Grant Title: Humanized vasculature in gene edited animals

Grant Number: RMM 11215 DS002

Principal Investigator: Daniel Garry, PhD, MD

Project Timeline: 4/1/2016 - 3/31/2018

Progress to Date:

Specific Aim #1: To define the functional role of ETV2 in the porcine model

Our laboratory discovered Etv2 as a direct downstream target of Nkx2-5 and using a gene disruption strategy we determined that Etv2 knockout mice were lethal early during development and completely lacked vasculature and blood. Recent studies from our laboratory and others have identified Etv2 as an essential transcription factor for the development of cardiac, endothelial and hematopoietic lineages.

ETV2 knockout pig embryos lack hematoendothelial lineages. Previous studies by our laboratory have demonstrated that Etv2 is essential for vasculogenesis and hematopoiesis in the mouse as embryos lacking Etv2 are lethal by E9.5 with an absence of vasculature and blood. To examine the role of ETV2 in the pig, we removed the entire ETV2 coding sequence, in a biallelic fashion, using CRISPR/Cas9 and two guide RNAs (gRNA) flanking the gene in porcine fibroblasts. These porcine mutant fibroblasts were then used for SCNT (i.e. cloning). Cloned, mutant pig embryos were transferred to synchronized gilts and sacrificed at E18. The embryos were genotyped (to assure that they lacked ETV2), immersion fixed in 4% paraformaldehyde, cryoprotected, frozen and 7 micron thick sections were obtained and processed for immunohistochemical or transcript analyses (using methods as previously described). Embryos were harvested and analyzed early during embryogenesis. After extensive analyses, we concluded that the ETV2 null pig embryo phenocopied the mouse.

Specific Aim #2: To define the capacity of wildtype GFP-labeled pig blastomeres to complement and rescue the ETV2 mutant porcine host.

Pig-pig complementation to define the highest efficiency chimerism in early developing porcine embryos. As a baseline study to define the very best chimerism that we could achieve and as a platform for the proposed interspecies chimera studies, we examined pig-pig chimeras in vitro. These studies verified our hypotheses and established a platform for future complementation experiments.
Publications:


Budget Update:
The funding was spent as outlined originally outlined in the grant proposal.

Reporting to all Minnesotans:
Cardiovascular diseases are both common and deadly. For example, peripheral artery disease affects more than 10M Americans resulting in more than 150,000 limb amputations each year in the U.S. In addition, more than 300,000 patients have coronary artery bypass grafting (surgical revascularization). These diseases collectively are amplified by the rising incidence of diabetes, obesity and cardiovascular disease. Importantly, these complications result in considerable morbidity and mortality. Current medical therapies for vascular disease include limb amputation, vascular bypass grafting (using the patient's diseased vasculature) or vascular grafts—all these therapeutic interventions have significant limitations. These diseases are chronic, debilitating, lethal and they warrant novel therapies. The results of our studies allowed us to engineer a novel large animal model that will serve as an important platform to engineer blood vessels. Given the tremendous morbidity and mortality of cardiovascular diseases in our society, the potential impact of this research is tremendous.