Reprogramming of vascular smooth muscle cells to multipotent progenitor cells contributes to progression of atherosclerosis

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OBJECTIVES/GOALS: Our lab previously identified a population of vascular smooth muscle (SM) derived progenitor cells (AdvSca1-SM) which expand robustly in response to disease and can differentiate into multiple cell types. We now aim to define the role of these AdvSca1-SM cells in atherosclerotic plaque progression.

METHODS/STUDY POPULATION: Goal one uses SM lineage tracing mice and a model of atherosclerosis to track reprogramming of SMCs to AdvSca1-SM cells in the setting of disease. Arteries are analyzed using flow cytometry and immunofluorescence to quantify changes in number of mature SMCs and AdvSca1-SM cells. Goal two uses AdvSca1-SM lineage tracing mice with high cholesterol-induced atherosclerosis and plaque neovascularization. Arteries are analyzed to quantify expansion of AdvSca1-SM cells, subsequent re-differentiation into mature SMC, endothelial cells, or macrophages, and contribution to plaque neovascularization. Mechanistic findings from both goals are being investigated in diseased human coronary arteries.

RESULTS/ANTICIPATED RESULTS: Flow cytometry from SMC lineage tracing mice revealed a 7-13-fold expansion of AdvSca1-SM cells in carotid arteries (p<0.001) and aortas (p=0.03) after 6 weeks of western diet; no differences in macrophage numbers were observed. Additional SMC and AdvSca1-SM cell lineage tracing mice are on atherogenic diets to assess early and advanced atherosclerosis. We predict that AdvSca1-SM cells will contribute to macrophage accumulation as well as plaque neovascularization in the setting of severe atherosclerosis. Translational relevance of mechanisms driving SM reprogramming and AdvSca1-SM cell contribution to plaque progression are being applied to studies of diseased human coronary arteries.

DISCUSSION/SIGNIFICANCE OF IMPACT: Our data suggest a role for AdvSca1-SM cells in atherosclerosis. Ongoing work will clarify the mechanisms driving plaque-associated AdvSca1-SM expansion and define the ultimate fates of these cells. In vivo modulation of this process could provide the basis for future anti-atherosclerotic therapies.

CONFLICT OF INTEREST DESCRIPTION: AD - CTCSS TOTTS TL1TR002533; SL - 18POST34030397 from the American Heart Association; AJ - no conflicts; KS - 1F31HL147393 from the National Heart, Lung, and Blood Institute, NIH; MM - no conflicts; RT - no conflicts; KSM - no conflicts; RAN - R01CA236222 from the National Cancer Institute, NIH, and 2018-03 from the Lungevity Foundation; and MCMW-E - R01 HL121877 from the National Heart, Lung, and Blood Institute, NIH, and 25A8679 from the Chernowit Foundation.
(Col1A1), tissue inhibitor of metalloproteinase 1 (TIMP1), or α-smooth muscle actin (ACTA2). CDAHFD and SD pHSCs were then treated for 48 hours with increasing doses of BMS-22 or MVC (range: 0.3-120 ng/mL) to determine (1) the degree of attenuation of the pro-fibrogenic response as measured by qPCR of fibrogenic genes (Col1A1, TIMP1, ACTA2); (2) enhancement of a fibrolytic response as measured by qPCR of matrix metalloproteinases (MMP) 2, 9 and 13 genes; and (3) pHSC migration using the scratch assay. Cell viability and CCR2 and CCR5 gene expression in response to escalating doses of antagonists were also measured. RESULTS/ANTICIPATED RESULTS: Plate- and TGF-b activated CDAHFD pHSCs had a 2-fold greater, dose-dependent attenuation of their pro-fibrogenic activity in response to BMS-22 and MVC, when compared with plate- and TGF-b activated SD pHSCs, as measured by reductions in collagen 1α1 (Col1A1) and α-smooth muscle actin (ACTA2) gene expression. TIMP1 gene expression was unaffected by drug treatment for 48 hours. Cell viability was not affected up to doses of 30 ng/mL of each drug. pHSCs also demonstrated a dose-dependent increase in CCR2, CCR5 and MMP-9 gene expression in response to surface receptor antagonism. Migration assays comparing CDAHFD and SD pHSCs in response to escalating doses of MVC and BMS-22 are ongoing and expected to demonstrate a significantly decreased migratory capacity of CDAHFD pHSCs than SD pHSCs in response to therapy, reflecting the increased susceptibility of the “fat-exposed” pHSCs to anti-fibrotic therapy than normal pHSCs. DISCUSSION/SIGNIFICANCE OF IMPACT: Anti-fibrotic drugs that dampen pro-fibrogenic activities of “fat-exposed” pHSCs are urgently needed. CCR2 and CCR5 antagonists, BMS-22 and MVC, respectively, can selectively dampen the pro-fibrogenic response of fat-exposed pHSCs, and must be considered for future trials in human NASH. CONFLICT OF INTEREST: Dr. Jill Smith has a patent licensing agreement with Immune Therapeutics, Inc.

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The role of creatine in developmental myelination and remyelination

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OBJECTIVES/GOALS: Oligodendrocytes (OL) are glial cells of the central nervous system (CNS) responsible for the energy demanding task of generating myelin sheaths during development and remyelination after demyelinating injury. One metabolite shown to significantly increase ATP production in OL is the nitrogeneous organic acid, creatine. Creatine plays an essential role in ATP buffering within tissues with highly fluctuating energy demands such as brain and muscle. Interestingly, mature OL, which are the cells capable of myelin production, are the main cells in the CNS expressing the rate-limiting enzyme for creatine synthesis, guanidinoacetate methyltransferase (Gamt). Patients with mutations in Gamt display intellectual disabilities, impaired myelination and seizures. Therefore, we hypothesize that creatine may be essential for developmental myelination and improve remyelination. METHODS/STUDY POPULATION: To investigate these hypotheses, we developed a new transgenic mouse model with LoxP sites flanking exons 2-6 of the Gamt gene where excision leads to expression of a green fluorescent tag allowing us to track the cells normally expressing Gamt. RESULTS/ANTICIPATED RESULTS: In this mouse model, we show a 95% (±0.47%, n = 3) co-localization of Gamt within mature OL during postnatal (P) day P14. Next, we show that knocking out Gamt leads to a significant reduction in OL in the major CNS white matter tract, the corpus callosum, at P14 and P21 (P14: 0.007, n = 3; P21: 0.04, n = 3). Here, we also investigate whether dietary creatine can enhance remyelination in the cuprizone model of toxic demyelination. DISCUSSION/SIGNIFICANCE OF IMPACT: These studies highlight the important role creatine plays in developmental myelination and investigate whether creatine can provide a therapeutic value during a CNS demyelinating insult.

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The Utilization of Polyethylene Glycol Fusion to Improve Facial Reanimation

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OBJECTIVES/GOALS: This study’s goal is to determine whether intraoperative treatment of facial nerves with polyethylene glycol (PEG) fusion technology improves facial paralysis outcomes. Improved facial nerve regeneration in facial paralysis patients would lead to improved recovery time and effectiveness. METHODS/STUDY POPULATION: 30 rats were utilized; 15 underwent facial nerve regeneration without PEG fusion, and 15 with PEG fusion. Facial paralysis was initiated on the left by transection of the buccal and marginal mandibular branches of facial nerve. The buccal branch was repaired through microsuture technique. Neuorrhaphy sites of rats in the PEG group were exposed to calcium free saline, methylene blue, and polyethylene glycol. Nerve continuity was assessed post-operative in 5 animals in each group through electron microscopy. Functionality was assessed in the other 10 per group by EMG and whisker analysis after surgery, and weekly for 8 weeks. At 8 weeks, nerves and distal muscles were histologically analyzed. RESULTS/ANTICIPATED RESULTS: PEG fusion technology immediately restored axonal continuity following surgery, demonstrated by electron microscopy. Electrophysiology was also similarly restored across the site immediately, determined through intraoperative nerve stimulation, in the PEG fusion group. The nonintervention group showed dramatically reduced functional recovery than the PEG fusion group following surgery, shown by lower whisking activity and poor electrophysiology outcomes. Furthermore, the PEG fusion group showed statistically significant higher fascicle counts, myelination diameter, axonal diameter, and distal muscle fibers histologically. DISCUSSION/SIGNIFICANCE OF IMPACT: This study demonstrates that polyethylene fusion technology may improve facial reanimation outcomes. PEG is already a FDA-approved drug, and thus the pathway to translational clinical application of this work may thus be streamlined, bringing new options to patients with facial paralysis.

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Utilization of swept source optical coherence tomography to optimize characterization of cystoid macular edema in preterm infants

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OBJECTIVES/GOALS: The goal of this study is to evaluate and optimize the characterization of cystoid macular edema (CME) using an investigational swept source (SS)-OCT system. Our knowledge of