonstrated that Cyclin/Cdk pathway and Aurora A as novel targets for rhabdoid tumors. In addition, we had discovered that INI1 harbors a masked nuclear export signal and that accumulation of this protein in the cytoplasm could be a tumor a tumor suppressor mechanism. Currently, in collaboration with international team of neuropathologists, we have found cytoplasmic INI1 in a significant number of rhabdoid tumors. Currently we are exploring the possibility that inhibiting nuclear export is a novel therapeutic strategy. In addition, we are investigating novel downstream targets of INI1, i.e. FoxM1 and GBP1, and their effect on senescence and their therapeutic potential.

Selected Publications:

Craig, E., Zhang, Z., Davies, K., and Kalpana, G. V. (2002) A masked NES in INI1/hSNF5 mediates hCRM1-dependant Nuclear Export: Implications for tumorigenesis. *EMBO J*. **21**, 31-42.

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Lee, S-J., Cimica, V., Zagzag, D., and Kalpana, G. V. (2011) Aurora A Is a Repressed Effector Target of the Chromatin Remodeling Protein INI1/hSNF5 Required for Rhabdoid Tumor Cell Survival. *Cancer Res.* **71**:3225-35. Epub 2011 Apr 26.

Mathew, S., Nguyen, M., Wu, X., Pal, A., Shah, V. B., Aiken, C., and Kalpana, G. V. (2013) INI1/hSNF5-interaction defective HIV-1 IN mutants exhibit impaired particle morphology, reverse transcription and integration *in vivo. Retrovirology.* **10**:66. [Epub ahead of print] PMID:23799881

Bhutoria, S., Kalpana G. V.*, and Acharya, S.* (2016) Computational Modeling of Repeat1 region of INI1/hSNF5: An evolutionary link with Ubiquitin. *Equal corresponding authors. *Protein Sci.* (PMID:27261671**)**

A. LaPorte, J. Cano, X. Wu, D. Mitra, G. V. Kalpana (2016) An essential role of INI1/hSNF5 chromatin remodeling protein in HIV-1 post-transcriptional events and Gag/GagPol stability. J Virol. 2016 Oct 14;90(21):9889-9904

David A., Rao V. R., Wu, W., Ramasamy, S., Pujato, M., Ruiz, A. P., Fiser, A., Bresnick, A., Kalpana,G. V., Prasad, V. R. (2019) CCL2 Mobilizes ALIX to Facilitate 1 Gag-p6 Mediated HIV-1 Virion Release. *eLife*;8:e35546

S. Bhutoria, U. Dixit, X. Wu, M. Spira, S. Mathew, R. Harris, L. Adams, S. Cahill, R. Pathak, R. Prakash, S. A. Acharya, M. Brenowitz, S. Almo, A. Steven, D. Cowburn, M. Girvin and G. V. Kalpana (2018) Structural mimicry of INI1/SMARCB1 Rpt1 domain to HIV-1 TAR RNA mediates its binding to HIV-1 integrase to facilitate viral replication **(*Submitted*)**

HERB LACHMAN, M.D.

Induced pluripotent stem (iPS) cells for disease modeling in schizophrenia and autism

A significant obstacle in studying the molecular basis of schizophrenia (SZ), autism spectrum disorders (ASD) and other neuropsychiatric disorders is the inaccessibility of the human brain, which has restricted molecular studies, such as gene expression profiling and epigenetic analysis, to autopsy samples. While some interesting findings have been made using postmortem brain, interpreting the data is associated with numerous confounding factors. In addition, since SZ is believed to be a developmental disorder, studying molecular events in postmortem samples is limiting. The discovery of iPS cells, which essentially allows investigators to reprogram somatic cells into pluripotent stem cells capable of differentiating into neurons and other cell types, provides an opportunity to create patient-specific neurons *in vitro*. The Lachman lab develops patient-specific iPS cells from controls and patients with SZ who have 22q11.2 deletions, which is found in ~1% of patients. Recently, an iPS cell model has been generated for Lowe Syndrome, a rare X-linked disorder that is associated with learning disability and epilepsy. In addition, an iPS cell model relevant to neuropsychiatric disorders is being generated using CRISPR-Cas9 gene editing targeting the ASD candidate gene CHD8. Gene expression profiling using RNA-seq showed that CHD8 haploinsufficiency leads to an increase in expression of genes involved in GABAergic differentiation in cerebral organoids, a property shared with another ASD candidate gene, FOXG1. CHD8 haploinsufficiency also leads to alterations in WNT/β-catenin signaling. Both GABAergic differentiation and WNT/β-catenin signaling are druggable targets; translating basic science findings into novel drug treatments for SZ and ASD is a major objective of the Lachman lab.

Selected Recent Publications:

Mingyan Lin, Erika Pedrosa, Abhishek K. Shah, Anastasia Hrabovsky, Shahina Maqbool, Deyou Zheng, **Herbert M. Lachman.** Deep sequencing transcriptome analysis of human neurons derived from induced pluripotent stem cells identifies candidate long non-coding RNAs involved in neurogenesis and neuropsychiatric disorders. PLoS One, 2011;6(9):e23356.

Mingyan Lin, Anastasia Hrabovsky, Erika Pedrosa, Tao Wang, Deyou Zheng, Herbert M. Lachman. Allele-biased expression in differentiating human neurons: implications for neuropsychiatric disorders. PLoS One. 2012;7(8):e44017. Epub 2012 Aug 30

Mingyan Lin, Dejian Zhao, Anastasia Hrabovsky, Erika Pedrosa, Deyou Zheng, Herbert M. Lachman. PLoS One. 2014 Apr 15;9(4):e94968. doi: 10.1371/journal.pone.0094968. eCollection 2014. Gene expression profiling in an induced pluripotent stem cell model of the developing human telencephalon: effects of heat shock and its potential consequences in the development of neuropsychiatric disorders.

Jian Chen, Mingyan Lin, Anastasia Hrabovsky, Erika Pedrosa, Jason Dean, Swati Jain Deyou Zheng, Herbert M. Lachman ZNF804A transcriptional networks in differentiating human neurons derived from induced pluripotent stem cells. PLoS One. 2015 Apr 23;10(4):e0124597.2015.

Zhao D, Lin M, Chen J, Pedrosa E, Hrabovsky A, Fourcade HM, Zheng D, Lachman HM.
PLoS One. 2015 Jul 14;10(7):e0132387. doi: 10.1371/journal.pone.0132387. eCollection 2015.PMID: 26173148 MicroRNA Profiling of Neurons Generated Using Induced Pluripotent Stem Cells Derived from Patients with Schizophrenia and Schizoaffective Disorder, and 22q11.2 Del.

Wang P, Lin M, Pedrosa E, Hrabovsky A, Zhang Z, Guo W, Lachman HM, Zheng D. CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. Mol Autism. 2015 Oct 19;6:55. doi: 10.1186/s13229-015-0048-6. eCollection 2015**.**

Nebel RA, Zhao D, Pedrosa E, Kirschen J, Lachman HM, Zheng D, Abrahams BS. Reduced CYFIP1 in Human Neural Progenitors Results in Dysregulation of Schizophrenia and Epilepsy Gene Networks. PLoS One. 2016 Jan 29;11(1):e0148039. doi: 10.1371/journal.pone.0148039. eCollection 2016.

Mingyan Lin, Erika Pedrosa, Ryan Mokhtari, Anastasia Hrabovsky, Jian Chen, Benjamin R. Puliafito, Stephanie R Gilbert, Deyou Zheng, Herbert M. Lachman. Integrative Transcriptome Network Analysis of iPSC-derived Neurons from Schizophrenia and Schizoaffective Disorder Patients with 22q11.2 Deletion. BMC Syst Biol. 2016 Nov 15;10(1):105.

Ping Wang, Ryan Mokhtari, Erika Pedrosa, Michael Kirschenbaum, Can Bayrak, Deyou Zheng, Herbert M. Lachman. CRISPR-Cas9 mediated knockout of the autism gene CHD8 and characterization of its transcriptional networks in cerebral organoids derived from iPS cells. Mol Autism. 2017 Mar 20;8:11. doi: 10.1186/s13229-017-0124-1

Dejian Zhao, Ryan Mokhtari, Erika Pedrosa, Rayna Birnbaum, Deyou Zheng, Herbert M. Lachman. Mol Autism. 2017 Mar 29;8:17. doi: 10.1186/s13229-017-0134-z. Transcriptome analysis of microglia in a mouse model of Rett Syndrome: differential expression of genes associated with microglia/macrophage activation and cellular stress

JACK LENZ, Ph.D.

Molecular Genetics of Retrovirus Induced Cancer and Other Diseases

Retroviruses are associated with a variety of diseases in humans and other vertebrates including cancer and immunodeficiency. The major goals of the laboratory are focused on understanding the molecular basis of retroviral diseases. Mouse retroviruses cause tumors by a mechanism of insertional activation of oncogenes where the viral DNA integrates adjacent to an oncogene, and enhancer elements in the virus activate transcription of the adjacent host gene. We use these viruses as tools for high-throughput identification of cancer-causing genes in the mouse genome. Using sophisticated PCR techniques combined with massively parallel DNA sequencing, the viruses have been used as molecular tags to identify over 60 different genes that cause lymphomas, many of which have not been associated with a cancer-causing role previously, and we are also investigating the molecular mechanisms by which these genes act. Since retrovirus gene therapy vectors cause tumors in human patients by the identical mechanism, we have developed strategies to prevent retroviruses (or any other gene therapy vector that integrates into the human genome) from activating oncogenes and causing cancer. Our newly developed strategies can block most tumors, and are being adapted to human gene therapy use, and we are striving for even greater success.

8% of the human genome is retrovirus DNA. Human endogenous retrovirus K (HERV-K) is the newest of all the retroviruses to enter the germline DNA of humans that is transmitted from parents to children. All humans are born with about 20 distinct HERV-K proviruses (the form of retroviral DNA that is integrated into the host genome) in their germlines. We are investigating whether this retrovirus can reinfect humans today. We have shown that most HERV-K proviruses in the human formed relatively recently in human evolution, long after the divergence of the human and chimpanzee lineages approximately 6 million years ago. We identified several proviruses that formed so recently that they are not yet fixed in the human genome. We have also identified two HERV-K proviruses that have full length open reading frames for all viral proteins, and are the best candidates to be infectious retroviruses in the human genome today. We are now asking whether HERV-K can indeed replicate in humans today, and whether it might be associated with any diseases.

In collaboration with Drs. Larry Herbst and Robert Burk, we are also studying a herpesvirus and a papillomavirus that are associated with fibropapillomas in endangered and threatened species of marine turtles. We are investigating the evolutionary histories of these viruses, how they are transmitted, the nature of turtle immune responses to them, and the roles of the viruses in causing tumors.

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Turner G, Barbulescu M, Su M, Jensen-Seaman MI, Kidd KK, Lenz J. Insertional polymorphisms of full-length endogenous retroviruses in humans. Curr Biol. 2001 Oct 2;11(19):1531-5.

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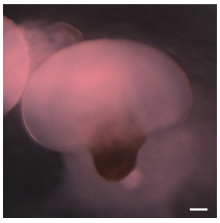
Herbst LH, Lemaire S, Ene AR, Heslin DJ, Ehrhart LM, Bagley DA, Klein PA, Lenz J. Use of baculovirus-expressed glycoprotein H in an enzyme-linked immunosorbent assay developed to assess exposure to chelonid fibropapillomatosis-associated herpesvirus and its relationship to the prevalence of fibropapillomatosis in sea turtles. Clin Vaccine Immunol. 2008 May;15(5):843-51.

Heslin DJ, Murcia P, Arnaud F, Van Doorslaer K, Palmarini M, Lenz J. A single amino acid substitution in a segment of the CA protein within Gag that has similarity to human immunodeficiency virus type 1 blocks infectivity of a human endogenous retrovirus K provirus in the human genome. J Virol. 2009 Jan;83(2):1105-143.

Mitra, K, Lenz, J. A combinatorial insulator strategy strongly reduces genome-wide oncogene utilization and tumorigenesis by retroviruses; implications for safer gene therapy. Manuscript submitted.

WEI LIU, Ph.D.

Retinal differentiation, inherited degenerations, and regeneration



- Elucidate the molecular and cellular mechanisms of retinal differentiation using engineered mice
- Model human retinal differentiation and inherited degenerations using pluripotent stem cells

The neuroretina, retinal pigment epithelium (RPE), ciliary body, and iris are structurally and functionally connected in the human adult retina. Inherited degenerations of any tissue will affect the others, leading to blinding retinal disease such as retinitis pigmentosa, age-related macular degenerations, and glaucoma. Macular degenerations affect vision the most, since the macula is responsible for central vision and visual acuity. Human adult neuroretina does not naturally regenerate. Regenerative medicine of the retina holds a promise to save and restore vision.

Elucidating the mechanisms of retinal differentiation is a prerequisite for retinal regeneration. Embryonic development of the neuroretina, RPE, ciliary body, and iris is an integrated process under the regulation of transcription factors and signal transduction molecules. In mice, morphogenesis of optic-cups leads to the specification of neuroretinal and RPE progenitor cells in the inner and outer layer of optic cups at E10.5, respectively. Neuroretina is continuous with RPE via epithelial sheet bending. Close to the bending region, peripheral neuroretina gradually reduces its thickness to form a tapered zone, which is subsequently specified as ciliary margin at E12.5. Neuroretinal progenitor cells are multipotent, producing all retinal neurons and Müller glial cells. Ciliary margin differentiates into ciliary body and iris. How multipotent retinal progenitor cells are regulated in coordination with ciliary margin specification is underexplored. We address the critical knowledge gap by dissecting the molecular functions of homeodomain transcription factors and signaling transduction molecules in retinal differentiation using engineered mice.

The macula is enriched for cone photoreceptors and is unique to primates. The availability and high cost of non-human primates limit their use in retinal disease studies. Macular degenerations are often not closely recapitulated in mouse models because mice do not have the macula. Notably, we recently generated and characterized cone-rich human retinal organoids reminiscent of the macula based on the ratio of cones to rods and single-cell transcriptomes. As a recognition by the field, we recently received an NEI prize for progress toward developing lab-made retinas. We now utilize retinal organoids to model human retinal differentiation and inherited degenerations.

Current projects in my lab:

- To elucidate the mechanisms underlying the regulation of multipotent neuroretinal progenitor cells;
- To determine the mechanisms of photoreceptor cell differentiation;
- To model human retinal differentiation and inherited degenerations using human embryonic stem cells.

Our studies will decipher the mechanisms of retinal differentiation and inherited degenerations, leading to therapeutic development for blinding retinal disease.

Recent publications

Kim S, Lowe A, Dharmat R…Zhou Z, Chen R, **Liu W** (2019). **Proc Natl Acad Sci U S A**, 116(22):10824-10833.

Diacou R, Zhao Y, Zheng D, Cvekl A, **Liu W** (2018). **Cell Reports**, 25: 2510-2523.

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Lowe A, Harris R, Bhansali P, Cvekl A, **Liu W** (2016). **Stem Cell Reports**, 6(5):743-756.

Liu W, LagutinO, SwindellE, JamrichM and OliverG (2010). **J of Clin Invest**,120: 3568-77.

SRIDHAR MANI, M.D.

Phenotyping Orphan Nuclear Receptors

Orphan nuclear receptors (those that lack a well defined physiologic ligand) control nearly every major physiologic and biochemical process in eukaryotes - cell metabolism (e.g., cholesterol, energy, bile acids), xenobiotic detoxification, cell differentiation (e.g., gastrulation, retinal development), circadian rhythm, and cancer cell growth and apoptosis (e.g., NURR77). Of these receptors, the steroid and xenobiotic receptor (SXR or PXR) is a key regulator of genes encoding drug metabolizing and transport proteins. In addition, PXR has been implicated in cancer drug resistance, carcinogenesis and pathophysiologic states like osteomalacia. Our laboratory has provided a first link of how this receptor controls normal intestinal physiology by co-opting bacterial metabolites. These studies have led to investigations into the microbiome, metabolites and PXR related receptor systems in illness and physiology.

SEE: https://sites.google.com/view/mani-lab/home

Recent Publications:

Wang H, Li H, Moore LB, Maglich JM, Goodwin B, Price R, Itoop ORR, Jones SA, Wisely B, Creech K, Parks DJ, Collins JL, Willson TM, Kalpana G, Xie W, Redinbo M, Moore JT, Mani S (2007). The Phytoestrogen Coumestrol is a Naturally Occurring Antagonist of the Pregnane X Receptor (PXR). *Mol Endocrinol.* 22:838-57.

Wallace B, Wang H, Lane KT, Scot JE, Oran E, Koo J, Jobin C, Yeh L, Mani S, Redinbo M. Alleviating Cancer Drug Toxicity by Inhibiting a Bacterial Enzyme. Science. 330(6005):831-5, 2010

Biswas A, Pasquel D, Tyagi RK, Mani S. Acetylation of Pregnane X Receptor protein determines selective function independent of ligand activation. Biochem Biophys Res Commun. 406(3): 371-6, 2011

Venkatesh M, Wang H,Cayer J, Leroux M, Salvail D, Das B, Mani S. Designing First-In Class Novel and Non-Toxic Azole Analogs that Target Pregnane X Receptor (PXR)-Mediated Drug Resistance. Mol Pharm 80(1):124-35, 2011

Wang H, Venkatesh M, Hao L, Goetz R, Mukherjee S, Biswas A, Zhu L, Kaubisch A, Wang L, Pullman J, Whitney K, Kuo-o M, Roig AI, Mohammadi M, Mani S. Pregnane X Receptor Activation Leads to Fibroblast Growth Factor-19 Dependent Tumor Regeneration after Cancer Chemotherapy. J. Clin Invest, 121(8):3220-32, 2011

Li H, Redinbo MR, Venkatesh M, Ekins S, Chaudhry A, Bloch N, Negassa A, Mukherjee P, Kalpana G, Mani S. Novel Yeast-based Strategy Unveils Antagonist Binding Regions on the Nuclear Xenobiotic Receptor PXR. J Biol Chem. 2013 May 10;288(19):13655-68

Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, Qiu Z, Maher L, Redinbo MR, Phillips RS, Fleet JC, Kortagere S, Mukherjee P, Fasano A, Le Ven J, Nicholson JK, Dumas ME, Khanna KM, Mani S. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity 41(2):296-310, 2014

Qiu Z, Cervantes JL, Cicek BB, Mukherjee S, Venkatesh M, Maher LA, Salazar JC, Mani S, Khanna KM. Pregnane X Receptor Regulates Pathogen-Induced Inflammation and Host Defense against an Intracellular Bacterial Infection through Toll-like Receptor 4. Sci Rep. 6:31936 (2016)

Pasquel D, Doricakova A, Li H, Kortagere S, Krasowski MD, Biswas A, Walton WG, Redinbo MM, Dvorak Z, Mani S. Acetylation of lysine 109 modulates pregnane X receptor DNA binding and transcriptional activity. Biochim Biophys Acta. (GRM) Feb 9. pii: S1874-9399(16)30003-7 (2016)

Huang K, Mukherjee S, DesMarais V, Albanese JM, Rafti E, Draghi Ii A, Maher LA, Khanna KM, Mani S, Matson AP. Targeting the PXR-TLR4 signaling pathway to reduce intestinal inflammation in an experimental model of necrotizing enterocolitis. Pediatr Res. 2018 May;83(5):1031-1040.

Pulakazhi Venu VK, Saifeddine M, Mihara K, Tsai YC, Nieves K, Alston L, Mani S, McCoy KD, Hollenberg MD, Hirota SA. The pregnane X receptor and its microbiota-derived ligand, indole 3-propionic acid, regulate endothelium-dependent vasodilation. Am J Physiol Endocrinol Metab. 2019 Jun 18. doi: 10.1152/ajpendo.00572.2018. [Epub ahead of print]

CRISTINA MONTAGNA, Ph.D.

Genetic, epigenetic and ploidy changes during cell differentiation in development and disease.

Project 1- Role of Septin 9 in Breast Carcinogenesis.

A comparative cytogenetic approach aimed to identify recurrent DNA copy number variations in a panel of murine models for breast cancer resulted in the identification of Septin 9 (Sept9) as potential novel oncogene. The septin family of genes codes for a highly redundant and conserved family of GTP-binding proteins that assemble into filaments and bind to microfilaments and microtubules. At the locus of genomic amplification deregulation of Sept9 expression occurs by a complex pattern of genetic and epigenetic alterations affecting several Sept9 isoform variants. Our hypothesis is that during malignant transformation, breast epithelial cells undergo genomic amplification of the Sept9 locus and over-express Sept9 mRNA and protein. Additionally, aberrant cytosine methylation occurs at specific alternative promoters within the Sept9 locus resulting in an abnormal pattern of Sept9 isoform variants. We are currently studying how the expression of various Sept9 isoforms is regulated in normal and cancer cells and the functional differences between these isoforms.

Project 2- Stage- and Cell Subtype-Specific Epigenetic Regulation of Mammary Gland Development and breast tumorigenesis.

We are interested in investigating the DNA methylation changes occurring in the development of the normal mammary gland during puberty, adult age, pregnant, lactating and undergoing mammary gland involution. This approach has the final goal of dissecting the molecular processes that mediate methylation changes in the morphogenesis and differentiation of the normal breast and to identify “hot spot” loci for gene silencing in breast carcinogenesis.

Project 3- Aneuploidy in aging.

Polyploidy and aneuploidy are the most frequent cytogenetic events observed in mammalian cells. Polyploidization is a widely accepted mechanism for increasing genetic variation in unicellular organisms and for the acquisition of new properties in a variety of cell types (e.g., osteoclast fusion in bone resorption and myoblast fusion in muscle development) and is considered a physiological process. Aneuploidy on the contrary is linked to pathological states. It is a hallmark of spontaneous abortions and birth defects and is observed virtually in every human tumor. While the catastrophic consequence of high levels of aneuploidy observed in abortions is self-explanatory, the role of aneuploidy under physiological conditions is a question waiting for answers. The major goal of this project is to explore a possible correlation between age-associated genome instability in a variety of tissues and functionality of these cells.

Recent Publications:

Acosta, D., M. Suzuki, D. Connolly, R. F. Thompson, M. J. Fazzari, J. M. Greally and C. Montagna (2011). "DNA methylation changes in murine breast adenocarcinomas allow the identification of candidate genes for human breast carcinogenesis." Mammalian genome : official journal of the International Mammalian Genome Society 22(3-4): 249-259.

Connolly, D., I. Abdesselam, P. Verdier-Pinard and C. Montagna (2011). "Septin roles in tumorigenesis." Biological chemistry 392(8-9): 725-738.

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BERNICE E. MORROW, Ph.D.

Mammalian Developmental Genetics

Our lab is interested in discovering genes required for human embryonic development to understand the cause of birth defects. Our research begins with collecting DNA samples from affected individuals with genetic disorders having known chromosomal gains or losses, and moves to looking at gene function in vertebrate model organisms. The reason for studying chromosomal disorders is that affected regions in the genome will pinpoint the location of causative genes whose function in organogenesis is sensitive to copy number.

Our main focus is on a disorder termed chromosome 22q11.2 deletion syndrome (22q11DS). Most affected individuals have a similar sized 3 million base pair (Mb) deletion encompassing 60 genes. The deletion occurs by a mistake during meiosis in forming the egg or sperm. Individuals with the syndrome have learning disabilities, psychiatric illness, cleft palate, hearing loss and cardiovascular defects. Many of these defects occur commonly in the general population in non-syndromic forms. This is why molecular genetic studies of this syndrome are particularly relevant to human health and disease.

One key gene in the 22q11.2 region is termed *TBX1* and it encodes a transcription factor that is responsible for many of the defects in patients with the syndrome. Using knockout and gain-of-function mutant mice, we have made headway to understand its function. Since it's a transcription factor, we are interested in genes it can regulate. Part of our mission is to understand the role of *Tbx1* in making cell fate decisions in mammalian embryos. We are doing this by taking single cell RNA-sequencing, chromatin accessibility and chromatin immunoprecipitation followed by genome sequencing from microdissected tissues from wildtype and mutant embryos followed by bioinformatics analysis.

Although most individuals with 22q11DS have the same sized deletion, the severity of malformations varies dramatically. For example, 60% have heart defects, many requiring surgery, while the rest have a normal heart. We hypothesize that the 22q11.2 deletion is the first hit in the genome and it uncovers other mutations that act as second hits to modify the overall phenotype of the disorder. We are taking candidate gene and unbiased whole genome sequencing approaches to identify genetic "modifiers" in 1,053 subjects with 22q11DS. We are identifying common and rare, copy number and single nucleotide variants. In order to interpret the genomic data, we are taking systems biology approaches. In this way, we will extract biologically important gene networks in a holistic sense. At the same time, genes in the networks will be tested for functional significance in mouse models.

Recent Publications:

Guo T, Chung JH, Wang T, McDonald-McGinn DM, Kates WR, Hawuła W, Coleman K, Zackai E, Emanuel BS, Morrow BE. Histone Modifier Genes Alter Conotruncal Heart Phenotypes in 22q11.2 Deletion Syndrome. Am J Hum Genet. 2015 Dec 3;97(6):869-77. PMID: 26608785

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Hasten E, Morrow BE. *Tbx1* and *Foxi3* genetically interact in the pharyngeal pouch endoderm in a mouse model for 22q11.2 deletion syndrome. PLoS Genet. 2019; In Press.

JAYANTA ROY-CHOWDHURY, MBBS, MRCP, AGAF, FAASLD

Our current focus is on developing cel-l and gene-based therapies for inherited hyperbilirubinemia (Crigler-Najjar syndrome, CN-1), α_1 antitrypsin (AAT) deficiency and primary hyperoxalurias.

Subproject 1. Hepatocyte-based therapies for genetic liver diseases. To overcome the hurdles of shortage of donor livers, inefficient hepatocyte engraftment and the need for prolonged immunosuppression, we are developing strategies to promote initial engraftment and augment subsequent proliferation of transplanted hepatocytes. To enhance initial; engraftment by transient disruption of the hepatic endothelial barrier we are evaluating drugs and low dose hepatic irradiation. Subsequently, proliferation of the engrafted cells is achieved by preparative regiospecific X-irradiation of the liver and mitotic stimulation using hepatocytes growth factor or thyroid hormone receptor-beta (TR-β) agonist drugs. Our work was translated into the first successful hepatocyte transplantation in a CN-1 patient. Regiospecific hepatic X-irradiation is being evaluated in a clinical trial in collaboration with University of Pittsburgh. We are also using gene transduction of donor primary hepatocytes to achieve their pharmacologically controlled proliferation after transplantation.

AAT deficiency (ATD) is one of the most common potentially lethal inherited liver disorders in the West. Classic ATD results from the expression of a misfolded AAT (ATZ) that is not secreted inefficiently and is retained within hepatocytes. Circulatory AAT deficiency leads to unrestrained neutrophil elastase activity in the lung, causing severe pulmonary emphysema, whereas ATZ accumulation within hepatocytes causes liver disease. We have shown that wildtype hepatocytes transplanted into transgenic mice expressing human ATZ competitively replace the host hepatocytes. Our current focus is to disrupt the ATZ expression in a fraction of the hepatocyte mass by DNA break-enhanced homologous recombination in vivo, permitting the gene-edited hepatocytes to repopulate the liver, thus providing normal AAT and correcting the liver disease.

Subproject 2. Human embryonic and pluripotent stem cells as sources of hepatocytes: A renewable source of hepatocytes from individual subjects could facilitate cell-based therapies of inherited liver diseases by alleviating the shortage of donor livers and circumventing the need for immunosuppression. Toward this goal, we have generated induced pluripotent stem cells (iPSCs) from skin fibroblasts or urinary epithelial cells of normal subjects and patients with inherited metabolic liver diseases, and differentiated the iPSCs into hepatocyte-like iHeps. Transplantation of normal human iHeps into jaundiced Gunn rats (model of human CN1), engraftment of the cells in the liver and their proliferation under appropriate stimuli. Partial repopulation of the Gunn rat liver with normal human iHeps ameliorated hyperbilirubinemia, providing the first example of effective therapy of an inherited metabolic liver disorder by transplanting iPSC-derived cells.

In ongoing studies we are correcting the genetic lesions in patient-derived iPSCs by CRISPR-cas or zinc finger nuclease enhanced homologous recombination to derive phenotypically corrected autologous iHeps for testing by transplantation into animal models of inherited human liver diseases.

Recent Publications:

Ding J, Yannam GR, Roy-Chowdhury N, Hidvegi T, Basma H, Rennard SI, Wong RJ, Avsar Y, Guha C,Perlmutter DH, Fox IJ, **Roy-Chowdhury J.** Spontaneous hepatic repopulation in transgenic mice expressingmutant human alpha 1-anti-trypsin by wildtype donor hepatocytes. J. Clin. Invest. 121:1930-4, 2011.

Chen Y, Li Y, Wang X, Zhang W, Sauer V, Chang CJ, Han B, Tchaikovskaya T, Avsar Y, Tafaleng E, Madhusudana Girija S, Tar K, Stephen S, Bouhassira E, Guha C, Fox IJ, **Roy-Chowdhury J** and **Roy-Chowdhury N**. Amelioration of hyperbilirubinemia in Gunn rats after transplantation of hepatocytes derived from human induced pluripotent stem cells. Stem Cell Reports 5:1-9, 2015.

Sauer V, Tchaikovskaya T, Wang X, Li Y, Zhang W Tar K, Polgar Z, Ding J, Guha C, Fox IJ, **Roy-Chowdhury N, Roy-Chowdhury J.** Human urinary epithelial cells as a source of engraftable hepatocyte-like cells using stem cell technology. Cell transplant, 2016, 25:2221-2243.

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Peterson EA, Polgar Z, Devakanmalai GS, Li Y, Jaber FL, Zhang W, Wang X, Iqbal NJ, Murray JW, Roy-Chowdhury N, Quispe Tintaya W, Maslov AY, Tchaikovskaya TL, Sharma Y, Rogler LE, Gupta S, Zhu L**, Roy-Chowdhury J**, Shafritz DA. Genes and pathways promoting long-term liver repopulation by ex vivo hYAP-ERT2 transduced hepatcytes and treatment of jaundice Gunn rats. Hepatology Communications 2019; 3:129-146

Barahman M, Zhang W, Harris HY, Aiyer A, Kabarriti R, Kinkhabwala M, Roy-Chowdhury N, Beck AP, Scanlan TS, **Roy-Chowdhury J**, Asp P, Guha C. Radiation-primed hepatocyte transplantation in murine monogeneic dyslipidemia normalizes cholesterol and prevents atherosclerosis. J Hepatol. 2019 70:1170-1179.

NAMITA ROY-CHOWDHURY, Ph.D.

I. Inherited Disorders of Bilirubin Glucuronidation

UGT1A1 is a member of UDP-glucuronosyltransferases (UGT) family of enzymes, which is concentrated in the hepatic endoplasmic reticulum (ER). UGT1A1, which mediates the glucuronidation of bilirubin and estrogens. is required for biliary excretion of bilirubin. We showed that the genetic lesions in any one of the five exons encoding UGT1A1 can abolish or reduce bilirubin glucuronidation, causing potentially lethal Crigler-Najjar syndrome type I (CN-I), or its less severe variant, Crigler-Najjar syndrome type II (CN-II). We also showed that Gilbert syndrome, a milder form of inherited hyperbilirubinemia, is caused by a promoter polymorphism. We have been studying the regulation of UGT1A1 gene expression. Our current objective is to develop novel gene and cell-based therapies to cure this disease. Fibroblasts or renal tubular epithelial cells present in urine of CN1 patients will be reprogrammed to iPS cells, genetically corrected, differentiated into hepatocytes and transplanted into jaundice Gunn rat model of CN1.

II. **Primary Hyperoxaluria Type 1 (PH1)**

PH1 is an autosomal recessive disease caused by mutations in the alanine:glyoxylate aminotransferase gene (*AGXT*). In humans, insufficient AGXT activity in liver peroxisomes leads to increased oxalate production that causes calcium oxalate stones in the kidney and then in blood, heart, bones, etc. It is a lethal disease unless combined liver and kidney transplantation is performed. We have developed a mouse model of PH1. Our plan is to cure this disease by (a) gene therapy (b) transplantation of adult primary hepatocytes or (c) hepatocytes derived from human embryonic (hESC) or induced pluripotent stem cell (iPSC). For the latter, fibroblasts from the skin of normal volunteers or patients with PH1 are used to generate iPS. Initially we used viral vectors to generate the iPSCs, but now use non-DNA integrating approaches to generate iPS cells. The cells are differentiated to hepatocyte-like iHep cells for transplantation into our mouse model of PH1

Publications:

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Roy-Chowdhury N, Roy-Chowdhury J. (2015**)** Liver physiology and energy metabolism. In *Sleisenger and Fordtran's Gastrointestinal and Liver Disease*, 10th edition. Feldman M., Friedman LS, Brandt LJ, editors. Saunders-Elsevier, Philadelphia.Page 1223-1232.

Chen Y, Li Y, Wang X, Zhang W, Sauer V, Chang CJ, Han B, Tchaikovskaya T, Avsar Y, Tafaleng E, Madhusudana Girija S, Tar K, Stephen S, Bouhassira E, Guha C, Fox IJ, Roy-Chowdhury J, **Roy-Chowdhury N.** (2015) Amelioration of hyperbilirubinemia in Gunn rats after transplantation of hepatocytes derived from human induced pluripotent stem cells. Stem Cell Reports 5:1-9.

Sauer V, Tchaikovskaya T, Wang X, Li Y, Zhang W Tar K, Polgar Z, Ding J, Guha C, Fox IJ, **Roy-Chowdhury N,** Roy-Chowdhury J (2016)**.** Human urinary epithelial cells as a source of engraftable hepatocyte-like cells using stem cell technology. Cell transplant, 25:2221-2243.

Polgar Z, Yanfeng Li Y, Wang X, Guha C, **Roy-Chowdhury N**, Roy-Chowdhury J (2017). Gunn rats as a surrogate model for evaluation of hepatocyte transplantation-based therapies of Crigler-Najjar syndrome type 1. Meth Mol Biol 1506:131-147.

Peterson EA, Polgar Z, Devakanmalai GS, Li Y, Jaber FL, Zhang W, Wang X, Iqbal NJ, Murray JW, **Roy-Chowdhury N**, Quispe Tintaya W, Maslov AY, Tchaikovskaya TL, Sharma Y, Rogler LE, Gupta S, Zhu L, Roy-Chowdhury J, Shafritz DA. Genes and pathways promoting long-term liver repopulation by ex vivo hYAP-ERT2 transduced hepatcytes and treatment of jaundice Gunn rats. Hepatology Communications 2019; 3:129-146

Roy-Chowdhury N, Wang X, Roy-Chowdhury J. Bile pigment metabolism and its disorders. Emery and Rimoins's Pinciple and Practice of Medical Genetics, 7th edition, Rimoin DL, Connor JM, Pyeritz RE, Korf BR, editors. Churchill-Livingstone-Elsevier, Philadelphia. 2019, in press

JULIE SECOMBE, Ph.D.

Transcriptional Regulation During Neuronal function and Aging.

In my lab, we are interested in understanding the transcriptional regulatory mechanisms that regulate the development and function of neurons in addition to those that mediate the aging process. To do this, we take advantage of the many genetic tools available by using the model organism *Drosophila melanogaster.*

There are currently two main projects in the lab:

(1) Defining the transcriptional and cellular defects caused by KDM5 mutations that result in intellectual disability. While many mutations in human KDM5 family genes have been found in intellectual disability patients, the link between KDM5 dysfunction and cognitive impairment remains unknown. Based on the hypothesis that intellectual disability-associated mutations in KDM5 are caused by aberrant transcription, we have generated fly strains harboring disease alleles. These alleles show defective learning and memory, in addition to morphological defects in several types of neurons. We are currently examining these fly strains for transcriptional defects and alterations to the recruitment of KDM5 to its target genes. We expect that this first *Drosophila* model of KDM5-induced intellectual disability will dramatically enhance our understanding of human intellectual disability.

(2) Activation of Endogenous Transposable Elements by Myc During Aging. A poorly explored potential contributor to aging is the mobilization of endogenous transposable elements (TEs), which can be highly mutagenic and promote genomic instability. We showed that increasing or decreasing levels of the oncoprotein Myc in *Drosophila* reduces or extends lifespan, respectively. More recently, we have shown Myc activates the expression of a subset of endogenous TEs. Because mobilization of TEs can cause insertional mutagenesis, genome rearrangements and DNA damage, they have been proposed to contribute to tumorigenesis and other phenotypes associated with aging. We are combining single cell analyses with strategies to attenuate or activate specific TEs to define their effect on normal and Myc-induce aging.

Recent Publications:

Drelon, C., Belalcazar, H.M. and J. Secombe (2019) The histone demethylase KDM5 controls developmental timing by promoting prothoracic gland endocycles. https://www.biorxiv.org/content/10.1101/617985v2

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Navarro-Costa, P., McCarthy, A., Prudêncio, P., Greer, C., Guilgur, L.G., Becker J., Secombe, J., Rangan, P and R. Martinho (2016). Early programming of the oocyte epigenome temporally controls late prophase I transcription and chromatin remodeling. *Nat. Comms*., 10;7:12331. PMCID:PMC4987523..

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Quinn, L.M., J. Secombe and G. Hime (2013) Myc in stem cell behavior: Insights from *Drosophila*. Adv Exp Med Biol. 2013;786:269-85.

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FRANK SOLDNER, M.D.

Novel approaches to investigate the genetic, cellular, and molecular basis of complex neurological disorders

Our laboratory focuses on modeling human brain development and function in a cell culture dish to understand the molecular and cellular basis of complex disorders such as Parkinson's and Alzheimer's disease. A significant challenge of studying complex human diseases is the lack of relevant model systems that combine know genetic elements with disease-associated phenotypic readouts. This is particularly problematic for sporadic neurodegenerative diseases that have no well-defined genetic etiology and do not follow Mendelian inheritance patterns. Epidemiology and population genetics suggest that such diseases result from a complex interaction between multiple risk factors, both genetic and non-genetic (lifestyle and environmental). Although genome wide association studies (GWAS) have identified genomic variations, such as single nucleotide polymorphisms (SNPs), deletions, and insertions associated with a higher risk to develop specific neurological disorders, the vast majority of such sequence variants have no established biological relevance to disease or clinical utility to prognosis or treatment.

Three major recent innovations have fundamentally changed our ability to study human neurological disorders in a cell culture dish: (i) Reprogramming of somatic cells into human induced pluripotent stem cells (hiPSCs) to generate patient-derived disease-relevant neuronal cells, (ii) the development of genome engineering technologies such as the CRISPR/Cas9 system to modify the genome in human cells, and (iii) the availability of tissue-type and disease-specific genome-scale genetic and epigenetic information. Our previous work demonstrated that an interdisciplinary approach, integrating these technologies, enables us to study neurological disorders in a genetically controlled and systematic manner in human neuronal cells. Using these previously unavailable molecular and cellular tools, we were able to dissect the functional role of diseases-associated sequence variations in non-coding regulatory elements such as distal enhancer sequences in the pathogenesis of Parkinson's disease. My lab is extending this novel experimental framework in human pluripotent stem cell (hPSC)-derived two-dimensional (2D) monolayer and three-dimensional (3D) organoid neuronal culture systems to systematically investigate the genetic, cellular, and molecular basis of neurodegenerative disorders. We are establishing robust disease-relevant phenotypic readouts to perform unbiased compound and CRISPR/Cas9-based genome-scale genetic screens and will exploit these approaches to understand how genetic, epigenetic, and environmental factors contribute to the development and progression of neurological diseases.

Selected publications

Soldner, F. & Jaenisch, R. Stem Cells, Genome Editing, and the Path to Translational Medicine. ***Cell***, 175, 615-632 (2018)

Soldner, F., Stelzer, Y., Shivalila, C. S., Abraham, B. J., Latourelle, J. C., Barrasa, M. I., Goldmann, J., Myers, R. H., Young, R. A. & Jaenisch, R. Parkinson-associated risk variant in distal enhancer of α-synuclein modulates target gene expression. ***Nature*** 533, 95–99 (2016).

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Soldner, F.*, Hockemeyer, D.*, Beard, C., Gao, Q., Bell, G. W., Cook, E. G., Hargus, G., Blak, A., Cooper, O., Mitalipova, M., Isacson, O. & Jaenisch, R. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. ***Cell*** 136, 964–977 (2009).

Hockemeyer, D.*, Soldner, F.*, Beard, C., Gao, Q., Mitalipova, M., Dekelver, R. C., Katibah, G. E., Amora, R., Boydston, E. A., Zeitler, B., Meng, X., Miller, J. C., Zhang, L., Rebar, E. J., Gregory, P. D., Urnov, F. D. & Jaenisch, R. Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. ***Nat Biotechnol*** 27, 851–857 (2009).

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(* Equally contributing authors)

SIMON SPIVACK, M.D., M.P.H.

Epigenetic Variability and Functional Impact on Gene Regulation in the Lung

The goal of the Spivack laboratory is to understand differences in gene regulation among individuals, using genetic and epigenetic techniques. The mechanistic goal is to understand the subtleties of how specific high-resolution patterns of DNA methylation and microRNA expression regulate gene expression. We have developed several new functional genetic technologies to examine epigenetic function. We have recently completed initial genome-wide searches of the transcriptome, methylome, and microRNAome of lung cancers. The translational goal is to use these functionally sifted epigenetic features, and detect them non-invasively, to identify individuals at particularly high risk for lung cancer and other lung disorders, to enhance prevention and early detection efforts.

Mechanistically, the role of promoter sequence and epigenetic variation in the regulatory region of carcinogenesis and oxidant pathway genes is being explored *in vitro. We develop* techniques in the lab, such as human genomic methyl-DNA reporter constructs, patch methylation strategies, and now CAS9-based methylome writing, in addition to studying native gene regulation models. Unique technologies include the laboratory's new microRNA:mRNA binding assay, and modelling the functional consequence of DNA methylation patterns reproduced in reporter constructs, and now in native chromatin.

Translationally, epigenetic and other biomarkers are being established in laser capture microdissected human lung and several unique, non-invasively collected surrogate specimens developed in the laboratory, such as mRNA expression signatures from brush-exfoliated buccal mucosa cells, and DNA methylation and microRNA analyses from exhaled breath condensate, which are first reports for a new airway biomarker class. These airway-derived specimens continue to accrue from our sampling (currently n>1000) of a lung cancer case-control study. The specimens are being studied for quantitative gene expression, and their regulatory substrates listed above, in multiple pathways. These expression, genetic, and epigenetic data are being linked to put a real metric to gene-environment interaction.

Selected Recent Publications/Manuscripts:

Khulan Batbayar, Weiguo Han, Yakov Peter, Miao Kevin Shi, Amos Yan, Mathew Levy, Simon D. Spivack. Targeted DNA methylation and inactivation of endogenous genes using a CRISPR-DNMT3a fusion protein strategy. *[Submission planned fall 2019].*

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Han W, Cauchi S, Herman JG, Spivack SD. (2006) DNA methylation mapping by tag-modified bisulfite genomic DNA sequencing *Analytic. Biochem.* 355: 50-61. PMID: 16797472.

YOUSIN SUH, Ph.D.

Functional Genomics of Aging

Our long-term research goal is to investigate the genetic components of aging and aging-related disease using functional genomics approaches. We focus on the identification of gene sequence variation, i.e. single nucleotide polymorphisms (SNPs), in candidate genes and the assessment of their potential functional impact on aging-related phenotypes. Candidate genes include categories of genes implicated in the modulation of common causes of aging, e.g. free radical production, antioxidant defense, genome maintenance, and apoptosis, or more targeted pathways involved in specific aging-related diseases such as breast cancer. Any genetic variation found to be significantly associated with one or more defined aging phenotypes is then further investigated in specific functional tests, utilizing in silico modeling, in vitro cell culture models, and mouse models. We consider the assessment of the functional impact of SNP haplotypes, i.e., allelic variation caused by multiple SNPs in the same gene, in vitro and animal models as essential to confirm the link between genotype and phenotype in aging studies. This should ultimately result in an integrated approach to study the genetics of aging at different levels ranging from genetic determinants in the form of DNA sequence variations, through cell type- and tissue-specific gene expression profiles, to molecular and cellular endpoints in tissues, to impacts on quality and duration of life span. The results are expected to lead to the identification of functional pathways that control basic aging processes and the onset of age-related diseases. Insight into the functional impact of individual genetic variation on the aging process will lead to a better understanding of phenotypic variation in aging human populations, including susceptibility to aging-related diseases and exceptional longevity. Importantly, it will help close the long-held gap between the population genetics and molecular genetics of aging.

Four systematic multidisciplinary studies are currently underway. *First*, we have initiated a population-based association study to test genotype-phenotype correlations of genome maintenance genes in a breast cancer cohort. We currently focus on the tumor suppressor BRCA1, which is involved in double strand break repair with broad effects on cellular physiology and genomic stability. We have established a high-throughput mouse embryonic stem cell transgenesis and TALEN-mediated cell engineering to knock-in human BRCA1 haplotype variants for functional analysis in vivo. *Second,* in a cohort of longitudinal study of aging, we are testing the hypothesis that genetic variation at loci involved in genome maintenance mechanisms (e.g., DNA repair, antioxidant defense, cell cycle control, and apoptosis) can be related to individual differences in the rate and severity of aging-related phenotypes. *Third,* we are focusing on identification of functional SNP haplotypes of genes involved in the Growth Hormone/Insulin-like Growth Factor-1 (GH/IGF-1) pathway. Down-regulation of the GH/IGF-1 pathway is well-known to extend life span in model organisms varying from worms and flies to mice. We are investigating whether this evolutionarily conserved pathway play a role in human longevity using Ashkenazi Jewish centenarian cohorts. *Fourth,* we are studying mouse models that harbor human gene variations in DNA repair/genome maintenance and as a consequence manifest premature aging phenotypes. Our results from transcriptome analysis delineate a complex genetic network of cellular responses to endogenous DNA damage and suggest it as the cause of the premature aging phenotypes in these mice.

Selected Publications:

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JAN VIJG, Ph.D.

Genome Dynamics in Aging

Aging is a universal process that brings life to a close at a rate that is specific for the species. In humans, life span has a limit of about 115 years. One process that has been implicated as a causal factor in the aging process is genome instability. Exactly how loss of genome integrity in normal somatic cells may lead to tissue degeneration, functional decline and increased risk of diseases, such as cancer, remains unknown. The main challenge in this respect is the lack of technology to analyze various types of DNA mutations in normal somatic cells. In the past we developed transgenic reporter systems in mouse and fruit fly, which allowed us to determine tissue-specific frequencies of various forms of genome instability, e.g., point mutations, deletions, translocations, as a function of aging. More recently, we developed new, single-cell whole genome sequencing methods to analyze these same types of mutations directly in normal cells. These and other methods, e.g., single-cell DNA methylomics and single-cell multi-omics whole genome sequencing, are now being used to comprehensively characterize the landscape of mutations and epimutations in relation to the aging process.

Selected Publications:

Bahar R, Hartmann CH, Rodriguez KA, Denny AD, Busuttil RA, Dollé MET, Calder RB, Chisholm GB, Pollock BH, Klein CA, Vijg J. Increased cell-to-cell variation in gene expression in aging mouse heart. *Nature* 2006;441:1011-1014.

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Gundry M, Li W, Maqbool SB, Vijg J. Direct, genome-wide assessment of DNA mutations in single cells. Nucleic Acids Res. 2012;40:2032-2040.

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DAVID Q.-H. WANG, M.D., Ph.D.

Lipid Metabolism and Lipid-related Diseases

Professional Interests: Dr. Wang has an active research interest in the molecular and genetic mechanisms of cholesterol homeostasis and the pathophysiology of cholesterol-related diseases. The long-term objectives of his research are (i) to identify gallstone (LITH) genes and investigate their genotypes and phenotypes in mice and humans, as well as elucidate the molecular, cellular, genetic and physical-chemical mechanisms of cholesterol gallstone disease at a fundamental level; (ii) to study molecular, cellular, genetic and physical-chemical factors that determine intestinal absorption efficiency of cholesterol and fatty acids; and (iii) to investigate the pathophysiology of the metabolic syndrome with a special focus on the relationship between insulin resistance and nonalcoholic fatty liver disease, and examine the pathogenesis of alcoholic liver disease and obesity, as well as explore novel approaches for the prevention and treatment of these lipid-related hepatobiliary and gastrointestinal diseases.

Selected Publications

Wang DQ, Portincasa P (Editors). Gallstones: Recent advances in epidemiology, pathogenesis, diagnosis and management. The first edition. Nova Science Publishers. New York, NY. 2017. pp. 1-676.

Wang HH, Li X, Patel SB, **Wang DQ**. Evidence that the ABCG5/G8-independent pathway plays a determinant role in cholesterol gallstone formation in mice. *Hepatology*. 2016; 64: 853-864.

Wang DQ, Afdhal NH. Chapter 65. Gallstone disease. In Sleisenger and Fordtran's Gastrointestinal and Liver Disease, the 10th Edition. Editors by Feldman M, Friedman LS, and Brandt LJ. Elsevier Saunders. 2016; 1100-1133.

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Wang DQ, Portincasa P, and Neuschwander-Tetri BA. Steatosis in the Liver. *Comprehensive Physiology*. 2013; 3: 1493-1532.

Wang HH, Portincasa P, Afdhal NH, **Wang DQ**. *Lith* genes and genetic analysis of cholesterol gallstone formation. *Gastroenterol. Clin. North Am*. 2010; 39: 185-207.

Wang HH, Portincasa P, Mendez-Sanchez N, Uribe M, **Wang DQ**. Effect of ezetimibe on the prevention and dissolution of cholesterol gallstones. *Gastroenterology*. 2008; 134: 2101-2110.

Wang DQ, Lee SP. Physical-chemistry of intestinal absorption of biliary cholesterol in mice. *Hepatology*. 2008; 48: 177-185.

TAO WANG, Ph.D.

Statistical Genetics and Genomics

The research field of my group is statistical genetics and genomics, with a strong focus on the analysis of genetic and genomic data from large-scale population based studies. Our research is focused on two highly related areas: the development of statistical genetics/genomics methodology and the application of statistical genetics/genomics methods to understand the complex genetic basis of common human diseases. Specifically, we are interested in developing statistical methods for multi-locus association analysis, multivariate genetic association analysis, family-based genetic association analysis, gene-gene and gene-environment analysis, genetic meta-analysis, and the estimation of genetic heritability and co-heritability between traits. Moreover, we have collaborated with scientists in many genetic/epigenetic studies of a variety of diseases, which include but are not limited to, congenital heart defects, aging, autism, cardiovascular diseases and cancers.

Recent publications:

Wang T, Lin CY, Rohan TE, Ye K. Resequencing of pooled DNA for detecting disease associations with rare variants. Genetic epidemiology. 2010; 34(5):492-501. NIHMSID: NIHMS587140 PubMed [journal] PMID: 20578089, PMCID: PMC4096227

Wang T, Pradhan K, Ye K, Wong LJ, Rohan TE. Estimating allele frequency from next-generation sequencing of pooled mitochondrial DNA samples. Frontiers in genetics. 2011; 2:51. PubMed [journal] PMID: 22303347, PMCID: PMC3268604

Wang T, Rohan TE, Gunter MJ, Xue X, Wactawski-Wende J, Rajpathak SN, Cushman M, Strickler HD, Kaplan RC, Wassertheil-Smoller S, Scherer PE, Ho GY. A prospective study of inflammation markers and endometrial cancer risk in postmenopausal hormone nonusers. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2011; 20(5):971-7. NIHMSID: NIHMS279677 PubMed [journal] PMID: 21415362, PMCID: PMC3096873

Ahn S, Wang T. A powerful statistical method for identifying differentially methylated markers in complex diseases. Pacific Symposium on Biocomputing. Pacific Symposium on Biocomputing. 2013; :69-79. NIHMSID: NIHMS441925 PubMed [journal] PMID: 23424113, PMCID: PMC3621641

Wang T, Zhou B, Guo T, Bidlingmaier M, Wallaschofski H, Teumer A, Vasan RS, Kaplan RC. A robust method for genome-wide association meta-analysis with the application to circulating insulin-like growth factor I concentrations. Genetic epidemiology. 2014; 38(2):162-71. NIHMSID: NIHMS592219 PubMed [journal] PMID:24446417, PMCID: PMC4049273

Guo T, Chung JH, Wang T, McDonald-McGinn DM, Kates WR, Hawuła W, Coleman K, Zackai E, Emanuel BS, Morrow BE. Histone Modifier Genes Alter Conotruncal Heart Phenotypes in 22q11.2 Deletion Syndrome. American journal of human genetics. 2015; 97(6):869-77. PubMed [journal] PMID: 26608785, PMCID: PMC4678435

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Loudig O, Wang T, Ye K, Lin J, Wang Y, Ramnauth A, Liu C, Stark A, Chitale D, Greenlee R, Multerer D, Honda S, Daida Y, Spencer Feigelson H, Glass A, Couch FJ, Rohan T, Ben-Dov IZ. Evaluation and Adaptation of a Laboratory-Based cDNA Library Preparation Protocol for Retrospective Sequencing of Archived MicroRNAs from up to 35-Year-Old Clinical FFPE Specimens. International journal of molecular sciences. 2017; 18(3). PubMed [journal] PMID: 28335433, PMCID: PMC5372640

Dong X, Zhang L, Milholland B, Lee M, Maslov AY, Wang T, Vijg J. Accurate dentification of single-nucleotide variants in whole-genome-amplified single cells. Nature methods. 2017; 14(5):491-493. NIHMSID: NIHMS855609 PubMed [journal] PMID: 28319112, PMCID: PMC5408311

DANIEL A. WEISER, MD

Childhood Cancer Translational Research

Our laboratory is focused on childhood cancer research with a goal of elucidating the underlying biology of the most aggressive malignancies. In such patients with typically incurable cancer, we are striving to identify new approaches to and types of treatment. We have multiple ongoing projects:

+ Identification of biologic drivers of neuroblastoma at ultra-high risk for treatment failure. Neuroblastoma is one of the most common and deadly childhood cancers. Despite intensive research, there are limited therapeutic strategies for patients with *de novo* chemotherapy resistance that leads to particularly poor outcomes. We have been studying neuroblastoma since 2009 and are identifying additional biologic drivers of highly lethal tumors. We assess features (genetic, transcriptomic, proteomic, histologic) from patients with early death from tumor progression compared with tumor features from those with a maintained complete response. This guides our workup of potential oncogenic targets and discovery of novel therapies for patients, including selinexor, a pharmacologic Exportin-1 (XPO1) inhibitor that limits nuclear export of key regulatory proteins in cancer cells.

+ Evaluation of novel combinatorial targeted therapeutic approaches in neuroblastoma. With expected outcomes lagging behind those of more common childhood cancers, children with neuroblastoma require new approaches to treatment. Our lab works with multiple international clinical and research consortium groups to perform preclinical studies that substantiate human clinical trials.

+ Repurposing of tenofovir, a reverse transcriptase inhibitor used in HIV, for treatment of neuroblastoma. We are exploring novel ways to target telomerase, the enzyme that maintains telomere length, for treatment of the most highly aggressive neuroblastoma. We are recruiting a post-doc to take the lead on this work and build upon our exciting preliminary data.

+ Detection of circulating tumor DNA in osteosarcoma. With no reliable non-invasive approach for disease monitoring during and after treatment, we are applying next-generation sequencing and bioinformatics approaches to identify solid tumors with blood-based "liquid" biopsies. This will allow clinicians to assess tumor responsiveness to chemotherapy and predict likelihood of recurrence.

+ Assessment of accelerated aging using miRNA-seq in survivors of childhood cancer. Chemotherapy has many untoward effects on healthy cells and leads to many signs of accelerated aging in children treated for cancer. Using a known microRNA "aging" signature discovered at Albert Einstein College of Medicine, we are studying what causes this phenotype in childhood cancer, with a goal of offering improved intervention to minimize long-term toxicity of treatment.

Lab website: https://sites.google.com/view/weiserlab/

Select publications:

- Niazi MKK, Chung JH, Heaton-Johnson KJ, Martinez D, Castellanos R, Irwin M, Master S, Pawel BR, Gurcan MN, **Weiser DA**. Advancing clinicopathologic diagnosis of high-risk neuroblastoma using computerized image analysis and proteomic profiling. *Pediatric and Developmental Pathology.* 2017 Sep-Oct;20(5):394. PMID 28420318.
- Bresler SC*, **Weiser DA***, Huwe PJ*, Park JH, Krytska K, Ryles H, Laudenslager M, Rappaport EF, Wood AC, McGrady PW, Hogarty MD, London WB, Radhakrishnan R, Lemmon MA, Mossé YP. ALK mutations confer differential oncogenic activation and sensitivity to ALK inhibition therapy in neuroblastoma. Cancer Cell. 2014 Nov 10;26(5):682-94. PMID: 25517749.
- Carpenter EL, Haglund EA, Mace EM, Deng D, Martinez D, Wood AC, Chow AK, **Weiser DA**, Belcastro LT, Winter C, Bresler SC, Vigny M, Mazot P, Asgharzadeh S, Seeger RC, Zhao H, Guo R, Christensen JG, Orange JS, Pawel BR, Lemmon MA, Mossé YP.Antibody targeting of anaplastic lymphoma kinase induces cytotoxicity of human neuroblastoma. Oncogene. 2012 Nov 15;31(46):4859-67. PMID: 22266870.
- Barris DM, Weiner SB, Dubin RA, Fremed M, Zhang X, Piperdi S, Zhang W, Maqbool S, Gill J, Roth M, Hoang B, Geller D, Gorlick R, **Weiser DA**. Detection of circulating tumor DNA in osteosarcoma. *Oncotarget*. 2018 Jan 18;9(16):12695. PMID 29560102.
- Moerdler S, Zhang L, Gerasimov E, Chong Y, Wolinsky T, Roth M, Goodman N, **Weiser DA**. Physician perspectives on compassionate use in pediatric oncology. *Pediatric Blood and Cancer.* 2019 Mar;66(3):e27545. PMID 30408307.

ZHENGDONG ZHANG, Ph.D.

Computational and Systems Biology of Cancer Metastasis and Human Aging

With recent resource and technology development, biology has entered a new data-driven phase in the 21st century. The research interest of my lab is computational biology and bioinformatics, focusing on algorithm development, data integration, and software implementation. With the advent of new DNA sequencing technologies, it is a particularly challenging and exciting time now to do such computational work, as more and more biological data are being generated at an ever-accelerating speed.

Gene expression in living cells is under strict spatial and temporal control, and its dysregulation is the direct cause of many human diseases. The primary focus of research in my lab is gene expression and its regulation, for which we take an integrated approach to study the following aspects on the whole genome scale:

- Gene expression profiles
- Transcriptional regulation of gene expression
- Epigenetic mechanisms and long range control of gene expression
- Gene copy number variation

The biological systems currently under investigation are breast cancer metastasis and human aging.

- Breast cancer metastasis is a complex multi-step process during which tumor cells spread from the primary tumor mass to distant organs. To study the genetic and biochemical determinations of this deadly aspect of cancer progression, we analyze various microarray and sequencing profiles to discover its regulatory sub-networks, DNA binding of key regulators, and copy number variations during the progression. This research project is supported by a grant from NIH/NLM.
- For reasons significant to individuals and the society as a whole, human aging is of great interest not only to the academic community but also to medicine and the public in general. However, despite much research progress made over the years, it still remains a poorly understood biological process. To gain novel insights, we use a systems-biology approach to analyze aging-related genes in the context of biological networks. This research project is supported by a New Scholar Award from the Ellison Medical Foundation.

Lab web site: www.zdzlab.org

Recent Publications:

MacArthur DG, Balasubramanian S, Frankish A, Huang N, Morris J, Walter K, Jostins L, Habegger L, Pickrell JK, Montgomery SB, Albers CA, Zhang ZD, et al (2012) A systematic survey of loss-of-function variants in human protein-coding genes. *Science*, **335**:806-807.

Zhang ZD, Du J, Lam H, Abyzov A, Urban A, Snyder M, Gerstein M (2011) Identification of genomic indels and structural variations using split reads. *BMC Genomics*, **12**:375.

Zhang ZD, Gerstein MB (2010) Detection of copy number variation from array intensity and sequencing read depth using a stepwise Bayesian model. *BMC Bioinformatics*, **11**:539

Zhang ZD, Frankish A, Hunt T, Harrow J, Gerstein M. (2010) Identification and analysis of unitary pseudogenes: historic and contemporary gene losses in humans and other primates. *Genome Biol.*, **11**, R26

Du J, Bjornson RD, Zhang ZD, Kong Y, Snyder M, Gerstein MB. (2009) Integrating Sequencing Technologies in Personal Genomics: Optimal Low Cost Reconstruction of Structural Variants. *PLoS Comput Biol.*, **5**, e1000432.

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Zhang ZD, Cayting P, Weinstock G, Gerstein M. (2008) Analysis of Nuclear Receptor Pseudogenes in Vertebrates: How the Silent Tell Their Stories. *Mol Biol Evol.*, **25**, 131-143.

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Zhang ZD, Rozowsky J, Snyder M, Chang J, Gerstein M. (2008) Modeling ChIP Sequencing In Silico with Applications. *PLoS Comput Biol.*, **4**, e1000158.

DEYOU ZHENG, Ph.D.

Bioinformatics and Computational Genomics

The research field of my group is Computational Genomics and Bioinformatics, with a strong focus of mining large-scale experimental genomic data to decipher the function of the human genome and the genomes of other model organisms. We develop and apply computational techniques for integrating data of comparative genomics and functional genomics (and epigenomics) to decode the structure, function, and evolution of the human genome. More generally, we are interested in bioinformatic and statistical approaches for exploiting novel and biologically significant patterns in big genomic data. Recently, we have become more focused on the expression, regulation, and evolution of human genes (both coding or non-coding) that are involved in the development, specification, maturation, and maintenance of human neural systems and hearts. Working extensively with experimentalists and by deep sequencing of the transcriptomes in human neurons or mouse hearts, our study has led to many interesting findings and will contribute important information to heart development, neuronal development, neurodegenerative diseases and other brain diseases. Please see our website for more details: http://www.einstein.yu.edu/labs/deyou-zheng/

Recent publications:

Zheng, D*, Zhao, K and Mehler, M. (2009) Profiling RE1/REST-mediated histone modifications in the human genome. *Genome Biol* 10:R9. (*corresponding author)

Guo X, Zhang Z, Gerstein MB, **Zheng D**. (2009). Small RNAs originated from pseudogenes: cis- or trans-acting? *PLoS Comput Biol* 5(7): e1000449.

Goldberg AD, Banaszynski LA, Noh KM, Lewis PW, Elsaesser SJ, Stadler S, Dewell S, Law M, Guo X, Li X, Wen D, Chapgier A, DeKelver RC, Miller JC, Lee YL, Boydston EA, Holmes MC, Gregory PD, Greally JM, Rafii S, Yang C, Scambler PJ, Garrick D, Gibbons R, Higgs DR, Cristea IM, Urnov FD, **Zheng D***, Allis CD* (2010). Distinct factors control histone variant H3.3 localization at specific genomic regions. *Cell* 140:678-691. (*co-corresponding authors)

Guo X, Freyer L, Morrow B, **Zheng D**. (2011) Characterization of the past and current duplication activities in the human 22q11.2 region. *BMC Genomics* 12:71

Lin M, Pedrosa E, Shah A, Hrabovsky A, Maqbool S, Zheng D, Lachman HM. (2011). RNA-Seq of human neurons derived from iPS cells reveals candidate long non-coding RNAs involved in neurogenesis and neuropsychiatric disorders. *PLoS ONE* 6: e23356.

Lin M. Hrabovsky A, Pedrosa, E, Wang T, Zheng D., Lachman HM. (2012) Allele-Biased Expression in Differentiating Human Neurons: Implications for Neuropsychiatric Disorders. *PLoS ONE* 7(8): e44017.

Guo X, Lin M, Rockowitz S, Lachman HM, Zheng D. (2014) Characterization of Human Pseudogene-derived Non-coding RNAs for Functional Potential. *PLoS One* 9: e93972

Adam RC, Yang H, Rockowitz S, Larsen SB, Nikolova M, Oristian DS, Polak L, Kadaja M, Asare A, Zheng D, Fuchs E. (2015). Pioneer factors govern super-enhancer dynamics in stem cell plasticity and lineage choice. *Nature* 521:366-370

Rockowitz S, Zheng D. (2015). Significant expansion of the REST/NRSF cistrome in human versus mouse embryonic stem cells: potential implications for neural development. *Nucleic Acids Res*. 43:5730-5743.

Wang P, Lin M, Pedrosa E, Hrabovsky A, Zhang Z, Guo W, Lachman HM*, Zheng D*. (2015). CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. *Mol Autism* 6:55. (*co-corresponding authors)

Lin M, Lachman HM, Zheng D. (2016). Transcriptomics Analysis of iPSC-derived Neurons and Modeling of Neuropsychiatric Disorders. *Mol Cell Neurosci* 73:32-42

Zhao D, Lin M, Pedrosa E, Lachman HM, Zheng D. (2017). Characteristics of allelic gene expression in human brain cells from single cell RNA-seq data analysis. *BMC Genomics* 18:860

Zhang D, Wang Y, Lu P, Wang P, Yuan X, Yan J, Cai C, Chang CP, Zheng D, Wu B, Zhou B. (2017) REST regulates the cell cycle for cardiac development and regeneration. *Nat Commun* 8:1979.

Wang P, Zhao D, Lachman HM, Zheng D. (2018). Enriched expression of genes associated with autism spectrum disorders in human inhibitory neurons. *Transl Psychiatry* 8:13.

BIN ZHOU, M.D., Ph.D.

Molecular Mechanisms in Heart Development, Disease and Regeneration

Our research focuses on molecular mechanisms in cardiac development, disease and regeneration. We apply an integrated approach of mouse genetics, developmental, cell, molecular and systems biology to study specification and lineage development of cardiac cells, transcriptional/genetic regulation of and signaling pathways in cardiac development and regeneration. Our main research projects are (1) Cardiac Cell Lineage Specification and Differentiation: We use cardiac cell specific Cre and reporter gene mouse lines, as well as single cell transcriptomics, to trace the cardiac cell lineage in developing and diseased hearts. Currently, we are applying single cell RNA-seq and single cell ATAC-Seq to reveal the cardiac cell heterogeneity and its functions in development. (2) Transcriptional Regulation of Cardiac Development: We study transcription factors NFATc1, SOX17 and REST in the regulation of heart development, including heart valve, chamber, and coronary vascular formation, using ChIP-seq, RNA-Seq, and ATAC-Seq technologies. (3) Modeling of Heart Valve Disease: Congenital heart valve disease is a major birth defect and aortic valve stenosis is a common disease in the elderly. We generate and study mouse models of these human heart valve diseases using Cre-LoxP and CRSPRI technologies. (4) Epigenetic Regulation of Heart Formation and Regeneration: We also study epigenetic regulation of heart formation and regeneration, specifically the roles of DNA methyltransferase (Dnmts) and RE1-silencing transcription factor (REST) in the regulation of fetal cardiac gene program, cardiomyocyte differentiation and maturation, using single cell RNA-seq and ChIP-seq for genetically-modified mice carrying either mutations. We collaborate with Drs Deyou Zheng and Bernice Morrow in these projects.

Selected Publications:

del Monte-Nieto G, Ramialison M,. Cherian AV, Wu B, Aharonov A, D'Uva G, Bourke LM, Pitulescu ME, Chen H, Shou W, Adams RH, Harten SK, Tzahor E, Zhou B, Harvey RP. (2018) Extracellular matrix dynamics reveals the building plan for cardiac trabeculation. *Nature* 557(7705):439-445

Zhang D, Wang Y, Lu P, Wang P, Xinchun Yuan X, Yan J, Cai C, Chang CP, Zheng D, Wu B, Zhou B. (2017) REST regulates the cell cycle for cardiac development and regeneration. *Nat Commun*; 8(1):1979.

Wang Y, Wu B, Lu P, Zhang D, Wu B, Varshney S, Del Monte-Nieto G, Zhuang Z, Charafeddine R, Kramer AH, Sibinga NE, Frangogiannis NG, Kitsis RN, Adams RH, Alittalo K, Sharp DJ, Harvey RP, Stanley P, Zhou B. (2017) Uncontrolled angiogenic precursor expansion causes coronary artery anomalies in mice lacking Pofut1. *Nat Commun* 18;8(1):578

Wu B, Wang Y, Xiao F, Butcher JT, Yutezy KE, Zhou B. (2017) Developmental mechanisms in aortic valve malformation and disease. *Annu Rev Physiol* 79:21-41

Zhang D, Wu B, Wang P, Wang Y, Nechiporuk T, Floss T, Greally JM, Zheng D, Zhou B. (2017) Non-CpG methylation by DNMT3B facilitates REST binding and gene silencing in developing mouse hearts. *Nucleic Acids Res* 45:3102-3115

Wang, Y, Wu B, Farrar E, Alfieri CM, Lui, W, Lu P, Zhang D, Mao K, Chu M, Yang D, Xu D, Rauchman M, Taylor V, Yutzey KE, Butcher JT, Zhou B. (2017) Notch-Tnf signaling is required for development and homeostasis of arterial valves. *Eur Heart J* 38:675-686

Goddard L, Chen M, Ramalingam H, Bamezai S, Yang, J, Morley M, Wang T, Jameson S, Morrisey E, Zhou B, Thomas C, Kahn M. (2017) Hemodynamic forces sculpt developing heart valves through a KLF2-Wnt9b paracrine signaling axis. *Dev Cell* 43(3):274-289

Han P, Li W, Lin CH, Yang J, Shang Q, Nuernberg S, Jin K, Xu W, Lin CY, Lin CJ, Xiong Y, Chien H, Zhou B, Ashley E, Bernstein D, Chen PS, Chen HS, Quertermous T, Chang CP. (2014) A long non-coding RNA protects the heart from pathological hypertrophy. *Nature*, 514:102-106

Wu B, Zhang Z, Lui W, Chen X, Moreno-Rodriquez RA, Wang Y, Chamberlain A, Markwald RA, O'Rourke B, Sharp DJ, Lenz J, Baldwin HS, Chang CP, Zhou B. (2012) Endocardial Cells Form the Coronary Arteries by Angiogenesis through Myocardial to Endocardial VEGF Signaling. *Cell* 151:1083-1096.

Lin CY, Lin CJ, Chen CH, Zhou B*, Chang CP*. (2012) Partitioning the heart: mechanisms of cardiac septation and valve development. Development 139:3277-99. *Corresponding authors.

Wu B, Wang Y, Lui W, Langworthy M, Tompkins KL, Hatzopoulos AK, Baldwin, HS, Zhou B. (2011) Nfatc1 Coordinates valve endocardial cell lineage development required for heart valve formation. *Circ Res* 109:183-192.

Hang CT, Yang J, Han P, Cheng HL, Shang C, Ashley E, Zhou B, Chang CP. (2010) Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature* 466:62-7