Dr. Hanrui Zhang

Lysosomal acid lipase (LAL), encoded by the *LIPA* gene, is the key lysosomal hydrolase that cleaves cholesteryl esters (CE) and triglycerides (TG). Loss-of-function (LOF) mutations in *LIPA* result in cholesteryl ester storage disease (CESD), which manifests with hyperlipidemia, hepatic and macrophage CE accumulation, and atherosclerosis. Recently, genome-wide association studies (GWASs) identified *LIPA* as a novel locus for coronary artery disease (CAD). Surprisingly, *LIPA* CAD-GWAS risk alleles do not associate with altered plasma lipids or hepatic *LIPA* mRNA levels but actually relate to higher monocyte *LIPA* mRNA expression. Our preliminary data also reveal a coincident increase in both *LIPA* mRNA and LAL enzymatic activity in monocyte-derived macrophages (HMDM) of CAD risk allele carriers, suggesting that monocyte/macrophage-specific gain-of-function (GOF) of *LIPA* may explain the GWAS CAD risk alleles. *LIPA* mRNA and LAL activity were markedly induced upon HMDM differentiation and mature HMDM secrets LAL. Extracellular LAL is abundant in the neointima of advanced human atherosclerotic lesions where LAL can remain enzymatically active in the lesion acidic microenvironment. Thus, macrophage *LIPA* GOF may aggravate atherosclerosis through accelerating intracellular LAL-induced lysosomal free cholesterol (FC) toxicity and extracellular LAL actions on low-density lipoprotein (LDL). My working hypotheses are that *LIPA* CAD risk alleles encode for macrophage-specific *LIPA* GOF, and therefore CAD risk alleles or macrophage *LIPA* overexpression will (i) increase intracellular LAL activity resulting in a shift toward greater lysosomal CE hydrolysis and FC accumulation and thus accelerate macrophage lysosomal dysfunction during modified-LDL loading; (ii) increase macrophage LAL secretion and extracellular LAL-mediated LDL modification driving atherogenic phenotypes in vascular smooth muscle cells (VSMC) and endothelial cells (EC); and (iii) macrophage *LIPA* overexpression will accelerate atherogenesis in *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice. These hypotheses will be addressed in primary HMDM of specific *LIPA* genotype, through causal modeling in a novel human iPSC-differentiated macrophage (IPSDM) system utilizing CRISPR/Cas knock-in of risk alleles, in HMDM with lentivirus-mediated *LIPA* overexpression (Lenti-LIPA), and in murine models with macrophage-specific *Lipa overexpression* by lentivirus-infected bone marrow (BM) transplantation driven by macrophage-specific promoter. These will be accomplished by pursuing the following three aims: Aim 1. K99 Phase: Determine the effects of *LIPA* CAD risk alleles and *LIPA* overexpression on intracellular macrophage *LIPA* expression, LAL activity, and phenotype of human macrophages. Aim 2. R00 Phase: Determine the effects of *LIPA* CAD risk alleles and *LIPA* overexpression on macrophage LAL secretion, extracellular LAL-modification of LDL and on atherogenic phenotypes in EC and VSMC. Aim 3. R00 Phase: Determine if macrophage *Lipa* overexpression accelerates atherosclerosis in *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice.