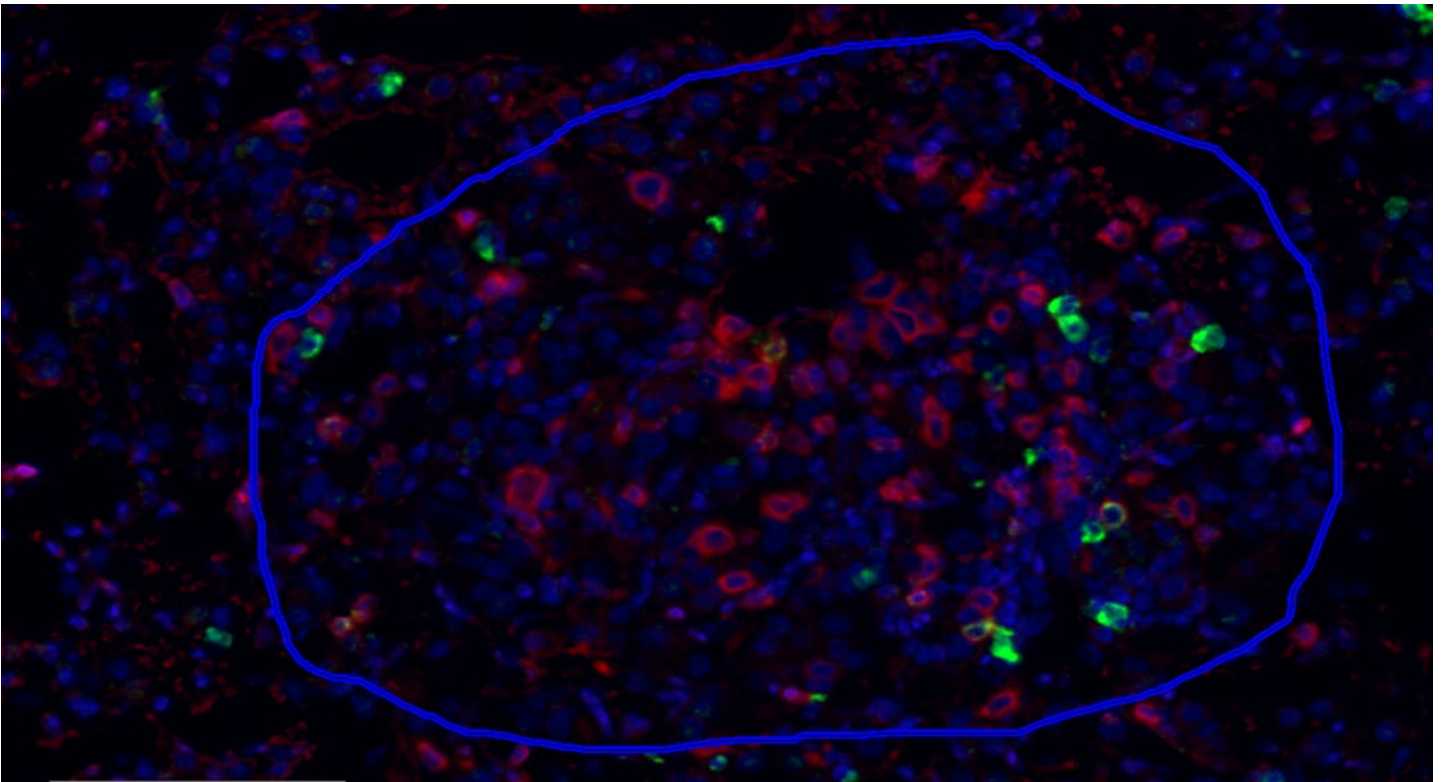# HUMAN BIOLOGY
2020-2021

About the cover: Provided by Assistant Professor, Shivani Srivastava PhD

Fluorescent image looking at CAR-T cell infiltration into lung tumors

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VIRAL MANIPULATION OF CHROMATIN

Our laboratory is focused on the mechanisms by which viruses hijack chromatin. Due to the major advancement in sequencing technologies and the expansion of the field of epigenetics, exploiting viruses to investigate chromatin biology has enormous potential. Our goal is to advance basic understanding of viral manipulation of chromatin and uncover new aspects of chromatin biology.

Much like the cellular genome, viral genomes must be compacted in virus particles with small basic molecules to maximize space and be poised for gene expression. Some DNA viruses use cellular histone proteins to compact their genomes whereas others use small basic molecules. Adenoviruses encode their own histone-like protein, called protein VII, that forms a 'beads on a string' assembly with the viral genome. By examining protein VII in host chromatin, we discovered that protein VII sequesters the immune danger signal, HMGB1, in chromatin thereby dampening downstream inflammation (Avgousti et al, Nature 2016). This discovery sets the framework for deciphering how adenovirus manipulates host chromatin and more broadly how DNA viruses use histones or histone-like proteins for dual function: to compact their genomes and control host genomes.

Research efforts in the lab use a multidisciplinary approach to address the following questions:

1. How does adenovirus protein VII impact nuclear architecture? The expression of protein VII in cells is sufficient to increase nuclear size and markedly disrupt the appearance and composition of cellular chromatin. In conjunction with chromatin fractionation proteomics, we have identified chromatin factor HMGB1 as well as linker histone H1 as potential key players in the chromatin distortion caused by protein

VII. Projects in the lab will use cell culture, microscopy, biochemistry and genomic techniques to asses this phenotype and define the role of host chromatin factors in viral pathogenesis.

    a. To which genomic sites does protein VII bind? What is the effect on transcription?

    b. How do host chromatin factors, such as HMGB1, contribute to protein VII's impact on host chromatin?

    c. Why is protein VII evolving while histones are not?

2. <u>How does herpes simplex virus (HSV-1) affect host chromatin</u>? HSV-1 is an enveloped DNA virus with well characterized lytic and latent stages. HSV-1 first replicates in epithelial cells then enters peripheral neurons where it establishes latency. We developed a novel proteomic approach for temporal analysis of the host and viral proteome, phosphoproteome and chromatin-associated proteins during HSV-1 lytic infection (Kulej and Avgousti et al, MCP 2017). These data reveal that hundreds of host proteins are regulated during lytic HSV-1 infection, including many chromatin factors. We also uncovered dynamic changes in histone post-translational modifications throughout the course of HSV-1 infection. In addition to mining this vast dataset, projects in the lab will use molecular biology and genome editing techniques to investigate chromatin-related proteins and their role during HSV-1 infection.

SELECTED PUBLICATIONS

Lynch KL, Gooding LR, Garnett-Benson C, Ornelles DA, Avgousti DC. Epigenetics and the dynamics of chromatin during adenovirus infections (2019) FEBS Lett. 10.1002/1873-3468.13697. PMID 31769503.

Avgousti DC, Della Fera AN, Otter CJ, Herrmann C, Pancholi NJ, and Weitzman MD. Adenovirus core protein VII down-regulates the DNA damage response on the host genome (2017) *J. Virol.* doi: 10.1128/JVI.01089-17.

Kulej K, Avgousti DC, Sidoli S, Herrmann C, Della Fera AN, Garcia BA, and Weitzman MD. Time- resolved global and chromatin proteomics during Herpes Simplex Virus Type 1 (HSV-1) infection (2017) *Mol Cell Proteomics.* doi:10.1074/mcp.M116.065987.

Avgousti DC, Herrmann C, Kulej K, Pancholi NJ, Sekulic N, Petrescu J, Molden RC, Blumenthal, D, Paris AJ, Reyes ED, Ostapchuk P, Hearing P, Seeholzer, SH, Worthen GS, Black BE, Garcia BA, and Weitzman MD. A core viral protein binds host nucleosomes to sequester immune danger signals (2016) *Nature.* doi:10.1038/nature/18317.

Avgousti DC and Weitzman MD. Stress flips a chromatin switch to wake up latent virus. (2015) *Cell Host Microbe.* 18(6):639-41.

Kulej K*, Avgousti DC*, Weitzman MD, and Garcia BA. Characterization of histone post-translational modifications during virus infection using mass spectrometry-based proteomics (2015) *Methods.* S1046-2023(15)00249-2. *Authors contributed equally.*

Avgousti DC, Palani S, Sherman Y, and Grishok A. CSR-1 RNAi pathway positively regulates histone expression in *C. elegans* (2012) *EMBO J.* 31(19):3821-32. PMID 22863779.

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My research program focuses on phylodynamic analysis of pathogen sequence data with an intent of making inferences that are actionable to public health. This research program spans a number of viral systems including seasonal and avian influenza, Ebola, Zika, SIV, MERS-CoV, dengue and mumps. This requires development of mathematical and statistical methods to integrate infectious disease sequence data into evolutionary and epidemiological models.

I've co-developed the open-source Nextstrain platform (nextstrain.org) that aims to harness the scientific and public health potential of pathogen genome data by providing a continually-updated view of publicly available data alongside powerful analytic and visualization tools. This platform is used by the World Health Organization Global Influenza Surveillance and Response System (GISRS) to aid in vaccine strain selection for seasonal influenza virus. This platform was also highlighted during the Zika epidemic in the Americas and the Ebola epidemic in West Africa as a central source for data sharing and up-to-date insights.

I have published over 50 scientific journal articles and my awards include a MIRA R35 investigator award from NIGMS, a Pew Biomedical Scholar Award and the NIH / HHMI / Wellcome Trust Open Science Prize.

SELECTED PUBLICATIONS

1. **Bedford T**, Suchard MA, Lemey P, Dudas G, Gregory V, Hay AJ, McCauley JW, Russell CA, Smith DJ, Rambaut A. 2014. Integrating influenza antigenic dynamics with molecular evolution. eLife 3: e01914.

2. Faria NR, Rambaut A, Suchard MA, Baele G, **Bedford T**, Ward MJ, Tatem AJ, Sousa JD, Arinaminpathy N, Pépin J, Posada D, Peeters M, Pybus OG, Lemey P. 2014. The early spread and epidemic ignition of HIV-1 in human populations. Science 346: 56–61.

3. Lemey P, Rambaut A, **Bedford T**, Faria N, Bielejec F, Baele G, Russell CA, Smith DJ, Pybus OG, Brockmann

D, Suchard MA. 2014. Unifying viral genetics and human transportation data to predict the global transmission dynamics of human influenza H3N2. PLoS Pathog 10: e1003932.

4. Roche B, Drake JM, Brown J, Stallknecht DE, **Bedford T**, Rohani P. 2014. Adaptive evolution and environmental durability jointly structure phylodynamic patterns in avian influenza viruses. PLoS Biol 12: e1001931.

5. **Bedford T**, Riley S, Barr IG, Broor S, Chadha M, …, Tashiro M, Wang D, Xu X, Lemey P, Russell CA (26 authors). 2015. Global circulation patterns of seasonal influenza viruses vary with antigenic drift. Nature 523: 217–220.

6. Frost SDW, Pybus OG, Gog JR, Viboud C, Bonhoeffer S, **Bedford T**. 2015. Eight challenges in phylodynamic inference. Epidemics 10: 88–92.

7. Neafsey DE, Juraska M, **Bedford T**, Benkeser D, Valim C, …, Yu Q, Lapierre D, Birren BW, Gilbert PB, Wirth DF (63 authors). 2015. Genetic diversity and protective efficacy of the RTS,S/AS01 malaria vaccine. New Engl J Med 373: 2025–2037.

8. Neher RA, **Bedford T**. 2015. nextflu: real-time tracking of seasonal influenza virus evolution in humans. Bioinformatics 31: 3546–3548.

9. Park DJ, Dudas G, Wohl S, Goba A, Whitmer SLM, …, **Bedford T**, Gnirke A, Ströher U, Rambaut A, Garry RF, Sabeti PC (86 authors). 2015. Ebola virus epidemiology, transmission, and evolution during seven months in Sierra Leone. Cell 161: 1–11.

10. **Bedford T**, Malik HS. 2016. Did a single amino acid change make Ebola virus more virulent? Cell 167: 892–894.

11. Lorenzo-Redondo R, Fryer HR, **Bedford T**, Kim EY, Archer J, …, Malim MH, Rambaut A, Haase AT, McLean AR, Wolinsky SM (16 authors). 2016. Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 530: 51–56.

12. Neher RA, **Bedford T**, Daniels RS, Russell CA, Shraiman BI. 2016. Prediction, dynamics, and visualization of antigenic phenotypes of seasonal influenza viruses. Proc Natl Acad Sci USA 113: E1701– E1709.

13. **Bell SM**, **Bedford T**. 2017. Modern-day SIV viral diversity generated by extensive recombination and cross-species transmission. PLoS Pathog 13: e1006466.

14. **Dudas G**, Max Carvalho L, **Bedford T**, Tatem AJ, Baele G, …, Ströher U, Wury I, Suchard MA, Lemey P, Rambaut A (93 authors). 2017. Virus genomes reveal the factors that spread and sustained the West African Ebola epidemic. Nature 544: 309–315.

15. Faria NR, Quick J, Morales I, Thézé J, Jesus JG, …, **Bedford T**, Teixeira MNR, Sabino EC, Alcantara LCJ, Loman N, Pybus OG (71 authors). 2017. Establishment and cryptic transmission of Zika virus in Brazil and the Americas. Nature 546: 406–410.

16. Grubaugh ND, Ladner JT, Kraemer MUG, **Dudas G**, Tan AL, …, **Bedford T**, Pybus OG, Isern S, Palacios G, Andersen KG (67 authors). 2017. Genomic epidemiology reveals multiple introductions of Zika virus into the United States. Nature 546: 401–405.

17. **Dudas G**, Max Carvalho L, Rambaut A, **Bedford T**. 2018. MERS-CoV spillover at the camel-human interface. eLife 7: e31257.

18. **Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C**, Sagulenko P, **Bedford T**, Neher RA. 2018. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics: bty407.

19. Lee JM, **Huddleston J**, Doud MB, Hooper KA, Wu NC, **Bedford T**, Bloom JD. 2018. Deep mutational scanning of hemagglutinin helps predict evolutionary fates of human H3N2 influenza variants. Proc Natl Acad Sci USA 115: E8276–E8285.

20. **Bell SM**, Katzelnick L, **Bedford T**. 2019. Dengue genetic divergence generates within-serotype antigenic variation, but serotypes dominate evolutionary dynamics. eLife 8: e42496.

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Genome sequencing studies have demonstrated that cancer develops as an evolutionary process of somatic mutation and clonal selection. The underlying genetic fingerprint of each cancer is different and the presence of specific mutations, e.g. *EGFR* activating mutations in lung cancer, can dramatically influence the response of tumors to specific therapies.

The goal of my laboratory is to enable precision medicine by systematically uncovering the molecular alterations in cancer, determining the function of these variant alleles, and understanding how these alleles modulate response to cancer therapies. Although many of the *genes* involved in cancer have now been identified, a major challenge is discovering which specific *alleles* of these genes are involved and how these alleles modulate therapeutic response. My group uses functional genomics, computational biology, biochemistry, and genetics to understand the mechanism of somatic cancer variants. Our goal is to identify drug targets and biomarkers and to translate this knowledge into clinical benefit for patients.

A central theme in the laboratory is understanding the mechanism and therapeutic targeting of cancer oncogenes in the RAS/MAPK pathway. Both *KRAS* and *RIT1* encode small GTPase proteins and are mutated in lung adenocarcinoma and in the germline of "Ras-opathy" patients. Although *KRAS* has been extensively studied, *RIT1* mutations were discovered only recently and the role of these mutations in cancer is poorly understood. We are using unbiased proteomic approaches and genome-wide CRISPR knockout screens to determine the functional similarities and differences between *KRAS* and *RIT1*, with the goal of identifying critical effectors of these proteins that might be targeted for therapeutic benefit. Candidate drugs will be tested in a genetically-engineered mouse model generated by our lab.

SELECTED PUBLICATIONS

Vichas A, Nkinsi NT, Riley A, Parrish PC, Duke F, Chen J, Fung I, Watson J, Rees M, Lee JK, Piccioni F, Hatch EM, **Berger AH**. **An integrative oncogene-dependency map identifies unique vulnerabilities of oncogenic EGFR, KRAS, and RIT1 in lung cancer**. [preprint]. 2020 July; [revised 2020 July]: Available from: https://www.biorxiv.org/content/10.1101/2020.07.03.187310v2.

Lo A, Holmes K, Mundt F, Moorthi S, Fung I, Fereshetian S, Watson J, Carr SA, Mertins P, **Berger AH**. **Mapping the unique and shared functions of oncogenic KRAS and RIT1 with proteome and transcriptome profiling.** [preprint]. 2020 April; Available from: https://www.biorxiv.org/content/10.1101/2020.04.10.030460v1.full.

Thomas JD, Polaski JT, Feng Q, De Neef EJ, Hoppe ER, McSharry MV, Pangallo J, Gabel AM, Belleville AE, Watson J, Nkinsi NT, **Berger AH**, Bradley RK. **RNA isoform screens uncover the essentiality and tumor-suppressor activity of ultraconserved poison exons.** Nat Genet. 2020 Jan;52(1):84-94. doi: 10.1038/s41588-019-0555-z. Epub 2020 Jan 7. PubMed PMID: 31911676; PubMed Central PMCID: PMC6962552.

Gelman H, Dines JN, Berg J, **Berger AH**, Brnich S, Hisama FM, James RG, Rubin AF, Shendure J, Shirts B, Fowler DM, Starita LM. **Recommendations for the collection and use of multiplexed functional data for clinical variant interpretation.** Genome Med. 2019 Dec 20;11(1):85. doi: 10.1186/s13073-019-0698-7. PubMed PMID: 31862013; PubMed Central PMCID: PMC6925490.

Chen M, Zhang J, **Berger AH**, Diolombi MS, Ng C, Fung J, Bronson RT, Castillo-Martin M, Thin TH, Cordon-Cardo C, Plevin R, Pandolfi PP. **Compound haploinsufficiency of Dok2 and Dusp4 promotes lung tumorigenesis**. J Clin Invest. 2019 Jan 2;129(1):215-222. doi: 10.1172/JCI99699. Epub 2018 Nov 26. PubMed PMID: 30475228; PubMed Central PMCID: PMC6307955.

Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, Dimitriadoy S, Liu DL, Kantheti HS, Saghafinia S, Chakravarty D, Daian F, Gao Q, Bailey MH, Liang WW, Foltz SM, Shmulevich I, Ding L, Heins Z, Ochoa A, Gross B, Gao J, Zhang H, Kundra R, Kandoth C, Bahceci I, Dervishi L, Dogrusoz U, Zhou W, Shen H, Laird PW, Way GP, Greene CS, Liang H, Xiao Y, Wang C, Iavarone A, **Berger AH**, Bivona TG, Lazar AJ, Hammer GD, Giordano T, Kwong LN, McArthur G, Huang C, Tward AD, Frederick MJ, McCormick F, Meyerson M; Cancer Genome Atlas Research Network, Van Allen EM, Cherniack AD, Ciriello G, Sander C, Schultz N. 2018. **Oncogenic Signaling Pathways in The Cancer Genome Atlas.** Cell 173(2):321-337.e10. doi: 10.1016/j.cell.2018.03.035.

Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, Gould J, Davis JF, Tubelli AA, Asiedu JK, Lahr DL, Hirschman JE, Liu Z, Donahue M, Julian B, Khan M, Wadden D, Smith IC, Lam D, Liberzon A, Toder C, Bagul M, Orzechowski M, Enache OM, Piccioni F, Johnson SA, Lyons NJ, **Berger AH**, Shamji AF, Brooks AN, Vrcic A, Flynn C, Rosains J, Takeda DY, Hu R, Davison D, Lamb J, Ardlie K, Hogstrom L, Greenside P, Gray NS, Clemons PA, Silver S, Wu X, Zhao WN, Read-Button W, Wu X, Haggarty SJ, Ronco LV, Boehm JS, Schreiber SL, Doench JG, Bittker JA, Root DE, Wong B, Golub TR. 2017. **A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles.** Cancer Cell 11;32(6):884.

Lu X, Peled N, Greer J, Wu W, Choi P, **Berger AH**, Wong S, Jen KY, Seo Y, Hann B, Brooks A, Meyerson M, Collisson EA. 2017. **MET Exon 14 Mutation Encodes an Actionable Therapeutic Target in Lung Adenocarcinoma.** Cancer Research 77(16):4498-4505.

Berger AH*, Brooks AN*, Wu X*, Shretha Y, Chouinard C, Piccioni F, Bagul M, Kamburov A, Imielinski M, Hogstrom L, Zhu C, Yang X, Pantel S, Sakai R, Watson J, Kaplan N, Campbell JD, Singh S, Root D, Narayan R, Natoli T, Lahr D, Tirosh I, Tamayo P, Getz G, Wong B, Doench J, Subramanian A, Golub T, Meyerson M, and Boehm J. 2016. **High-throughput phenotyping of lung cancer somatic mutations.** Cancer Cell 30(2): 214-228. *equal contribution

Campbell JD, Alexandrov A, Kim J, Wala J, **Berger AH**, Pedamallu CS, Shukla SA, Guo G, Brooks AN, Murray BA, Imielinski M, Hu X, Ling S, Akbani R, Rosenberg M, Cibulskis C, Ramachandran A, Collisson EA, Kwiatkowski DJ,

Lawrence MS, Weinstein JN, Verhaak RG, Wu CJ, Hammerman PS, Cherniack AD, Getz G; Cancer Genome Atlas Research Network, Artyomov MN, Schreiber R, Govindan R, Meyerson M. 2016. **Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas.** Nature Genetics 48(6):607-16.

The Cancer Genome Atlas Network (including **Berger AH**). 2014. **Comprehensive Molecular Profiling of Lung Adenocarcinoma.** Nature 511(7511):543-50.

Berger AH, Imielinski M, Duke F, Wala J, Kaplan N, Shi G, Andres D, Meyerson M. 2014. **Oncogenic *RIT1* mutations in lung adenocarcinoma.** *Oncogene* 33(35):4418-23.

Berger AH, Chen M, Morotti A, Janas JA, Niki M, Bronson RT, Taylor BS, Ladanyi M, Van Aelst L, Politi K, Varmus HE, Pandolfi, PP. 2013. **DOK2 inhibits EGFR-mutated lung adenocarcinoma.** PLOS One 8(11):e79526.

Imielinski, M*, **Berger AH***, Hammerman PS*, Hernandez B*, Pugh TJ*, Hodis E, Cho J, Suh J, Capelletti M, Sivachenko A, Sougnez C, Auclair D, Lawrence MS, Stojanov P, Cibulski K, Choi K, de Waal L, Sharifnia T, Brooks A, Greulich H, Banerji S, Zander T, Seidel D, Leenders F, Ansén S, Ludwig C, Engel-Riedel W, Stoelben E, Wolf J, Goparju C, Thompson K, Winckler W, Kwiatkowski D, Johnson BE, Janne P, Miller VA, Pao W, Travis WD, Pass HI, Gabriel SB, Lander ES, Thomas RK, Garraway LA, Getz G, Meyerson M. 2012. **Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing.** Cell 150(6):1107-20. *equal contribution

Greulich H, Kaplan B, Mertins P, Chen TH, Tanaka KE, Yun CH, Zhang X, Lee SH, Cho J, Ambrogio L, Liao R, Imielinski M, Banerji S, **Berger AH**, Lawrence MS, Zhang J, Pho NH, Walker SR, Winckler W, Getz G, Frank D, Hahn WC, Eck MJ, Mani DR, Jaffe JD, Carr SA, Wong KK, Meyerson M. 2012. **Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2.** PNAS 109(36): 14476-81.

Berger AH, Knudson AG, Pandolfi PP. 2011. **A continuum model for tumour suppression.** Nature 476(7359):163-9.

Berger AH*, Niki M*, Morotti A, Taylor BS, Socci ND, Viale A, Brennan C, Szoke J, Motoi N, Rothman PB, Teruya-Feldstein J, Gerald WL, Ladanyi M, Pandolfi PP. 2010. **Identification of DOK genes as lung tumor suppressors.** Nature Genetics 42(3):216-23. *equal contribution

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REGULATION OF EPITHELIAL GROWTH IN DEVELOPMENT AND CANCER

My laboratory studies molecular and cellular mechanisms that regulate tissue growth in development and tumorigenesis. Our goal is to identify genes and gene pathways that can be used as novel targets in cancer therapy, with a particular focus on regulators of the balance between stem cell renewal and differentiation.

We use mouse skin epidermis and epidermal squamous cell carcinoma (SCC) as models of tissue growth in development and disease. The skin epidermis is particularly suited for our investigations for the following reasons:

A. It is a well-defined physiological system: The skin consists of an epithelial compartment, the epidermis, and mesenchymal compartment, the dermis, separated by a basement membrane. Epidermal growth during the embryonic development and its maintenance in the adult are achieved through continuous cycles of progenitor cell self-renewal and differentiation under the control of cell extrinsic signals from the surrounding mesenchyme.

B. It has implications to human health: SCC of the skin is the second most common cancer in people, with an estimated 700,000 new cases in the US each year. Fortunately, most lesions are detected early and surgically removed, accounting for the disease's high survival rate. Importantly, ontogeny of epidermal SCC parallels cancers with much higher mortality rates, including the SCC of the head and neck, and the lung SCC.

C. There are mature tools for analysis of gene function: In addition to established methods of creating transgenic animals, we have shown that mouse epidermis can be efficiently and stably targeted through *in*

utero injection of lentivirus. Using lentiviral vectors for RNAi-mediated gene knockdown or gene overexpression, we can rapidly assess gene function and complex genetic interactions *in vivo.*

Research efforts in the lab are divided between several approaches:

<u>1. Candidate-based analysis of gene function in regulation of epidermal tissue growth.</u> We have completed an RNAi screen of ~16,000 mouse genes and uncovered putative regulators of epidermal tissue growth during embryonic development and oncogenic hyperplasia. We are now testing the precise cellular and molecular mechanisms behind the observed growth effects, with a focus on genes that specifically operate within the physiological environment by altering the balance between stem cell renewal and differentiation.

<u>2. Large–scale investigation of modifiers of epidermal tumor initiation.</u> We have successfully combined pooled-format, lentiviral-mediated RNAi and quantitative Illumina sequencing in a rapid, comprehensive, and relatively low-cost approach to genome-wide gene function analysis during embryogenesis. We have now extended the use of this approach to identify *bona fide* enhancers of tumor initiation and progression in the oncogenic Ras animal model postnatally. The complexity of our lentiviral pools vary from patient-specific to genome-wide.

<u>3. Development of a general model of epithelial growth and tumorigenesis.</u> Our technique of injecting lentivirus *in utero* can be modified to produce efficient transduction of other tissues, including the oral, mammary and airway epithelium. These are distinct from the skin in their organization, physiological environment, and rate of developmental and regenerative growth, and carcinomas in these epithelia are the leading cause of tumor-associated deaths worldwide. Using RNAi-mediated gene knockdown, we test the general applicability of molecular mechanisms uncovered in our studies of epidermal growth and tumorigenesis.

SELECTED PUBLICATIONS

Cai, E.Y., Kufeld, M.N., Schuster, S., Arora, S., Larkin, M., Germanos, A.A., Hsieh, A.C., and Beronja, S. (2020). Selective translation of cell fate regulators mediates tolerance to broad RAS activation. <u>Cell Stem Cell</u> *In Press*.

Ying, Z., and Beronja, S. (2020). Embryonic barcoding of equipotent mammary progenitors functionally identifies breast cancer drivers. <u>Cell Stem Cell</u> 403-419.e4.

Ying, Z., Sandoval, M., and Beronja, S. (2018). Oncogenic activation of PI3K induces progenitor cell differentiation to suppress epidermal growth. <u>Nature Cell Biology</u>, 20:1256-66.

Brown, J.A, Yonekubo, Y., Hanson, N., Sastre-Perona, A., Basin, A., Rytlewski, J.A., Dolgalev, I., Meehan, S., Tsirigos, A., Beronja, S. and Schober, M. (2017). Defining Cellular Quiescence as a Drug Resistance Mechanism in Squamous Cell Carcinoma. <u>Cell Stem Cell</u> 21:650-64.

Brown, S., Pineda, C.M., Xin, T., Boucher, J., Suozzi, K.C., Park, S., Matte-Martone, C., Gonzalez, D.G., Rytlewski, J., Beronja, S.* and Greco, V.* (2017). Correction of aberrant growth preserves tissue homeostasis. <u>Nature</u> 548: 334-7. (*co-corresponding author).

Mallarino, R., Henegar, C., Mirasierra, M., Manceau, M., Shradin, C., Vallejo, M., Beronja, S., Barsh, G.S. and Hoekstra, H.E. (2016). Developmental mechanisms of stripe patterns in rodents. *Nature*, 539: 518-523.

Rytlewski J, Beronja S. (2015). RNAi in the mouse: rapid and affordable gene function studies in a vertebrate system. *WIREs Developmental Biology,* 4: 45-57.

Schramek D, Sendoel A, Segal JP, Beronja S, Heller E, Oristan D, Reva B, Fuchs E. (2014). Direct in vivo RNAi screen unveils myosin IIa as a tumor suppressor of squamous cell carcinomas. *Science*, 343: 309-13.

Beronja S, Janki P, Heller E., Lien WH, Keyes B, Oshimori N, Fuchs E. (2013). RNAi screens in mice identify physiological regulators of oncogenic growth. *Nature*, 501: 185-90.

Beronja S, Fuchs E. (2013). RNAi-mediated gene function analysis in skin. *Methods Molecular Biology* 961: 351-61.

Chen T, Heller E, Beronja S, Oshimori N, Stokes N, Fuchs E. (2012). An RNA interference screen unveils a new molecule in stem sell self-renewal and long-term regeneration. *Nature* 485: 104-8.

Beronja S, Fuchs E. (2011). A breath of fresh air in lung regeneration. *Cell* 147: 485-7.

Beronja S, Fuchs E. (2011). Tracing the roots of squamous cell carcinoma. *Nature Medicine* 17: 668-9.

Williams S E, Beronja S, Passoli HA,Fuchs E. (2011). Asymmetric cell divisions promote Notch-dependent epidermal differentiation. *Nature* 470: 353-8.

Beronja S, Livshits G, Williams SE, Fuchs E. (2010). Rapid functional dissection of genetic networks via tissue-specific transduction and RNAi in mouse embryos. *Nature Medicine* 16: 821-7.

Laprise P*, Beronja S*, Silva-Gagliardi NF, Pellikka M, Jensen AM, McGlade CJ, Tepass U. (2006). The FERM protein Yurt is a negative regulatory component of the Crumbs complex that controls epithelial polarity and apical membrane size. *Developmental Cell* 11: 363-74. (*co-first author).

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TRANSLATIONAL RESEARCH PROGRAM

Our mission is to prevent disease, advance treatment, and increase patient survival. To this end, we pursue a broad-based methodological approach to elucidate the fundamental and clinical implications of nuclear and mitochondrial DNA mutations in the pathogenesis of cancer and age-related disease.

The majority of our projects set out to address long-standing intractable questions in mutation research, which have remained unanswered, largely due to technical limitations. Thus, our first step toward their resolution typically involves the development of new methods and technologies (featured examples highlighted below) to sensitively measure biological information. The application of these tools to the question at hand, more often than not, reveals new insights into biology that exceed the scope of the hypothesis being tested, driving us down new and exciting pathways of discovery in pursuit of our overarching mission.

As such, while mutagenesis remains at the core of our research program, the focus of the laboratory continues to diversify and expand. Current and ongoing areas of interest include nuclear and mitochondrial genomics, DNA repair, transcriptomics, metabolomics, single cell biology, tumor immunology, cancer therapeutics and diagnostics.

SELECTED PUBLICATIONS

Massively parallel digital transcriptional profiling of single cells. Zheng GXY, Terry JM, Belgrader P, Ryvkin P, Bent ZW, Wilson R, Ziraldo SB, Wheeler TD, McDermott GP, Gregory MT, Shuga J, Montesclaros L, Masquelier DA, Nishimura SY, Schnall-Levin M, Wyatt PW, Hindson CM, Bharadwaj R, Wong A, Ness KD, Beppu LW, Deeg HJ, McFarland C, Loeb KR, Valente WJ, Ericson NG, Stevens EA, Radich JP, Mikkelsen TS, Hindson BJ, Bielas JH. Nat Commun. 2017 Jan 16;8:14049. doi: 10.1038/ncomms14049.

Mitochondrial DNA Exhibits Resistance to Induced Point and Deletion Mutations. Valente WJ, Ericson NG, Long AS , White PW, Marchetti F, Bielas JH. Nucleic Acids Res. 2016 Oct 14;44(18):8513-8524. *NAR Breakthrough Article

Targeted single molecule mutation detection with massively parallel sequencing.
Gregory MT, Bertout JA, Ericson NG, Taylor SD, Mukherjee R, Robins HR, Drescher CW, **Bielas JH**.

Nucleic Acids Research. 2015 Sep 17. doi: 10.1093/nar/gkv915.

[Digital genomic quantification of tumor-infiltrating lymphocytes.](#)
Robins HS, Ericson NG, Guenthoer J, O'Briant KC, Tewari M, Drescher CW, **Bielas JH**.
Sci Transl Med. 2013 Dec 4;5(214):214ra169. doi: 10.1126/scitranslmed.3007247.
PMID:
24307693

[Targeted enrichment and high-resolution digital profiling of mitochondrial DNA deletions in human brain.](#)
Taylor SD, Ericson NG, Burton JN, Prolla TA, Silber JR, Shendure J, **Bielas JH**.
Aging Cell. 2014 Feb;13(1):29-38. doi: 10.1111/acel.12146. Epub 2013 Sep 11.

[Heterogeneity of tumor-induced gene expression changes in the human metabolic network.](#)
Hu J, Locasale JW, **Bielas JH**, O'Sullivan J, Sheahan K, Cantley LC, Vander Heiden MG, Vitkup D.
Nat Biotechnol. 2013 Jun;31(6):522-9. doi: 10.1038/nbt.2530. Epub 2013 Apr 21.

[Decreased mitochondrial DNA mutagenesis in human colorectal cancer.](#)
Ericson NG, Kulawiec M, Vermulst M, Sheahan K, O'Sullivan J, Salk JJ, **Bielas JH**.
PLoS Genet. 2012;8(6):e1002689. doi: 10.1371/journal.pgen.1002689. Epub 2012 Jun 7.

Proc Natl Acad Sci U S A. 2003 Oct 28;100(22):12853-8. Epub 2003 Oct 20.
PMID:
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The Tumor Associated Microbiota: A role in cancer progression and treatment

My lab focuses firstly on understanding the translational impact of the tumor microbiota in human cancers, and secondly on the delineation of specific mechanisms involved in the pathogenesis of microbe-associated human cancers. We combine molecular microbiology, computational biology, biochemistry, and genetics to understand host-microbial interactions within the tumor microenvironment. Through such efforts, we seek to make discoveries that have both a scientific and clinical impact in the emerging area of bacterial-associated malignancies. Bacterial agents that have a role in cancer initiation or progression provide a viable route for prevention and treatment of these cancers.

We have a particular interest in the colorectal cancer associated bacterium, *Fusobacterium nucleatum*. Utilizing state-of-the-art functional and computational approaches, we aim to improve the understanding of processes that contribute to *Fusobacterium*-associated cancer, its contribution to the tumor microenvironment and accelerate the development of new targeted therapeutics or personalized approaches that take in to account the tumor microbiota for cancer treatment. Our current projects touch on two broad areas, noted below.

Host-Microbe Interactions:
  (i)   Spatial analyses of the microbiota within the tumor microenvironment
  (ii)   Reductionist approach to functionally characterizing the capabilities of tumor-associated microbiota

Microbe-Drug Interactions:
  (i)   Assessing microbiota modulation by routine chemotherapeutics
  (ii)   Assessing chemotherapeutic modulation by tumor microbiota
  (iii) Development of novel targeted antimicrobials against selected tumor enriched bacteria

SELECTED PUBLICATIONS:

Jin C, Lagoudas G, Zhao C, **Bullman S**, Bhutkar A, Hu B, Ameh S, Sandel D, Liang XS, Mazzilli S, Whary MT, Meyerson M, Germain R, Blainey PC, Fox JG, Jacks T. Commensal Microbiota Promote Lung Cancer Development via γδ T Cells. *Cell.* 2019 Feb 21; 176(5), 998-1013.

Walker M, Pedamallu CS, Ojesina AI, **Bullman S**, Sharpe T, Whelan CA, Meyerson M. GATK PathSeq: A customizable computational tool for the discovery and identification of microbial sequences in libraries from eukaryotic hosts. *Bioinformatics*. 2018 Jul 4. doi: 10.1093/bioinformatics/bty501.

Hamada T*; Zhang X*; Mima K*; **Bullman S*** *et al*., The relationship between *Fusobacterium nucleatum* and immune response to colorectal cancer differs by tumor microsatellite instability status. *Cancer Immunology Research. 2018 Nov;6(11):1327-1336 *Equal contribution*

Liu, Y., ..., **Bullman, S**., ..., The Cancer Genome Atlas Network, Laird, P.W. Comparative Molecular Analysis of Gastrointestinal Adenocarcinomas. *Cancer Cell.* 2018*; 721-735 (33). e728.

Bullman S, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, Neuberg D, Huang K, Guevara F, Nelson T, Chipashvili O, Hagan T, Walker M, Ramachandran A, Diosdado B, Serna G, Mulet N, Landolfi S, Ramon Y Cajal S, Fasani R, Aguirre AJ, Ng K, Élez E, Ogino S, Tabernero J, Fuchs CS, Hahn WC, Nuciforo P, Meyerson M. *Science.* 2017 Dec 15;358(6369):1443-1448.

Mehta RS, Nishihara R, Cao Y, Song M, Mima K, Qian ZR, Nowak JA, Kosumi K, Hamada T, Masugi Y, **Bullman S**, Drew DA, Kostic AD, Fung TT, Garrett WS, Huttenhower C, Wu K, Meyerhardt JA, Zhang X, Willett WC, Giovannucci EL, Fuchs CS, Chan AT, Ogino S. Association of dietary patterns with risk of colorectal cancer subtypes classified by *Fusobacterium nucleatum* in tumor tissue. *JAMA Oncol* 2017, 2016.6374.

Bullman S, Meyerson M, Kostic AD. Emerging Concepts and Technologies for the Discovery of Microorganisms Involved in Human Disease. *Annual review of pathology*. 2017; 12:217-244.

Mima K, Sukawa Y, Nishihara R, Qian ZR, Yamauchi M, Inamura K, Kim SA, Masuda A, Nowak JA, Nosho K, Kostic AD, Giannakis M, Watanabe H, **Bullman S**, Milner DA, Harris CC, Giovannucci E, Garraway LA, Freeman GJ, Dranoff G, Chan AT, Garrett WS, Huttenhower C, Fuchs CS, Ogino S. *Fusobacterium nucleatum* and T-cells in colorectal carcinoma. *JAMA Oncol* 2015 Aug;1(5):653-61.

Mima K, Nishihara R, Qian ZR, Cao Y, Sukawa Y, Nowak JA, Yang J, Dou R, Masugi Y, Song M, Kostic AD, Giannakis M, **Bullman S,** Milner DA, Baba H, Giovannucci EL, Garraway LA, Freeman GJ, Dranoff G, Garrett WS, Huttenhower C, Meyerson M, Meyerhardt JA, Chan AT, Fuchs CS, Ogino S. *Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. *Gut.* 2015 Aug 26 2015-310101.

Bullman S, Kearney K, O' Mahony M, Kelly L, Whyte P, Fanning S, Morgan JG. Identification and genetic characterization of a novel picornavirus from chickens. *J Gen Virol*, 2014, vir.0.061085-0.

Bullman S, Lucid A, Lucey B, Sleator RD. Genomic investigation into strain heterogeneity and pathogenic potential of the emerging GI pathogen *Campylobacter ureolyticus*. *PLoS ONE* 2013, 8 (8): 1-17.

Bullman S, O'Leary J, Corcoran D, Sleator RD, Lucey B. Molecular- based detection of non- culturable and emerging campylobacteria in patients presenting with gastroenteritis. *Epidemiol and Infect* 2011, 1-5.

Bullman S, Corcoran D, O'Leary J, Lucey B, Byrne D, Sleator RD: *Campylobacter ureolyticus*: an emerging gastrointestinal pathogen? *FEMS Immunol Med Micobiol* 2011, 61: 228-230.

Bullman S, Corcoran D, O'Leary J, Derry O'Hare, Lucey B, Byrne D, Sleator RD: Emerging dynamics of human campylobacteriosis in Southern Ireland. *FEMS Immunol Med Microbiol* 2011, 63.

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Molecular and cell biology of collective metastasis. A grand challenge in cancer biology is the problem of metastasis, the process by which tumor cells colonize distant organs. For decades, there has been a focus on understanding how tumor cells transition to become individually invasive and why this transition might be advantageous for tumor dissemination and metastasis. More recently, it has become increasingly recognized that cancer cells occur as multicellular clusters at every step of the metastatic cascade. Tumor cell clusters are potent metastatic seeds: associated with over 500-fold increase in metastatic potential compared with individual tumor cells, and giving rise to the majority of metastases in different mouse models. Further, the presence of circulating tumor cell clusters is associated with rapid disease progression and increased risk of death in many cancer types. Compared with single cell metastasis, the mechanisms driving collective metastasis remain largely unknown.

In my lab, we approach the problem of metastasis from a collective angle. Rather than focusing on how and why tumor cells become individualized, we are studying how and why tumor cells maintain their multicellular organization during metastasis. This perspective yields new questions such as what cell types cooperatively promote collective metastasis? Does heterogeneity pre-exist or emerge in clustered tumor cells? What biochemical signals support cooperative metastatic seeding? What are the cellular events and milieu driving collective dissemination? By gaining robust answers to these questions, we hope to learn more about the mechanistic basis of emergent cell-cell interactions. Our ultimate aim is to contribute to the discovery of more effective treatments and prevention strategies for metastatic breast cancer.

Kevin Cheung, MD

Our lab is making unique contributions to three fields: 1) cooperative signaling mechanisms determining the ability of tumor cell clusters to form metastases (Wrenn et al. Cell, in press); 2) advances in organoid technology for improved breast cancer metastatic modeling (Wrenn et al. under review); and 3) properties and origins of circulating tumor cells and CTC-clusters. Most recently we have discovered that clustered tumor cells utilize nanolumenal signaling, a mechanism by which clusters generate their own collective signaling factors and concentrate them within intercellular compartments. Potential rotation projects include understanding the molecular composition and regulation of the nanolumen signaling compartment.

SELECTED PUBLICATIONS

Wrenn ED, Yamamoto A, Moore, BM, Huang S, McBirney M, Thomas AJ, Greenwood E, Rabena YF, Rahbar H, Partridge SC, **Cheung KJ**. Regulation of collective metastasis by nanolumenal signaling. Cell, in press.

Cheung KJ, Ewald AJ. A collective route to metastasis: seeding by tumor cell clusters. Science 2016. 352, 167-169.

Cheung KJ*, Padmanaban V, Silvestri V, Schipper K, Cohen JD, Fairchild AN, Gorin MA, Verdone JE, Pienta KJ, Bader JS, Ewald AJ*. Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. Proceedings of the National Academy of Science 2016. * Co-Corresponding Authors.

Cheung KJ*, Ewald AJ*. Illuminating breast cancer invasion: diverse roles for cell-cell interactions. Current Opinion in Cell Biology 2014; 30C:99-111. * Co-Corresponding Authors.

Cheung KJ, Gabrielson E, Werb Z, Ewald AJ. Collective invasion in breast cancer requires a conserved basal epithelial program. Cell 2013; 155(7):1639-51

Nguyen-Ngoc KV*, **Cheung KJ***, Brenot A, Shamir ER, Gray RS, Hines WC, Yaswen P, Werb Z, Ewald AJ. The ECM microenvironment regulates collective migration and local dissemination in normal and malignant mammary epithelium. Proceedings of the National Academy of Science 2012; * Co-First Authors.

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CELL DIVISION AND THE UBIQUITIN-PROTEASOME SYSTEM IN CANCER DEVELOPMENT AND THERAPY

The Clurman lab studies protein degradation, cell division, and carcinogenesis. This research has led to fundamental discoveries about how cell proliferation and protein ubiquitylation are normally regulated, and how defects in these pathways are associated with cancer. We employ diverse methods, including biochemistry, functional genomics, cell biology, proteomics, and gene targeting in human cells and mice. We have had a particularly sustained impact in two areas: 1) studies on the Fbw7 ubiquitin ligase, which sits at the crossroads of cell division, protein degradation by the ubiquitin system, and tumorigenesis; and 2) understanding the functions and regulation of cyclin E and CDK2 in cell division and tumorigenesis.

Examples of Current Studies:

The Fbw7 Tumor Suppressor – Fbw7 is the substrate recognition component of a ubiquitin ligase that targets a remarkable group of proteins for degradation by the proteasome. Many Fbw7 substrates are critical oncoproteins and Fbw7 is one of the most frequently mutated human tumor suppressors. Our lab has made numerous contributions to understanding this complex pathway. One area has been the discovery of new substrates and the pathways that regulate their degradation, and these have included Myc, Notch, cyclin E, and Mediator. Another area has entailed and mouse gene targeting models to understand Fbw7 functions and the consequences of Fbw7 mutations in cancer. We also use approaches such as high-throughput screening and metabolomics to understand the consequences of Fbw7 mutations in cancer and how they may be targeted in novel therapeutic strategies. We have recently been focusing on a novel regulatory mechanism of Myc degradation by Fbw7, a collaborative project with Steve Henikoff to use genome-scale mapping to study how Fbw7 regulates oncogenic transcription factor chromatin occupancy, the discovery of new Fbw7 substrates,

and the development of novel therapeutic strategies that employ the Fbw7 pathway to target undrugable oncology targets.

CDKs and cell division. CDKs regulate cell division by phosphorylating their substrates, and identifying these substrates is key to understanding normal and neoplastic CDK functions. One continuing aspect of our work developing proteomic methods to identify these substrates. We have developed novel proteomic methods using substrate thiophosphorylation and engineered CDKS that utilize ATP analogs to discover more than a hundred new CDK2 substrates. We have adapted these methods to highly physiologic contexts, which has revealed a novel landscape of nuclear CDK2 substrates, including the discovery that CDK2 regulates a host of chromatin-modifying enzymes that we speculate coordinate cell division and epigenetic control of gene expression. We are now studying how CDK2 regulates chromatin and gene expression, as well as many new CDK2 substrates with roles in DNA replication, DNA damage and repair, transcription, and replication stress. In light of therapies targeting CDK4/6 in breast and other cancers, we have adapted these methods to identify new CDK4/6 substrates and are studying the functions of CDK4/6 in cancer and the mechanisms of cancer treatment with CDK4/6 inhibitors.

Another area of interest involves the Wee1 kinase, which inhibits CDKs. In one area of work. We are focusing on the on the importance of CDK2 inhibitory phosphorylation by Wee1 in replication stress. For example, we made a human knockin cell line to show that CDK2 inhibitory phosphorylation has essential roles in cell cycle control, in DNA replication dynamics, and in genome instability. Most importantly, we found that the inability to inhibit CDK2 during stalled S-phase leads to massive DNA damage, which has formed the basis for an exciting new chemotherapy strategy employing pharmacologic Wee1 inhibition and S-phase toxins.

Related recent work builds upon the work of Eddie Mendez on the role of targeting Wee1 in the treatment of head and neck squamous cell cancers (HNSCC). These studies have focused on HNSCC associated with HPV, which is rapidly increasing in incidence. We have found that these cancers are remarkably sensitive to Wee1 inhibition and that this involves premature activation of pro-mitotic gene expression driven by FoxM1.

SELECTED PUBLICATIONS

Chi Y, Carter J, Swanger J, Mazin A, Moritz R, and Clurman B.E. 2020. A novel landscape of nuclear human CDK2 substrates revealed by in situ phosphorylation. 2020. Science Advances. Apr 17; 6(16):eeaz9899.

Loeb KR, Hughes BT, FIssel BM, Osteen NJ, Knoblaugh SE, Grim JE, Drury LJ, Sarver A, Dupuy AJ, Clurman BE. Insertional mutagenesis using the Sleeping Beauty transposon system identifies drivers of erythroleukemia in mice. Sci Rep 2019; 9(1):5488.

Dye KN, Welcker M, Clurman BE, Roman A, Galloway DA. Merkel cell polyomarvirus Tumor atnigens expressed in Merkel cell carcinoma function independently of the ubiquitin ligases Fbw7 and β-TrCP. PLoS Pathog 2019; 15(1):e1007543.

Davis RJ, Gőnen M, Margineantu, DH, Handeli S, Swanger J, Hoellerbauer P, Paddison PJ, Gu H, Raftery D, Grim JE, Hockenbery DM, Margolin AA, Clurman BE. Pan-cancer transcriptional signatures predictive of oncogenic mutations reveal that Fbw7 regulates cancer cell oxidative metabolism. Proc Natl Acad Sci USA 2018; 115(21):5462-5467.

Goyal N, Rossi MJ, Mazina OM, Chi Y, Moritz, RL, Clurman BE, Mazin AV. RAD54 N-terminal domain is a DNA sensor that couples ATP hydrolysis with branch migration of Holliday junctions. Nat Commun. 2018; 9(1):34.

Davis RJ, Swanger J, Hughes BT, Clurman BE. 2017. The PP2A-B56 phosphatase opposes cyclin E autocatalytic degradation via site-specific dephosphorylation. Mol Cell Biol. March 31;37(8):e00657-16

Kuppers DA, Schmitt TM, Hwang H, Clurman BE, Fero M. 2017. The miR 106a~363^{Xpcl1} miRNA cluster induces murine T cell lymphoma despite transcriptional activation of the p27^{Kip1} cell cycle inhibitor". Oncotarget; 8(1):50680-50691

Calses PC, Dhillon KK, Tucker N, Chi Y, Huang JW, Kawasumi M, Nghiem P, Wang Y, Clurman BE, Jacquemont C, Gafken PR, Sugasawa K, Saijo M, Taniguchi T. DGCR8 Mediates Repair of UV-Induced DNA Damage Independently of RNA Processing. Cell Rep. 2017 Apr 4;19(1):162-174.

Park E, Lee B, Clurman BE, Lee K. NUP50 is necessary for the survival of primordial germ cells in mouse embryos.Reproduction. 2016 Jan;151(1):51-8.

Toledo CM, Ding Y, Hoellerbauer P, Davis RJ, Basom R, Girard EJ, Lee E, Corrin P, Hart T, Bolouri H, Davison J, Zhang Q, Hardcastle J, Aronow BJ, Plaisier CL, Baliga NS, Moffat J, Lin Q, Li XN, Nam DH, Lee J, Pollard SM, Zhu J, Delrow JJ, Clurman BE, Olson JM, Paddison PJ. Genome-wide CRISPR-Cas9 Screens Reveal Loss of Redundancy between PKMYT1 and WEE1 in Glioblastoma Stem-like Cells. Cell Rep. 2015 Dec 22;13(11):2425-2439.

Davis RJ, Welcker M, Clurman BE. 2014. Tumor Suppression by the Fbw7 Ubiquitin Ligase: Mechansisms and Opportunities. Cancer Cell.; 26(4):455-464

Zhao, H., Faltermeier, C.M., Mendelsohn, L., Porter, P.L., Clurman, B.E.*, and Roberts, J.M.* 2014. Mislocalization of p27 to the cytoplasm of breast cancer cells confers resistance to anti-HER2 targeted therapy. Oncotarget. Dec 30;5(24):12704-14.

Welcker, M., Larimore, E., Swanger, J., Grim, J., Ericsson, E, Zheng, N., and B. Clurman, and Bruce E Clurman. 2013. Fbw7 dimerization determines the specificity and robustness of substrate degradation. Genes and Development. 27(23):2531-6.

Hughes, B.T. Sidorova, J., Swanger, J., Monnat, Jr., R., and Clurman, B. 2013. An essential role for Cdk2 inhibitory phosphorylation during replication stress revealed by a human Cdk2 knockin mutation. Proc Natl Acad Sci, 110(22):8954-9.

Pierce, N. Lee, J., Liu J., Sweredoski, M., Graham, R., Larimore, E., Rome' M., Zheng,N., Clurman, B., Hess, S. Shan, , and Deshaies, R. 2013. Cand1 Promotes Assembly of New SCF Complexes through Dynamic Exchange of F Box Proteins. Cell. Feb 26. pii: S0092-8674 (13)00213-4. [Epub ahead of print].

Davis, M.A., Larimore, E, Fissel, B., Taatjes, D., and Clurman, B. 2013. The SCF- Fbw7 ubiquitin ligase degrades MED13 and MED13L and regulates CDK8 association with Mediator. Genes and Development, Jan 16, 2013, [Epub ahead of print].

Hizli, A., Chi, Y., Swanger, J., Welcker, M., Ryazanov, A., and Clurman, B. Phosphorylation of Elongation Factor 2 by Cyclin A-CDK2 Regulates Its Inhibition by eEF2 Kinase. Mol Cell Biol. 2012 Nov 26. [Epub ahead of print].

Grim, JE, Knoblaugh SE, Guthrie KA, Hagar A, Swanger J, Hespelt J, Delrow JJ, Small T, Grady WM, Nakayama KI et al. 2012 Fbw7 and p53 Cooperatively Suppress Advanced and Chromosomally Unstable Intestinal Cancer. Molecular and Cellular Biology. 32(11):2160-7.

Minella, A.C., Loeb, K.R., Knecht, A., Welcker., M., Varnum-Finney, B., Bernstein, I., Roberts, J., and Clurman, B.E. 2008. Cyclin E phosphorylation regulates cell proliferation in hematopoietic and epithelial lineages in vivo. Genes and Development. Jun 15;22(12):1677-89.

Welcker, M., Larimore, E.A., Frappier, L., and Clurman, B.E. 2011. Nucleolar targeting of the Fbw7 ubiquitin ligase by a pseudosubstrate and glycogen synthase kinase 3.   Mol Cell Biol.   Mar;31(6):1214-24

Grim, J.E., Gustafson, M.P., Hirata, R., Hagar, A.C., Swanger, J., Welcker, M., Hwang, H.C., Ericsson, J., Russell, D.W., and Bruce E. Clurman. 2008. Isoform- and cell cycle-dependent substrate degradation by the Fbw7 ubiquitin ligase. Journal of Cell Biology, 181(6):913-20

Welcker, M., Orian, A., Jin, J., Grim, J., Harper, W., Eisenman, R., and Bruce E. Clurman. 2004 The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. Proc Natl Acad Sci 2004 Jun 15; 101(24): 9085-90.

Hwang, H., Martins, C., Bronkhorst, Y., Randel, E., Berns, A., Fero, M., and Clurman, B.E. 2002. Identification of oncogenes collaborating with p27Kip1 loss by insertional mutagenesis and high-throughput insertion site analysis. Proc. Natl. Acad. Sci. Aug 20;99(17):11293-8.

Sheaff, R., Singer, J., Smitherman, M., Swanger, J., Roberts, J., and Clurman, B.E. 2000. Proteasome-dependent turnover of p21^{cip1} does not require p21 ubiquitination. Molecular Cell. Feb;5(2):403-10

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HIV AND THE EVOLUTIONARY HISTORY OF VIRUS-HOST INTERACTIONS

The Emerman lab studies host-cell interactions of the human immunodeficiency virus (HIV) and related viruses in order to understand the molecular and evolutionary basis of virus replication and pathogenesis. We do this by studying the evolution and function of host antiviral genes. Our goal is to determine how HIV adapted to humans to be become a pandemic virus.

Host restriction factors are potent, widely expressed, intracellular blocks to viral replication that are an important component of the innate immune response to viral infection. However, viruses have evolved mechanisms of antagonizing restriction factors. Through evolutionary pressure for both host survival and virus emergence, an evolutionary—arms race has developed that drives continuous rounds of selection for beneficial mutations in restriction factor genes. Thus, we use the evolutionary history of restriction factors and their functional interactions with viral proteins to understand modern viral pathogens such as HIV-1. We are using this information combined with the evolution of restriction factors to create novel, more potent variants on existing antiviral proteins that we call "super restriction factors".

We have developed a powerful new high-throughput screen for HIV restriction factors and host other host factors based on incorporation of CRISPR guide RNAs into HIV virions. We are using these screens to discover the interferon-stimulated genes that inhibit HIV and to comprehensively discover and characterize human genes that used for HIV for productive infection. We are also using similar screens for uncovering pathways used by HIV to maintain itself in a latent state in which it avoids elimination by the immune system. Such efforts are aimed at stratagies towards HIV Cure.

RECENT SELECTED PUBLICATIONS

OhAinle, M., Helms, L., Vermeire, J., Roesch, F., Humes, D., Basom, R., Delrow, J. J., Overbaugh, J., and Emerman, M. A Virus-Packageable CRISPR Screen Identifies Host Factors Mediating Interferon Inhibition of HIV. eLIFE (2018), doi: 10.7554/eLife.39823

Colón-Thillet, R., Hsieh, E., Graf, L., McLaughlin Jr, R.N., Young, J.M., Kochs, G., **Emerman, M**., and Malik, H.S. Combinatorial mutagenesis of rapidly-evolving residues yields super-restrictor antiviral proteins. PLOS Biol (2019) 17(10): e3000181. https://doi.org/10.1371/journal.pbio.3000181

Binning, J.M., Chesarino, N.M., **Emerman, M.,** and Gross, J.D. Structural basis for a species-specific determinant of an SIV Vif protein towards hominid APOBEC3G antagonism. Cell Host Microbe, (2019) 26(6):739-747. doi:10.1016/j.chom.2019.10.014

Chesarino, N.M., and **Emerman, M.** Polymorphisms in human APOBEC3H differentially regulate ubiquitination and antiviral activity. Viruses (2020**)**, *12*(4), 378;

OhAinle, M., Kim, K., Keceli, S. K., Felton, A., Campbell, E., Luban, J., and **Emerman, M**. TRIM34 restricts HIV-1 and SIV capsids in a TRIM5α -dependent manner. PLOS Pathog (2020) 16(4): e1008507. https://doi.org/10.1371/journal.ppat.1008507

McDonnell MM, Crawford KHD, Dingens AS, Bloom JD, **Emerman M.**. APOBEC3C tandem domain proteins create super restriction factors against HIV-1. mBio (2020) 11:e00737-20. https://doi.org/10.1128/mBio.00737-20.

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VIRUSES AND CANCER

The Galloway Lab studies how human papillomaviruses (HPVs) and human polyomaviruses (HPyV), specifically Merkel Cell polyomavirus (MCPyV) contribute to the development of cancers, and how that information can be used to better prevent, diagnose or treat malignancies. High-risk papillomaviruses (HR-HPVs) are causative agents in nearly all cervical cancers and the majority of other anogenital and oropharyngeal cancers. By identifying the targets of the viral oncoproteins, E6 and E7, we have revealed key regulatory pathways that control proliferation, senescence, apoptosis and DNA damage repair. These pathways are also targets of somatic mutation in other epithelial tumors. We are particularly interested in the mechanisms by which the HR-HPV E6 and E7 proteins disrupt the repair of DNA damage. In addition to studying the mechanism we plan to exploit this vulnerability to develop therapeutic approaches to treat HPV associated neoplasia. Development of cervical cancer occurs over decades and the precursor intraepithelial lesions can be readily obtained. Analysis of the staged clinical lesions, coupled with ectopic expression of E6 and E7 in otherwise normal cells, allows us to distinguish the proximal consequences of E6/E7 expression from the myriad changes that result from genetic instability. Additionally, the requirement for HPV infection and gene expression in anogenital malignancy provides a clear target for prophylactic and therapeutic immune intervention.

A longstanding interest has been in characterizing the humeral immune response to HPV following natural infection or vaccination. We have mapped epitopes involved in virus neutralization and continue to characterize the breadth of the response. Current studies are characterizing the B cell memory response by identifying HPV-specific memory B cells, plasmablasts and the antibodies they express. These studies are important in determining the most effective vaccine regimens, whether fewer doses are effective and whether natural immune responses could be improved by vaccination. Understanding memory responses and the protective antibodies they generate will have broad implications for vaccine design. We are collaborating with others to conduct an efficacy and immunogenicity trial of a single dose of HPV vaccines in Kenya and are also determining effective dosing regimens in HIV+ children in Peru.

Denise Galloway, PhD

We have also become interested in the role that the Merkel Cell polyomavirus (MCPyV) plays in the etiology of Merkel Cell carcinoma (MCC), a rare but aggressive skin cancer. Interestingly we demonstrated that antibodies to the common region of large and small T antigen are present in about 60% of patients with MCC and that increases in antibody titer are prognostic of recurrence. We also showed that MCPyV encodes another T antigen in an alternate reading frame to large T antigen, which we named ALTO. ALTO is evolutionarily related to the middle T antigen of rodent PyVs. A major focus is to determine the mechanisms by which the viral T antigens play in tumorigenicity and to develop suitable cell based and mouse models.

SELECTED PUBLICATIONS

Dye, K.N., Welcker, M., Clurman, B.E., Roman, A., **Galloway, D.A.** Merkel Cell polyomavirus tumor antigens expressed in Merkel Cell carcinoma function independently of the ubiquitin ligases Fbw7 and β-TrCP. PLoS Pathogens, Jan 28;15(1):e10075. 2019

Khanal, S., **Galloway, D.A.** High risk human papillomavirus oncogenes disrupt the Fanconi Anemia DNA repair pathway by impairing foci formation and de-ubiquitinylation of FancD2. PLoS Pathogens PLoS Pathog. Feb 28;15(2):e1007442. 2019 PMID: 30818369

Xi LF, Schiffman M, Hughes JP, **Galloway, DA**, Koutsky LA, Kiviat NB Changes in DNA level of oncogenic human papillomaviruses other than types 16 and 18 in relation to risk of cervical intraepithelial neoplasia grades 2 and 4. Cancer Epidemiol. Biomarkers Prev. Aug 28 (8)1388-1394 Epub 2019 May 7 2019

Scherer, E.M., Smith, R.A., Carter, J.J., Wipf, G.C., Gallego, D.F., Stern, M., Wald, A., Galloway, D.A. Analysis of memory B cell responses reveals suboptimal dosing schedule of a licensed vaccine. J. Infec. Dis. 2018 Jan 30;217(4):572-580

Scherer, E.M., Smith, R.A., Carter, J.J., Wipf, G.C., Gallego, D.F., Stern, M., Wald, A., **Galloway, D.A.** Analysis of memory B cell responses reveals suboptimal dosing schedule of a licensed vaccine. J. Infec. Dis. 2018 Jan 30;217(4):572-580

Wallace, N.A., Khanal, S., Robinson, K.L., Wendel, S.O., Messer, J.J., Galloway, D.A., High risk alpha papillomavirus oncogenes impair the homologous recombination pathway. J Virol. 2017 Sep 27;91(20). pii: e01084-17. doi: 10.1128/JVI.01084-17. Print 2017 Oct 15

Scherer, E.M., Smith, R.A., Gallegos, D.F., Carter, J.J., Wipf, G., Hoyos, M., Stern, M., Wald, A. Galloway, D.A. A single HPV vaccine dose Improves B cell memory in previously infected subjects. eBiomedicine. doi: 10.1016/j.ebiom.2016.06.042. 2016

Wallace, N.A., Robinson, K.L., Galloway, D.A. Beta-HPV 5 and 8 E6 disrupt homology dependent double strand break repair by attenuating BRCA1 and BRCA2 expression and foci formation. PLoS Pathog. 2015 Mar;11(3):e1004687

Scherer, E., M., Smith, R.A., Simonich, C.A., Niyonzima, N., Galloway, D.A. Characteristics of memory B cells elicited by a highly efficacious HPV vaccine in subjects with no pre-existing immunity. PLoS Pathogens. Oct 16;10(10):e1004461. doi: 10.1371/journal.ppat.1004461. eCollection 2014 Oct. 2014

Carter JJ*, Daugherty MD*, Qi X., Bheda-Malge A, Wipf GC, Robinson K, Roman A, Malik HS, Galloway DA. Identification of an overprinting gene in Merkel Cell polyomavirus provides evolutionary insight into the birth of viral genes.   Proc Natl Acad Sci USA. 30; 110 (31):12744-9. 2013

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Paulson KG, Carter JJ, Johnson LG, Cahill KW, Iyer JG, Schrama D, Becker JC, Madeleine MM, Nghiem P, Galloway DA. 2010. Antibodies to Merkel cell polyomavirus T antigen oncoproteins reflect tumor burden in Merkel cell carcinoma patients. Cancer Research, 70(21)8388-97. 2010

Professional Highlights

National Cancer Institute, Outstanding Investigator Grant   2017 – 2024

Director, Pathogen Associated Malignancies Integrated Research Center   2017

Washington State Life Sciences Hall of Fame 2017

Paul Stephanus Memorial Endowed Chair   2018

Association of Women in Science (AWIS) Science Achievement and Leadership Award 2019

American Academy of Arts and Sciences   2019

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DNA VIRUS ADAPTATION TO HOST CELL DEFENSES

In response to myriad host defenses, viruses have evolved mechanisms that counteract cellular anti-viral factors. These host-virus conflicts result in an evolutionary "arms race," in which the structure and specificities of the participating genes change with surprising rapidity. Research in the Geballe lab focuses on identifying the factors, dissecting the mechanisms, and understanding the evolutionary pathways used by large DNA viruses, such as cytomegaloviruses and poxviruses, to enable viral replication in the face of host defenses.

Human cytomegalovirus (HCMV), a member of the herpesvirus family, typically produces few if any symptoms in otherwise healthy individuals, but often causes life-threatening infections in newborns, solid organ and hematopoietic stem cell transplant recipients, and other immuncocompromised patients. In addition to its medical importance, HCMV is also a useful model system for the study of viral mechanisms for circumventing host defenses such as the shut off of translation mediated by the interferon-induced, double-stranded RNA-activated protein kinase R (PKR). A genetic screen identified two essential HCMV genes that participate in maintaining translational capacity in the infected cell by inhibiting the PKR pathway. Comparisons between these HCMV genes and related ones encoded by nonhuman primate and rodent CMVs have revealed surprising specificity and complexity in the interactions and mechanisms by which these factors act. We are now dissecting the molecular basis for these differences and investigating additional critical roles these proteins play in the viral life cycle.

A broadly applicable strategy for studying viral mechanisms that counteract host defenses is to force viral adaptations in cell culture and then to sequence the resulting viruses to identify the genetic basis of the

adaptation. Such an experimental evolutionary approach revealed an unexpected ability of the model poxvirus vaccinia to adapt by amplification of a weak antagonist of the human PKR, followed by mutation and collapse back down to a single copy gene. Current efforts are underway to explore whether this "accordion-like" mechanism is a general property of large DNA viruses and to assess its role in adaptation of these viruses to other host defenses.

SELECTED PUBLICATIONS

Duke, E.R., M. Boeckh, and A.P. Geballe. 2020. Cytomegalovirus. In: Encyclopedia of Gastroenterology, 2nd edition (E.J. Kuipers, ed) pp. 807-814.

Bayer, A., G. Brennan and A.P. Geballe. 2018. Adaptation by copy number variation in monopartite viruses. Current opinion in virology. [Epub ahead of print] Jul 13;33:7-12. doi: 10.1016/j.coviro.2018.07.001. NIHMS980090

Hickson, S.E., D. Margineantu, D.M. Hockenbery, J.A. Simon, and A.P. Geballe. 2018. Inhibition of vaccinia virus replication by nitazoxanide. Virology 518:398-405

Child, S.J., S.E. Hickson, A. Bayer, D. Malouli, K. Fruh, and A.P. Geballe. 2018. Antagonism of 1 the protein kinase R pathway in human cells by rhesus cytomegalovirus. J. Virol. 92, PMCID: PMC5827409

Gray, E.E., D. Winship, J.M. Snyder, S.J. Child, A. P. Geballe, D.B. Stetson. 2016. The AIM2-like receptors are dispensable for the interferon response to intracellular DNA. Immunity 45:255-266. PMCID: PMC4988931

Carpentier, KS., N.M. Esparo, S.J. Child, and A.P. Geballe. 2016. A single amino acid dictates Protein Kinase R susceptibility to unrelated viral antagonists. PLoS Pathogens. 12(10):e1005966. doi: 10.1371/journal.ppat.1005966. PMCID: PMC5079575

Daugherty MD, Schaller AM, Geballe AP, Malik HS. 2016. Evolution-guided functional analyses reveal diverse antiviral specificities encoded by IFIT1 genes in mammals. Elife. 31;5. PubMed Central PMCID: PMC4887208

Braggin JE, Child SJ, Geballe AP. 2016. Essential role of protein kinase R antagonism by TRS1 in human cytomegalovirus replication. Virology. 489:75-85. PubMed Central PMCID: PMC4761322

Mouna L, Hernandez E, Bonte D, Brost R, Amazit L, Delgui LR, Brune W, Geballe AP, Beau I, Esclatine A. Analysis of the role of autophagy inhibition by two complementary human cytomegalovirus BECN1/Beclin 1-binding proteins. Autophagy. 2016, 12(2):327-42. PubMed Central PMCID: PMC4836022

Carpentier KS, Geballe AP. 2016. An Evolutionary View of the Arms Race between Protein Kinase R and Large DNA Viruses. J Virol. 90(7):3280-3. PubMed Central PMCID: PMC4794663

Schleiss MR, Bierle CJ, Swanson EC, McVoy MA, Wang JB, Al-Mahdi Z, Geballe AP. 2015. Vaccination with a Live Attenuated Cytomegalovirus Devoid of a Protein Kinase R Inhibitory Gene Results in Reduced Maternal Viremia and Improved Pregnancy Outcome in a Guinea Pig Congenital Infection Model. J Virol. 89(19):9727-38. PMCID: PMC4577886

Adam Geballe, MD

Brennan, G, Kitzman JO, Rothenburg S, Shendure J, and Geballe AP. 2014. Adaptive gene amplification as an intermediate step in expansion of virus host range. *PLoS Pathogens,* 10(3): e1004002. doi:10.1371/journal.ppat.1004002. PMCID: PMC3953438

Bierle, CJ, Semmens KM, and Geballe AP. 2013. Double-stranded RNA binding by the human cytomegalovirus PKR antagonist TRS1. *Virology,* 442:28-37. NIHMS470480

Bierle, CJ, Schleiss MR, and Geballe AP. 2012. Antagonism of the protein kinase R pathway by the guinea pig cytomegalovirus US22-family gene gp145. *Virology,* 433:157-166

Elde, NC., Child SJ, Eickbush MT, Kitzman JO, Rogers KS, Shendure J, Geballe AP, and Malik HS. 2012. Poxviruses deploy genomic accordions to adapt rapidly against antiviral defenses. *Cell,* 150:485-489. NIHMSID #402637

Chaumorcel, M, Lussignol M, Mouna L, Cavignac Y, Fahie K, Cotte-Laffitte J, Geballe A, Brune W, Beau I, Codogno P et al. 2012. The Human Cytomegalovirus Protein TRS1 Inhibits Autophagy via Its Interaction with Beclin 1. *Journal of Virology*, 86(5):2571-2584

Child, SJ, Brennan G, Braggin JE, Geballe AP, 2012. Species specificity of protein kinase R antagonism by cytomegalovirus TRS1 genes. *Journal of Virology,* 86(7):3880-3889

Grantt S, Carlsson J, Ikoma M, Gachelet E, Gray M, Geballe AP, Corey L, Casper C, Lagunoff, M, Vieira J. 2011. The HIV protease inhibitor Nelfinavir inhibits Kaposi"s Sarcoma-associated herpes virus replication in vitro. *Antimicrobial Agents and Chemotherapy*, 55(6)2696-703

Boeckh M, Geballe AP. 2011. Cytomegalovirus: pathogen, paradigm, and puzzle. *The Journal of Clinical Investigation*, 121(5)1673-80

Strang BL, Geballe AP, Coen DM. 2010. Association of human cytomegalovirus proteins IRS1 and TRS1 with the viral DNA polymerase accessory subunit UL44. *Journal of General Virology*.

Marshall EE, Geballe AP. 2009. Multifaced evasion of the interferon response by cytomegalovirus. *Journal of Interferon & Cytokine Research,* NIHMS132275

Marshall EE, Bierle CJ, Brune W, Geballe AP. 2009. Essential role for either of TRS1 or IRS1 in human cytomegalovirus replication. *Journal of Virology,* 83, 4112-4120. Pervasive and Mobile Computing Journal

Child SJ, Geballe AP. 2009. Binding and relocalization of protein kinase R by murine cytomegalovirus, Journal of Virology, 83, 1790-1799. *Pervasive and Mobil Computing Journal*

Elde N, Child SJ, Geballe AP, Malik HS. 2009. Protein kinase R reveals an evolutionary model for defeating viral mimicry. *Nature,* 457, 485-489. NIHMS73372

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REGULATION OF DISSEMINATED TUMOR CELL DORMANCY

Cyrus Ghajar directs the Laboratory for the Study of Metastatic Microenvironments (LSM^2). The goal of his laboratory is to understand how microenvironments within distant tissues regulate the four "hallmarks" of disseminated tumor cells (DTCs): long-term survival, reversible growth arrest, immune evasion and therapeutic resistance. Solving these puzzles is key to extending metastasis-free survival of cancer patients; with the ultimate goal of preventing metastasis altogether.

Four major research directions are underway in our laboratory:

1. What puts DTCs to sleep? Do the underlying mechanisms change from tissue to tissue?

Breast cancer stereotypically yields metastases in the brain, lung, liver, bones and lymph nodes. But in a substantial fraction of patients, metastases may not emerge for five, 10, or even 20 years after treatment. How are DTCs kept at bay in the interim?

In 2013, we established that dormant breast cancer cells localize to the outer surface of microvessels- a microenvironment called the perivascular niche. There, endothelial-derived factors effect quiescence. Specifically, we reported that endothelial derived thrombospondin-1 suppresses DTC outgrowth in lung and in bone marrow. Interestingly, although DTCs are found on microvessels within the brain as well, brain endothelium does not express thrombospondin-1. This is but one minor reflection of how vascular and perivascular environments vary by tissue. Our hypothesis is that these unique perivascular environments effect dormancy in unique ways. To elucidate the tissue-

specificity of the perivascular niche and of dormancy, we engineer mimetics of microvascular beds of a number of different tissues, including those where disseminated breast tumor cells commonly emerge (e.g., brain, lung, bone marrow and liver) as well as those where they rarely do.

2. What do DTCs rely on for survival? DTCs transit into totally 'foreign' microenvironments, where they are able to survive in patients for a decade or more. What biologies are they relying on to survive?

To address this question, we have adopted a high-content/high-throughput approach leveraging organotypic vascular niches, reporters of quiescence and apoptosis, and a 5,500 compound library. Our findings have revealed— unsurprisingly— that the drugs that target quiescent *vs.* proliferative tumor cells are completely different. By combining sequencing with therapeutic pressures, we have begun to unravel the pathways targeted by these compounds, with the hopes that this will inform us about the unique molecular and metabolic requirements of dormant DTCs. The goal is to define safe and specific therapies we can apply to eradicate dormant DTCs.

3. How do dormant disseminated tumor cells resist therapies?

It is assumed commonly that quiescent DTCs do not respond to genotoxic therapy because such therapies only target rapidly dividing cells. We challenged this notion, showing that chemotherapeutic regimens employed in the treatment of invasive breast cancer select for perivascular tumor cells in the bone marrow. Using organotypic vascular niches, we discovered that endothelium protects DTCs from chemotherapy, and that the mechanisms are cell cycle-independent, relying instead on interactions between integrins and molecules present within the vascular niche. Targeting integrins sensitized the vast majority of DTCs to chemotherapy, yielding drastic enhancements to metastasis-free survival in pre-clinical models.

This finding has motivated us to measure how chemotherapy impacts other non-dividing cells; especially endothelium. We hypothesized that chemotherapy causes DNA damage within the endothelium, and that this damage is linked to a vascular secretome that paradoxically protects DTCs from chemotherapy. We are now working to thoroughly define this secretome, and the signaling that links it to DNA damage. Targeting what we have called the chemotherapy-associated vascular secretome at its root or at its stem may yield efficacious therapies that can be applied with or without integrin inhibitors to eradicate DTCs.

4. How do dormant DTCs escape immunity?

We have used model antigens and immune-competent models to establish that dormant DTCs evade immunity, and uncover intrinsic and extrinsic mechanisms that allow them to do so. These findings have motivated us— in collaboration with Stan Riddell's Lab— to explore engineered T cell receptor (TCR)- and chimeric antigen receptor (CAR)-based approaches to target dormant DTCs. Our goals are to: (i) identify the formant of immunotherapy best suited to eradicate dormant DTCs, (ii) re-engineer these cells so that they are optimized to traffic to and eradicate rare cell populations, and (iii) profile *human* DTCs so that we can identify realistic (neo)antigens that we can target with engineered T cells.

SELECTED PUBLICATIONS

C.M. Ghajar, H. Peinado, H. Mori, I.R. Matei, K.J. Evason, H. Brazier, D. Almeida, A. Koller, K.A. Hajjar, D.Y.R. Stainier, E.I. Chen, D. Lyden, M.J. Bissell. The perivascular niche regulates breast tumour dormancy. *Nature Cell Biology* 15 (7), 2013 (cover article).

C.M. Ghajar. *Perspective*: Metastasis prevention by targeting the dormant niche. *Nature Reviews Cancer* 15 (4), 2015.

P. Carlson*, A. Dasgupta*, C.A. Grzelak*, J. Kim*, A. Barrett, I.M. Coleman, R.E. Shor, E.T. Goddard, J. Dai, E.M. Schweitzer, A.R. Lim, Sarah B. Crist, D.A. Cheresh, P.S. Nelson, K.C. Hansen, C.M. Ghajar. Targeting the perivascular niche sensitizes disseminated tumour cells to chemotherapy. *Nature Cell Biology* 21 (2), 2019 (*cover article)*.

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The Gujral lab is focused on understanding the molecular details of how cells respond to growth factors and how these signaling networks can be targeted therapeutically. To advance investigation of pathological signaling events among cells within the microenvironment, the Gujral lab is developing techniques for culturing organotypic slices of tumor tissue and monitoring the behavior of specific cell populations. Dr. Gujral discovered a pathway involving the growth factor Wnt5 and the receptor Fzd2. His lab explores how this pathway contributes to liver fibrosis and cancer metastasis by triggering changes in cell fate. The molecular networks that let cells respond to stimuli are complex, dynamic, interconnected, and context dependent. Therefore, the Gujral lab uses systems biology and computational biology along with cell biological, genetic, and pharmacological approaches to investigate tissue-specific signaling networks, identify molecular targets for drug discovery, and identify new uses for existing medicines.

Current Studies

Wnt Signaling in Disease: Metastasis is responsible for ~90% of cancer-associated death, yet progress has been slow in developing drugs that either specifically target metastasis or target cells with metastatic potential. The process of epithelial-to-mesenchymal transition (EMT) is associated normal embryonic development, but this process is aberrantly activated and contributes to metastatic potential in cancer. EMT is a reversible process in which epithelial cells adopt mesenchymal properties, which include changes in cell shape, proliferative capacity, and increased motility. Our lab identified the Wnt5-Fzd2 pathway as a key signaling network driving EMT and tumor metastasis in several challenging cancers, including liver, breast, lung, and colon. This pathway is one of several growth factor-mediated pathways that trigger EMT in both embryonic development and normal and transformed cell lines. Our lab uses a combination of cell biological, biochemical, and genetic approaches to uncovering the signaling networks by which growth factors stimulate EMT, which could guide the development of new therapies directed at cancer metastasis.

Developing New Technologies for Studying Signaling within a Tissue Microenvironment: A main focus of our lab is the development of new technologies that enable investigation of cellular interactions within the tissue microenvironment. Such methods need to (i) preserve the tissue microenvironment and maintain cell-cell interactions; (ii) study the properties of individual cells within this microenvironment, (iii) expose individual cells within a tissue to stimuli in a spatially and temporally controlled manner, and (iv) evaluate the response

of single cells within a tissue to stimuli. We are developing new experimental approaches to study cell-cell interactions and single cell responses in the complex tissue microenvironment. We are developing approaches relevant to investigating cancer within the tumor tissue context, as well as approaches that will enable new insights into physiological regulation of cells within normal or disease tissues.

Developing Computational Tools for Performing Network Pharmacology. Another area of investigation is in the application of computational strategies to explore novel pharmacological options. Many drugs affect multiple targets, and many effective therapies target multiple regulators of a physiological process or multiple mediators of an intracellular signaling pathway. Network pharmacology is a new field of science focused on targeting multiple steps in a physiological regulatory network. Goals of this field include facilitating the design of drugs with specific multi-target profiles and exploiting the existing polypharmacology of many currently used medicines. We are developing computational tools for evaluating the many potential clinical applications of kinase inhibitors, because kinases represent a core group of enzymes involved in most cellular responses to stimuli and represent an important target for existing therapeutics for cancer, inflammation, and other disease states. Additionally, most available kinase inhibitors lack specificity, making attributing the effects of an inhibitor to an individual kinase difficult. Finally, the physiological and pathological roles of many kinases remain to be discovered. Thus, the tools we are developing will enhance our understanding the basic biology of kinases as well as advance pharmacological exploitation of these key cellular regulators.

PUBLICATIONS (2017- present)

1. Golkowski, M., Lau, HT., **Chan, M**., Kenerson, H., Vidadala,V.N., Shoemaker, A., Maly, D.J., Yeung, R.S., **Gujral, T.S.*,** and Ong, S*. Pharmacoproteomics identifies kinase pathways that drive the epithelial-mesenchymal transition and drug resistance in hepatocellular carcinoma. *Co-corresponding. **Cell Systems**. 2020. In press.
2. Szulzewsky, F., Arora,S., Hoellerbauer,P., King, C., Nathan, E., **Chan, M**., Cimino, P.J., Ozawa, T., Kawauchi, D., Pajtler, KW., Gilbertson, R.J., Paddison, P.J., Vasioukhin, V., **Gujral, T.S**, and Holland, E.C. Comparison of tumor-associated YAP1 fusions identifies a recurrent set of functions critical for oncogenesis. **Genes & Development**. 2020. 34: 1051-1064.
3. **Xue, A**., **Chan, M**., and **Gujral, T.S**. Pan-Cancer Analysis of the Developmental Pathways Reveals Non-canonocal Wnt signaling as a Driver of Mesenchymal-type Tumors. **Translational Research**. 2020. S1931-5244 (20)30140-7
4. **Siddharth, V**., and **Gujral, T.S**. Non-linear Deep Neural Network for Rapid and Accurate Prediction of Phenotypic Responses to Kinase Inhibitors. **iScience**. 2020. 23 (5). 101129
5. **Nishida-Aoki**, N., **Bondesson, A.J**., and **Gujral, T.S**. Measuring Real-time Drug Response in Organotypic Tumor Tissue Slices. **J. Vis. Exp**. 2020. (159), e61036
6. **Sivakumar, R**., **Chan, M**., **Shin, J.S**., **Nishida-Aoki, N**., Kenerson, H.L., Elemento, O., Beltran, H., Yeung, R and **Gujral, T.S**. Organotypic tumor tissue slice cultures provide a versatile platform for immuno-oncology and drug discovery. **OncoImmunology**. 2019. 8(12): e1670019
7. **Nishida-Aoki, N**., and **Gujral. T.S**. Emerging approaches to study cell-cell interactions in tumor microenvironment. **Oncotarget**. 2019. 10:785-797
8. **Bello, T**., and **Gujral. T.S**. KInhibition: A Kinase Inhibitor Selection Portal. **iScience**. 2018. 8, 49–53
9. Arang, N., Kain, H., Glennon, E., **Bello, T**., Dudgeon, D., Walter, E., **Gujral, T.S**. and Kaushansky, A. Identifying host regulators and inhibitors of liver stage malaria infection using kinase activity profiles. **Nature Communications**. 2017. 1;8(1):1232
10. Susman MW, Karuna EP, Kunz RC, **Gujral TS**, Cantú AV, Choi SS, Jong BY, Okada K, Scales MK, Hum J, Hu LS, Kirschner MW, Nishinakamura R, Yamada S, Laird DJ, Jao LE, Gygi SP, Greenberg ME, Ho HH. Kinesin superfamily protein Kif26b links Wnt5a-Ror signaling to the control of cell and tissue behaviors in vertebrates. **Elife**. 2017. 8;6. e26509

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Description of your research

My laboratory's research is focused on studying the role of genomic alterations in cancer and expanding applications for precision medicine. We combine research in two complementary areas: (1) We develop and apply novel **computational methods** to comprehensively profile and study **cancer genomes** from patient tumors and (2) we develop approaches for non-invasive **liquid biopsies**, such as **circulating tumor DNA** from blood, to monitor genomic changes in cancer patients. Our goals are to uncover the genetic and epigenetic alterations in treatment resistance, identify blood-based genomic biomarkers, and translate these findings and innovations to advance cancer precision medicine.

We are interested in understanding the roles of tumor evolution and abnormal genome structure in cancer. We apply cutting-edge whole genome DNA sequencing technologies, particularly platforms that generate long-range genomic information, such as linked-read sequencing. These technologies enhance the reconstruction of genomic rearrangements and enable the study of alterations in non-coding genomic regions. Our recent work applying this technology to study advanced prostate cancer includes the discoveries of non-coding alterations to an enhancer of the androgen receptor and a genome-wide tandem duplication signature associated with CDK12-loss.

A key focus of our research is to accelerate the development of new approaches to exploit liquid biopsies for studying cancer. We leverage insights from the analysis of tumor genomes to inform the design of circulating tumor DNA applications to study treatment response in cancer patients. We have established strong collaborations with experimental and clinical scientists at Fred Hutch and UW to apply our approaches to study treatment resistance in prostate and other cancers.

The development of novel computational algorithms for analyzing human cancer genomes is a primary research focus of our group. Probabilistic, machine learning algorithms that we have developed include TITAN, HMMcopy, and ichorCNA for predicting genome-wide alterations from tumor and cell-free DNA sequencing data. These software tools are widely used by the cancer research community. We are actively developing new methods to expand our suite of tools to analyze and discover new signatures in tumor and circulating tumor DNA.

SELECTED PUBLICATIONS

Viswanathan SR*, **Ha G***, Hoff AM*, Wala JA, Carrot-Zhang J, Whelan CW, Haradhvala NJ, Freeman SS, Reed SC, Rhoades J, Polak P, Cipicchio M, Wankowicz SA, Wong A, Kamath T, Zhang Z, Gydush G, Rotem D, International PCF/SU2C Prostate Cancer Dream Team, Love JC, Getz G, Gabriel S, Zhang C-Z, Dehm SM, Nelson PS, Van Allen EM, Choudhury AD, Adalsteinsson VA, Beroukhim R, Taplin M-E, Meyerson M. Structural alterations driving castration-resistant prostate cancer revealed by linked-read genome sequencing. *Cell* 2018 Jul 12;174(2):433-447.e19.

Schweizer MT*, **Ha G***, Gulati R, Brown L, McKay RR, Dorff T, Hoge ACH, Reichel J, Vats P, Kilari D, Patel V, Oh WK, Chinnaiyan A, Pritchard CC, Armstrong AJ, Montgomery RB, Alva A. CDK12-Mutated Prostate Cancer: Clinical Outcomes with Standard Therapies and Immune Checkpoint Blockade. *JCO Precision Oncology* 2020 Apr 21;4:382-392.

Stover DG*, Parsons HA*, **Ha G***, Freeman S, Barry WT, Guo H, Choudhury AD, Gydush G, Reed SC, Rhoades J, Rotem D, Hughes ME, Dillon DA, Partridge AH, Wagle N, Krop IE, Getz G, Golub TR, Love JC, Winer EP, Tolaney SM, Lin NU, Adalsteinsson VA. Association of cell-free DNA tumor fraction and somatic copy number alterations with survival in metastatic triple-negative breast cancer. *Journal of Clinical Oncology* 2018 Feb 20;36(6):543-553.

Adalsteinsson, VA *, **Ha G***, Freeman SS*, Choudhury AD, Stover DG, Parsons HA, Gydush G, Reed SC, Rotem D, Rhoades J, Loginov D, Livitz D, Rosebrock D, Leshchiner I, Kim J, Stewart C, Rosenberg M, Francis JM, Zhang CZ, Cohen O, Oh C, Ding H, Polak Paz, Lloyd M, Mahmud S, Helvie S, Merrill MS, Santiago RA, O'Connor EP, Jeong SH, Leeson R, Barry RM, Kramkowski JF, Zhang Z, Polacek L, Lohr J, Schleicher M, Lipscomb E, Saltzman A, Oliver NM, Marini L, Waks AG, Harshman LC, Tolaney SM, Van Allen EM, Winer EP, Lin NU, Nakabayashi M, Taplin ME, Johannessen CM, Garraway LA, Golub RE, Boehm JS, Wagle N, Getz G*, Love JC*, Meyerson M*. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nature Communications* 2017 8:1324.

Ha G, Roth A, Khattra J, Ho J, Yap D, Prentice LM, Melnyk N, McPherson A, Bashashati A, Laks E, Biele J, Ding J, Le A, Rosner J, Shumansky K, Marra MA, Gilks CB, Huntsman DG, McAlpine JN, Aparicio S, Shah SP. TITAN: inference of copy number architectures in clonal cell populations from tumor whole-genome sequence data. *Genome Research* 2014; 24(11):1881-93.

Ha G, Roth A, Lai D, Bashashati A, Ding J, Goya R, Giuliany R, Rosner J, Oloumi A, Shumansky K, Chin SF, Turashvili G, Hirst M, Caldas C, Marra MA, Aparicio S, Shah SP. Integrative analysis of genome-wide loss of heterozygosity and monoallelic expression at nucleotide resolution reveals disrupted pathways in triple-negative breast cancer. *Genome Research* 2012; 22(10):1995-2007.

https://www.ncbi.nlm.nih.gov/myncbi/gavin.ha.1/bibliography/public/

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I am a physician-scientist and as a practicing pathologist I gain a lot of insights and motivation for my laboratory-based research from observations made as part of my clinical practice.

A defining feature of almost all solid tumors is that cancer cells show dramatic changes in cell shape and size compared to benign cells. Yet, we know little about the principles that govern cell shape and their alterations in cancer. Every cell has a structural scaffold, a skeleton, termed the cytoskeleton. It consists of a complex interlinked network of proteins that supports the cell and helps to maintain its shape. To invade other tissues, cancer cells need to alter their cytoskeletal properties to become malleable enough to change their shape and to move through tissues. However, the processes by which cancer cells become such "shape shifters" are not well understood. To explore this further, my laboratory will focus on elucidating alterations of the cytoskeleton in solid tumors with a particular focus on prostate cancer.

For instance, we have recently described a novel protein, named AIM1 that regulates cytoskeletal organization by binding to actin, a major component of the cytoskeleton. When AIM1 is present, the cells' scaffolding keeps it rigid and correct shape. When AIM1 is lost, cells can remodel their cytoskeleton more frequently, change their shape and become capable of invading and migrating to distant locations. Notably, AIM1 function is disrupted in many solid tumors and genomic alterations of *AIM1* are associated with aggressive tumor growth.

The goal of these studies is to understand the mechanisms that govern changes in cell architecture to explore such cancer specific changes for therapeutic targeting.

I am very interested in having a graduate student join my laboratory in 2020-2021.

SELECTED PUBLICATIONS

Hughes RM, Simons BW, Khan H, Miller R, Kugler V, Torquato S, Theodros D, **Haffner MC**, Lotan T, Huang J, Davicioni E, An SS, Riddle RC, Thorek DLJ, Garraway IP, Fertig EJ, Isaacs JT, Brennen WN, Park BH, Hurley PJ. Asporin Restricts Mesenchymal Stromal Cell Differentiation, Alters the Tumor Microenvironment, and Drives Metastatic Progression. <u>Cancer Res</u>. 2019 Jul 15;79(14):3636-3650.

Haffner MC, Taheri D, Luidy-Imada E, Palsgrove DN, Eich ML, Netto GJ, Matoso A, Nirschl TR, Zheng Q, Hicks JL, Nelson WG, De Marzo AM, Marchionni L, Drake CG, Yegnasubramanian S. Hypomethylation, endogenous retrovirus expression, and interferon signaling in testicular germ cell tumors. <u>Proc Natl Acad Sci U S A</u>. 2018 Sep 11;115(37):E8580-E8582.

Haffner MC, Esopi DM, Chaux A, Gürel M, Ghosh S, Vaghasia AM, Tsai H, Kim K, Castagna N, Lam H, Hicks J, Wyhs N, Biswal Shinohara D, Hurley PJ, Simons BW, Schaeffer EM, Lotan TL, Isaacs WB, Netto GJ, De Marzo AM, Nelson WG, An SS, Yegnasubramanian S. AIM1 is an actin-binding protein that suppresses cell migration and micrometastatic dissemination. <u>Nat Commun</u>. 2017 Jul 26;8(1):142.

De Marzo AM, **Haffner MC**, Lotan TL, Yegnasubramanian S, Nelson WG. Premalignancy in Prostate Cancer: Rethinking What we Know. <u>Cancer Prev Res</u> (Phila). 2016 Aug;9(8):648-56.

Haffner MC, Weier C, Xu MM, Vaghasia A, Gürel B, Gümüşkaya B, Esopi DM, Fedor H, Tan HL, Kulac I, Hicks J, Isaacs WB, Lotan TL, Nelson WG, Yegnasubramanian S, De Marzo AM. Molecular evidence that invasive adenocarcinoma can mimic prostatic intraepithelial neoplasia (PIN) and intraductal carcinoma through retrograde glandular colonization. <u>J Pathol.</u> 2016 Jan;238(1):31-41.

Haffner MC, De Marzo AM, Yegnasubramanian S, Epstein JI, Carter HB. Diagnostic challenges of clonal heterogeneity in prostate cancer. <u>J Clin Oncol.</u> 2015 Mar 1;33(7):e38-40.

Haffner MC, Mosbruger T, Esopi DM, Fedor H, Heaphy CM, Walker DA, Adejola N, Gürel M, Hicks J, Meeker AK, Halushka MK, Simons JW, Isaacs WB, De Marzo AM, Nelson WG, Yegnasubramanian S. Tracking the clonal origin of lethal prostate cancer. <u>J Clin Invest</u>. 2013 Nov;123(11):4918-22.

Aryee MJ, Liu W, Engelmann JC, Nuhn P, Gurel M, **Haffner MC**, Esopi D, Irizarry RA, Getzenberg RH, Nelson WG, Luo J, Xu J, Isaacs WB, Bova GS, Yegnasubramanian S. DNA methylation alterations exhibit intraindividual stability and interindividual heterogeneity in prostate cancer metastases. <u>Sci Transl Med.</u> 2013 Jan 23;5(169):169ra10.

Haffner MC, De Marzo AM, Meeker AK, Nelson WG, Yegnasubramanian S. Transcription-induced DNA double strand breaks: both oncogenic force and potential therapeutic target? <u>Clin Cancer Res</u>. 2011 Jun 15;17(12):3858-64.

Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, Isaacs WB, Bova GS, Liu W, Xu J, Meeker AK, Netto G, De Marzo AM, Nelson WG, Yegnasubramanian S. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. <u>Nat Genet</u>. 2010 Aug;42(8):668-75.

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My lab studies the structure and dynamics of the nuclear envelope (NE) to understand how changes in this compartment cause human genetic diseases and drive cancer pathogenesis. Our previous work characterized a new NE dynamic in cancer cells where the nuclear membrane ruptures, which causes mislocalization of proteins and even organelles, and then either repairs or collapses. Remarkably, cancer cell nuclei that undergo many rounds of nucleus rupture and repair are viable, but these NE dynamics can increase genome instability, cause massive chromosome rearrangements, and activate signaling pathways that lead to inflammation and metastasis. However, we are just at the beginning of redefining the NE as a dynamic structure and determining how defects in NE stability impacts cell function. My lab uses a combination of fluorescent microscopy, biochemistry, and genomics tools to investigate the following questions:

1) What controls NE rupture and repair in cancer cells?

Our current model is that disorganization of the nuclear lamina leads to areas of weak membrane that are prone to both chromatin herniation and membrane rupture when force is applied to the nucleus. However, our understanding of why the lamina becomes disrupted and what types of forces cause instability is limited. In addition, the mechanisms of NE repair are almost completely uncharacterized. To understand these mechanisms better, we are taking a candidate approach to ask how previously characterized nuclear lamina proteins affect NE stability and a large-scale approach to identify new factors that affect nuclear lamina structure and NE membrane dynamics. We are also collaborating with cancer researchers to examine NE stability in *in vivo* and treatment contexts.

2) Why are micronuclei so unstable?

Our previous work found that when cancer cell chromosomes missegregate and recruit their own NE at the end of mitosis, forming compartments called micronuclei, they make a highly unstable NE that is prone to rupture and collapse. We showed that NE rupture in micronuclei frequently causes massive DNA damage and cytoplasmic chromatin is thought to be a key trigger of innate immune and invasion signaling. Yet why micronuclei fail to assemble a stable NE, why they can't repair after rupture, and the mechanism of DNA damage are still unclear. We are both taking a hypothesis-driven approach and developing new tools to address these questions.

3) What are the consequences of losing nucleus compartmentalization and how does this contribute to human disease?

Although loss of nucleus compartmentalization has been shown to cause substantial changes in genome organization and cell signaling, we expect that many additional responses occur that have gone undetected. Thus we are developing new tools to comprehensively identify the effect of NE instability on the proteome and transcriptome, as well as at the level of chromatin structure. Our overall goal is to define causal links between NE rupture, misregulation of cellular functions, and cancer development to identify potential therapeutic opportunities.

Selected Publications

Alexandra M Young, Amanda L Gunn, Emily M Hatch. "BAF facilitates interphase nuclear membrane repair through recruitment of nuclear transmembrane proteins." *Mol Biol Cell.* 2020. 31(15):1551–60

John Maciejowski, and Emily M Hatch. "Nuclear Membrane Rupture and Its Consequences." *Annu Rev Cell Dev Biol.* 2020. 36(1):annurev–cellbio–020520–120627

Emily M Hatch and Martin W Hetzer "Nuclear envelope rupture is induced by actin-based nucleus confinement." *JCB*, 2016 Oct 10; 215 (1): 27.

Emily M Hatch, Andrew H Fisher, Thomas J Deerinck, Martin W Hetzer "Catastrophic nuclear envelope collapse in cancer cell micronuclei." *Cell*, 2013 Jul 3;154(1):47-60.

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Members of Dr. Hockenbery's laboratory study programmed cell death (apoptosis) pathways, and the role of cell metabolism in apoptosis, oncogene functions, and environmental/dietary risk factors, including excess nutrient supply. We have identified small molecule inhibitors of Bcl-2 and published the first structure of a Bcl-xL homodimer. My lab has also published studies of c-Myc regulation of mitochondrial gene expression and central carbon metabolism, and discovered a novel pathway of ubiquitin-mediated protein turnover in mitochondria, known as MAD (for mitochondria-associated degradation). We are currently pursuing metabolomic studies directed toward identifying unique metabolic pathways in chemoresistant breast cancers, and biochemical markers of mitochondrial injury in serum and urine samples. **The following projects are currently active in the lab:**

a) Activation of the Fanconi Anemia/BRCA DNA damage response by endogenously produced aldehydes.

b) Metabolic dependencies on nicotinamide metabolism in glioblastomas (collaboration with Patrick Paddison).

c) Functional genomics and metabolomics of Tasmanian Devil Facial Tumor cell lines.

d) In vivo CRISPR-Cas9 screens to interrogate tumor metabolism specific to obese hosts.

SELECTED PUBLICATIONS

Margres MJ, Ruiz-Arevena M, Hamede R, Chawla K, Patton AH, Lawrance MF, Fraik AK, Stahlke AR, Davis BW, Ostrander EA, Jones ME, McCallum H, Paddison PJ, Hohenlohe PA, Hockenbery D, Storfer A. Spontaneous tumor regression in Tasmanian devils associated with RASL11A activation. Genetics 215: 1143-52, 2020.

Centini R, Tsang M, Iwata T, Park H, Delrow J, Margineantu D, Iritani BM, Gu H, Liggitt D, Kang J, Kang L, Hockenbery DM, Raftery D, Iritani BM. Loss of Fnip1 alters kidney developmental transcriptional program and synergizes with TSC1 loss to promote mTORC1 activation and renal cyst formation. PLoS One 13: e0197973, 2018.

Nagana Gowda GA, Barding GA Jr, Dai J, Gu H, Margineantu DH, Hockenbery DM, Raftery D. A metabolomics study of BPTES altered metabolism in human breast cancer cell lines. Front Mol Biosci 5: 49, 2018.

Davis RJ, Gonen M, Margineantu DH, Handeli S, Swanger J, Hoellerbauer P, Paddison PJ, Gu H, Raftery D, Grim JE, Hockenbery DM, Margolin AA, Clurman BE. Pan-cancer transcriptional signatures predictive of oncogenic mutations reveal that Fbw7 regulates cancer cell oxidative metabolism. Proc Natl Acad Sci U S A 115: 5262-67, 2018.

Hickson SE, Margineantu D, Hockenbery DM, Simon JA, Geballe AP. Inhibition of vaccinia virus replication by nitazoxanide. Virology 518: 398-405, 2018.

Amankulor NM, Kim Y, Arora S, Kargl J, Szulzewsky F, Hanke M, Margineantu DH, Rao A, Boulouri H, Delrow J, Hockenbery D, Houghton AM, Holland EC. Mutant IDH1 regulates the tumor-associated immune system in gliomas. Genes Dev 31: 774-86, 2017.

Berger S, Procko E, Margineantu D, Lee EF, Shen BW, Zelter Z, Silva DA, Chawla K, Herold MJ, Garnier JM, Johnson R, MacCoss MJ, Lessene G, Davis TN, Stayton PS, Stoddard BL, Fairlie WD, Hockenbery DM, Baker D. Computationally designed high specificity inhibitors delineate the roles of BCL2 family proteins in cancer. Elife 5. Pii: e20352, 2016.

Iwata TN, Ramirez JA, Tsang M, Park H, Margineantu DH, Hockenbery DM, Iritani BM. Conditional disruption of Raptor reveals an essential role for mTORC1 in B cell development, survival, and metabolism. J Immunol 197: 2250-60, 2016.

Margineantu DH, Hockenbery DM. Mitochondrial functions in stem cells. *Curr Opin Genet Dev* 38: 110-117, 2016.

Aguilar E, Marin de Mas I, Zodda E, Marin S, Morrish F, Selivanov V, Meca-Cortes O, Delowar H, Pons M, Izquierdo I, Celia-Terrassa T, de Atauri P, Centelles JJ, Hockenbery D, Thomson TM, Cascante M. Metabolic reprogramming and dependencies associated with epithelial cancer stem cells independent of the epithelial-mesenchymal transition program. *Stem Cells* 34: 1163-76, 2016.

Bluestein BM, Morrish F, Graham DJ, Guenthoer J, Hockenbery D, Porter PL, Gamble LJ. An unsupervised MVA method to compare specific regions in human breast tumor tissue samples using ToF-SIMS. _Analyst_ 141: 1947-57, 2016.

Sperber H, Mathieu J, Wang Y, Ferreccio A, Hesson J, Xu Z, Fischer KA, Devi A, Detraux D, Gu H, Battle SL, Showalter M, Valensisi C, Bielas JH, Ericson NG, Margaretha L, Robitaille AM, Margineantu D, Fiehn O, Hockenbery D, Blau CA, Raftery D, Margolin AA, Hawkins RD, Moon RT, Ware CB, Ruohola-Baker H. The metabolome regulates the epigenetic landscape during naïve-to-primed human embryonic stem cell transition. *Nat Cell Biol* 17: 1523-35, 2015.

Procko E, Berguig GY, Shen BW, Song Y, Frayo S, Convertine AJ, Margineantu D, Booth G, Correia BE, Cheng Y, Schief WR, Hockenbery DM, Press OW, Stoddard BL, Stayton PS, Baker D. A computationally

designed inhibitor of an Epstein-Barr viral Bcl-2 protein induces apoptosis in infected cells. *Cell* 157: 1644-56, 2014.

Morrish F, Hockenbery D. MYC and mitochondrial biogenesis. *Cold Spring Harb Perspect Med* 225-240, 2014.

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MOUSE MODELS OF BRAIN TUMORS

The main goal of my laboratory is the use of genetically-accurate mouse models of glioblastoma to understand the molecular basis for the genesis of these tumors and their response to standard therapy. In the process, we have developed preclinical trial infrastructure and imaging that supports the development of novel therapeutic approaches. My laboratory developed the RCAS/tv-a system of post-natal somatic cell-type specific gene transfer to study cancer formation in mice, and used this system to model the formation of gliomas and medulloblastomas. We demonstrated that stem cells are more sensitive to transforming events than differentiated cells, that Akt activity is elevated in human glioblastomas, and that deletion of *PTEN* (as occurs in human glioblastoma) in mice was causal in glioma formation and progression. Brain tumor cells that are resistant to radiation therapy occupy the perivascular niche and have stem-cell characteristics driven by a combination of Akt and notch activities. We have shown that nitric oxide produced by endothelial cells promote stem-cell characteristics in perivascular cells through cGMP, PKG and Notch signaling. We have further characterized therapeutic DNA Damage Response pathway in gliomas and compared this data with that from human GBM. Finally, one recent breakthrough in the field is the molecular subdivision of the GBMs. Our contribution to this was demonstrating proteomic evidence that specific signaling pathway activity characterizes these subgroups and that the mouse models were specific mimics of the molecular GBM subgroups. The human and mouse glioma data has led to molecularly-stratified clinical trials for GBM patients.

The biology of immunotherapy response in gliomas
We have been using our immunocompetent models of gliomas to better understand the immunologic response to the tumor and to its response to standard therapy. Different glioma subtypes have different immune characteristics, a feature mimicked by our mouse models.   The Abscopal effect, where radiation or other localized therapy induces the body's immune system to recognize and target tumors outside the field of therapy as foreign. We have created experimental paradigms that have bilateral tumors of different subtypes,

or where one tumor is treated and the immunologic response measured on the other side. Ultimately, we hope to better understand when and how checkpoint inhibitors, CAR T cells and oncolytic vectors can be used in combination with standard therapy.

The biology of stem-ness in tumors and its consequences in gliomas *in vivo*
One topic in this area is the issue of stem-ness in tumor cells and what drives this character. The work evolves from our previous work showing that stem like cells are located in the PVN and are driven by NO signaling mentioned in the abstract among others.

Mathematical and mouse modeling
My laboratory has a long-standing collaboration with Franziska Michor of the Computational Biology department at the Dana Farber. We combine mathematical modeling with mouse modeling to understand the likelihood of events in the evolution of gliomas development or in optimizing therapy based on parameters obtained from mouse models. In these projects we have: 1. identified the most probable cell of origin for PDGF-induced gliomas, 2. determined the order of genetic events in the evolution of these tumors, 3. identified the first events in gliomas formation, and identified an optimized schedule for delivery of radiation therapy based on parameters obtained from our PDGF-induced gliomas model.

The biology of therapeutic response in gliomas
Many laboratories are studying the biology of these tumors (and other tumor types), but few are trying to understand the biology of how these tumors respond to therapy. This is conceptually important because the disease that kills people in the western world is a treated and recurrent tumor, not an untreated tumor. Therefore, we have spent effort in developing the technologies to understand how these tumors respond to standard therapy using the same rigor that we have studied the biology of the tumor in the first place.

MRI and bioluminescence imaging and preclinical trial drug development
In order to perform preclinical trials in mice, we need to identify tumors, quantify their size, and follow them over time non-invasively. One approach that we have used is by MRI scanning with T2 weighted images or with T1 weighted images with and without contrast as is done in people. However, MRI only measures anatomic structure and not biologic processes. Therefore, we have developed bioluminescence imaging strategies for use in preclinical trials of brain tumor-bearing mice. We initially developed a reporter mouse that expressed luciferase from the E2F1 promoter that measures proliferation and a Gli responsive promoter measuring SHH signaling. We are now developing genetic backgrounds that activate luciferase expression by cre recombinase activity that will allow us to "see" the tumor cells *in vivo* that have been deleted for PTEN, or that have knocked down INK4a/arf. This will allow us to easily identify mice with tumors and to count live tumor cells *in vivo* non-invasively.

The glioma tumor microenvironment
Gliomas are composed of not only tumor cells per se but also reactive astrocytes, microglia, endothelial cells and pericytes. Multiple lines of evidence indicate that many if not all of the cells that make up the stroma in these tumors contribute to the tumor biology and may be valid therapeutic targets.

Novel models of gliomas subtypes and ependymomas
We have also developed a modified version of the RCAS/tv-a system that achieves loss-of-function combined with lineage tracing using short hairpins and florescent tags. This system is able to mimic the mesenchymal GBMs by combining knockdown the combination of NF1 and p53 while lineage tracing each of these two events from specific cell types, with a penetrance of essentially 100%. We are using this model to understand the evolution of mesenchymal GBM from proneural ones and understand the complexity of these tumors. This type of lineage tracing allows us to appreciate the cellular heterogeneity in ways that germline strategies are unable to. We also have developed a new model of ependymoma by expressing a commonly occurring gene fusion (C11orf95/RELA) with this system.

PDGFR inhibition as a therapeutic strategy for PDGF-driven GBM

PDGF signaling characterizes the proneural subgroup of GBM and is sufficient to induced similar tumors in mice. One might think that inhibition of PDGFR would be a good therapeutic strategy for at least the proneural GBM subgroup. However, several trials of PDGFR inhibitors have been done in humans with GBM and none have been successful. A simple explanation is that the patients were not stratified to PDGFR active tumors prior to enrolling in these trials. However, there are several additional more interesting possibilities as to why this might be the case, and we are investigating under what circumstances PDGFR inhibition might be effective. One contributing factor is likely to be cellular heterogeneity of these tumors where subclones of cells within the tumor express PDGFR while others express EGFR in humans, and in mice similar results can be seen. A second contributing factor in the resistance to PDGFR inhibition is the fact that most of the gene expression changes that accompany the oncogenic transformation of olig2 expressing cells by PDGFR in vivo are not reversed by PDGFR inhibitors in vivo, even when that inhibition achieves a full cycle arrest. Additionally, mutant forms of PDGFR alpha found in some GBM appear to reduce effect of PDGFR inhibition. Finally, we have found that additional alterations found in human gliomas such as loss of Ink4a/arf, p53 or PTEN enhance oncogenic character of these tumors and prevent PDGFR inhibition of achieving full cell cycle arrest.

SELECTED PUBLICATIONS

Holland EC, Hively W, DePinho R, Varmus H. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. Genes Dev. 1998 Dec 1;12(23):3675-85.

Dai C, Celestino J, Okada Y, Louis DN, Fuller GN, Holland EC. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. Genes Dev. 2001 Aug 1;15(15):1913-25.

Holland EC, Celestino J, Dai C, Schaefer L, Sawaya RE, Fuller GN. Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. Nat Genet. 2000 May;25(1):55-7.

Rajasekhar VK, Viale A, Socci ND, Wiedmann M, Hu X, Holland EC. Oncogenic Ras and Akt signaling contribute to glioblastoma formation by differential recruitment of existing mRNAs to polysomes. Mol Cell. 2003 Oct;12(4):889-901.

Uhrbom L, Nerio E, Holland EC. Dissecting tumor maintenance requirements using bioluminescence imaging of cell proliferation in a mouse glioma model. Nat Med. 2004 Nov;10(11):1257-1260.

Hambardzumyan D, Becher OJ, Rosenblum MK, Pandolfi PP, Manova-Todorova K, Holland EC. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. Genes Dev. 2008 Feb 15;22(4):436-48.

Bleau AM, Hambardzumyan D, Ozawa T, Fomchenko EI, Huse JT, Brennan CW, Holland EC. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell. 2009 Mar 6;4(3):226-35.

Charles N, Ozawa T, Squatrito M, Bleau AM, Brennan CW, Hambardzumyan D, Holland EC. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. Cell Stem Cell. 2010 Feb 5;6(2):141-152.

Squatrito M, Brennan CW, Helmy K, Huse JT, Petrini JH, Holland EC. Loss of ATM/Chk2/p53 pathway components accelerates tumor development and contributes to radiation resistance in gliomas. Cancer Cell. 2010 Dec 14; 18(6):619-29.

Ozawa T, Brennan CW, Wang L, Squatrito M, Sasayama T, Nakada M, Huse JT, Pedraza A, Utsuki S, Yasui Y, Tandon A, Fomchenko EI, Oka H, Levine RL, Fujii K, Ladanyi M, Holland EC. PDGFRA gene rearrangements are frequent genetic events in PDGFRA-amplified glioblastomas. Genes Dev. 2010 Oct 1;24(19):2205-18.

Kircher MF, de la Zerda A, Jokerst JV, Zavaleta CL, Kempen PJ, Mittra E, Pitter K, Huang R, Campos C, Habte F, Sinclair R, Brennan CW, Mellinghoff IK, Holland EC, Gambhir SS. A brain tumor molecular imaging strategy using a new triple-modality MRI-photoacoustic- Raman nanoparticle. Nat Med. 2012 Apr 15.

Leder K, Pitter K, LaPlant Q, Hambardzumyan D, Ross B, Chan T, Holland EC, Michor F. Mathematical Modeling of PDGF-Driven Glioblastoma Reveals Optimized Radiation Dosing Schedules. Cell. 2014 Jan 30; 156(3):603-616.

Pietras A, Katz A, Ekström E, Wee B, Halliday J, Pitter K, Werbeck J, Amankulor N, Huse J, Holland EC. Osteopontin-CD44 Signaling in the Glioma Perivascular Niche Enhances Cancer Stem Cell Phenotypes and Promotes Aggressive Tumor Growth. Cell Stem Cell. 2014 March 6; 14(3):357-369.

Halliday J, Helmy K, Pattwell S, Pitter K, LaPlant Q, Ozawa T, Holland EC. In vivo radiation response of proneural glioma characterized by protective p53 transcriptional program and proneural-mesenchymal shift. Proc Natl Acad Sci U S A., 2014 March 24; 111(14): 5248–5253.

Ozawa T, Riester M, Cheng YK, Huse JT, Squatrito M, Helmy K, Charles N, Michor F, Holland EC. Most Human Non-GCIMP Glioblastoma Subtypes Evolve from a Common Proneural-like Precursor Glioma. Cancer Cell. 2014 Aug 11;26(2):288-300.

Bolouri H, Zhao LP, Holland EC. Big data visualization identifies the multidimensional molecular landscape of human gliomas. Proc Natl Acad Sci U S A. 2016 May 10;113(19):5394-9.

Olig2-Dependent Reciprocal Shift in PDGF and EGF Receptor Signaling Regulates Tumor Phenotype and Mitotic Growth in Malignant Glioma. Lu F, Chen Y, Zhao C, Wang H, He D, Xu L, Wang J, He X, Deng Y, Lu EE, Liu X, Verma R, Bu H, Drissi R, Fouladi M, Stemmer-Rachamimov AO, Burns D, Xin M, Rubin JB, Bahassi el M, Canoll P, Holland EC, Lu QR. Cancer Cell. 2016 May 9;29(5):669-83.

Amankulor NM, Kim Y, Arora S, Kargl J, Szulzewsky F, Hanke M, Margineantu DH, Rao A, Bolouri H, Delrow J, Hockenbery D, Houghton AM, Holland EC. Mutant IDH1 regulates the tumor-associated immune system in gliomas. Genes Dev. 2017 May 2.

Cimino PJ, Kim Y, Wu HJ, Alexander J, Wirsching HG, Szulzewsky F, Pitter K, Ozawa T, Wang J, Vazquez J, Arora S, Rabadan R, Levine R, Michor F, Holland EC. Increased *HOXA5* expression provides a selective advantage for gain of whole chromosome 7 in IDH wild-type glioblastoma. Genes Dev. 2018 Apr 9. 1;32(7-8):512-523.

Ozawa T, Arora S, Szulzewsky F, Juric-Sekhar G, Miyajima Y, Bolouri H, Yasui Y, Barber J, Kupp R, Dalton J, Jones TS, Nakada M, Kumabe T, Ellison DW, Gilbertson RJ, Holland EC. A De Novo Mouse Model of C11orf95-RELA Fusion-Driven Ependymoma Identifies Driver Functions in Addition to NF-κB. Cell Rep. 2018 Jun 26;23(13):3787-3797.

McFerrin LG, Zager M, Zhang J, Krenn G, McDermott R, Horse-Grant D, Silgard E, Colevas K, Shannon P, Bolouri H, Holland EC. Analysis and visualization of linked molecular and clinical cancer data by using Oncoscape. Nat Genet. 2018 Sep;50(9):1203-1204.

Szulzewsky F, Arora S, Hoellerbauer P, King C, Nathan E, Chan M, Cimino PJ, Ozawa T, Kawauchi D, Pajtler KW, Gilbertson RJ, Paddison PJ, Vasioukhin V, Gujral TS, Holland EC. Comparison of tumor-associated YAP1 fusions identifies a recurrent set of functions critical for oncogenesis. Genes Dev. 2020 Aug 1;34(15-16):1051-1064.

Alexander J, LaPlant QC, Pattwell SS, Szulzewsky F, Cimino PJ, Caruso FP, Pugliese P, Chen Z, Chardon F, Hill AJ, Spurrell C, Ahrendsen D, Pietras A, Starita LM, Hambardzumyan D, Iavarone A, Shendure J, Holland EC. Multimodal single-cell analysis reveals distinct radioresistant stem-like and progenitor cell populations in murine glioma. Glia. 2020 Jul 4. doi: 10.1002/glia.23866. Online ahead of print.

Pattwell SS, Arora S, Cimino PJ, Ozawa T, Szulzewsky F, Hoellerbauer P, Bonifert T, Hoffstrom BG, Boiani NE, Bolouri H, Correnti CE, Oldrini B, Silber JR, Squatrito M, Paddison PJ, Holland EC. A kinase-deficient NTRK2 splice variant predominates in glioma and amplifies several oncogenic signaling pathways. Nat Commun. 2020 Jun 12;11(1):2977.

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RESEARCH FOCUS

The focus of our group is the study of the tumor microenvironment with an emphasis on the role of the neutrophil lineage. We are currently investigating the role of immune cells within the lung tumor microenvironment in a comprehensive fashion, beginning with how they have been recruited, and ending with a detailed understanding of the mechanism(s) by which a specific immune cell effector has impacted tumor growth.

We have recently developed a program to personalize immune modulatory therapies for non-small cell lung cancer (NSCLC) patients. Using a combination of mouse models of lung cancer and human NSCLC specimens, we have been able to determine the exact composition of the immune response to lung cancer. Our findings show that neutrophils are the most common immune cell population present in human NSCLC and inversely correlate with $CD8^+$ cellular content and access to malignant tumor. Ongoing studies in our lab have been designed to understand the mechanistic basis of neutrophil antagonism of lymphocyte responses and to investigate the efficacy of neutrophil depletion in conjunction with immune checkpoint inhibitor therapy.

SELECTED PUBLICATIONS

Mark NM, Kargl J, Busch SE, Yang GHY, Metz HE, Zhang H, Hubbard JJ, Pipavath SNJ, Madtes DK, Houghton AM. COPD alters immune cell composition and immune checkpoint inhibitor efficacy in NSCLC. *Am J Respir Crit Care Med*. 197:325-336 (2018). PMID: 28934595.

Veatch JR, Lee SM, Fitzgibbon M, Chow IT, Jesernig B, Schmitt T, Kong YY, Kargl J, Houghton AM, Thompson JA, McIntosh M, Kwok WW, Riddell SR. Tumor infiltrating BRAFV600E-specific CD4 T cells correlated with complete clinical response in melanoma. *J Clin Invest* (2018). [Epub ahead of print]. PMID: 29360643.

Lastwika KJ, Kargl J, Zhang Y, Zhu X, Lo E, Shelley D, Ladd JJ, Wu W, Kinahan P, Pipavath S, Lampe PD, Houghton AM. Tumor-derived autoantibodies identify malignant pulmonary nodules. *Am J Respir Crit Care Med*. 2018 Nov 13. Doi: 10.1164/rccm.201804-0628OC. [Epub ahead of print]. PMID: 30422669.

Kargl J, Busch SE, Yang GHY, Kim KH, Hanke ML, Metz HE, Hubbard JJ, Lee SM, Madtes DK, McIntosh MW, Houghton AM (2017). Neutrophils Dominate the Immune Cell Composition in NSCLC. *Nature Communications*. 8, 14381, doi: 10.1038/ncomms14381. PMID 28146145.

Busch SE, Hanke ML, Kargl J, Metz HE, MacPherson D, Houghton AM (2016). Lung Cancer Subtypes Generate Unique Immune Responses. *J Immunol.* 197:4493-4503. PMID 27799309.

Metz HE, Kargl J, Busch SE, Kim KH, Kurland BF, Abberbock SR, Randolph-Habecker J, Knoblaugh SE, Kolls JK, White MF, Houghton AM. (2016). Insulin Receptor Substrate-1 Deficiency Drives a Pro-Inflammatory Phenotype in KRAS-Mutant Lung Adenocarcinoma. *Proc Natl Acad Sci USA*. 113:8795-8800. PMID 27439864.

Houghton AM. Mechanistic links between COPD and lung cancer. *Nat Rev Cancer.* 2013, 13:233-45.

Gregory AD, Hale P, Perlmutter DH, Houghton AM. Clathrin pit-mediated endocytosis of neutrophil elastase and cathepsin G by cancer cells. *J Biol Chem.* 2012, 287:35341-50.

Gregory AD, Houghton AM. Tumor-associated neutrophils: new targets for cancer therapy. *Cancer Res.* 2011, 71:2411-6.

Houghton AM, Rzymkiewicz DM, Ji H, Gregory AD, Egea EE, Metz HE, Stolz DB, Land SR, Marconcini LA, Kliment CR, Jenkins KM, Beaulieu KA, Mouded M, Frank SJ, Wong KK, Shapiro SD. Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. *Nat Med*. 2010, 16:219-23.

Houghton AM, Hartzell WO, Robbins CS, Gomis-Ruth FX, Shapiro SD. Macrophage elastase kills bacteria within murine macrophages. *Nature*. 2009, 460:637-641.

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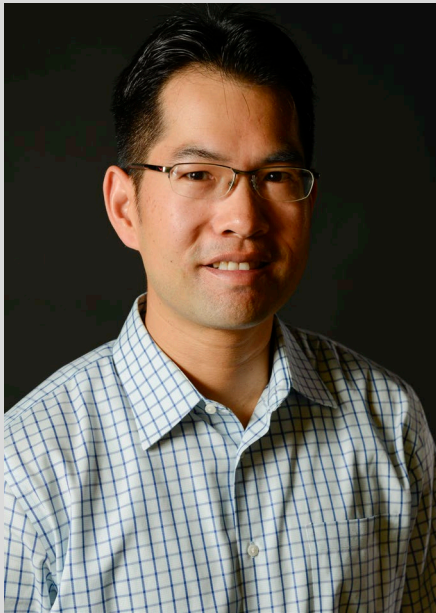
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The goal of the Hsieh Lab at the Fred Hutchinson Cancer Research Center is to comprehensively delineate the fundamental role of mRNA translation in normal cell physiology, cancer etiology, and cancer progression. Armed with this knowledge we are defining the next generation of therapeutic vulnerabilities in disorders associated with translation deregulation such as cancer.

Dissecting the functional interface between transcription and translation in genitourinary malignancies. The process by which mRNA is translated into a protein is a highly energetic and meticulous process that is essential for life. However, protein synthesis can also be usurped by cancer to drive cellular transformation, uncontrolled proliferation, evasion of apoptosis, metastasis, and drug resistance (Hsieh et al. Cancer Cell 2010, Hsieh et al. Nature 2012, Hsieh et al. Science Signaling 2015). Work from our laboratory indicates that transcription factors utilize the translation apparatus to shape the cellular proteome (Liu and Horn et al. Science Translational Medicine 2019). Interestingly, this relationship can be co-opted to drive specific cancer behavior at a molecular, cellular, and organismal level in prostate cancer.

Key questions:

1) How does the translation apparatus interface with transcription factors and other regulators of gene expression in the context of genitourinary malignancies?

2) What are the key downstream translational drivers necessary for cancer phenotypes such as lineage plasticity?

3) How can we therapeutically disrupt oncogenic translation?

Understanding mechanisms of oncogenic mRNA specific translation. mRNA specific translation is the mechanism by which distinct mRNAs are preferentially translated to control cellular phenotypes. This can be mediated through the protein synthesis apparatus or changes in mRNA sequence and structure. Our laboratory has been fascinated by the untranslated regions (UTRs) of mRNAs, which are necessary for mRNA metabolism and efficient protein synthesis (Hsieh et al. Nature 2012, Liu and Horn et al. Science Translational Medicine 2019, Schuster and Hsieh Trends in Cancer 2019). Surprisingly, their functionality remains poorly understood particularly in the disease context. Thus, we are deeply investigating how UTR dynamics tune gene expression to impact the multistep process of cancer initiation and progression.

Key questions:

1) How are UTRs usurped to promote cancer pathogenesis?

2) What are the underlying *cis*- and *trans*-regulatory mechanisms that enable oncogenic mRNA specific translation?

Advance stage bladder cancer as a platform for biological and therapeutic discoveries. In 2015, our laboratory along with Drs. Ming Lam (UW Urology), Jonathan Wright (UW Urology), Bruce Montgomery (UW Oncology), and Funda Vakar-Lopez (UW Pathology) nucleated the first bladder cancer focused rapid autopsy program in the world. We have used this precious resource of late stage tumor specimens to interrogate the genomic underpinning of aggressive bladder cancer and to develop patient derive xenografts and primary cell-based models. Through this work, we have identified distinctions between upper tract urothelial carcinoma and lower tract urothelial carcinoma as well as the potential therapeutic implications of druggable genetic lesions in patients with metastatic bladder cancer (Winters et al. JCI Insight 2019). There are also ongoing projects focused on dissecting translation deregulation in bladder cancer.

Key questions:

1) To what extent and how does bladder cancer heterogeneity influence disease aggressiveness and response to therapeutics?

2) How is the translation apparatus usurped in urothelial cells to drive the process of transformation?

Advancing our understanding of translation regulation in normal cell physiology through collaboration. Chemical modifications to RNA such as a methylation of adenines and isomerization of uridines have been shown to impact the process of mRNA translation. Work from our laboratory in collaboration with the Bellodi Lab (Lund University) and the Paddison Lab (Fred Hutch) have demonstrated a central role for these types of modifications in shaping the cellular proteome. Importantly, these processes are essential for the maintenance of normal stem cell physiology and the dynamic transitions that occur during erythrocyte differentiation (Guzzi et al. Cell 2018, Kuppers et al. Nature Communications 2019). In addition, through work with the Beronja Lab (Fred Hutch) we are unraveling the critical role of mRNA specific translation in cell fate choice (Cai et al. Cell Stem Cell 2020).

Key questions:

1) How do m6A modifications enable the select translation of mRNA essential for the various stages of erythrocyte differentiation?

2) How is mRNA-specific translation directed in basal epithelial cells of the skin to control self-renewal and differentiation?

SELECTED PUBLICATIONS

Cai EY, Kufeld MN, Schuster SL, Arora S, Larkin M, Germanos AA, **Hsieh AC,** Beronja S. Oncogenic translation drives progenitor cell differentiation to restrain tumorigenesis. **Cell Stem Cell** doi: 10.1016/j.stem.2020.05.007

Liu Y, Horn JL, Banda K, Hardin WR, Lim Y, Jana S, Arora S, Germanos AA, Goodman AZ, Yang YC, Coleman IM, Cai EY, Uo T, Pillai SPS, Corey E, Morrissey C, Chen Y, Carver BS, Plymate SR, Beronja S, Nelson PS, **Hsieh AC.** (2019) Androgen receptor regulates a druggable translational regulon in advance prostate cancer. **Science Translational Medicine** doi: 10.1126/scitranslmed.aaw4993

Schuster SL, **Hsieh AC**. (2019). The untranslated regions of mRNAs in cancer. **Trends in Cancer** doi: 10.1016/j.trecan.2019.02.011

Winters BR, De Sakar N, Arora S, Bolouri H, Vakar-Lopez F, Jana S, Cheng HH, Schweizer M, Yu EY, Kollath L, Grivas P, McFerrin L, Montgomery RB, Wright JL[#], Lam H[#], **Hsieh AC[#]**. (2019) Genomic distinctions between metastatic lower and upper tract urothelial carcinoma revealed through rapid autopsy. **JCI Insight.** doi: 10.1172/jci.insight.128728 [#]co-corresponding author

Kuppers DA, Arora S, Lim Y, Lim A, Carter L, Basom R, Delrow J, Torok-Storb B, **Hsieh AC[#],** Paddison PJ[#]. N6-methyladenosine mRNA marking promotes selective translation of regulons required for human erythropoiesis. **Nature Communications** doi: 10.1038/s41467-019-12518-6 [#]co-corresponding author

Guzzi N, Ciesla M, Lang S, Arora S, Dimitrious M, Munita R, Lubas M, Lim Y, Okuyama K, Soneji S, Jonsson G, Lund AH, Sigvardsson M, Hellstrom-Lindberg E, **Hsieh AC,** Bellodi C. (2018). Pseudouridylation of tRNA-derived fragments steers translational control in stem cells. **Cell** 173, 1204-1216. doi:10.1016/j.cell.201803.008

Nguyen HG, Conn CS, Kye Y, Xue L, Cowan JE, Forester CM, **Hsieh AC,** Cunningham JT, Truillet C, Simko J, Tameire F, Evans MJ, Koumenis C, Walter P, Carroll PR, Ruggero D. (2018) Development of a stress response therapy targeting aggressive prostate cancer. **Science Translational Medicine** 10, eaar2036. doi: 10.1126/scitranslmed.aar2036

Hsieh AC*, Wen L, Nguyen HG, Edlind MP, Kim W, Ruggero D. (2015). Cell type-specific abundance of 4EBP1 primes prostate cancer sensitivity or resistance to PI3K pathway inhibitors. **Science Signaling** 8, 1-10*. doi:10.1126/scisignal.aad5111. *co-corresponding author

Hsieh AC*, Liu Y*, Edlind MP, Ingolia, NT, Janes MR, Sher A, Shi EY, Christensen C, Stumpf CR, Bonham MJ, Wang S, Ren P, Martin M, Jessen K, Feldman ME, Weissman JS, Shokat KM, Rommel C, Ruggero D. (2012). The translational landscape of mTOR signaling steers cancer initiation and metastasis. **Nature** 485, 55-61. doi:10.1038/nature10912. *co-first author

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CANCER TARGET DISCOVERY AND DEVELOPMENT

Our lab's research program has a long-standing interest in using mouse models to address the cellular, molecular, and genetic mechanisms of tumor progression. The functions of oncogenes and tumor suppressor genes are remarkably conserved between mice and humans and mouse models have and will continue to provide fundamental insights into the causes, prevention, and treatment of human cancer.

More recently we have pivoted direction towards more translation approaches, specifically discovering and developing novel drugs and drug targets for the treatment of cancer. Using arrayed well-based siRNA high throughput screens, we are identifying the complement of genes that are required for survival of cancer cells but not normal cells. By combining this functional genomic data with small molecule drug screens and genomic characterization within the same cells, we arrive at a set of prioritized targets and novel drug candidates. Because we can do this in patient derived tumor cells we can anchor results to patient treatment history thereby enhancing successful clinical translation. . To date, we have identified novel targets for head and neck cancer, pancreatic cancer, breast cancer and ovarian cancer. We are collaborating with clinician scientists, computational biologists and patient advocates at a number of cancer centers and precision medicine initiatives to broaden and deepen this cancer drug target search engine. We are confident that this grass roots and cross disciplinary approach will accelerate the discovery of safer, more effective cancer treatments in the near future.

PUBLICATIONS

Kemp, C.J. Multistep skin cancer in mice as a model to study the evolution of cancer cells. *Semin. Cancer Biol.*, 15:460-473, 2005

Kelly-Spratt, K.S., Philipp-Staheli, J., Gurley, K.E., Hoon-Kim, K., Knoblaugh, S., and Kemp, C.J. Inhibition of P13K restores nuclear p27/Kip expression in a mouse model of Kras-driven lung cancer. *Oncogene*, 28: 3652-3662, 2009

Pitteri,S.J., Kelly-Spratt,K.S., Gurley, K.E., Kennedy, J., Buson, T.B., Chin, A., Wang, H., Zhang, Q., Wong, C.H., Chodosh, L.A., Nelson, P.L., Hanash, S. M., and Kemp,C.J.. Tumor microenvironment derived proteins dominate the plasma proteome during breast cancer induction and progression. *Cancer Res*., 71, 5090-5100, 2011

Taguchi, A., Politi, K., Pitteri, S.J., Lockwood, W.W., Faca, V.M., Kelly-Spratt, K.S., Wong, C.H., Zhang, Q., Chin, A., Park K.S., Goodman, G., Gazdar, A.F., Sage, J., Dinulescu, D.M., Kucherlapati, R., DePinho, R.A., Kemp, C.J., Varmus, H. E., and Hanash, S.M. Lung cancer signatures in plasma based on proteome profiling of mouse tumor models. *Cancer Cell*, 20, 289-299, 2011

Yang, F., Kemp, C.J. and Henikoff, S. Doxorubicin enhances nucleosome turnover around promoters. *Current Biology*, 23,782-7, 2013

Kemp, C.J. Animal models of chemical carcinogenesis: driving breakthroughs in cancer research for 100 years. In: Mouse Models of Cancer, K. Politi, K. Olive, and L. Chodosh, Eds. *Cold Spring Harbor Laboratory Press*, pgs 51-60, 2013

Busch, S.E., Gurley, K.E., Moser, R., Kelly-Spratt, K.S., Liggitt, D., and Kemp, C.J. p19Arf inhibits the growth and malignant progression of carcinogen induced non small cell lung cancer. Oncogene, 33: 2665-2673, 2014

Kemp, C.J., Moore, J. M., Moser, R., Bernard, B., Teater, M., Smith, L. E., Rabaia, N., Gurley, K., Guiney, J., Busch, S.E., Shaknovich, R., Lobanenkov, V.V., Liggitt, D., Shmulevich, I., Melnick A., and Filippova, G.N. CTCF haploinsufficiency destabilizes DNA methylation and predisposes to cancer. *Cell Report*s, 7, 1020-1029, 2014

Moser, R., Xu, C., Kao, M., Annis, J., Lerma, L.A., Schaupp, C., Gurley, K.E., Jang, I.S., Margolin, A., Biktasova, A., Yarbrough, W.G., Grandori, C., Kemp, C.J.*, and Mendez, E.* Functional kinomics identifies candidate therapeutic targets in head and neck cancer. *Clin. Cancer Res.*, 20, 4274-4288, 2014 , * Co-corresponding authors and equal contribution

Westcott, P.M.K., Halliwill, K.D., To, M.d., Mamunar, R., Rust, A.G., Keane, T.M., Delrosario, R., Jen, K.U., Gurley, K.E., Kemp, C.J., Fredlund, E., Quigley, D.A., Adams, D.J., Balmain, A., The mutational landscapes of genetic and chemical models of Kras-driven non-small cell lung cancer. *Nature*, 517, 489-492, 2015

Yang, F., Kemp, C.J. and Henikoff, S. Anthracyclines induce double-strand DNA breaks at active gene promoters. *Mutation Res,* 773, 9-15, 2015

Gerhard, D.S., Clemons, P.A., Shamji, A.F., Hon, C., Wagner, B.K., Schreiber, S.L., et al. Transforming big data into cancer-relevant insight: an initial multitier approach to assess reproducibility and relevance. *Mol. Cancer Res.*,14, 675-682, 2016

Nikolova, O., Moser, R., Kemp, C.J., Gonen, M., Margolin, A. M. Modeling gene-wise dependencies improves the identification of drug response biomarkers in cancer studies. *Bioinformatics*, 33, 1362-1369, 2017

Pauli C., Hopkins B., Prandi D., Shaw R., Fedrizzi T., Sboner A., Sailer V., Augello M., Puca L., Rosati R., McNary T., Churakova Y., Cheung C., Triscott J., Pisapia D., Rao R., Mosquera J.M., Robinson B., Faltas B., Emerling B., Gadi V.K., Bernard B., Elemento O., Beltran H., Dimichelis F., Kemp C.J., Grandori C., Cantley L., Rubin M., Personalized in vitro and in vivo cancer models to guide precision medicine. *Cancer Discovery* 7 (5) 462-77, 2017. Featured as highlight for this issue and profiled in Nature Medicine and Nature

Gurley, K.E., Ashley, A. K., Moser, R., and Kemp, C.J. Synergy between Prkdc and Trp53 regulates stem cell proliferation and GI-ARS after irradiation. *Cell Death and Differentiation*, 24, 1853-1860, 2017

Nguyen, H. H., Tilton, S.C., Kemp, C.J., Song, M. Non-monotonic pathway gene expression analysis reveals oncogenic role of p27/Kip1 at intermediate dose. *Cancer Informatics*, 16, 1-16, 2017

Xu, C.*, Nikolova, O.*, Basom, R.S., Mitchell, R.M., Shaw, R., Moser, R.D., Park, H., Gurley, K.E., Kao, K.C., Green, C.L., Schaub, F.Z., Diaz, R.L., Swan, H.S., Jang, I.S., Guinney, J., Gadi, V.K., Margolin, A.A., Grandori, C., Kemp, C.J., Méndez, E., § (*co-first authors; §co-corresponding authors). Functional precision medicine identifies novel targets and therapeutic options in head and neck cancer. *Clin. Cancer Res*., 24, 2828-2843, (2018)

Grandori, C. and Kemp, C.J. Personalized cancer models for target discovery and precision medicine. *Trends in Cancer*, 4, 634-642, 2018

Lui, G., Grandori, C., and Kemp, C.J. CDK12: An emerging therapeutic target for cancer. J. Clin. Path., 0, 1-6, 2018

Ishibashia, M., Toyoshimaa, M., Zhang, X., Hasegawa-Minatoa, J., Shigeta, S., Usuib, T., Kemp, C.J., Grandori, C., Kitatania, K., Yaegashia, N. Tyrosine kinase receptor TIE-1 mediates platinum resistance by promoting nucleotide excision repair in ovarian cancer. Scientific Reports, 8, 13207, 2018

Ashley, A.K., Kemp, C.J. Etiology of Cancer: Chemical Factors. In: Cancer Principles and Practice of Oncology, DeVita, V.T., Lawrence, T. S., and Rosenberg, S.A., Eds. *Walters Kluwer*, 2019

Kemp, C.J. and Grandori, C. The promise of functional precision medicine. Trends in Cancer, 5, 569-573, 2019

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EPIGENETIC REPROGRAMMING OF PANCREATICOBILIARY CANCER

The central theme of our research group is to study how the dysregulation of chromatin modifying enzymes contributes to pancreaticobiliary cancer pathogenesis and, further, whether these pathways present liabilities that could be exploited for cancer therapy.

Although chromatin-remodeling proteins are frequently dysregulated in human cancer, little is known about how they control tumorigenesis. This question is particularly relevant given that oncogenic transformation often involves epigenetic rewiring to meet the demands of uncontrolled proliferation, survival and metastasis. An imbalance in chromatin dynamics can lead to cancer by inactivating tumor suppressors, activating oncogenes, or by reactivating pathways that inhibit differentiation or favor stem cell self-renewal.

A challenge of the next decade will be to not only chronicle the altered expression and mutations of chromatin factors but to also define the phenotypic ramifications and the epigenetic abnormalities for each in cancer. Exploring chromatin factor dysregulation in cancer also provides a tractable system to address a more fundamental question of how tumor cells evolve when epigenetic barriers are altered, what characteristics are selected for to enhance tumor cell growth and the plasticity of these tumor cells in response to environmental perturbations.

A more in depth study of the chromatin factors that are lost or gained during tumorigenesis and how they remodel the epigenome are likely to form the basis for innovative approaches to cancer therapy and the development of novel biomarkers.

Currently, the laboratory is investigating the following questions:

1) What are the epigenetic barriers to the development and pathogenesis of pancreaticobiliary cancer?

2) What is the role of epigenetic dysregulation in defining transcriptional and genetic subtypes of pancreaticobiliary cancer?
3) How does the aberrant expression of developmental programs drive pancreaticobiliary growth, progression and metastasis?

SELECTED PUBLICATIONS

Kugel S, Sebastián C, Fitamant J, Ross KN, Saha SK, Jain E., Gladden A, Arora KS, Kato Y, Rivera MN, Ramaswamy S, Sadreyev RI, Goren A, Deshpande V, Bardeesy N & Mostoslavsky R. (**2016**). SIRT6 suppresses pancreatic cancer through control of Lin28b. *Cell*. 165(6):1401-15.

***Kugel S,** *Feldman JL, Klein AM, Silberman DM, Sebastián C, Dobersch S, Clark AR, Getz G, Denu JM & Mostoslavsky R. (**2015**). Identification and molecular basis for SIRT6 loss-of-function point mutations in cancer. *Cell Reports*. 13(3):479-88. ***These authors contributed equally to this work.

Etchegaray JP, Chavez L, Huang Y, Ross KN, Choi J, Martinez-Pastor B, Walsh RM, Sommer CA, Lienhard M, Gladden A, **Kugel S,** Silberman DM, Ramaswamy S, Mostoslavsky G, Hochedlinger K, Goren A, Rao A., and Mostoslavsky R. (**2015**). The histone deacetylase SIRT6 controls embryonic stem cell fate via Tet-mediated production of 5-hydroxymethylcytosine. *Nat. Cell Biol*. 17, 545-557.

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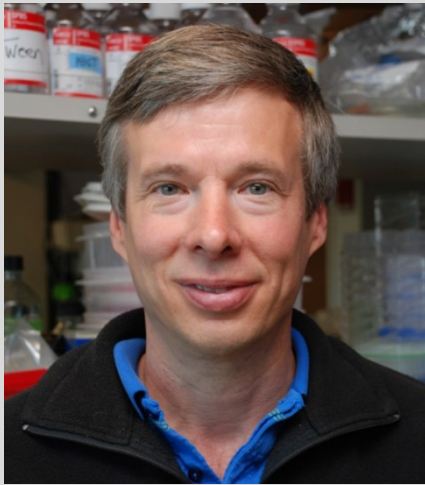
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CANCER BIOMARKERS

The Lampe laboratory investigates the control of cell growth both at the cell biological/mechanistic level and through cancer biomarker discovery. The advent of new screening methodologies has expanded our efforts into broad proteomic screens for potential cancer biomarkers using high density antibody array technologies produced by our lab to discover proteomic, autoantibody and glycomic biomarkers of cancer. We study potential biomarkers for pancreas, colon, breast and lung cancer. Our colon cancer biomarkers are most advanced and are involved in industry sponsored studies hopefully heading towards regulatory approval. The lung cancer work has the best chance to have a real impact on lung cancer mortality through better methods of screening. We already have discovered and preliminarily validated excellent markers for both non-small cell (NSCLC) and small cell (SCLC). We need to formally validate them in larger sample sets. For NSCLC this means testing them in both prediagnostic (i.e., NLST, DECAMP1) and diagnostic sample sets combined with Computed Tomography (CT) imaging as part of our funded Lung SPORE project 4. For SCLC we have a panel of excellent autoantibody markers that were already twice validated that we will further validate in NLST, Vanderbilt and WHI samples via a recently funded NCI R01 grant. Some of these autoantibodies are to proteins inappropriately expressed in SCLC and thus are targets for both imaging (e.g., immunoPET) and immunotherapy (CAR-T and targeted drug delivery).

SELECTED PUBLICATIONS

Mirus, JE *, Zhang, Y, Li, CI, Prentice, RL, Hingorani, SR, **Lampe, PD** (2015) "Cross-species antibody microarray interrogation identifies a 3-protein panel of plasma biomarkers for the early detection of pancreas cancer" *Clinical Cancer Research*. **21**(7):1764-71. PMC4391639.

Laird, DW, **Lampe, PD**, Johnson, RG (2105) "CELLULAR SMALL TALK" *Scientific American*. 312(5):70-7.

Aasen T, Mesnil M, Naus CC, **Lampe PD**, Laird DW. (2016) "Gap junctions and cancer: communicating for 50 years." *Nature Reviews Cancer*. 16(12):775-788. PMC5279857.

Garrison CB**, Lastwika KJ**, Zhang Y, Li CI, **Lampe PD**. Proteomic Analysis, Immune Dysregulation, and Pathway Interconnections with Obesity. *J Proteome Res.* 2017 Jan 6;16(1):274-287. PubMed PMID: 27769113; PubMed Central PMCID: PMC5234688.

Laird, DW, Naus, CC, **Lampe PD** (2017) "Snapshot: Connexin Gap Junction Proteins and Linked Diseases" *Cell,* **170**, 1260-1260.e1.

Rho JH**, Ladd JJ**, Li CI, Potter JD, Zhang Y, Shelley D, Shibata D, Coppola D, Yamada H, Toyoda H, Tada T, Kumada T, Brenner DE, Hanash SM, **Lampe PD.** Protein and glycomic plasma markers for early detection of adenoma and colon cancer. *Gut.* 2018 67: 473-484. PMC5420499.

Laird, DW, **Lampe, PD** (2018) "Therapeutic strategies targeting connexins" *Nature Reviews Drug Disc.* **17** (12) 905–921. PMC6461534 [Available on 2020-04-12].

Lastwika KJ**, Kargl J, Zhang Y, Zhu X, Lo E**, Shelley D, Ladd JJ**, Wu W, Kinahan P, Pipavath SNJ, Randolph TW, Shipley M, **Lampe PD**, Houghton AM (2019) Tumor-derived Autoantibodies Identify Malignant Pulmonary Nodules. Am J Respir Crit Care Med. 2019 May 15;**199**(10):1257-1266.

Solan JL**, Márquez-Rosado L**, **Lampe PD.** "Cx43 phosphorylation mediated effects on ERK and Akt protect against ischemia reperfusion injury and alter stability of stress-inducible protein NDRG1". *J. Biol. Chem*. 2019 June. In press PMID:31189653 DOI:10.1074/jbc.RA119.009162.

Lastwika, KJ**, Dunn CA**, Solan JL**, **Lampe PD** (2019) Phosphorylation of Connexin43 at MAPK, PKC or CK1 sites each distinctly alter the kinetics of epidermal wound repair. *J Cell Sci*. 2019 Sep 23;132(18). doi: 10.1242/jcs.234633. [Epub ahead of print] PubMed PMID: 31427427.

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The aim of my lab is to identify molecular drivers and biological properties of prostate cancer that may be exploited for the development of new and effective treatments. In our research we employ cutting-edge technologies including mouse and human prostate epithelial transformation systems; functional genomics; multi-omic data integration; high-throughput screening; small molecule drug discovery; and immuno-oncology to develop new approaches to stratify and treat prostate cancer.

The research in the lab is divided into three main areas:

1. <u>Functional characterization of drivers of prostate and bladder cancer:</u> Next-generation sequencing has enabled the large-scale profiling of aberrant genetic events associated with cancer. However, functional annotation of this rich information in relevant, genetically-defined cancer models is limited. To address this, we use a forward genetic approach with mouse and human epithelial organoid transformation systems. Benign epithelial cells are modified to stably express specific oncogenic factors, cultured briefly in the permissive environment of organoid cultures, and transplanted into immune-deficient mice. The resultant tumors are characterized to gain insight into the mechanisms by which the interaction of specific oncogenic events generate certain phenotypes of prostate and bladder cancer.

2. <u>Immunotherapeutic targeting of prostate cancer differentiation-specific antigens:</u> We have established a platform integrating RNA-seq and proteomics to nominate tumor-associated antigens enriched in subtypes of advanced prostate cancer with limited systemic expression in normal tissues. Candidate cell surface and intracellular antigens undergo multi-level validation including immunohistochemistry of microarrays of metastatic prostate cancers and benign human tissues. From the validated targets, we engineer and test both humoral and cellular immunotherapies in cell line- and patient-derived xenograft models of advanced prostate cancer.

3. <u>Disrupting the protein stability of Myc and androgen receptor (AR) in advanced prostate cancer:</u> Myc and AR are transcription factors with essential roles in the pathogenesis and maintenance of typical prostate cancer. We have developed Myc and AR reporter assays that facilitate the dynamic assessment of both subcellular protein localization and protein turnover. We are interrogating these assays with genome-wide CRISPR loss-of-function and gain-of-function screens to uncover the genetic/biologic pathways that modulate Myc and AR localization and stability. These reporters have also been used to complete high-throughput, high-content imaging screens with diverse chemical libraries to identify small molecule Myc and AR protein destabilizers. Lead optimization is ongoing in collaboration with prominent medicinal chemistry and prostate cancer biology colleagues.

SELECTED PUBLICATIONS

VanDeusen HR, Ramroop JR, Morel KL, Bae S, Sheahan AV, Sychev Z, Lau NA, Cheng LC, Tan VM, Li Z, Petersen A, Lee JK, Park JW, Yang R, Hwang JH, Coleman I, Witte ON, Morrissey C, Corey E, Nelson PS, Ellis L, Drake JM. Targeting RET Kinase in Neuroendocrine Prostate Cancer. Mol Cancer Res. 2020 May 27.

DeLucia DC and Lee JK. Identification of Cell Surface Targets for CAR T Cell Immunotherapy. Methods Mol Biol. 2020;2097:45-54.

Corella AN, Victoria Andrea Cabiliza Ordnio M, Coleman I, Lucas JM, Kaipainen A, Nguyen HM, Sondheim D, Brown L, True LD, Lee JK, MacPherson D, Nghiem P, Gulati R, Morrissey C, Corey E, Nelson PS. Identification of Therapeutic Vulnerabilities in Small Cell Neuroendocrine Prostate Cancer. Clin Cancer Res. 2019 Dec 5.

Shapovalova M, Lee JK, Li Y, Vander Griend DJ, Coleman I, Nelson PS, Dehm SM, LeBeau AM. PEG10 promoter-driven expression of reporter genes enables molecular imaging of lethal prostate cancer. Cancer Res. 2019 Sep 17.

Beltran H, Hruszkewycz A, Scher HI, Hildesheim J, Isaacs J, Yu EY, Kelly K, Lin D, Dicker AP, Arnold JT, Hecht TT, Wicha MS, Sears RC, Rowley DR, White RM, Gulley JL, Lee JK, Diaz-Meco MT, Small EJ, Shen MM, Knudsen KE, Goodrich DW, Lotan TL, Zoubeidi A, Sawyers CL, Rudin CM, Loda M, Thompson TC, Rubin MA, Tawab-Amiri A, Dahut W, Nelson PS. The role of lineage plasticity in prostate cancer therapy resistance. Clin Cancer Res. 2019 Jul 30.

Lee JK, Priceman SJ. Precision Medicine-Enabled Cancer Immunotherapy. Cancer Treat Res. 2019;178:189-205.

Park JW, Lee JK, Sheu KM, Wang L, Balanis NG, Nguyen K, Smith BA, Cheng C, Tsai BL, Cheng D, Huang J, Kurdistani SK, Graeber TG, Witte ON. Reprogramming normal human epithelial tissues to a common, lethal neuroendocrine cancer lineage. Science. 2018 Oct 5;362(6410):91-95.

Lee JK, Bangayan NJ, Chai T, Smith BA, Pariva TE, Yun S, Vashisht A, Zhang Q, Park JW, Corey E, Huang J, Graeber TG, Wohlschlegel J, Witte ON. Systemic surfaceome profiling identifies target antigens for immune-based therapy in subtypes of advanced prostate cancer. Proc Natl Acad Sci U S A. 2018 May 8;115(19)E:4473-E4482.

Park JW, Lee JK, Witte ON, Huang J. FOXA2 is a sensitive and specific marker for small cell neuroendocrine carcinoma of the prostate. Mod Pathol. 2017 Sep;30(9):1262-1272.

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Lee JK, Phillips JW, Smith BA, Park JW, Stoyanova T, McCaffrey EF, Baertsch R, Sokolov A, Meyerowitz JG, Mathis C, Cheng D, Stuart JM, Shokat KM, Gustafson WC, Huang J, Witte ON. N-Myc Drives Neuroendocrine Prostate Cancer Initiated from Human Prostate Epithelial Cells. Cancer Cell. 2016 Apr 11;29(4):536-547.

Park JW, Lee JK, Phillips JW, Huang P, Cheng D, Huang J, Witte ON. Prostate epithelial cell of origin determines cancer differentiation state in an organoid transformation assay. Proc Natl Acad Sci U S A. 2016 Apr 19;113(16):4482-7.

Drake JM*, Paull EO*, Graham NA, Lee JK, Smith BA, Titz B, Stoyanova T, Faltermeier CM, Uzunangelov V, Carlin DE, Fleming DT, Wong CK, Newton Y, Sudha S, Vashisht AA, Huang J, Wohlschlegel JA, Graeber TG, Witte ON, Stuart JM. Phosphoproteome Integration Reveals Patient-Specific Networks in Prostate Cancer. Cell. 2016 Aug 11;166(4):1041-1054.

Stoyanova T, Riedinger M, Lin S, Faltermeier CM, Smith BA, Zhang KX, Going CC, Goldstein AS, Lee JK, Drake JM, Rice MA, Hsu EC, Nowroozizadeh B, Castor B, Orellana SY, Blum SM, Cheng D, Pienta KJ, Reiter RE, Pitteri SJ, Huang J, Witte ON. Activation of Notch1 synergizes with multiple pathways in promoting castration-resistant prostate cancer. Proc Natl Acad Sci U S A. 2016 Oct 18;113(42):E6457-E6466.

Drake JM, Lee JK, Witte ON. Clinical targeting of mutated and wild-type protein tyrosine kinases in cancer. Mol Cell Biol. 2014 May;34(10):1722-32.

Drake JM, Graham NA, Lee JK, Stoyanova T, Faltermeier CM, Sud S, Titz B, Huang J, Pienta KJ, Graeber TG, Witte ON. Metastatic castration-resistant prostate cancer reveals intrapatient similarity and interpatient heterogeneity of therapeutic kinase targets. Proc Natl Acad Sci U S A. 2013 Dec 3;110(49):E4762-9.

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USE OF IN VIVO APPROACHES TO STUDY LUNG CANCER INITIATION, PROGRESSION AND RESPONSE TO THERAPY

Our laboratory investigates the mechanisms through which cancer-mutated genes drive tumorigenesis. We focus on small cell lung carcinoma (SCLC), a highly aggressive neuroendocrine cancer that responds well to chemotherapy but rapidly becomes chemoresistant. Typically, SCLC has metastasized by the time of diagnosis, and survival rates are dismal. We identified major driver genes mutated in human SCLC using next-generation sequencing approaches. To explore key activities of SCLC-mutated genes we use mouse genetics and functional studies. We have generated a panel of new mouse models of SCLC (e.g. see Augert et al, 2020, *Cancer Cell*; Grunblatt et al, 2020, *Genes and Development*; and Jia et al, 2018, *Cancer Discovery*). These models, along with derived cell lines, are employed to understand how mutations in certain genes promote SCLC and to identify vulnerabilities conferred by these mutations. We collaborate closely with our clinical colleagues at the Seattle Cancer Care Alliance to generate and study patient derived xenograft models of SCLC and identify therapeutic strategies to target subsets of SCLC (see Augert et el, 2019 *Science Signaling*). Genomic analyses and functional genomics, including genome-scale CRISPR inactivation and cDNA overexpression screens are used in these efforts. We perform genetic screens in cell culture and in vivo to identify oncogenes and tumor suppressor genes and to identify new therapeutic approaches that can be tested in our in vivo models. Our ultimate aim is to translate an increased understanding of the basic biology of SCLC driver genes to the development of novel therapies for a cancer type greatly in need of new therapies.

SELECTED PUBLICATIONS

Augert A, Mathsyaraja H, Ibrahim AH, Freie B, Geuenich MJ, Cheng P, Alibeckoff SP, Wu N, Hiatt JB, Basom R, Gazdar A, Sullivan LB, Eisenman RN#, MacPherson D#. MAX functions as a tumor suppressor gene in small cell lung cancer. 2020 *Cancer Cell* 38:97-114.e7. PMID: 32470392 # Co-corresponding authors

Grunblatt E, Wu, N, Zhang H, Liu X, Norton JP, Ohol Y, Leger P, Hiatt JB, Eastwood EC, Thomas R, Ibrahim AH, Jia D, Basom R, Eaton KD, Martins R, Houghton AM, and MacPherson D. MYCN drives chemoresistance in small cell lung cancer while USP7 inhibition can restore chemosensitivity. 2020 *Genes and Development* (in press)

Rudin CM, Poirier JT, Byers LA, Dive C, Dowlati A, George J, Heymach JV, Johnson JE, Lehman JM, MacPherson D, Massion PP, Minna JD, Oliver TG, Quaranta V, Sage J, Thomas RK, Vakoc CR, Gazdar AF. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. *Nat Rev Cancer*. 2019 19:289-297. PMID: 30926931

Augert A, Eastwood E, Ibrahim AH, Wu N, Grunblatt E, Basom R, Liggitt D, Eaton KD, Martins R, Poirier JT, Rudin CM, Milletti F, Cheng WY, Mack F, MacPherson D. Targeting NOTCH activation in small cell lung cancer through LSD1 inhibition. *Science Signaling*. 2019 12(567).

Jia D, Augert A, Kim DW, Eastwood E, Wu N, Ibrahim AH, Kim KB, Dunn CT, Pillai SPS, Gazdar AF, Bolouri H, Park KS, MacPherson D. Crebbp Loss Drives Small Cell Lung Cancer and Increases Sensitivity to HDAC Inhibition. *Cancer Discovery*. 2018 8(11):1422-1437.

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EXPLOITING CANCER GENOMICS AND THE TUMOR MICROENVIRONMENT TO GUIDE ONCOLOGY TREATMENT

The Nelson laboratory focuses on prostate cancer as a „test case" that dramatically illustrates the variation in cancer behavior between individuals and the need for prevention, better screening methods and the potential for personalized approaches to revolutionize oncology care. Prostate cancer is the most common malignancy in men with more than 160,000 new cases diagnosed yearly in the US. There is a clear genetic predisposition with higher rates of aggressive cancer in certain families. Environmental and dietary factors also contribute. Major technological advances in DNA sequencing now provide an opportunity to comprehensively detail every molecular change that occurs in a given tumor. This information has the potential to avoid "one-size-fits-all" treatments, eliminate ineffective therapy, and tailor interventions to individual tumor vulnerabilities.

Areas of current work include:

Developing New Therapeutic Strategies for Early and Late Stage Cancer
We aim to determine the molecular features that associate with response and resistance mechanisms to pathway-targeted agents and conventional chemotherapy. Several clinical (translational) trials are underway including studies incorporating neoadjuvant therapies and large-scale tumor genome sequencing. Tissue samples are acquired pre- and post-therapy and molecular correlates of direct drug effects are identified to define tumor and host signatures: (a) predictive of therapeutic response and (b) predictive of disease outcome (relapse). Mechanism-based assessments of specific oncogenic mutations serve to focus further drug development. We are exploring new minimally-invasive approaches to assess the molecular composition of tumors using circulating tumor cells and cell-free tumor DNA biomarkers that allow for iterative sampling of tumor biology.

Characterization of the Cellular Androgen Receptor (AR) Program
A major focus of the lab has been the identification of down-stream "effector" genes that are responsible for

cellular events (e.g. proliferation) after androgen receptor (AR) activation. The AR is the first known example of a „precision medicine" target that continues to be the major focus of treatment in advanced prostate cancer. We identified a network of genes that are regulated by androgens in prostate cancer cells. Systematic studies involving the genetic and pharmacological modulation of these genes are designed to determine cellular functions to identify roles in proliferation, anti-apoptosis, differentiation, and treatment resistance. Genes with prostate-restricted expression serve as therapeutic targets for immunological and pharmacological strategies.

Determining the Role of the Tumor Microenvironment (TME) in Cancer Biology

The macro and microenvironments within which malignant neoplasms arise can exert profound influences on tumor behaviors that range from a complete reversion of the malignant phenotype to the promotion of tumor cell invasion and metastatic growth. In addition to tumor cells, the architecture of most solid tumors includes an assortment of non-malignant cell types derived from distinct developmental lineages that carry out structural or functional roles including fibroblasts, muscle cells, nerves, and vasculature. We have determined that components of tumor microenvironments (TME) also contribute to *de novo* and acquired treatment resistance. In current practice, the majority of cancer-directed therapeutics do not exclusively target malignant cells, but also injure benign cells in the local, and potentially the distant host microenvironments. Such collateral damage is quite evident for non-specific therapies that involve DNA-damaging modalities such as genotoxic drugs and ionizing radiation. Ongoing work centers on characterizing a DNA Damage Secretory Program in the TME that is comprised of a remarkable spectrum of proteases, growth factors and cytokines. The composite effects of this program promote tumor cell proliferation, metastasis, and also resistance to therapeutics.

Cancer Predisposition.

Prostate cancer is one of the most heritable malignancies: it is estimated that ~50% of prostate cancer risk is due to genetic factors. In addition to common polymorphisms that influence cancer predisposition, we have recently determined that rare highly-penetrant cancer predisposition genes are frequently mutated in men with aggressive/advanced prostate cancer. These predisposition genes link prostate cancer with other heritable cancers such as breast and ovarian cancer in the context of *BRCA1/2* mutations and colon cancer in the context of mismatch repair gene mutations. Importantly, mutations in these DNA repair genes identify families at risk for cancer and support precision oncology strategies that exploit responses to specific therapeutics.

SELECTED PUBLICATIONS

Sun Y et al**.** Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. *Nat Med*. 2012 Sep; 18(9):1359-68. PMID: 22863786.

Kumar A, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. *Nat Med*. 2016 Apr;22(4):369-78. PMID: 26928463.

Pritchard CC, et al Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. *New England Journal of Medicine*. 2016 Aug 4;375(5):443-53. PMID: 27433846.

Bluemn EG et al Androgen Receptor Pathway-Independent Prostate Cancer is Sustained through FGF Signaling. *Cancer Cell* 2018 Oct 9;32(4):474-489. PMID: 29017058.

Chatterjee P et al Supraphysiological androgens suppress prostate cancer growth through androgen receptor-mediated DNA damage. *J Clin Invest* 2019 Jul 16:130. PMID: 31310591

Labrecque MP et al Molecular profiling stratifies diverse phenotypes of treatment-refractory metastatic castration-resistant prostate cancer. *J Clin Invest* 2019 Jul 30;130. PMID: 31361600

Nyquist M et al Combined TP53 and RB1 Loss Promotes Prostate Cancer Resistance to a Spectrum of Therapeutics and Confers Vulnerability to Replication Stress. *Cell Reports*. 2020 May 26;31(8). PMID: 32460015.

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HIV-1 TRANSMISSION AND PATHOGENESIS

Dr. Overbaugh's laboratory has a long-standing interest in understanding the mechanisms of HIV transmission and pathogenesis and the role of innate and adaptive immunity in these outcomes. The lab seeks to understand what immune responses contribute to whether a productive infection is established and why certain viruses are selected in that process. In the case of antibody responses, much of the work is on defining the types of antibodies that are associated with protection in HIV exposed humans. The lab also studies the functional properties of these antibodies, including their ability to mediate killing of infected cells and neutralize virus. The lab has made the surprising finding that infants more rapidly develop neutralizing antibody responses to HIV than adults and the HIV-specific antibodies have less somatic hypermutation. Efforts are underway to identify the target of these responses and to define the evolutionary pathway that leads to their emergence of HIV antibody responses in infants. Similar studies are focused on individuals who are superinfected with a second strain of HIV and develop robust antibody responses.

She is currently extending her work on antibodies to the study of SARS-CoV-2. There the focus is on identifying the key antibody responses to infection and their association with outcome.

The studies of innate immunity seek to define host cells factors that target the replication of circulating, transmitted variants of HIV. These studies include defining the interferon induced genes that respond to HIV as well as determining which of these factors restrict viral replication, particularly transmitted strains. Her laboratory has more recently begun exploring similar questions for Zika virus and SARS-CoV-2 and is conducting screens to identify antiviral host factors to these important global pathogens.

Much of the HIV research in the lab is focused on populations in Africa because this is where the AIDS epidemic is most severe. The laboratory is part of a larger team, comprising researchers in both Seattle and Kenya (The Kenya Research Project), that is studying the molecular epidemiology of HIV transmission.

SELECTED PUBLICATIONS (2015-2020)

Milligan C, Richardson BA, John-Stewart G, Nduati R, Overbaugh J. Passively Antibody-Dependent Cellular Cytotoxicity (ADCC) Activity in HIV-Infected Infants Is Associated with Reduced Mortality. *Cell Host Microbe* 17(4): 500-506, 2015.

Cortez V, Chen MM, Ronen K, Georgiev IS, McClelland RS, Overbaugh J. The broad neutralizing antibody responses in HIV-1 superinfection are not dominated by antibodies directed to epitopes common in single infection. *PLoS Pathog,* 11(7): e1004973, 2015.

Boyd DF, Sharma A, Humes D, Cheng-Mayer C and Overbaugh J. Adapting SHIVs *in vivo* selects for Envelope-mediated Interferon-□ resistance. PLos Pathogens 12(7): e1005727, 2016.

Williams KL, Wang B, Arenz D, Williams JA, Dingens AS, Cortez V, Simonich CA, Lehman DA, Rainwater S, Lee KK, Overbaugh J. Superinfection drives HIV neutralizing antibody responses from several B cell lineages that contribute to a polyclonal response. Cell Reports, 23(3):682-691, 2018.

Simonich CA, Williams KL, Verkerke HP, Williams JA, Nduati R, Lee KK and Overbaugh J. HIV-1 neutralizing antibodies with limited hypermutation from an infant. *Cell*, 166:77-87, 2016.

Dingens AS, Arenz D, Weight H, Overbaugh J, Bloom JD. An antigenic atlas of HIV-1 escape from broadly neutralizing antibodies. Immunity. :520-532.e3, 2019.

Simonich CA, Doepker LN, Ralph D, Williams JA, Dhar A, Yaffe Z, Gentles L, Small CT, Oliver B, Vigdorovich V, Prasad VM, Nduati R, Sather DN, Lee KK, Matsen FA, Overbaugh J. Rapid development of an infant-derived HIV-1 broadly neutralizing antibody lineage. Nature Communication, 10: article 2190, 2019.

Naiman NE, Slyker J, Richardson BA, John-Stewart G, Nduati R, Overbaugh J. Antibody-dependent cellular cytotoxicity targeting CD4-inducible epitopes predicts mortality in mother-to-child transmission of HIV-1. EBioMedicine. 2019 Sep;47:257-268.

Sharma A, McLaughlin RN, Basom RS, OhAinle M, Kikawa C, Yount JS, Emerman M, Overbugh J. Macaque IFITMs limit replication of SHIVs derived from circulating HIV-1 in an Envelope-dependent manner. PLoS Pathogens, in press, 2019.

Gobillot T, Kikawa C, Lehman D, Kinuthia J, Drake A, Jaoko W, Mandaliya K, John-Stewart G, McClelland RS, **Overbaugh J**. Zika virus circulates at low levels in Western and Coastal Kenya. J Infect Dis. 2020 Apr 3. pii: jiaa158. doi: 10.1093/infdis/jiaa158. Online ahead of print.

REVIEWS AND COMMENTARIES

Overbaugh J. 24/7 isn't the only way: A healthy work-life balance can enhance research. *Nature,* 477: 27-28, 2011.

Overbaugh J. Mother-Infant HIV Transmission: Do maternal HIV-specific antibodies protect the infant? *PLoS Pathog*. 10(8): e1004283, 2014.

Deeks S, Overbaugh J, Phillips A, Buchbinder S. HIV Infection and Disease. *Nature Disease Primers*, 1:1-22 2015.

Ronen K, Sharma A, Overbaugh J. HIV transmission biology: translation for HIV prevention. *AIDS* 29: 2219-2227, 2015.

Overbaugh J. Defining the barriers to women publishing in high-impact journals. *J Virol*, 92(7), 2018.

Clynes M, Corbett A, Overbaugh J. Why we need good mentoring. Nature Reviews Cancer, July, 2019.

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FUNCTIONAL GENOMICS OF STEM CELL AND CANCER CELL BIOLOGY

The Paddison Lab uses genomic screening technologies to probe human gene activity in mammalian stem and progenitor cells and cancer cells.

Functional Genomics
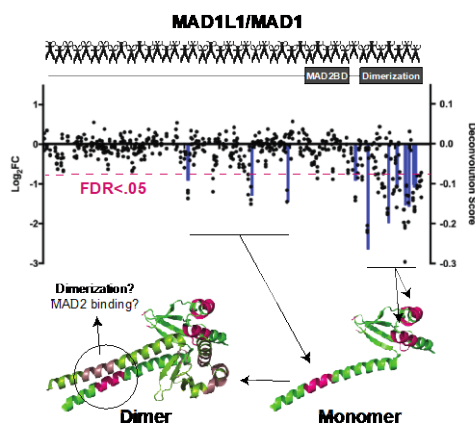
Fig 1. Identifying predicted and new essential protein domains using mutagenic sgRNA tiling libraries.

Functional genomics is the study of the function of genes contained within an organism's genome, or, put another way, an approach to figure out what roles genes have in an organism. In the last fifteen years, two powerful homology-based gene targeting technologies have come along that have revolutionized functional genomics in mammals. These are RNAi and CRISPR-Cas9. The Paddison Lab routinely uses both to power studies regarding the underlying biology of human stem and progenitors, by targeting each gene in the genome and determining their contribution to a phenotype of interest.

In addition, we have leveraged the mutagenic properties of CRISPR-Cas9 to test models of the human genome's protein coding structure. Although the human genome is currently predicted to contain 5494 conserved protein family (Pfam) domains (e.g., methyltransferase-like domain), most of these domains have not been validated while ~45% of protein coding regions lack Pfam domains altogether. However, by tiling sgRNAs across protein-coding genes we are able to identify essential domains and motifs in phenotypically constrained regions. As a result, genes required for particular phenotypes (e.g., viability) can produce a unique mutational signature,

with constrained regions scoring as phenotypic "peaks" (Fig. 1). We are currently applying this technique to >400 genes in the human genome spanning the epigenome, mitotic factors, and the spliceosome.

Biological Focus Areas

Precision oncology. The promise of "precision oncology" relies on decoding the molecular signatures of tumors to make predictions about effective therapies. The prevailing wisdom is that precision therapies will arise from identifying and targeting "drivers" of oncogenic transformation (e.g., mutated oncogenes). However, this approach has met with limited clinical success, particularly for some of the most devastating and difficult to treat cancers. Glioblastoma multiforme (GBM) is the most aggressive and common form of brain cancer in adults: approximately 90% of GBM patients die within two years of diagnosis with current standard of care therapy. We used GBM stem-like cells in combination with functional genomic screening to identify novel GBM therapeutic targets (**Fig. 2**).
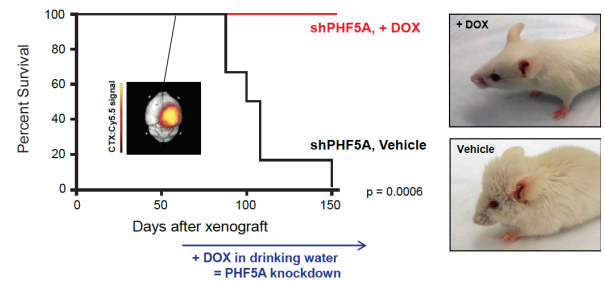
Fig 2. Inhibition of PHF5A causes human GBM tumor regression and survival in immune-compromised mice. PHF5A is important for 3' splice site recognition and was identified as a key GBM vulnerability driven by MYC activity.
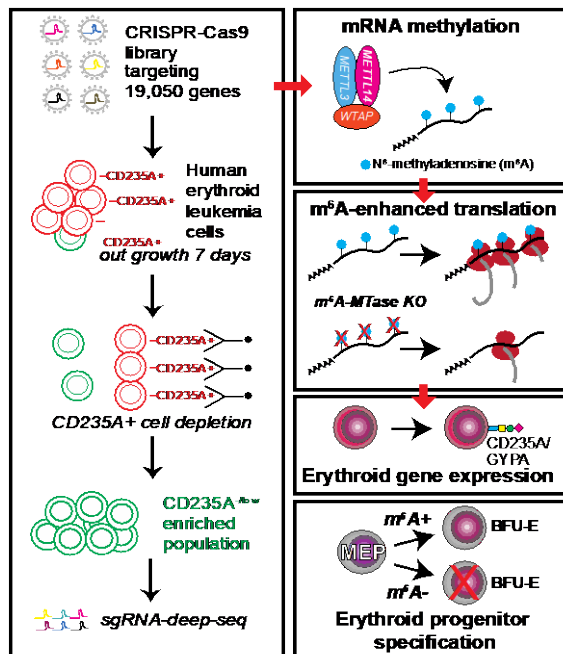

Fig. 3: Overview of screen for regulators of erythroid gene expression program and roles for m^{6}A mRNA marking during lineage specification.

Epitranscriptomics. Epitranscriptomics generally pertains to chemical modifications of mRNA occurring during or after gene transcription. N6-methyladenosine (m^{6}A) is among the most frequent post-transcriptional chemical modifications found in mammalian mRNA. In cell-based models, m^{6}A has been suggested to participate in numerous types of mRNA regulation (e.g., turnover, splicing, translation, or miRNA targeting). However, while m^{6}A-mRNA likely exists in most if not all eukaryotes, physiologically relevant roles for m^{6}A-mRNA have yet to be well established in mammals. Recently, in collaboration with Dr. Beverly Torok-Storb (Clinical Research Division), we performed a genome-wide CRISPR-Cas9 genome-wide screens to identify genes required for human erythroid (red blood cell) lineage specification. Among the novel hits were m^{6}A mRNA regulatory machinery, including core methyltransferase subunits *METTL14*, *METTL3*, and *WTAP*. Through a collaboration with Dr. Andrew Hsieh's group (Human Biology), we have now shown that m^{6}A mRNA marks promote the *translation* of a network genes required for human erythropoiesis, including factors that control epigenetic patterning in chromatin (**Fig. 3**) (Kuppers et al., 2019).

Regulation of Cell Identity and Cell Growth. Cancer cells may arise from maligned development programs, hijacking molecular pathways that are normally involved in developmental processes such as cell fate determination. The existence of cancer stem cells, which may play vital roles in tumor progression, maintenance, and recurrence, underscores this notion. One of the current projects in lab in this space is understanding how normal and cancer-causing progenitor cells enter and exit quiescent-like states. Through single cell RNA sequence analysis and functional genomic screens, we have identified classes of genes that block or promote entry into quiescent-like states. The results from these studies will better help define our notions of cell cycle regulation and maintenance of progenitor cell identity. In collaboration with Drs. Anoop Patel and Eric Holland, we recently received NIH funding for study of tumor quiescent states and genes that regulate tumor cell quiescence ingress and egress.

SELECTED PUBLICATIONS

Kuppers DA, Arora S, Lim Y, Lim A, Carter L, Corrin P, Plaisier CL, Basom R, Delrow J, Wang S, He H, Torok-Storb B, Hsieh AC, and Paddison PJ. N6-methyladenosine mRNA marking promotes selective translation of regulons required for human erythropoiesis. *Nature Communications* in press, 2019.

Mathieu J, Detraux D, Kuppers D, Wang Y, Cavanaugh C, Sidhu S, Levy S, Robitaille AM, Ferreccio A, Bottorff T, McAlister A, Somasundaram L, Artoni F, Battle S, Hawkins RD, Moon RT, Ware CB, Paddison PJ, and Ruohola-Baker H. Folliculin regulates mTORC1/2 and WNT pathways in early human pluripotency. *Nature Communications* 10:632, 2019.

Ding Y, Herman JA, Toledo CM, Lang JM, Corrin P, Girard E, Basom R, Delrow JJ, Olson JM, and Paddison PJ. ZNF131 suppresses centrosome fragmentation in Glioblastoma stem cells through regulation of HAUS5. *Oncotarget* 10.18632/oncotarget.18153, 2017.

Plaisier CL, Bluvstein S, Bernard B, Reynolds S, Simon Z, Toledo CM, Ding Y, Reiss DJ, Paddison PJ, and Baliga NS. Rational prediction of combinatorial interventions for glioblastoma multiforme based on patient-derived regulatory networks, *Cell Systems* 3:172-86, 2016.

Toledo CM, Ding Y, Hoellerbauer P, Davis RJ, Basom R, Girard EJ, Lee E, Corrin P, Hart T, Bolouri H, Davison J, Zhang Q, Hardcastle J, Aronow BJ, Plaisier CL, Baliga NS, Moffat J, Lin Q, Li X-N, Nam D-H, Lee J, Pollard SM, Zhu J, Delrow JJ, Clurman BE, Olson JM, and Paddison PJ. Genome-wide CRISPR-Cas9 screens reveal loss of redundancy between PKMYT1 and WEE1 in Glioblastoma stem-like cells. *Cell Reports*. 13:2425-39, 2015.

Toledo CM, Herman J, Olsen JB, Ding Y, Corrin P, Girad E, Olson JM, Emili A, DeLuca J, and Paddison PJ. BuGZ is required for Bub3 stability, Bub1 kinetochore function, and chromosome alignment. *Dev. Cell.* 28:282-94, 2014.

Hubert CG, Bradley RK, Ding Y, Toledo CM, Herman J, Skutt-Kakaria K, Girard EJ, Davison J, Berndt J, Corrin P, Hardcastle J, Basom R, Delrow JJ, Webb T, Pollard SM, Lee J, Olson JM, and Paddison PJ. Genome-wide RNAi screens in human brain tumor isolates reveal a novel viability requirement for PHF5A. *Genes Dev.* 27:1032-45, 2013.

Betschinger J, Nichols J, Dietmann S, Corrin P, Paddison PJ, Smith A. Exit from pluripotency is gated by intracellular redistribution of the bHLH transcription factor Tfe3. *Cell*. 153: 335-47, 2013.
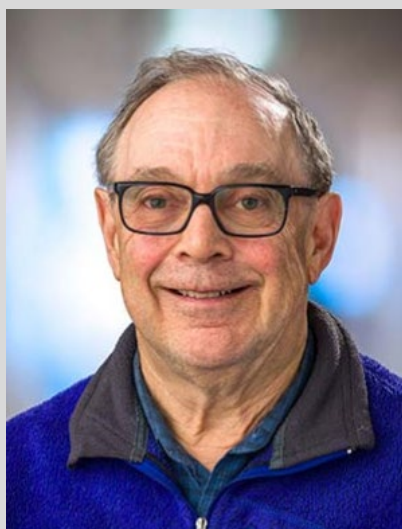
Ding Y, Hubert CG, Herman J, Corrin P, Toledo CM, Skutt-Kakaria K, Vazquez J, Basom R, Zhang B, Risler JK, Pollard SM, Nam DH, Delrow JJ, Zhu J, Lee J, DeLuca J, Olson JM, Paddison PJ. Cancer-Specific requirement for BUB1B/BUBR1 in human brain tumor isolates and genetically transformed cells. *Cancer Discovery.* 3:198-211, 2013.

Chen X, Skutt-Kakaria K, Davison J, Ou YL, Choi E, Malik P, Loeb K, Wood B, Georges G, Torok-Storb B, Paddison PJ. G9a/GLP-dependent histone H3K9me2 patterning during human hematopoietic stem cell lineage commitment. *Genes Dev.* 26:2499-511, 2012.

Paddison PJ. RNA interference in mammalian cell systems. In: RNA interference. *Current Topics in Microbiology and Immunology.* Paddison PJ, Vogt PK (eds.). Springer Press, 2008.

Paddison PJ, Silva JM, Conklin DS, Schlabach M, Li M, Gnoj L, Balija V, O'Shaughnessy A, Scobie K, Aruleba S, Chang K, Westbrook T, Sachidanandam R, McCombie WR, Elledge SJ, Hannon GJ. A resource for large-scale RNAi based screens in mammals. *Nature*. 428: 427-431, 2004.

Paddison PJ, Hannon GJ. RNA interference: The New Somatic Cell Genetics? *Cancer Cell.* 2:17-23, 2002.

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NEOPLASTIC EVOLUTION IN SPACE AND TIME
CONTROLLING COMPLEXITY AND CONTEXT

Personal Statement

Our team has been funded by our Program Project Grant (P01 CA091955), a NCI Provocative Question Grant (R01 CA179949) and a NIH Challenge Grant (RC1 CA146973). Advances that we made with these NIH grants are described below.

Background. I was trained as a geneticist (PhD). Lee Hartwell and I discovered yeast cell cycle mutants when I was an undergraduate researcher in the University of Washington Department of Genetics. This discovery led to two groundbreaking papers that became the basis for Lee's well deserved Nobel Prize.

I became interested in cancer from the research of Drs. Stan Gartler and the late Phil Fialkow in the University of Washington Department of Genetics. They both taught me that cancer was a disease of somatic genome evolution. This concept was later articulated eloquently by the late Dr. Peter Nowell in his 1976 _Science_ classic article. The insights of these pioneers led me to enter medical school to study cancer as an evolutionary process. The lessons of every class help guide me to a subspecialty (gastroenterology), in which endoscopy allowed access to visually detect and biopsy neoplastic lesions of the GI tract where genomic and evolutionary insights could be investigated to prevent or detect cancer early when it was curable.

Research focus. The focus of our Fred Hutch research team and our collaborators is Barrett's esophagus (BE) and its evolution to esophageal adenocarcinoma (EA). However, the vast majority of patients with BE do not progress to EA and BE instead remain in a benign state that patients die with, not of. Conversely, EAs typically

arise in individuals who were not known to have BE before presentation of an advanced, symptomatic EA. The outcomes of population screening for BE and EA have been well documented in Denmark by Hvid-Jensen et al (Figure 1) as well as many other investigators.
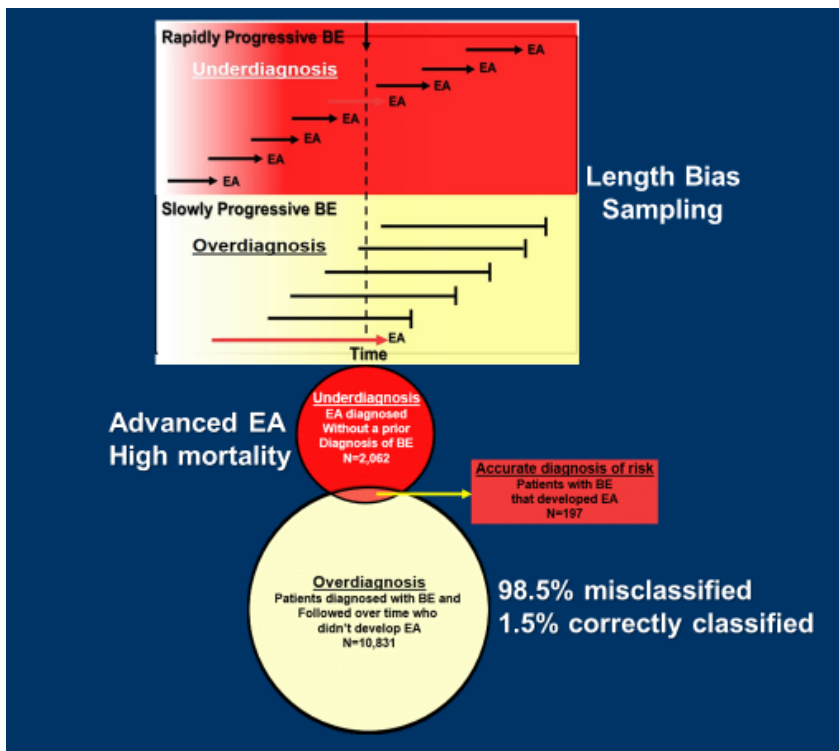
This conundrum has also been documented in many other "premalignant" conditions and cancers, including those of the breast and prostate, as well as many other organs. NIH sponsored a "Think Tank" on the topic. I was a member of this Think Tank, which resulted in two major publications:

(1) "*Overdiagnosis and overtreatment in cancer: an opportunity for improvement.*" Esserman LJ, Thompson IM Jr, **Reid B** JAMA. 2013 Aug 28;310(8):797-8 and

(2) "*Addressing overdiagnosis and overtreatment in cancer: a prescription for change.*" Esserman LJ, Thompson IM, **Reid B**, Nelson P, Ransohoff DF, Welch HG, Hwang S, Berry DA, Kinzler KW, Black WC, Bissell M, Parnes H, Srivastava S. Lancet Oncol. 2014 May;15(6):e234-42. doi: 10.1016/S1470-2045(13)70598-9.

Our research on BE is designed to elucidate and control the evolution of BE and EA over time and space in the esophagus. The goals include (1) accurately distinguishing aggressive BE that evolves rapidly to EA from benign BE that does not progress to EA over the lifetime of a patient and who will instead die of unrelated causes. Other goals include (2) elucidation of the evolutionary mechanisms that cause some BE to rapidly progress to EA while others remain indolent. These mechanisms may also be aberrant in other "CIN" cancers. Finally, (3) we seek to elucidate the evolutionary mechanisms that can render EA resistant to all known therapies, including immunotherapy.

Challenges for EA control.

The fundamental challenges of BE and EA include (1) <u>complexity</u> of the EA genome compared to the typical non-progressing BE genome, (2) the <u>speed</u> with which this genomic complexity evolves and (3) absence of a <u>known effective treatment for advanced EA</u>. The complexity of the cancer genome has recently become widely appreciated through advances in DNA sequencing and genomics but there have been no major advances in treatment of advanced EAs.

The clinical implications of the speed at which cancers evolve has been the subject of discussion for decades. Cancers that evolve rapidly will be harder to detect because rapid progression decreases the "window of opportunity" for early detection of EA (Figure 1). In this case, slowly or non-progressing BE will be easy to detect because they persist for sufficiently prolonged periods that the patient will die of competing causes of mortality before EA develops. This concept has been called "length bias sampling" (Figure 1). The outcomes in BE are poor with only a small proportion of patients who benefit from currently available approaches to prevention, early detection or therapy for EA (Figure 1). Length-bias sampling has also been implicated in screening for many other types of cancer, but very few studies have compared the temporal evolutionary progression to

cancer with a control population of patients who do not progress to cancer. Our team has done this in BE. Some of our results have been published during the current review period and others are currently being analyzed.

Although there was initial optimism that EA might respond to immunotherapy, subsequent studies have reported that EA and many other cancers with high rates of aneuploidy and chromosome instability are resistant to current immune therapies. Our goals are to provide research advances that will improve current outcomes for prevention, early detection and therapy of EA. We have either recently completed or are currently analyzing three large data sets. I have also participated in a number of Think Tanks and other venues to generate new concepts, ideas, initiatives and approaches to address these vexing challenges that characterize many cancers in addition to EA.

Below I review three major studies that our team has been engaged over the past 5 years. The first two studies have been completed and published. The third is currently in analysis.

Study 1 (Early Detection) was a case-cohort study design that used high density (1M) SNP arrays to track evolving somatic genomes in space and time in 248 patients with BE. As progressors approached the diagnosis of EA, they developed chromosome instability with gain or loss of whole chromosomes or large regions of chromosomes, whole genome doublings and massive chromosome instability. This genome instability increased diversity in the Barrett's epithelium that resulted in evolution to EA over a 2-4 year period (Figure 1). In contrast, non-progressors had only localized regions of genomic changes including 9p loss or copy neutral LOH, and damage in fragile sites, including frequent losses of CDKN2A, FHIT, and WWOX that remained remarkably stable over time (Figure 1) (NIH P01 CA091955, NIH Challenge Grant RC1 CA146973).
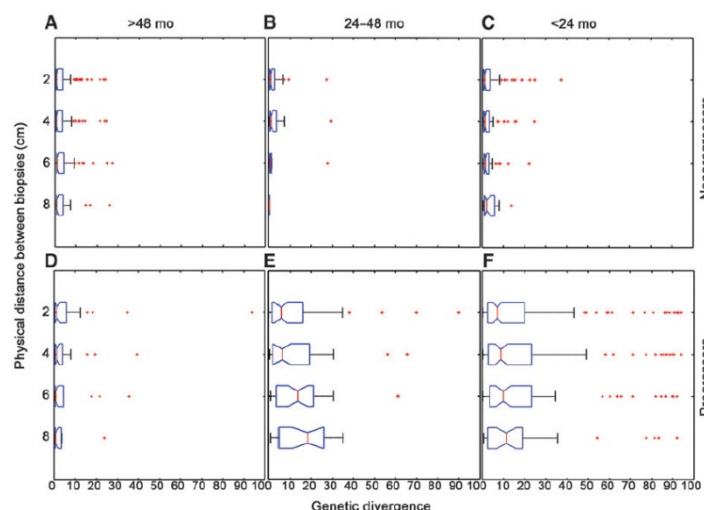

Figure 1. Genetic divergence in a case-cohort study of evolving BE genomes over space and time using 1M SNP arrays.. Non-progressors (N=169) maintain a consistently low level of large chromosome alterations. In contrast, progressors developed marked chromosome instability (CIN) including whole genome doublings (WGD) and genome-wide gains and losses of whole chromosomes, and large regions of chromosome beginning 24-48 weeks before the diagnosis of EA,

Study 2 (Prevention). Our recent study of chemoprevention with aspirin or other NSAIDs was funded in response to the NCI Provocative Question: "Given the evidence that some drugs commonly and chronically used for other indications, such as an anti-inflammatory drug, can protect against cancer incidence and mortality, can we determine the mechanism by which any of these drugs work?" (Funding: P01 CA091955, NIH Provocative Question Grant R01 CA179949).

We hypothesized that use of aspirin and other NSAIDs exerted their protective effects through an evolutionary mechanism: decreasing the mutation rate in the BE epithelium. We tested this hypothesis in a well-designed study of NSAID users vs. nonusers with input collaboration and joint authorship from epidemiology and biostatistics colleagues. This study found that differences in 96-trinuceotide somatic base substitutions between NSAID users and nonusers were highly significant ($p<3\times10^{-16}$). The study also found that two Cosmic Signatures

(1 and 17) were dominant in BE and were sensitive to NSAID use and smoking status. We also found that NSAID use decreased copy number alterations, especially copy gains, which are more common in EAs than BE. Finally, a pathway analysis found that NSAID users had significantly lower diversity of functional mutations in genes across nine pathways compared to non-users (p=0.007). Interestingly, the two pathways with the greatest reduction in mutations are known to interact with the immune system. These results have been recently published (Galipeau et al *Genome Medicine Feb 27; 10(1): 17, 2018)* and published in the Science Spotlight (http://www.fredhutch.org/en/news/spotlight/2018/04/hb_galipeau_genomemedicine.html). (Funding: NIH P01 CA091955; NCI Provocative Question R01 CA179949)
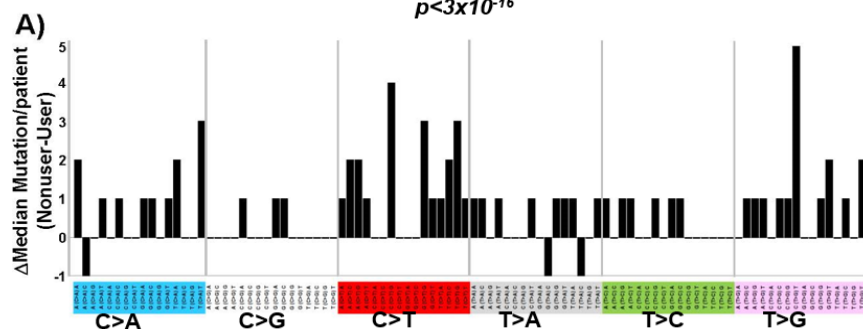

Figure 2A. NSAID use and somatic mutations. Differences in 96-trinucleotide somatic base substitutions between NSAID users and non-users. The difference in median mutation load at each 96 trinucleotide somatic base substitution is shown between NSAID non-users and users. Medians above zero are higher in NSAID non-users.
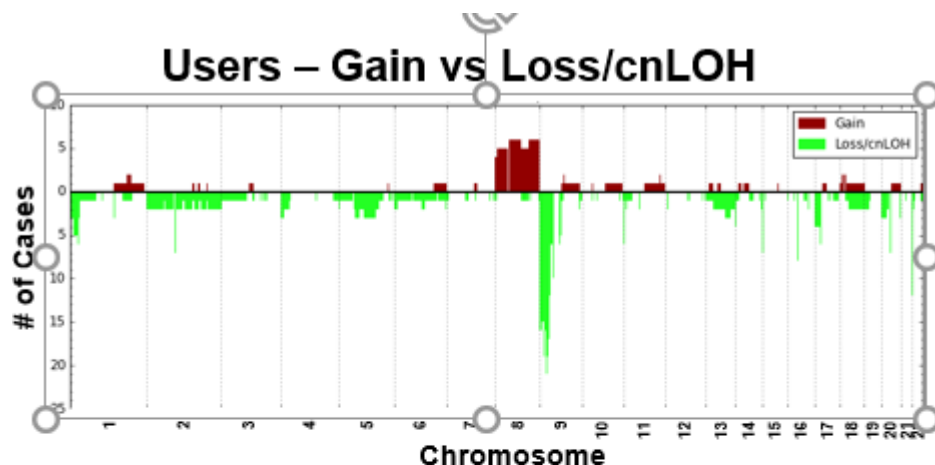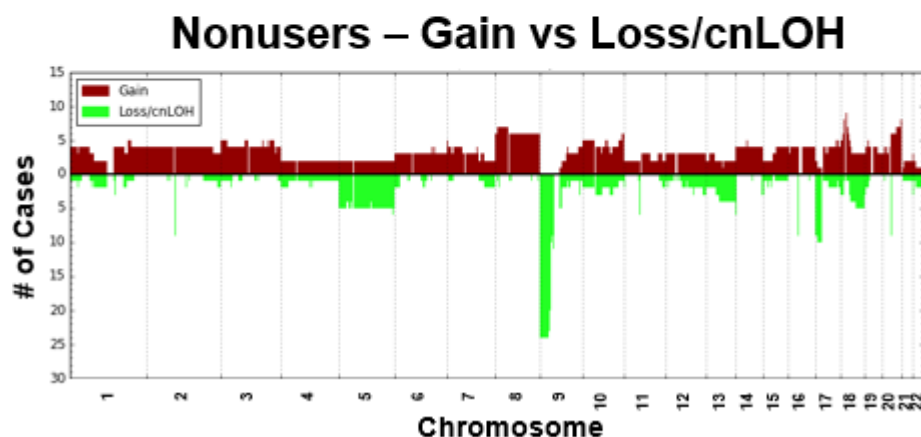

Figure 2B. NSAID use and copy number alterations, Red: Number of patients with allele specific copy gain, balanced gain, or high gain. Green: Any homozygous deletion, loss, or cnLOH. X axis: chromosome location. Y-axis: Number of samples with any event (41 user and 41 nonusers samples). Homozygous deletions are too small to be represented in this plot.

Study 3 (Early Detection and Therapy). This is a well-designed case-control study using Whole Genome Sequencing (WGS) to compare mutation rates in patients who p78rogressed from BE to EA compared to control patients with BE who did not progress. This study is currently in analysis. However, we have already discovered that patients that progress to EA continue to accumulate mutations in the BE segment while patients who do not progress to EA do not develop more mutations over time (Figure 3) (Funding: P01 CA091955).
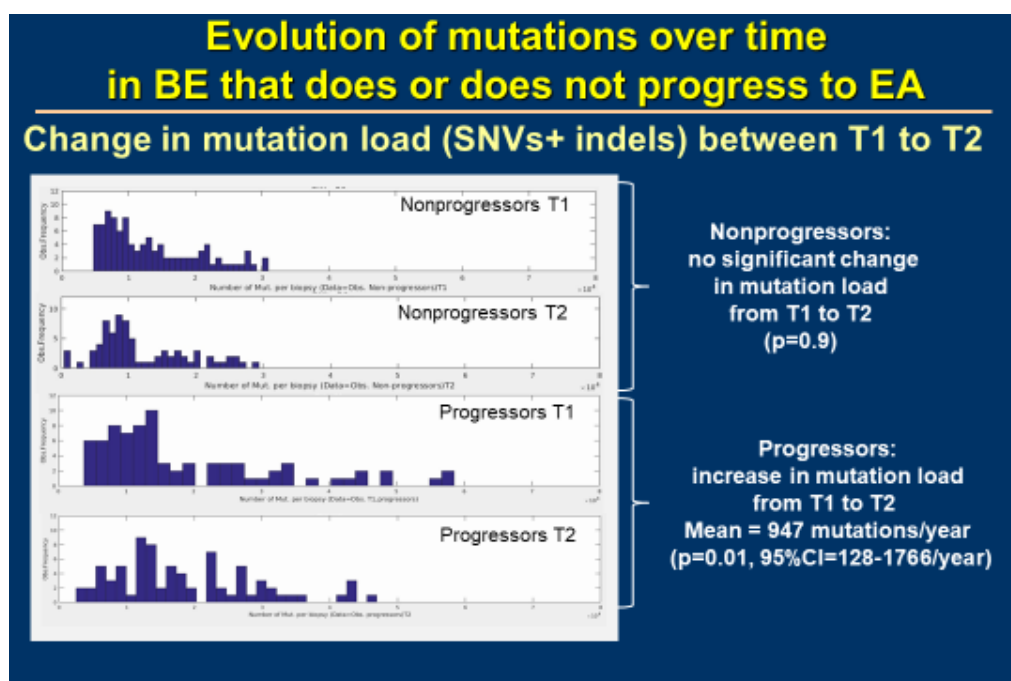
Figure 3. Whole Genome Sequencing in a case control study of 40 patients with Barrett's esophagus who progressed from BE to EA and 40 control patients with BE who did not progress to EA. The total mutation load (SNVs plus indels) remained stable in patients who did not progress to EA. However mutation load increased over time in patients who progressed to EA. The mean increase in mutation load was 947 mutations per year in progressors. This study is still being analyzed.
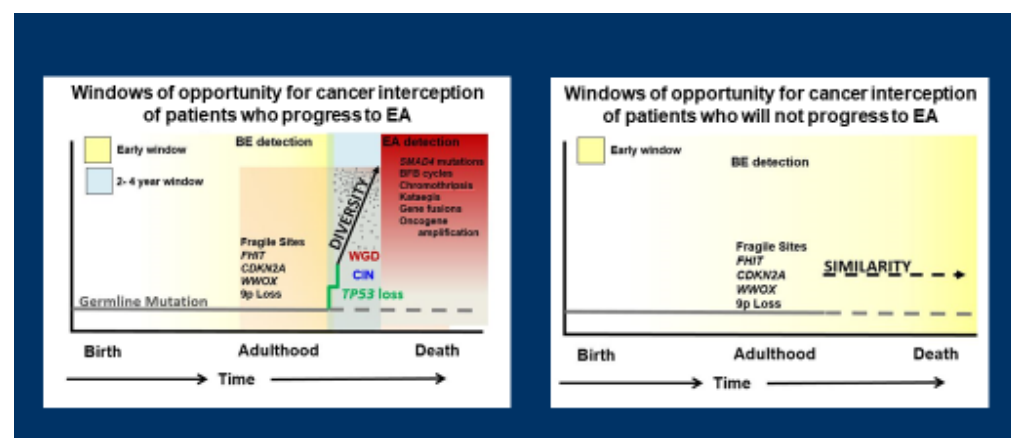

Figure 4. Summary: Progressors have about a 2-4 year window of opportunity for detection of TP53 mutation, CIN and WGD. In contrast non-progressors have similar early abnormalities but do not develop TP53, CIN and WGD.

Think Tanks, Leadership, Citizenship
Over the course of my career, I have been privileged to meet and work with investigators representing a broad range of disciplines to improve outcomes for patients who have or are at risk for developing cancer. These are all listed in my CV, but they include:

2004   NCI/NHGRI Workshop – "Exploring Cancer Through Genomic Sequence Comparisons"
2005-2007   NCI Translational Research Working Group – Co-chair Funding and Organization Committee
2008   Santa Fe Institute Working Group "Integrating Evolutionary Theory into Cancer Biology"
2008   Program Committee for NCI Translational Science Meeting
2008   NCI Think Tank: A New Look at Evolution and Evolutionary Theory in Cancer
2008   NCI Think Tank: The Coding, Decoding, Transfer, and Translation of Information in Cancer
2008   NCI/AACR Think Tank: Charting the Future of Cancer Prevention
2009-2013   NCI Intergovernmental Personnel Act Mobility Program with Division of Cancer Prevention
2010   NCI Think Tank: Rethinking the Role of Infectious Agents in Cancer
2011-2016   Esophageal Cancer Disease Working Group in the Cancer Genome Atlas (TCGA)
2011   National Cancer Institute, Gastric and Esophageal Cancer Meeting "Esophageal and Gastric Cancers, Solving Tough Problems"
2011   Center for Evolution and Cancer (Founding Member)

2011	International Evolution and Cancer Conference
2011- 2012	NCI DCP Think Tank "Defining the Molecularly-Informed Natural History of Occult Neoplasms Meeting," Organizing Committee
2014	Preventing Overdiagnosis, University of Oxford, UK
2014	MD Anderson Advisor: Epidemiology and Cancer Prevention Meeting
2014	EDRN Scientific Workshop, Panel Discussion Leader – Integrative Biology Session
2016	AACR Cancer Prevention Summit
2017	Publication of TCGA Esophageal Cancer Sequencing
2017	Special Session AACR National Meeting: Cancer Interception in Space and Time
2017	NCI Think Tank: Pre Cancer Atlas
2017	Introductory presentation, NCI Think Tank: PreCancerAtlas (PCA)
2017	Think Tank Panel Discussion: "Evolutionary Biomarkers: A Key Strategy for Precision Cancer Medicine"
2018	Think Tank: "Evolutionary Biomarkers II: Advancing the Field via Innovative Strategies and Novel Clinical Trials"

In summary, our team focuses on BE and EA as complex, adaptive systems that arise through genome instability, selection and evolution. We collaborate widely with other disciplines including epidemiologists, statisticians, math modelers, clinicians and others to improve the care of these patients.

SELECTED PUBLICATIONS (17 from a total of 193)

1. Hartwell, L.H., Culotti, J.C., Pringle, J.R. and Reid, B.J. Genetic control of the cell-division cycle in yeast: A model. Science 183:46-51, 1974. This was first genetic model of the eukaryotic cell cycle. This model became the basis for the 2001 Nobel Prize in Physiology or Medicine.

2. Galipeau, P.C., Cowan, D.S., Sanchez C.A., Barrett, M.T., Emond, M.J., Levine, D.S., Rabinovitch, P.S. and Reid, B.J. 17p (p53) allelic losses, 4N (G2/tetraploid) populations, and progression to aneuploidy in Barrett's esophagus. This was the first manuscript to report the spatial and temporal evolution of *TP53* loss, chromosome instability and genome doublings during progression to cancer. Proc Nat Acad Sci USA, 93: 7081-7084, 1996. This manuscript and our other research was prominently referenced in a recent publication reporting that the sequence of gains or losses of chromosome arms followed by genome doublings occurs commonly in human cancers (Carter et al Nature Biotechnology 2012; 30: 413). It is now recognized that nearly 40% of all human cancers studied by TCGA have undergone a whole genome doubling (Zach et al Nature Genetics 2013; 45: 1134).

3. Barrett, M.T., Sanchez, C.A., Prevo, L.J., Galipeau, P.C., Wong, D.J., Paulson, T.G., Rabinovitch, P.S. and Reid, B.J. Evolution of neoplastic cell lineages in Barrett's esophagus. Nature Genetics, 22: 106-109, 1999. This was the first model of genomic clonal evolution based on analyses over space and time in individuals at risk for cancer. It showed that clonal evolution was branching in contrast to linear models that have dominated clinical and cancer thought for more than a century. These branches underlie many aspects of cancer that we find frustrating clinically, including relapse after therapy, underdiagnosis and overdiagnosis. The method used in manuscripts 2 and 3 was used to construct phylogenetic trees based on somatic genomic alterations in advanced cancers (Gerlinger et al NEJM 2012; 366:883) and many others subsequently.

4. Reid, B.J., Levine, D.S., Longton, G., Blount, P.L. and Rabinovitch, P.S. Predictors of Progression to Cancer in Barrett's Esophagus: Baseline Histology and Flow Cytometry Identify Low and High Risk Patient Subsets. American Journal of Gastroenterology, 95:7 1669-76, 2000. To our knowledge, this was the first prospective cohort study to evaluate somatic genomic alterations (tetraploidy {whole genome doubling}, aneuploidy) as predictors of progression from a premalignant condition to cancer.

5. Maley, C.C., Galipeau, P.C., Li, X., Sanchez, C.A., Paulson, T.G., Blount P.L. and Reid, B.J. The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. Cancer Research 64: 7629-33, 2004. Prior to this manuscript, there was an intense debate in the literature as to whether genomic instability or clonal expansion was the key driver in progression to cancer. This manuscript found that both interacted to produce an increased risk of evolving to esophageal adenocarcinoma.

6. Vaughan, T.L., Dong, L.M., Blount, P.L., Ayub, K., Odze, R.D., Sanchez, C.A., Rabinovitch, P.S. and Reid, B.J. Non-steroidal anti-inflammatory drugs and risk of neoplastic progression in Barrett's oesophagus: a prospective study. Lancet Oncol 6: 945-52, 2005. This manuscript found that use of aspirin and other NSAIDs was associated with decreased risk of progression to somatic genomic abnormalities (tetraploidy and aneuploidy) as well as esophageal adenocarcinoma.

7. Maley, C.C., Galipeau, P.C., Finley, J.C., Wongsurawat, V.J., Li, X, Sanchez, C.A., Paulson, T.G., Blount, P.L., Rabinovitch, P.S. and Reid, B.J. Genetic clonal diversity predicts progression to esophageal adenocarcinoma, Nature Genet 38: 468-473, 2006. This paper reported that clonal genetic diversity, a measure derived from evolutionary biology, was an independent risk factor for progression from Barrett's esophagus to esophageal adenocarcinoma. This manuscript was subsequently cited in a study of breast cancer that highlighted the importance of genetic diversity as a cause of intratumor heterogeneity to more effectively plan cancer treatment strategies (Park et al J. Clin Invest 2010; 120:636). This concept that somatic genetic diversity is the fuel for cancer evolution is now well established for many other human cancers.

8. Galipeau, P.C., Li, X., Blount, P.L., Maley, C.C., Sanchez, C.A., Odze, R.D., Ayub, K, Rabinovitch, P.S., Vaughan, T.L. and Reid, B.J. NSAIDs Modulate CDKN2A, TP53, and DNA Content Risk for Progression to Esophageal Adenocarcinoma. PLoS Medicine 4: 342-54, 2007. This was a ten-year prospective cohort study in which we found that 9p LOH, 17p LOH and DNA content abnormalities, assessed by STR polymorphisms and DNA content flow cytometry, identified individuals with Barrett's esophagus at high and low risk for progression to esophageal adenocarcinoma. We also found that the 10 year risk of progression to cancer could be reduced from 80% to 30% by use of aspirin and other NSAIDs even in very high risk patients with advanced genomic abnormalities in their Barrett's esophagus.

9. Reid, B.J., Li, X. Galipeau, P.C. and Vaughan, T.L. "Barrett's Oesophagus and Oesophageal Adenocarcinoma: time for a new synthesis" Nat. Rev. Cancer 10: 87-101, 2010. PMCID: PMC2879265. In this manuscript, we reviewed the world literature on Barrett's esophagus and esophageal adenocarcinoma to identify the overarching problems and critical barriers to progress in prevention and early detection of this highly lethal cancer. We found that current clinical and research paradigms lead to overdiagnosis of slowly or non-progressing Barrett's esophagus and underdiagnosis of life threatening early esophageal adenocarcinoma. We then proposed a global research strategy to develop risk models to reduce both over- and underdiagnosis. The research and risk models for the general population and primary care settings have been addressed using the international BEACON consortium of which Dr. Reid is a founding member.

10. Esserman, L.J., Thompson, I.M. and Reid, B.J. Addressing the Emergence of Overdiagnosis and Overtreatment in Cancer: An Opportunity for Improvement and Change. Journal of the American Medical Association 310: 797-798, 2013. PMID 23896967. Altmetric, which tracks blogs, social media, government policy documents, newspapers, magazines, and downloads, ranked this manuscript as 31st of the top 100 scholarly articles that received the most public attention in 2013. This article was also featured on the front page of the New York Times (July 30, 2013).

11. Li, X., Galipeau, P.C., Paulson, T.G., Sanchez, C.A., Arnaudo, J., Liu, K., Sather, C.L., Kostadinov, R.L., Odze, R.D., Kuhner, M.K., Maley, C.C., Self, S.G., Vaughan, T.L., Blount, P.L., and Reid B.J. Temporal and spatial evolution of somatic chromosomal alterations: A case-cohort study of Barrett's

esophagus. Cancer Prev Res 7(1): 114-127, 2014. PMCID: PMC 3904552. This was the first manuscript to evaluate the evolving neoplastic genome over both space and time in patients who do and do not progress to cancer. The article became the basis for an article by Yael Waknine in MEDSCAPE (December 23, 2013). Barrett's that did not progress to cancer typically had only small, localized changes that remained stable without generating diversity over long periods of follow-up. In contrast, Barrett's esophagus that progressed to cancer was characterized by *punctuated* gains or losses of whole chromosomes or chromosome arms beginning about four years prior to the diagnosis of cancer followed by *catastrophic genome doublings* beginning about two years prior to cancer diagnosis. Recent studies have inferred a similar sequence of events in many other cancers based on data at one point in space and time (See for example, Carter et al Nature Biotechnology 30: 413, 2012). These recent papers acknowledge the seminal contribution of Galipeau et al Manuscript 2 above, which showed that 17p LOH could be detected in diploid biopsies and was highly associated with genome doubling events that were rapidly followed by progression to aneuploidy.

12. Li, X., Paulson, T.G., Galipeau, P.G., Sanchez, C.A. Liu, K., Kuhner, M.K., Maley, C.C., Self, S.G., Vaughan, T.L., Reid, B.J., and Blount, P.L. Assessment of Esophageal Adenocarcinoma Risk Using Somatic Chromosomal Alterations in Longitudinal Samples in Barrett's Esophagus. Cancer Prev Res 2015 September 8:845-856. PMCID:PMC 4560605. This manuscript reported that a risk model based on chromosome instability and evolution from Barrett's esophagus to esophageal adenocarcinoma as reported in the above manuscript (11 in "Selected publications") outperformed histopathologic assessment of dysplasia and flow cytometric DNA content in predicting future progression from Barrett's esophagus to esophageal adenocarcinoma.

13. Reid, B.J., Paulson, T.G., and Li, X. Genetic Insights in Barrett's Esophagus Adenocarcinoma. 2015 Gastroenterology 2015 Oct;149(5):1142-1152. PMCID:PMC 4589483.

14. Reid, B.J, Culotti, J.G., Nash, R.S., Pringle, J.R. Forty-five years of cell-cycle genetics. Mol Biol Cell. 2015 Dec 1;26(24):4307-12. PMCID:PMC 4666127. This invited retrospective reviewed the history of the events that led to Lee Hartwell's 2001 Nobel Prize in physiology or medicine. It provided a vehicle to report the contributions of a large number of scientists that enriched our research on the yeast cell cycle and made an excellent eukaryotic model system.

15. Kuhner MK, Kostadinov R, Reid BJ. Limitations of the Driver/Passenger Model in Cancer Prevention. Cancer Prev Res (Phila). 2016 May;9(5):335-8. doi: 10.1158/1940-6207.CAPR-15-0343. PubMed PMID: 26932841; PubMed Central PMCID: PMC4856031. This manuscript challenged the concept that mutations could be categorized into two classes (passengers/drivers) and proposed a 4 tier model.

16. Integrated genomic characterization of oesophageal carcinoma. Cancer Genome Atlas Research Network. Nature. 2017 541(7636): 169-175. doi: 10.1038/nature20805. PubMed PMID: 28052061.

17. Galipeau, P.C., Oman, K.M., Paulson, T.G., Sanchez, C.A., Zhang, Q., Marty, J.A., Delrow, J.J., Kuhner, M.K., Vaughan, T.L., Reid, B.J., and Li, X. NSAID use and somatic exomic mutations in Barrett's Esophagus. Genome Medicine Feb 27; 10(1):17, 2018.

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HOST-PATHOGEN INTERACTIONS AND GENETIC DIVERSITY OF HUMAN PATHOGEN, *HELICOBACTER PYLORI*

In the mid 1990's, a bacterium, *Helicobacter pylori*, was linked to gastric cancer, the third leading cancer killer worldwide. *H. pylori* establishes lifelong infection in the stomach of half the human population world-wide. The consequence of this infection ranges from undetected gastritis (inflammation of the stomach), to ulcer disease, and gastric cancer. Our lab is interested in the mechanisms by which this bacterium can establish and maintain a chronic infection and the molecular cross talk between the host and the bacteria during the decades-long infection that can lead to disease. Our current projects include:

1. ***H. pylori* genomic diversity:** *H. pylori* clinical isolates show extensive heterogeneity in both sequence and the presence and absence of whole genes. Even in the context of a single human stomach there exist multiple clones with unique gene complements. We are currently investigating how this diversity is generated and the consequences of this diversity on bacterial properties related to pathogenesis and patient outcome. This includes efforts to track genetic changes that accumulate during chronic infection of humans using noninvasive samples (like stool), which we test with molecular methods (like ddPCR) to track virulence and antibiotic resistance genes.

2. **Cell wall modification and cell shape:** Shape mutants (straight or slightly curved rods instead of helical rods) have stomach colonization defects in our mouse infection model. Most of these cell shape factors alter the peptide content of the peptidoglycan cell wall. We are testing motility in viscous solutions, susceptibility to various stresses and peptidoglycan-mediated innate immune signaling to tease out how these proteins and cell morphology contribute to survival in the stomach. To understand how changes in cell wall peptides drive shape changes at size scale of the cell, we are using super-resolution microscopy of cell shape proteins and cell wall synthesis probes combined with mathematical modeling to understand how *H. pylori* builds its helical shape.

3. **Host tissue responses to chronic infection**: We have developed several bacterial mutant libraries, including random transposon mutant libraries and a sequenced-defined mutant library encompassing most non-essential genes. We are using these libraries in a variety of *in vitro* and *in vivo* systems to probe *H. pylori* phenotypes important for pathogenesis. We use gastric epithelial tissue culture cells and primary gastric tissue organoids to monitor wild-type and mutant bacteria binding to host cells and stimulation of host cell signaling pathways, including those activating innate immunity and host cell morphological changes. To understand bacterial-host interactions in the complex environment of the stomach, which includes many cell types, we employ a mouse model of infection, testing both wild-type mice and genetically modified mice with altered immune pathways or stomach epithelial differentiation. This allows us to look at the relative fitness of different bacterial mutants, their location within the gastric epithelium, and their ability to induce host inflammation and pathology associated with gastric cancer.

SELECTED PUBLICATIONS

1. Jackson, LK, Potter B,.Schneider, S. Fitzgibbon M, Blair K, Farah H, Krisna U, Bedford T, Peek RM, Salama NR, *Helicobacter pylori* diversification during chronic infection within a single host generates sub-populations with distinct phenotypes. bioRxiv, 2020: 2020.06.10.144071 ; doi: https://doi.org/10.1101/2020.06.10.144071

2. Taylor JA, Bratton BP, Sichel SR, Blair KM, Jacobs HM, DeMeester KE, Kuru E, Gray J, Biboy J, VanNieuwenhze MS, Vollmer W, Grimes CL, Shaevitz JW, Salama NR. 2020. Distinct cytoskeletal proteins define zones of enhanced cell wall synthesis of *Helicobacter pylori*. *eLife* 9:e52482. PMC7012605

3. Yang DC, Blair KM, Taylor JA, Petersen TW, Sessler T, Tull CM, Leverich CK, Collar AL, Wyckoff TJ, Biboy J, Vollmer W, **Salama NR**. 2019. A genome-wide *Helicobacter pylori* morphology screen uncovers a membrane spanning helical cell shape complex. *J. Bacteriol.* 201(14). pii: e00724-18. doi: 10.1128/JB.00724-18. Print 2019 Jul 15

4. Blair KM, Mears KS, Taylor JA, Fero J, Jones LA, Gafken PR, Whitney JC, Salama NR**. 2018. The *Helicobacter pylori* cell shape promoting protein Csd5 interacts with the cell wall, MurF, and the bacterial cytoskeleton. *Mol Microbiol.* 2018Oct;110(1):114-127. doi: 10.1111/mmi.14087. Epub 2018 Sep 28

5. Sun L, Talarico S, Yao L, He L, Self S, You Y, Zhang H, Zhang Y, Guo Y, Liu G, Salama NR, Zhang J. 2018. Droplet Digital PCR detection of *Helicobacter pylori* clarithromycin resistance reveals frequent heteroresistance. *J Clin Microbiol.* 56:e00019-18. *https://doi.org/10.1128/JCM.00019-18*. PMC6113488

6. Talarico S, Leverich C, Wei B, Ma J, Cao X, Guo Y, Han G, Yao L, Self S, Zhao Y, Salama NR. 2018. Increased *H. pylori* stool shedding and EPIYA-D *cagA* alleles are associated with gastric cancer in an East Asian hospital. PLoS ONE 13(9): e0202925. PMC6135355

7. Gall A, Gaudet RG, Gray-Owen SD, Salama NR. TIFA Signaling in Gastric Epithelial Cells Initiates the *cag* Type 4 Secretion System-Dependent Innate Immune Response to *Helicobacter pylori* Infection. *MBio.* 2017 Aug 15;8(4). pii: e01168-17. doi: 10.1128/mBio.01168-17. PMC5559637

8. Gall A, Fero J, McCoy C, Claywell BC, Sanchez CA, Blount PL, Li X, Vaughan TL, Matsen FA, Reid BJ, Salama NR. 2015. Bacterial Composition of the Human Upper Gastrointestinal Tract Microbiome Is Dynamic and Associated with Genomic Instability in a Barrett's Esophagus Cohort. *PLOS One* 10(6):e0129055. doi: 10.1371/journal.pone.0129055. PMC4468150

9. Sycuro LK, Pincus Z, Gutierrez KD, Biboy J, Stern CA, Vollmer W, Salama NR. 2010. Pepti dogly can crosslinking relaxation promotes *Helicobacter pylori's* helical shape and stomach colonization. Cell. 141(5)822-33

10. Dorer MS, Fero J, Salama NR. 2010. DNA damage triggers genetic exchange in Helicobacter pylori. PLOS Pathogens. 6(7)e1001026

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CHEMICAL BIOLOGY, DRUG DISCOVERY AND TRAGET VALIDATION

The overarching goal of research in the Simon laboratory is the development of small molecules as mechanistic probes for a variety of cellular processes linked to disease and as potential lead compounds drug development. To this end we use interdisciplinary approaches ranging from chemical synthesis and medicinal chemistry to pharmacology, genetics and cell biology. The compounds we are studying have been identified from large collections of synthetic, drug-like compounds and from natural sources. A majority of drug screens are phenotypic and unbiased in terms of specific targets. While screening compound libraries is a significant part of what we do the majority of our efforts go into target identification, mechanistic studies to understand the biology and pharmacology of lead compounds and target validation.

The clinical use of several therapeutically important drugs is limited because of drug-induced hearing loss. Aminoglycoside antibiotics (e.g. amikacin) and platinum-based cancer drugs (e.g. cisplatin) are among drugs that can cause significant and in many cases irreversible hearing loss due to selective toxicity to the auditory hair cells. The mechanism of hair cell death, also called ototoxicity, is poorly understood. In collaboration with Ed Rubel's laboratory at the Bloedel Center for Hearing Research and Department of Otolaryngology and Dave Raible's laboratory in the Department of Biological Structure both at the University of Washington, we carried out a screen using zebrafish mechanosensory hair cells as a model for mammalian auditory hair cells. This screen identified a family of small molecule inhibitors of aminoglycoside-induced hair cell death. Dr. Rubel's laboratory showed that our lead compound, PROTO1, also protects rat auditory hair cells and preserves hearing following doses of kanamycin that induce significant hearing loss in control animals. We have optimized the protective activity as well as pharmacological properties of PROTO compounds through medicinal chemistry and structure activity relationship (SAR) studies. An optimized analogue of PROTO-1 called ORC-13661 recently completed phase I safety trials and is about to enter phase 2 efficacy trials in patients with cystic fibrosis and non-tuberculous mycobacterium lung infection. Preliminary studies with ORC-13661 indicate that the compound also protects mammalian auditory hair cells against cisplatin-induced death.

Julian Simon, PhD

We previously identified inhibitors of yeast (*S. cerevisiae*) and human NAD-dependent deacetylases using yeast cell-based phenotypic screens. In humans, there are seven NAD-dependent deacetylases called the sirtuins. These ubiquitous enzymes have been shown to play roles in functions ranging from transcriptional modulation to DNA damage responses and modulators of specific sirtuins have been suggested as therapeutic agents for a variety of human disease. In collaboration with Toni Bedalov's laboratory (FHCRC), we are working to optimize our sirtuin-2 inhibitors using medicinal chemistry strategies for use as anti-lymphoma therapeutics. Sirtuin 2, or SIRT2, plays a unique role in the biology of B-cell development. Immature B-cell precursors have to undergo genetic rearrangements to mount an antigen-specific response to pathogens. The genetic rearrangements, such as V(D)J recombination and somatic hypermutation, would normally be perceived as DNA damage and lead to apoptosis were it not for the suppression of the DNA damage response regulator p53 and upregulation of the transcriptional repressor BCL6. SIRT2-mediated deacetylation of p53 and BCL6 accomplishes these functions. In B-cell lymphoma, mutation of histone acetyl transferases (HATs) and consequent hypo-acetylation of p53 and BCL6 accomplishes the same ends allowing DNA damage to go undetected. Inhibition of SIRT2 is B-cell lymphoma harboring HAT mutations restores p53 and BCL6 acetylation homeostasis and leads to cell death. We hope small molecule SIRT2 inhibitors will be effective B-cell lymphoma therapeutics.

SELECTED PUBLICATIONS

Chowdhury, S., Sripathy, S., Webster, A.A., Park, A., Lao, U., Hsu, J.H., Loe, T., Bedalov, A., Simon, J.A., "Discovery of Selective SIRT2 Inhibitors as Therapeutic Agents in B-Cell Lymphoma and Other Malignancies" *Molecules* **25,** 455; doi:10.3390/molecules25030455, 2020.

Chowdhury, S., Owens, K.N., Herr, J.R., Jiang, Q., Chen, X., Johnson, G., Groppi, V.E., Raible, D. W., Rubel, E.W, Simon, J.A., "Phenotypic Optimization of Urea-Thiophene Carboxamides To Yield Potent, Well-Tolerated, and Orally Active Protective Agents against Aminoglycoside-Induced Hearing Loss." *Journal of Medicinal Chemistry* **61**: 84-97. doi: 10.1021/acs.jmedchem.7b00932.2017, NIHMS ID#: NIHMS953119, 2017.

Mahajan, S.S., Scian, M., Sripathy, S., Posakony, J., Lao, U., Loe, T.K., Leko, V., Thalhofer, A., Schuler, A.D., Bedalov, A., Simon, J.A., "Development of Pyrazolone and Isoxazol-5-one Cambinol Analogs as Sirtuin Inhibitors" *Journal of Medicinal Chemistry* **57**:3283-94, DOI: 10.1021/jm401064 PMID: 24697269, PMCID# PMC4002067, 2014.

Thomas, A.J., Hailey, D.W., Stawicki, T.M., Wu, P., Coffin, A.B., Rubel, E.W., Raible, D.W., Simon, J.A., Ou, H.C. "Functional mechanotransduction is required for cisplatin-induced hair cell death in zebrafish lateral line" *Journal of Neurosci*ence **33**, 4405-14. doi: 10.1523/JNEUROSCI.3940-12.2013. PMID: 23467357, 2013.

Owens, K.N., Santos, F., Roberts, B., Linbo, T., Coffin, A.B., Knisley, A.J., Simon, J.A., Rubel, E.W., and Raible, D.W. "Identification of Genetic and Chemical Modulators of Zebrafish Mechanosensory Hair Cell Death" PLoS Genetics **4**(2):e1000020, PMCID: PMC2265478, 2008.

Heltweg, B., Gatbonton, T., Schuler, A. D., Posakony, J., Li, H., Goehle, S., Kollipara, R., DePinho, R. A., Gu, Y., Simon, J. A., Bedalov A., "Antitumor activity of a small molecule inhibitor of human Silent Information Regulator 2 enzymes" *Cancer Res*earch **66:**4368-77, 2006.

Posakony, J., Hirao, M., Stevens, S., Bedalov, A., and Simon, J.A., "Inhibitors of Sir2p: Evaluation of Splitomicin Analogues" *Journal of Medicinal Chemistry* **47:**2635, 2004.

Bedalov, A., Gatbonton, T., Irvine, W.P., Gottschling, D.E., and Simon, J.A., "Identification of a small molecule inhibitor of Sir2p" *Proceedings of the National Academy of Sciences USA,* **98:**15113-15118, 2001.

Li, K.W., Wu, J., Xing, W., and Simon, J.A., "Total Synthesis of the Anti-tumor Depsipeptide FR-901", 228." *Journal of the American Chemical Society*, **118:**7237-7238, 1996.

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Immunotherapy using CAR-T cells has shown impressive efficacy in B cell malignancies but has largely been ineffective when targeting antigens on epithelial cancers, which account for 80-90 percent of all cancers. The Srivastava lab is focused on using clinically relevant animal models of human cancers to define the mechanisms limiting the activity of CAR- and TCR-engineered T cells in solid tumors and to evaluate strategies to overcome these barriers. A major focus of the lab is in understanding the principles that govern basic T cell trafficking, persistence, and activity and applying these principles to engineer more effective adoptive T cell therapies for human cancers. Our long-term goal is to work with clinical colleagues and industry partners to translate the most promising strategies to the clinic.

Developing genetically-engineered mouse models to study CAR-T cell therapy for solid tumors
Transplantable and tumor xenograft models lack clinically relevant tumor microenvironments (TME), making it difficult to study the mechanisms that limit activity of transferred T cells in solid tumors. We recently adapted the $Kras^{LSL-G12D/+}p53^{fl/fl}$ (KP) genetically engineered mouse (GEM) model of non-small cell lung cancer, which mimics the initiation, progression, and immunosuppressive TME of human lung cancer, to express a target for CAR-T cells, and demonstrated that this model mirrors many of the barriers to effective CAR-T cell therapy observed in patients, including poor CAR-T cell infiltration into tumors and acquired dysfunction. Using this model, we identified a novel lymphodepletion regimen that induces immunogenic tumor cell death and activates tumor macrophages to express T cell-recruiting chemokines, resulting in improved CAR-T cell infiltration, remodeling of the TME, and increased tumor sensitivity to anti-PD-L1 checkpoint blockade and improved survival. However, the infiltrating CAR-T cells still became dysfunctional over time, suggesting that additional strategies to recruit greater numbers of T cells that retain function are needed to achieve durable efficacy. Current research in the lab is focused on addressing two major barriers to effective T cell therapy:

1) Improving trafficking of engineered T cells to solid tumors
Inefficient trafficking of CAR-T cells into solid tumors is a barrier to efficacy in both humans and mice, but it is unclear why migration of CAR-T cells into non-lymphoid tissues is so poor or how trafficking can be enhanced. Conventional T cells acquire their tissue-homing potential in draining lymph nodes (LN), where interactions with antigen-presenting cells imprint specific homing receptor expression patterns on antigen-specific T cells that direct them towards specific tissues. By contrast, CAR-T cells only encounter their cognate antigen on tumor cells and do not receive this instructive programming in the periphery prior to tumor entry. Indeed, preliminary

experiments indicate that tumor-specific conventional T cells (Tconv) strongly out-compete CAR-T cells in infiltrating the same lung tumors, indicating a fundamental impairment in the ability of CAR-T cells to access solid tumors. We hypothesize that, relative to Tconv, CAR-T cells are inferior at accessing solid tumors in non-lymphoid tissues because they do not receive activating and/or co-stimulatory signals from APCs prior to tumor entry. Current work is focused on understanding: 1) the signals normally delivered to conventional T cells that mediate successful trafficking to the lung, and 2) whether delivering these signals to CAR-T cells can improve their infiltration into solid tumors and subsequent tumor control.

2) Preserving the function of engineered T cells in immunosuppressive tumor microenvironments
The pathways mediating the development of T cell dysfunction and "exhaustion" are beginning to be elucidated, and recent work suggests that exhausted CAR-T cells exhibit an imbalance in AP-1 family transcription factors, with a relative deficiency in the AP-1 subunit c-Jun that drives inhibitory signaling. We found that overexpression of c-Jun significantly improves the function and persistence of CAR-T cells in KP mice, but anti-tumor activity is only enhanced in a subset of mice. Our work is focused on understanding: 1) the mechanisms by which c-Jun improves CAR-T cell activity, 2) what pathways mediate tumor progression in a subset of mice, and 3) what combination therapies synergize with c-Jun overexpression to durably enhance CAR-T cell activity.

SELECTED PUBLICATIONS

Srivastava S, Salter AI, Liggitt D, Yechan-Gunja S, Sarvothama M, Cooper K, Smythe KS, Dudakov JA, Pierce RH, Rader C, Riddell SR. Logic-Gated ROR1 Chimeric Antigen Receptor Expression Rescues T Cell-Mediated Toxicity to Normal Tissues and Enables Selective Tumor Targeting. *Cancer Cell*. 2019 Mar 18; 3(35): 489-503.

Srivastava S, Riddell SR. Chimeric Antigen Receptor T Cell Therapy: Challenges to Bench-to Bedside Efficacy. *Journal of Immunology*. 2018 Jan; 200(2): 459-468.

Paszkiewicz PJ, Fräβle SP, **Srivastava S**, Sommermeyer D, Hudecek M, Drexler I, Sadelain M, Liu L, Jensen MC, Riddell SR, Busch DH. Targeted antibody-mediated depletion of CD19 CAR-T cells permanently reverses B cell aplasia. *Journal of Clinical Investigation*. 2016 Nov: 126(11): 4262-4272.

Srivastava S, Riddell SR. Engineering CAR-T Cells: Design Concepts. *Trends in Immunology*. 2015 Aug; 36(8): 494-502.

Srivastava S, Koch LK, Campbell DJ. IFNαR signaling in effector but not regulatory T cells is required for immune dysregulation during type I IFN-dependent inflammatory disease. *Journal of Immunology*. 2014 Sep; 193(6): 2733-42.

Srivastava S, Koch MA, Pepper M, Campbell DJ. Type I interferons directly inhibit regulatory T cells to allow optimal antiviral T cell responses during acute LCMV infection. *Journal of Experimental Medicine,* 2014 May; 211(5): 961-74.

Smigiel KS*, **Srivastava S*,** Stolley JM, Campbell DJ. Regulatory T cell homeostasis: steady-state maintenance and modulation during inflammation. *Immunological Reviews.* 2014 May; 259(1): 40-59. ***These authors contributed equally.**

Smigiel KS, Richards E, **Srivastava S**, Thomas KR, Klonowski KD, Campbell DJ. CCR7 provides localized access to IL-2 and defines homeostatically distinct regulatory T cell subsets. *Journal of Experimental Medicine.* 2014 Jan; 211(1): 121-136.

Koch MA, Thomas KN, Perdue NR, Smigiel KS, **Srivastava S**, Campbell DJ. T-bet(+) Treg cells undergo abortive Th1 cell differentiation due to impaired expression of IL-12 receptor B2. *Immunity.* 2012 Sep; 37(3): 501-10.

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Understanding the Metabolic Constraints of Cell Proliferation

Cancer cells have altered cell metabolism compared to the parental cells from which they arise. To maintain aberrant proliferation, cancer cells enact changes in metabolic fluxes to support the increased synthesis of proteins, nucleotides and lipids needed to replicate cell biomass and divide. Thus, exploiting the metabolic differences between normal cells and cancer cells is a promising approach to improve cancer therapy. Indeed, many chemotherapeutic agents are cytotoxic due to their ability to interfere with metabolic pathways in cancer cells. The fact that these treatments can be curative provides strong evidence that cancer cells have targetable metabolic vulnerabilities, making it critical to better understand how cell metabolism supports proliferation and to determine which metabolic pathways are required for cancer growth. My laboratory uses mass spectrometry, isotopic tracing, metabolic flux measurements, and cancer models to broadly understand how metabolism supports cell proliferation.

The current goals of my laboratory range from testing metabolic targets in preclinical cancer models to discovery of novel metabolic interactions and pathways:

1. Investigating mechanisms of aspartate metabolism for cancer therapy

The amino acid aspartate is a critical substrate for protein and nucleotide synthesis that must be synthesized intracellularly to support cancer cell proliferation. Aspartate production is metabolically costly, and our work has shown that bolstering aspartate levels in cancer cells in vivo can increase tumor growth rate. These data indicate aspartate is an endogenous metabolic limitation for tumor growth and that any further suppression of aspartate would therefore inhibit cancer growth. Since aspartate levels are dependent on various synthesis pathways and metabolic fates in different conditions, all of which can modified by changes in gene expression and signaling, we will seek to understand how different cancer relevant biological processes including

signaling cascades, metabolic adaptations, and drug sensitivities converge on aspartate metabolism to promote tumor growth and sensitize tumors to therapies.

2. Determining the roles of coenzyme homeostasis to support proliferative metabolism

While metabolism is classically viewed through the lens of efficient ATP production, maximization of biomass synthesis in proliferation requires balancing several other coenzyme systems in addition to ATP. Our work has shown that maintenance of NAD+/NADH by mitochondrial respiration is essential for supporting proliferation, however, there are many other coenzyme systems in cells, e.g. NADP+/NADPH, Biopterins, ubiquinone/ubiquinol, etc., that are interrelated and are also used to support biosynthesis. Further complication arises when considering that these coenzymes are also compartmentalized into organelles which have distinct roles in using these coenzymes to support local and global metabolism. Thus, we will determine how modifying coenzyme homeostasis causes network effects throughout metabolism and how these consequences can affect cell proliferation.

3. Discovery of metabolic products and pathways

The canonical map of metabolic reactions is often perceived as a complete list of all reactions that occur in all human cells. However, there is no reason to assume that this list is comprehensive in all tissue types and conditions. Our work has identified previously uncharacterized metabolites in a subset of cancer cells, providing a proof of concept that there may be many metabolites yet to be discovered. Using state-of-the-art mass spectrometry and a novel isotopic tracing technique we will seek to identify new metabolic products, and potentially entire new metabolic pathways, with the hope of identifying biomarkers and metabolic modifiers of disease.

SELECTED PUBLICATIONS

Quon E, Hart ML, **Sullivan LB.** Redox debt leads to metabolic bankruptcy in tumors. *Trends Cancer.* 2020 May; 6(5):359-61

Sullivan LB. (2018) Metabolic frugality marks cancer cells for immune targeting. *Cell*. 2018 Sep 6; 275(6):1344-1346.

Sullivan LB*, Luengo A, Danai LV, Bush LB, Diehl FF, Hosios AM, Lau AN, Elmiligy S, Malstrom S, Lewis CA, Vander Heiden MG*. (2018) Aspartate is an Endogenous Metabolic Limitation for Tumour Growth. *Nature Cell Biology*. Jul;20(7):782-788 (*Co-Corresponding Author).

Sullivan LB. Extracellular Vesicles: Taking Metabolism on the Road. (2017) *Nature Chemical Biology*. Aug 18;13(9):924-925.

Sullivan LB, Gui DY, Vander Heiden MG. (2016) Altered metabolite levels in cancer: implications for tumor biology and cancer therapy. *Nature Reviews Cancer*. Nov;16(11):680-693.

Gui DY*, **Sullivan LB***, Luengo A, Davidson SM, Hosios AM, Bush LB, Gitego N, Thomas CJ, Vander Heiden MG. (2016) Environment dictates dependence on mitochondrial complex I for NAD+ and aspartate production and determines cancer cell sensitivity to metformin. *Cell Metabolism*. Nov 8;24(5):716-727. (*Co-First Author).

Olenchock BA, Moslehi J, Baik AH, Davidson SM, Williams JL, Gibson WJ, Pierce KA, Miller CM, Hanse EA, Kelekar A, **Sullivan LB**, Wagers AJ, Clish CB, Vander Heiden MG, Kaelin WG, Jr. (2016) Inhibition of the EglN1 Oxygen Sensor and Rerouting of α-Ketoglutarate are Sufficient for Remote Ischemic Protection. *Cell*. Feb 25; 164(5):884-95.

Hecht VC*, **Sullivan LB***, Kimmerling RJ, Kim DH, Hosios AM, Stockslager MA, Stevens MM, Kang JH, Wirtz D, Vander Heiden MG, Manalis SR. (2016) Biophysical changes reduce energetic demand in growth factor-deprived lymphocytes. *Journal of Cell Biology*. Feb 15;212(4):439-47. (*Co-First Author).

Sullivan LB*, Gui DY*, Hosios AM, Bush LN, Freinkman E, Vander Heiden MG. (2015) Supporting aspartate biosynthesis is an essential function of respiration in proliferating cells. *Cell*. Jul 30; 162(3):552-63. (*Co-First Author).

Sullivan LB, Chandel NS. Mitochondrial reactive oxygen species and cancer. (2014) *Cancer & Metabolism*. Nov 28, 2:17.

Wheaton WW, Weinberg S, Hamanaka RB, Soberanes S, **Sullivan LB**, Anso E, Glasauer A, Dufour E, Mutlu GM, Budinger GRS, Chandel, NS. (2014) Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. *eLife*. May 13;3.

Sullivan LB, Garcia-Martinez E, Mullen AR, Nguyen H, Dufour E, Sudarshan S, Yang Y, Linehan WM, Licht JD, Deberardinis RJ, Chandel NS. (2013) The proto-oncometabolite fumarate binds glutathione to amplify ROS dependent signaling. *Molecular Cell*. Jul 25;51(2):236-48.

Mullen AR, Wheaton WW, Jin ES, Chen PH, **Sullivan LB**, Cheng T, Yang Y, Linehan WM, Chandel NS, DeBerardinis RJ. (2011) Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature*. Nov 20;481(7381):385-8.

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TRANSCRIPTIONAL REGULATION OF MYOGENESIS AND NEUROGENESIS IN NORMAL DEVELOPMENT AND DISEASE

Cell specification, differentiation, and trans-differentiation:
The conversion of a non-muscle cell into a skeletal muscle cell by the expression of the transcription factor MyoD was the first demonstration of genetically engineered trans-differentiation. We have been using this as a model system to study how a single initiating event, in this case the expression of the MyoD transcription factor, can orchestrate the chromatin and transcriptional changes necessary to switch cell specification, and how this process might be subverted in rhabdomyosarcomas, cancers that express MyoD but do not differentiate into muscle cells. Similar to myogenesis, neurogenesis is regulated by the related NeuroD transcription factors. We have been able to demonstrate that non-neuronal cells can be converted into neurons by the forced expression of NeuroD family members, and we are comparing MyoD and NeuroD factors to determine how they achieve distinct transcriptional programs despite having very similar DNA binding regions. These studies are beginning to show how master regulatory factors drive programs of cell differentiation.

DUX4 regulation of totipotency in development, muscular dystrophy, and cancer:
We have identified the double-homeodomain transcription factor DUX4 as a gene that drives expression of the totipotent signature in the early cleavage stage embryo at the time of the initial wave of zygotic gene activation. Mis-expression of DUX4 in skeletal muscle causes facioscapulohumeral dystrophy (FSHD), a common form of muscular dystrophy. DUX4 mis-expression in muscle is caused by the inefficient epigenetic repression of the DUX4-containing D4Z4 macrosatellite repeat on chromosome 4, either because of deletions that decrease the number of the macrosatellite units in the array or mutations in SMCHD1, a cohesion-family member protein that epigenetically represses repetitive regions, including the D4Z4 repeat array. Recently, we have also identified DUX4 expression in many solid cancers where it promotes immune evasion by down-regulating MHC Class I protein expression, possibly related to a normal role in the immune evasion of the early embryo. Future work seeks to further understand the role of DUX4 in normal development, FSHD muscular dystrophy, and cancer; as well as seeking mechanisms of suppressing DUX4 Tapression for therapeutic interventions.

SELECTED PUBLICATIONS

Diede SJ, Guenthoer J, Geng LN, Mahoney SE, Marotta M, Olson JM, Tanaka H, Tapscott SJ. DNA methylation of developmental genes in pediatric medulloblastomas identified by de-naturation analysis of methylation differences. *Proc Natl Acad Sci U S A*. 2010 Jan 5;107 (1):234-9. Epub 2009 Dec 4. PMCID: PMC2806770.

Geng LN, Yao Z, Snider L, Fong AP, Cech JN, Young JM, van der Maarel SM, Ruzzo WL, Gentleman RC, Tawil R, Tapscott SJ. DUX4 activates germline genes, retroelements, and immune mediators: implications for facioscapulohumeral dystrophy. *Dev Cell.* 2012 Jan 17;22 (1):38-51. Epub 2011 Dec 29. PMID: 22209328; PMCID: PMC3264808.

Fong AP, Yao Z, Zhong J-W, Cao Y, Ruzzo WL, Gentleman RC, Tapscott SJ. Genetic and epigenetic determinants of neurogenesis and myogenesis. *Dev Cell.* 2012; 22:721-735. PMID: 22445365; PMCID: PMC3331915.

MacQuarrie KL, Yao Z, Young JM, Cao Y, Tapscott SJ. miR-206 integrates multiple components of differentiation pathways to control the transition from growth to differentiation in rhabdomyosarcoma cells. *Skeletal Muscle*. 2012 Apr 29;2(1):7. PubMed PMID: 22541669; PMCID: PMC3417070.

Lemmers RJ, Tawil R, Petek LM, Balog J, Block GJ, Santen GW, Amell AM, van der Vliet PJ, Almomani R, Straasheijm KR, Krom YD, Klooster R, Sun Y, den Dunnen JT, Helmer Q, Donlin-Smith CM, Padberg GW, van Engelen BG, de Greef JC, Aartsma-Rus AM, Frants RR, de Visser M, Desnuelle C, Sacconi S, Filippova GN, Bakker B, Bamshad MJ, Tapscott SJ, Miller DG, van der Maarel SM. Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nat Genet.* 2012 Dec;44(12):1370-4. doi: 10.1038/ng.2454. Epub 2012 Nov 11. PubMed PMID: 23143600.

MacQuarrie KL, Yao Z, Fong AP, Diede SJ, Rudzinski ER, Hawkins DS, Tapscott SJ. Comparison of genome-wide binding of MyoD in normal human myogenic cells and rhabdomyo- sarcomas identifies regional and local suppression of promyogenic transcription factors. *Mol Cell Biol.* 2013 Feb;33(4):773-84. doi: 10.1128/MCB.00916-12. Epub 2012 Dec 10. PubMed PMID: 23230269; PubMed Central PMCID: PMC3571334.

Diede SJ, Yao Z, Keyes CC, Tyler AE, Dey J, Hackett CS, Elsaesser K, Kemp CJ, Neiman PE, Weiss WA, Olson JM, Tapscott SJ. Fundamental differences in promoter CpG island DNA hypermethylation between human cancer and genetically engineered mouse models of cancer. *Epigenetics.* 2013 Dec;8(12):1254-60.

Young JM, Whiddon JL, Yao Z, Kasinathan B, Snider L, Geng LN, Balog J, Tawil R, van der Maarel SM, Tapscott SJ. DUX4 binding to retroelements creates promoters that are active in FSHD muscle and testis. *PLoS Genet.* 2013 Nov;9(11):e1003947.

Yao Z, Snider L, Balog J, Lemmers RJ, Van Der Maarel SM, Tawil R, Tapscott SJ. DUX4-induced gene expression is the major molecular signature in FSHD skeletal muscle. *Hum Mol Genet.* 2014 May 26. PMID: 24861551.

Fong AP, Yao Z, Zhong JW, Johnson NM, Farr GH 3rd, Maves L, Tapscott SJ. Conversion of MyoD to a neurogenic factor: binding site specificity determines lineage. Cell Rep. 2015 Mar 31;10(12):1937-46. doi: 10.1016/j.celrep.2015.02.055. Epub 2015 Mar 19. PubMed PMID: 25801030.

Lim JW, Snider L, Yao Z, Tawil R, Van Der Maarel SM, Rigo F, Bennett CF, Filippova GN, Tapscott SJ. DICER/AGO-dependent epigenetic silencing of D4Z4 repeats enhanced by exogenous siRNA suggests

mechanisms and therapies for FSHD. Hum Mol Genet. 2015 Sep 1;24(17):4817-28. doi: 10.1093/hmg/ddv206. Epub 2015 Jun 3. PubMed PMID: 26041815; PubMed Central PMCID: PMC4527486.

Conerly ML, Yao Z, Zhong JW, Groudine M, Tapscott SJ. Distinct Activities of Myf5 and MyoD Indicate Separate Roles in Skeletal Muscle Lineage Specification and Differentiation. Dev Cell. 2016 Feb 22;36(4):375-85. doi:10.1016/j.devcel.2016.01.021. PubMed PMID: 26906734; PubMed Central PMCID: PMC4769793.

Whiddon JL, Langford AT, Wong CJ, Zhong JW, Tapscott SJ. Conservation and innovation in the DUX4-family gene network. Nat Genet. 2017 Jun;49(6):935-940. doi: 10.1038/ng.3846. Epub 2017 May 1. PubMed PMID: 28459454; PubMed Central PMCID: PMC5446306.

Hendrickson PG, Doráis JA, Grow EJ, Whiddon JL, Lim JW, Wike CL, Weaver BD, Pflueger C, Emery BR, Wilcox AL, Nix DA, Peterson CM, Tapscott SJ, Carrell DT, Cairns BR. Conserved roles of mouse DUX and human DUX4 in activating cleavage-stage genes and MERVL/HERVL retrotransposons. Nat Genet. 2017 Jun;49(6):925-934. doi: 10.1038/ng.3844. Epub 2017 May 1. PubMed PMID: 28459457.

Chew GL, Campbell AE, De Neef E, Sutliff NA, Shadle SC, Tapscott SJ, Bradley RK. DUX4 Suppresses MHC Class I to Promote Cancer Immune Evasion and Resistance to Checkpoint Blockade. Dev Cell. 2019 Jul 17. pii: S1534-5807(19)30529-5. doi:10.1016/j.devcel.2019.06.011. [Epub ahead of print] PubMed PMID: 31327741.

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CELL POLARITY AND CELL ADHESION IN MAMMALIAN DEVELOPMENT AND CANCER

Dr. Vasioukhin's laboratory has a long-standing interest in understanding the mechanisms of normal tissue homeostasis, transformation, tumor progression and metastasis. The laboratory focuses on mouse prostate gland epithelium and skin epidermis as primary in vivo research model systems. It is also extensively using primary human organoid cultures. The laboratory employs a combination of mouse genetic, molecular, and microscopic methods to uncover the mechanisms underlying cancer initiation and progression, as well as to explore new avenues for therapeutic treatments.

SELECTED PUBLICATIONS

Lien W-H, Klezovitch O, Fernandez T, Delrow J, Vasioukhin V. α-catenin controls cerebral cortical size by regulating the hedgehog signaling pathway. *Science.* 2006. 311(5767):1609-12.

Nechiporuk T, Fernandez T, Vasioukhin V.   Failure of epithelial tube maintenance causes hydrocephalus and renal cysts in Dlg5-/- mice. *Dev Cell,* 2007. Sep;13(3):338-50, 2007.

Klezovitch O, Risk M, Coleman I, Lucas J, Null M, True L, Nelson PS, Vasioukhin V.   A causal role for ERG in neoplastic transformation of prostate epithelium. *Proc Natl Acad Sci U S A.* 2008, 12;105(6):2105-10.

Sripathy S, Lee M, Vasioukhin V. Mammalian *Llgl2* is necessary for proper branching morphogenesis during placental development. *Mol Cell Biol.* 2011, 31(14):2920-33. Epub 2011 May 23.

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Schlegelmilch K, Mohseni M, Kirak O, Pruszak J, Rodriguez JR, Zhou D, Kreger BT, Vasioukhin V, Avruch J, Brummelkamp TR, Camargo FD. Yap1 Acts Downstream of α-Catenin to Control Epidermal Proliferation. *Cell,* 2011, March 4, 144, 782–795.

Silvis M., Kreger B., Lien W-H, Klezovitch O, Rudakova M, Camargo FD, Lantz D, Seykora JT and Vasioukhin V.   α-Catenin is a tumor suppressor that controls cell accumulation by regulating the Hippo pathway. *Science Signaling,* 2011, 24;4(174):ra33.doi:10.1126/scisignal. 2001823.

Nechiporuk T, Klezovitch O, Nguyen L, Vasioukhin V.   Dlg5 maintains apical aPKC and regulates progenitor differentiation during lung morphogenesis. *Dev Biol.* 2013 May 15;377(2):375-84.

Heidel FH, Bullinger L, Arreba-Tutusaus P, Wang Z, Gaebel J, Hirt C, Niederwieser D, Lane SW, Döhner K, Vasioukhin V, Fischer T, Armstrong SA. The cell fate determinant Llgl1 influences HSC fitness and prognosis in AML. *J Exp Med.* 2013 Jan 14;210(1):15-22.

Tang X, Mahajan SS, Nguyen LT, Béliveau F, Leduc R, Simon JA and Vasioukhin V.   Targeted inhibition of cell-surface serine protease Hepsin blocks prostate cancer bone metastasis. *Oncotarget.* 2014. Mar 15;5(5):1352-62.

Nguyen LT, Tretiakova MS, Silvis MR, Lucas J, Klezovitch O, Coleman I, Bolouri H, Kutyavin VI, Morrissey C, True LD, Nelson PS, Vasioukhin V.   ERG Activates the YAP1 Transcriptional Program and Induces the Development of Age-Related Prostate Tumors. *Cancer Cell.* 2015 Jun 8;27(6):797-808.

Li P, Silvis MR, Honaker Y, Lien W-H, Arron ST, Vasioukhin V.   αE-catenin inhibits a Src-YAP1 oncogenic module that couples tyrosine kinases and the effector of Hippo signaling pathway. *Genes & Development,* 2016 Apr 1;30(7):798-811.

Kwan J, Sczaniecka A, Arash EH, Nguyen L, Chen CC, Ratkovic S, Klezovitch O, Attisano L, McNeill H, Emili A, and Vasioukhin V. Dlg5 negatively regulates the Hippo signaling pathway and links Mst1/2 with Par-1 polarity proteins. *Genes & Development,* 2016, 2016 Dec 15;30(24):2696-2709.

Beattie R, Postiglione MP, Burnett LE, Laukoter S, Streicher C, Pauler FM, Xiao G, Klezovitch O, Vasioukhin V, Ghashghaei TH, Hippenmeyer S. Mosaic Analysis with Double Markers Reveals Distinct Sequential Functions of Lgl1 in Neural Stem Cells. *Neuron.* 2017 May 3;94(3):517-533.

Jossin Y, Lee M, Klezovitch O, Kon E, Cossard A, Lien WH, Fernandez TE, Cooper JA, Vasioukhin V. Llgl1 Connects Cell Polarity with Cell-Cell Adhesion in Embryonic Neural Stem Cells. *Dev Cell.* 2017 May 25. 41(5), p481-495. PMID: 28552558.

Scott J, Thakar S, Mao Y, Qin H, Hejran H, Lee SY, Yu T, Klezovitch O, Cheng H, Mu Y, Ghosh S, Vasioukhin V, Zou Y. Apical-Basal Polarity Signaling Components, Lgl1 and aPKCs, Control Glutamatergic Synapse Number and Function. *iScience.* 2019 Oct 25;20:25-41. PMID: 31546104.

Szulzewsky F, Arora S, Hoellerbauer P, King C, Nathan E, Chan M, Cimino PJ, Ozawa T, Kawauchi D, Pajtler KW, Gilbertson RJ, Paddison PJ, Vasioukhin V, Gujral TS, Holland EC. Comparison of tumor-associated YAP1 fusions identifies a recurrent set of functions critical for oncogenesis. *Genes Dev.* 2020 Aug 1;34(15-16):1051-1064. PMID: 32675324