**ABSTRACT.**

Age-related vascular conditions have had a devastating negative impact on the morbidity and mortality of elderly patients. In the U.S. alone, the aged population >65 years old encompasses ~56 million individuals, and this large cohort shows disproportionately increased risk for vascular disease. Improved understanding of the molecular underpinnings of vascular aging is urgently warranted to inform new anti-senolytic therapies for these at-risk individuals.

Our extramurally-funded research lab uses patient-centered approaches to study how endothelial cells (ECs), cells that line blood vessels, contribute to the longevity of tissue allografts. Using humanized models and patient specimens, we discovered a new molecule in ECs called ZFYVE21. ZFYVE21 is an ancient protein whose functions are virtually unknown. We found that ZFYVE21 was expressed on intracellular vesicles called Rab5 endosomes. ZFYVE21 was capable of modifying the protein constituency of Rab5 endosomes to elicit inflammatory signaling, a process causal for vascular senescence, a key mediator of healthspan.

To explore ZFYVE21 in vascular senescence, we generated ZFYE21 $EC^{-/-}$ mice using gene targeting technology. Mice lacking ZFYVE21 in ECs developed vascular dysfunction and sequelae of age-related vascular disease including failure to thrive, renal insufficiency, hepatic insufficiency, and HFpEF. Multi-system organ dysfunction in ZFYVE21 $EC^{-/-}$ mice developed by 8-12 wks of age, equivalent to 20-30 yrs of age in humans, and occurred in association with increased markers of cellular senescence. Rab5 endosomes isolated from ZFYVE21 $EC^{-/-}$ mice showed dramatically reduced levels of various anti-senolytic proteins including pENOS. pENOS is an EC-specific enzyme that catalyzes the formation of nitric oxide (NO), a bioactive gas well known to support EC health by upregulating genes promoting growth and tissue repair. ZFYVE21 $EC^{-/-}$ mice showed systemic deficits in NO generation, and NO supplementation using isosorbide improved renal insufficiency.

Our data showed that an altered cohort of endosomes which we call senescence-associated endosomes (SAEs) were unable to support the stability of anti-senolytic molecules including pENOS, resulting in accelerated aging and decreased healthspan. NO-modifying drugs including isosorbide and sildenafil are used in clinical practice to treat vascular conditions including heart failure and pulmonary hypertension, respectively. Our findings open the possibility that these FDA-approved therapies could be repositioned to support vascular healthspan in aged individuals by blocking the negative systemic effects of SAEs.

Based on our studies we explore the hypothesis that SAEs regulate vascular senescence. In Specific Aim 1 we will characterize changes in SAEs in aged ZFYVE21 $EC^{-/-}$ mice which developed clinically relevant vascular disease. Informed by these studies, in Specific Aim 2, we will examine frequencies of SAEs isolated from patient tissues from solid organ transplant recipients, and we will calculate correlations of these molecules with patient parameters including use of NO-modifying therapies.

My lab has had long-standing, multi-disciplinary collaborations with Dr. George Tellides in the Dept of Cardiovascular Surgery, Dr. Jordan Pober in the Dept of Pathology, Dr. Arnar Geirsson in the Dept of Surgery, and Dr. Sanjay Kulkarni in the Dept of Transplant Surgery. My lab has numerous publications and co-PI funding awards with all these investigators who are included in this proposal. Our proposal carries important implications for age-related vascular conditions and reflects a significant divergence from our current projects. By analyzing SAEs in patient specimens and focusing on new anti-senoltyic molecules amenable to drug manipulation, our application addresses vascular senescence, a problem relevant to translational geroscience.