LAB OVERVIEW

Our lab is interested in developing zebrafish models of human diseases to interrogate the disease mechanisms and to identify potential therapeutics against these diseases. Using both transgenesis and several gene-editing nuclease technologies that we have developed in our lab, we can generate unique disease models in zebrafish, enabling high-throughput chemical screening for small molecules that modify the disease phenotypes in a whole organism.

Selected review articles:

Helenius IT, Yeh JR. Small zebrafish in a big chemical pond. *J. Cell Biochem*. 2012;113(7):2208-16.

Zhang Y, Yeh JR. In vivo chemical screening for modulators of hematopoiesis and hematological diseases. *Advances in Hematology* 2012;2012:851674.

Yeh JR and Crews CM. Chemical genetics: adding to the developmental biology toolbox. *Dev. Cell* 2003;5:11-19

SCIENCE

Currently, there are four major projects in our lab.

1. Use a chemical suppressor screen to identify novel pathways involved in leukemogenesis

AML1-ETO is the fusion product of t(8;21) chromosomal translocation that can be found in approximately 12% of the acute myelogenous leukemia (AML) patients. AML1-ETO, as many other leukemic oncogenes, causes dysregulation of hematopoietic differentiation. Current treatments against AML rely heavily on non-specific cytotoxic drugs that kill proliferating cells, resulting in unbearable side effects, and yet most of the patients relapse after a brief remission. Compared to conventional cytotoxic therapeutics, targeted cancer therapies hold great promise to be more effective and less toxic. Thus, we generated a zebrafish model of AML by inducing AML1-ETO expression in zebrafish embryos, and showed that AML1-ETO exerts AML-like hematopoietic differentiation defects within just hours. We have developed a chemical suppressor screen using this zebrafish model of AML. Several classes of chemical suppressors have been identified in the screen and they are currently being evaluated for therapeutic potential using human cells and mouse models of AML.

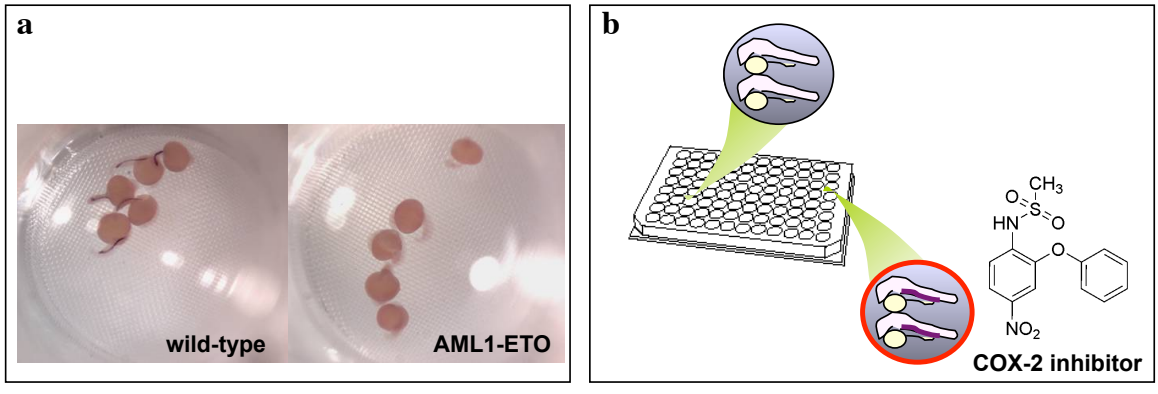

Figure 1. A chemical suppressor screen conducted in the zebrafish model of AML1-ETO identifies a potential role for COX-2 in AML leukemogenesis. (a) Expression of AML1-ETO results in a hematopoietic differentiation defect, suppressing the erythroid cell fate in zebrafish embryos. This effect can be readily detected using the erythroid cell marker, gata1. Each panel shows five zebrafish embryos per well in a 96-well screening plate after *gata1* staining. (b) We found that COX-2 inhibitors reverse the hematopoietic defect caused by AML1-ETO, pointing to potential therapeutic benefits of COX inhibitors for treating AML.

Selected publications:

Zhang Y, Wang J, Wheat J, Chen X, Jin S, Sadrzadeh H, Fathi AT, Peterson RT, Kung AL, Sweetser DA, Yeh JR. AML1-ETO mediates hematopoietic self-renewal and leukemogenesis through a COX/β-catenin signaling pathway. *Blood* 2013;121(24):4906-16.

Cunningham L, Finckbeiner S, Hyde RK, Southall N, Marugan J, Yedavalli VR, Dehdashti SJ, Reinhold WC, Alemu L, Zhao L, Yeh JR, Sood R, Pommier Y, Austin CP, Jeang KT, Zheng W, Liu P. Identification of benzodiazepine Ro5-3335 as an inhibitor of CBF leukemia through quantitative high throughput screen against RUNX1-CBFβ interaction. *Proc Natl Acad Sci USA* 2012;109(36):14592-7.

Yeh JR, Munson KM. Zebrafish small molecule screen in reprogramming/cell fate modulation. *Methods Mol. Biol.* 2010;636:317-27.

Yeh JR, Munson KM, Elagib KE, Goldfarb AN, Sweetser DA, Peterson RT. Discovering chemical modifiers of oncogene-regulated hematopoietic differentiation. *Nat. Chem. Biol.* 2009;5:236-243.

Dayyani F, Wang J, Yeh JR, Ahn EY, Tobey E, Zhang DE, Berstein ID, Peterson RT, Sweetser DA. Loss of TLE1 and TLE4 from the del(9q) commonly deleted region in AML cooperates with AML1-ETO to affect myeloid cell proliferation and survival. *Blood* 2008;111:4338-4347.

Yeh JR, Munson K, Chao YL, Peterson QP, MacRae CA, Peterson RT. AML1-ETO reprograms hematopoietic cell fate by down-regulating scl expression. *Development* 2008;135:401-410.

2. Develop gene-editing nuclease technologies for the generation of targeted mutations in zebrafish

In collaboration with the Joung lab in the Department of Pathology and the Peterson lab in the Cardiovascular Research Center of MGH, we have developed several customizable nucleases platforms, including the zinc finger nucleases (ZFNs), the transcription activator-like effector (TALE) nucleases and the CRISPR-Cas system, that can be used for generating targeted mutations in zebrafish. We have created a number of zebrafish mutant lines for the studies of iron homeostasis, angiogenesis and neurological/psychiatric disease mechanisms. By exploiting the unique capabilities of the zebrafish for disease modeling and small molecule screening, these zebrafish mutant lines will provide powerful tools for dissecting signaling pathways involved in a broad range of biological processes and may uncover promising new therapeutic approaches for treating human diseases.

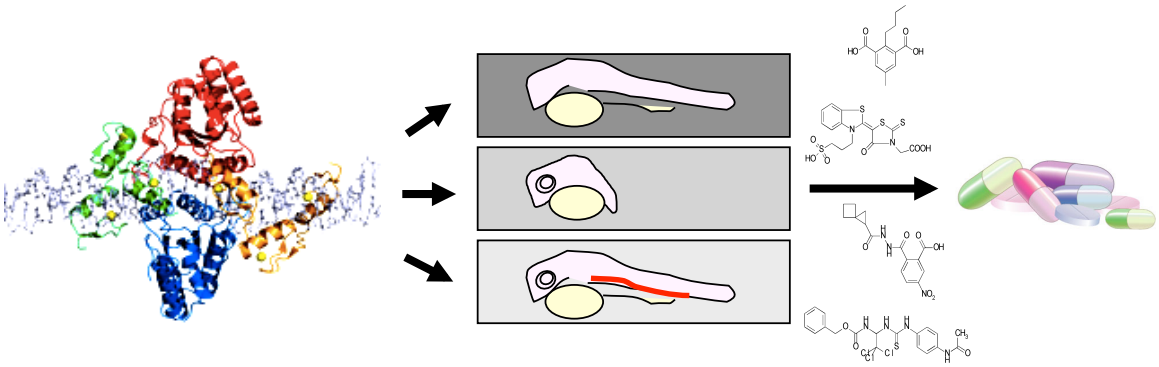

Figure 2. The ZFN technology facilitates the creation of human disease models in zebrafish for the discovery of novel therapeutic agents. First, we engineer gene-specific zinc-finger nucleases (ZFNs) for generating zebrafish mutants of interest. Second, we identify zebrafish mutant lines that manifest specific disease phenotypes similar to humans. Third, zebrafish mutant embryos are subjected to large-scale chemical screening to identify compounds that can modify the disease phenotypes. Lastly, we identify the compounds' underlying mechanisms of action, which may provide new insights into the development of therapeutic agents.

Selected publications:

Hwang WY, Fu Y, Reyon D, Maeder ML, Kaini P, Sander SD, Joung JK, Peterson RT, Yeh JR. Heritable and precise zebrafish genome editing using a CRISPR-Cas system. *PLos One* 2013;8(7):e68708.

Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JR, Joung JK. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat. Biotechnol.* 2013;31(3):227-9.

Cade L, Reyon D, Hwang WY, Tsai SQ, Patel S, Khayter C, Joung JK, Sander JD, Peterson RT, Yeh JR. Highly efficient generation of heritable zebrafish gene mutations using homo- and heterodimeric TALENs. *Nucleic Acids Res.* 2012;40(16):8001-10.

Sander JD, Cade L, Khayter C, Reyon D, Peterson RT, Joung JK, Yeh JR. Targeted gene disruption in somatic zebrafish cells using engineered TALENs. *Nat. Biotechnol.* 2011;29:697-8.

Sander JD, Yeh JR, Peterson RT, Joung JK. Engineering zinc finger nucleases for targeted mutagenesis of zebrafish. *Methods Cell Biol*. 2011;104:51-8.

Sander JD, Dahlborg EJ, Goodwin MJ, Cade L, Zhang F, Cifuentes D, Curtin SJ, Blackburn JS, Thibodeau-Beganny S, Qi Y, Pierick CJ, Hoffman E, Maeder ML, Khayter C, Reyon D, Dobbs D, Langenau DM, Stupar RM, Giraldez AJ, Voytas DF, Peterson RT, Yeh JR, Joung JK. Seletion-free zinc-finger-nuclease engineering by context-dependent assembly (CoDA). *Nat. Methods* 2011;8:67-69.

Foley JE, Maeder ML, Pearlberg J, Joung JK, Peterson RT, Yeh JR. Targeted mutagenesis in zebrafish using customized zinc-finger nucleases. *Nat. Protoc.* 2009;4:1855-1867.

Foley JE, Yeh JR, Maeder ML, Reyon D, Sander JD, Peterson RT, Joung JK. Rapid mutation of endogenous zebrafish genes using zinc finger nucleases made by Oligomerized Pool Engineering (OPEN). *PLos One* 2009;4:e4348.

3. Investigate the roles of metabolic oncogenes and tumor suppressor genes

Cancer metabolism is a field currently under intensive invesitigations partly due to the recent discoveries of cancer-causing mutations in several metabolic genes, such as *IDH1* and *IDH2* in glioblastoma and AML, *FH* in hereditary leiomyomatosis and renal cell cancer (HLRCC) and *SDH* in paraganglioma and phaeochromocytoma. Mutations in these genes can affect not only metabolism, but also DNA methylation, histone methylation and cellular hypoxia response due to the important roles of α-KG-dependent dioxygenases in these processes (Figure 3). Understanding the metabolic transformation mechanisms may shed light on new and promising targeted therapeutic approaches. Interestingly, these mutations are only found in some specific cancer types, suggesting their tissue-specific functions. We are interested in investigating the disease mechanisms of these mutations using zebrafish models in conjunction with human cell culture systems.

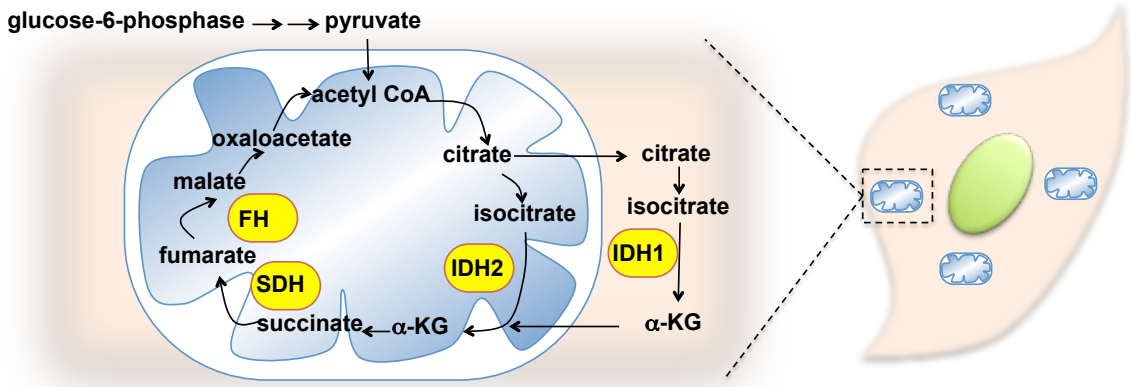

Figure 3. Metabolic oncogenes and tumor suppressor genes in cancer. Gain-of-function mutations in *isocitrate dehydrogenase 1* (*IDH1*) and *IDH2* as well as loss-of-function mutations in *succinate dehydrogenase* (*SDH*) and *fumarate hydratase* (*FH*) have been found in human cancers. The diagram depicts the subcellular localizations of the enzymes encoded by these genes and their normal enzymatic functions in the Kreb Cycle. The dashed box shows a mitochondria. A nucleus is indicated by a green oval. It has been shown that accumulations of fumarate, succinate and 2-hydroxyglutarate in the presence of *FH*, *SDH* and *IDH* mutations, respectively, can suppress the activities of α-KG-dependent enzymes.

4. Use zebrafish to identify dietary modulations that may reduce hyperlipidemia

The circulating metabolites in the blood may represent the well-being of an individual and report for certain disease states. However, diets and changes in plasma metabolites can also play causative roles in disease mechanisms. Previously, our collaborator Dr. Gerszten's group in the Cardiovascular Research Center at MGH has discovered several plasma metabolites that show significant associations with various metabolic risk factors including hyperlipidemia (*Circulation* 2012; 125(18):2222-31). Using zebrafish, we plan to identify how certain amino acids may affect triglycerides/cholesterol profiles in a whole orgamism.

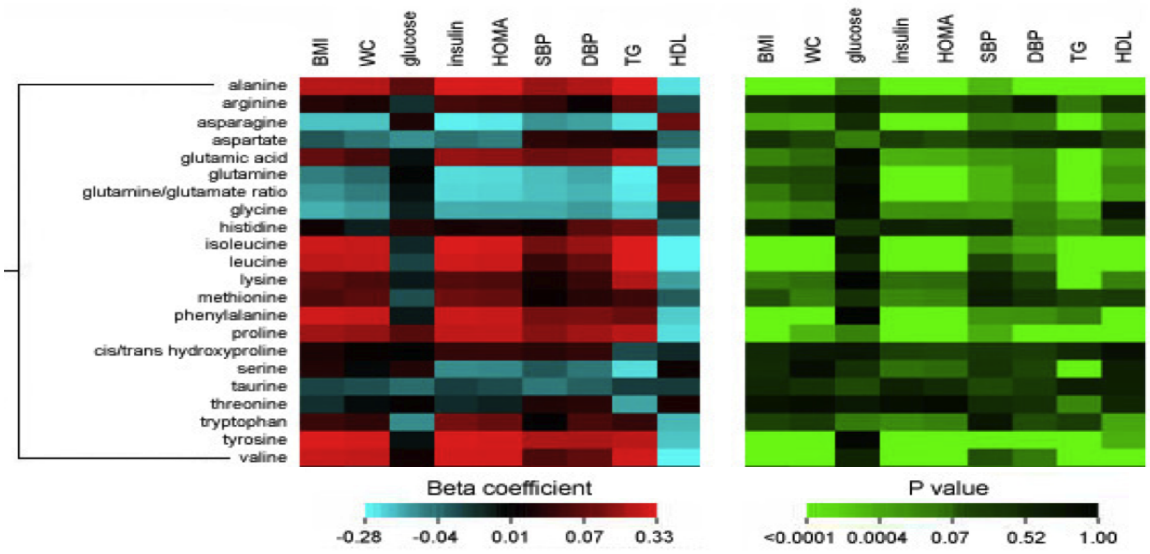

Figure 4. The associations between plasma amino acids and metabolic traits in the Framingham Heart Study participants. β coefficients and P values generated from age- and sex-adjusted regression analyses of the relation of each metabolite (standardized and log transformed) with insulin resistance phenotypes and metabolic traits (standardized) in the Framingham Heart Study sample. BMI indicates body mass index; WC, waist circumference; HOMA, homeostasis model assessment; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HDL, high-density lipoprotein. (Cheng, et al. *Circulation* 2012; 125(18):2222-31.)

Selected publications:

Rhee EP, Ho JE, Chen MH, Shen D, Cheng S, Larson MG, Ghorban A, Shi X, Helenius IT, O'Donnell CJ, Souza AL, Deik A, Pierce KA, Bullock K, Walford GA, Vasan RS, Florez JC, Clish C, Yeh JR, Wang TJ and Gerszten RE. A Genome-Wide Association Study of the Human Metabolome in a Community-Based Cohort. *Cell Metabolism* 2013;18(1):130-43.