Project Summary/Abstract

The arteriovenous fistula (AVF) is the lifeline for Veterans with end-stage kidney disease (ESKD) requiring hemodialysis therapy. Arteriovenous fistulas (AVF) are the preferred vascular access for hemodialysis patients with end stage kidney disease. A large proportion of AVFs (30-60%) created in the United States fail to successfully mature. The high failure rates after AVF creation reflect our poor understanding of the mechanisms leading to AVF maturation failure; and the lack of therapies to prevent or treat this problem represents an unmet clinical need. Our Collaborative Merit Award (CMA) is an integrated and synergistic proposal assembling a group of VA investigators focused on improving AVF maturation failure for Veteran hemodialysis patients. The Overall Research Strategy is to elucidate mechanisms of AVF maturation failure in order to develop novel therapies to mitigate this unmet clinical need. The overall rationale for this CMA is that fibrotic venous remodeling influences intimal hyperplasia development and successful outward remodeling, and mitigating these biological problems could improve successful AVF maturation. Specifically, the CMA will define those mechanisms by which aging (Project 1), Type VIII collagen in smooth muscle cells (Project 2), and endothelial cell autophagy (Project 3) determines venous remodeling after AVF anastomosis. The overarching hypothesis of this CMA project is that the biology of AVF maturation is influenced by mechanisms regulating venous fibrotic remodeling. Specific to project 3, we will investigate the role of endothelial cell (EC) autophagy in fibrotic venous remodeling in AVF maturation. The mechanisms leading to AVF maturation failure are poorly understood; and the lack of therapies to treat this clinical problem in hemodialysis patients represents an unmet clinical need. Our central hypothesis is that impaired endothelial cell autophagy, after AVF creation, promotes venous fibrotic remodeling, leading to AVF maturation failure. We will address our central hypothesis with three specific aims: Specific Aim I: Demonstrate the role of EC autophagy in the setting of disturbed flow and its impact on profibrotic signaling, Specific Aim II: Demonstrate the role of EC autophagy in venous fibrosis and AVF remodeling, and Specific Aim III: Demonstrate the association of EC autophagy with venous fibrosis and AVF maturation.

SPECIFIC AIMS

Arteriovenous fistulas (AVF) are the preferred vascular access for hemodialysis patients with end stage kidney disease. **A large proportion of AVFs (30-60%) created in the United States fail to successfully mature (i.e. lack sufficiently large lumen and blood flow) for dialysis use.** The high failure rates after AVF creation reflect our poor understanding of the mechanisms leading to AVF maturation failure; and the lack of therapies to prevent or treat this problem represents an *unmet clinical need*. Our ***Collaborative Merit Award (CMA)*** is an integrated and synergistic proposal focused on improving AVF maturation failure for Veteran hemodialysis patients. The ***Overall Research Strategy*** is to elucidate mechanisms of AVF maturation failure in order to develop novel therapies to mitigate this unmet clinical need. The ***overall rationale for this CMA*** is that fibrotic venous remodeling promotes intimal hyperplasia development to restrict lumen area for blood flow, and mitigating these biological problems could improve successful AVF maturation. The ***specific aims of this CMA*** will define mechanisms by which senescence and aging in endothelial cells (EC)(Project 1), Type VIII collagen in smooth muscle cells (Project 2), and autophagy in endothelial cells (Project 3) determine venous remodeling after AVF creation (**Figure 1**). The ***overarching hypothesis*** of this CMA project is that the biology of AVF maturation is influenced by mechanisms regulating venous fibrotic remodeling.

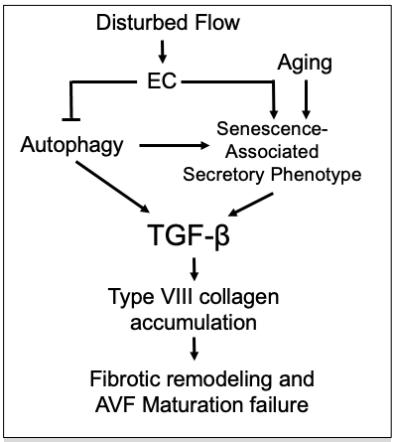

Figure 1: Overall Collaborative Merit Award Concept (New)

Specific to project 3, we will investigate the role of EC autophagy in fibrotic venous remodeling in AVF maturation. Autophagy is a multi-step process that regulates protein and organelle quality to preserve cellular homeostasis. We focus on EC autophagy because the endothelium plays a critical role in the regulation of intimal hyperplasia, outward remodeling, and the regulation of flow, but its role in development of venous fibrotic remodeling has not been previously studied. Repressed autophagy is associated with pathologic arterial conditions (e.g. atherosclerosis) but is poorly studied in *venous* pathologies. Our proposal is *novel* and supported by strong preliminary data pointing to a causal role for repressed EC autophagy and profibrotic signaling. Key preliminary data include: (1) In cultured human umbilical vein ECs autophagy is repressed and profibrotic signaling increased by disturbed flow vs. laminar flow, and senescence activated in repressed EC autophagy (New). (2) AVFs created in mice with defective EC autophagy, autophagy-related gene 7 knockout mice (ecAtg7KO), have increased intimal hyperplasia and venous fibrotic remodeling. (3) Human veins from failed AVFs exhibit repressed EC autophagy and increased profibrotic signaling in ECs from single cell RNAseq (scRNAseq). *Based on these results, our* ***central hypothesis*** *for project 3 is that disturbed flow impairs EC autophagy and results in profibrotic signaling, which promotes venous fibrotic remodeling and leads to AVF maturation failure (***Figure 1***).* We will test our hypothesis with three *specific aims*.

Specific Aim I: Demonstrate the role of EC autophagy in the setting of disturbed flow and its impact on profibrotic signaling. Hypothesis: Disturbed flow impairs EC autophagy and activates TGFβ signaling and senescent pathways leading to increased type VIII collagen synthesis (New). We will use a customized in vitro perfusion system exposing human vein ECs, co-cultured with vascular smooth muscle cells (SMC), to laminar vs. disturbed flow to dissect how autophagy influences profibrotic and collagen signaling pathways in EC.

Specific Aim II: Demonstrate the role of EC autophagy in venous fibrosis and AVF remodeling. Physiologic Hypothesis: Repressed EC autophagy promotes venous fibrotic remodeling after AVF creation characterized by impaired hemodynamic adaptation and outward remodeling, and intimal hyperplasia development**.** Mechanistic Hypothesis: Repressed EC autophagy activates TGFβ signaling and senescent pathways leading to increased type VIII collagen synthesis (New)**.** We will create AVFs in ecAtg7KO mice and measure AVF flow and diameter with ultrasound, assess intimal hyperplasia development, characterize AVF hemodynamics with fluid dynamic modeling from magnetic resonance imaging, and elucidate profibrotic signaling pathways and Type VIII collagen synthesis from AVF vein.

Specific Aim III: Demonstrate the association of EC autophagy with venous fibrosis and AVF maturation. Hypothesis: Impaired EC autophagy following AVF creation is associated with increased TGFβ signaling and Type VIII collagen in ECs and AVF maturation failure (New). We will use a biorepository of human AVF veins from Dr. Vazquez-Padron (Project 2 leader and co-investigator on project 3) to perform scRNAseq, bioinformatic analysis, and tissue validation of scRNAseq. This shared resource, used in all 3 projects, will be leveraged to investigate associations in vein autophagy from mature and failed human AVFs with (i) fibrotic remodeling and (ii) clinical AVF outcomes.