the degeneration. DISCUSSION/SIGNIFICANCE OF IMPACT: These results suggest that the attenuated Q344X VMR is a result of the rod photoreceptor death. This behavioral phenotype can be taken advantage of to develop a drug screening assay. Future steps will screen chemical libraries to identify compounds that ameliorate the rod degeneration. Compounds that prevent degeneration are expected to result in a significant increase in locomotion in response to the dim visual stimulus.

2229

Development of an angiogenic proteoglycan mimic to accelerate ischemic diabetic foot ulcer repair

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OBJECTIVES/SPECIFIC AIMS: This project aims to synthesize an angiogenic decorin mimic (VEGFp-DS-SILY) with varying densities of QK and characterize its angiogenic potential and synergism with VEGF by evaluating (1) endothelial cell (EC) migration and proliferation, (2) EC VEGF receptor activation, (3) EC tubule formation in collagen scaffolds, and (4) angiogenesis from a chick chorioallantoic membrane (CAM assay) growing into the scaffold, reflecting the ability of the collagen scaffold to integrate into existing vasculature. The next main goal is to develop and characterize an MMP-degradable nanoparticle system for controlled release of VEGF. Future work will evaluate in vivo effects of VEGFp-DS-SILY bound to a 3D collagen scaffold on ischemic wound repair in a combined excisional wound/bipedical dorsal skin flap rat model. METHODS/STUDY POPULATION: Peptide hydrazides are conjugated to the free carboxylic acid functional groups on dermatan sulfate using EDC chemistry. We added a 3 amino acid spacer (-Gly-Ser-Gly) to the C-terminus of the established QK sequence before the hydrazide functional group and refer to this modified QK as "VEGFp." VEGFp, SILY, and *N*-terminal biotinylated versions were synthesized using standard Fmoc solid-phase peptide synthesis protocols and purified using reverse phase HPLC. Coupling efficiencies of peptides to dermatan sulfate were determined spectroscopically at 280 nm measuring the aromatic residues (Trp or Tyr) using a NanoDrop system. Dermatan sulfate with 1 or 4 VEGFp peptides coupled were termed DSV1 and DSV4, respectively. After further conjugation with SILY, we will blend this VEGFp-DS-SILY with unmodified DS-SILY to a total 10 μM to test increasing densities of VEGFp. To verify that the collagen-binding properties of VEGFp-DS-SILY are not compromised by the addition of VEGFp, we will use a streptavidin-HRP system to detect bound biotinylated VEGFp-DS-SILY on collagen-coated plates by established protocols. DSV1 and DSV4 were tested for their effects on endothelial VEGFR2 phosphorylation using an MSD ELISA-type assay and endothelial proliferation using an MTS assay. Cell migration was monitored using an ORIS assay where cells are grown to confluence around a silicone stopper that is then removed to allow cells to migrate inward. Tubulogenesis was evaluated by examining tubule formation on matrigel. Finally, in vivo angiogenesis will be evaluated using a chorioallantoic membrane assay. For extracellular VEGF release, hollow MMP-degradable thermoresponsive nanoparticles [NIPAM, 5 mol% 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), 1% Acrylic Acid (AAc), 2 mol% MMP-degradable peptide diacrylate, and potassium persulfate initiator] will be synthesized around noncross-linked polymer cores. The cores will then be diffused out through the shell by dialysis prior to drug loading. SILY (and some biotinylated SILY for visualization) will be conjugated with EDC chemistry for targeting nanoparticles to collagen. NPs size and zeta-potential will be measured on a Malvern Zetasizer. VEGF will be loaded into NPs by co-incubating a loading solution of 1 μg/mL VEGF with 1 mg of NPs, incubating overnight at 4°C. VEGF loading and release will be measured by ELISA. Biological activity of the released VEGF from particles will be determined on ECs using assays similar to those outlined previously. RESULTS/ANTICIPATED RESULTS: Preliminary data have verified the synthesis and purification of SILY and VEGFp (QK-Gly-Ser-Gly-hydrazide), as well as an *N*-terminal biotinylated version, through mass spectrometry and reverse-phase HPLC, respectively. For proof-of-concept, we have verified binding of VEGFp to the VEGF receptor 2 using a ForteBio Blitz interferometry instrument. In addition to support based on published reports showing retained bioactivity of QK after conjugation using other spacers, our preliminary data suggests that VEGFp still binds to VEGF receptor 2, albeit with decreased affinity like QK as compared with VEGF. Circular dichroism also shows that VEGFp has retained its α-helical structure necessary for bioactivity; however it appears that it has some uncoiling when conjugated to dermatan sulfate. We hypothesize that varying densities of VEGFp conjugated to the decorin mimetic (DS-SILY) will modulate the degree of angiogenic activity and synergy with VEGF. We determined that we can achieve ~70% VEGFp conjugation completion to dermatan sulfate after 3.5 hours. We have quantified VEGFR2 phosphorylation after 5 minute treatments by using phospho-specific antibodies and an ELISA-type protocol in a mesoscale discovery system. Preliminary data with human umbilical vein endothelial cells shows that VEGFp exhibits synergism with VEGF at levels similar to QK. DSV1 and DSV4 data suggests synergy with VEGF, although free-peptides and engineered compounds alone did not show effects similar to VEGF in the conditions tested. Prelminary data with 30 minute treatments suggests that the peptides and compounds may require longer exposures to induce activation, as they may have slower binding rates. In contrast, prolonged stimulation with VEGF causes a sharp increase in receptor activation, peaking around 10 minutes and decreasing significantly by 30 minutes. Peptides QK and VEGFp both slightly increased proliferation of dermal microvascular endothelial cells (HMVECs) after 60 hours incubation. However, incubation with dermatan sulfate and DSV caused significant cell death after 24 hours in reduced growth factor media, likely due to sequestering of growth factors. It is possible that VEGFp-DS-SILY may better stimulate proliferation since it would be presented as a surface bound proteoglycan mimic, rather than as a soluble factor. HMVECs migrated farther for all treatment groups (10 μM QK, 10 μM VEGFp, 1 μM DSV4, and 10 μM DSV4) than the 10 ng/mL VEGF positive control, although more cells migrated in response to VEGF. This may be accounted at least in part by the more pronounced proliferation induced by VEGF. Migration will also be tested in 3D culture within a collagen gel. We are currently testing a 2D matrigel system for tubulogenesis. We have found that 10 μM DSV4 forms qualitatively more well-defined tubules than the untreated control on reduced growth factor matrigel. However, we were not able to quantify the improved tubule formation and are still troubleshooting the tubule analysis. After seeding ECs and culturing for 4, 8, and 12 hours, cells will be fluorescently stained with anti-CD31 and imaged for 3D tubule formation. CAM assay angiogenesis growing into a collagen scaffold. In brief, fertilized chicken embryos are incubated for 2 days before exposing the CAM. VEGFp-DS-SILY bound to a collagen gel will be placed onto the CAM. Some treatment groups will receive additional VEGF to investigate synergistic effects. Light microscope images of angiogenesis into the collagen gel coated with VEGFp-DS-SILY, taken every day from days 10 to 13, will reflect the ability of the collagen scaffold to integrate into existing vasculature and 3D angiogenic potential of VEGFp-DS-SILY with or without VEGF. We expect that VEGFp-DS-SILY treatment will increase the number of vessels formed on the CAM. Preliminary data using a Fluoraldehyde assay indicates that loading of ~300 ng VEGF per mg of nanoparticles can be achieved. We expect that using an MMP-degradable peptide diacrylate crosslinker will allow nanoparticles to degrade in protease-rich environments like the chronic wound bed and release VEGF. Adjustments to the formulation, such as crosslinker density, may need to be modified to control the rate of VEGF release. DISCUSSION/SIGNIFICANCE OF IMPACT: We expect that our angiogenic decorin mimetic will lead to a novel treatment to accelerate healing of ischemic diabetic foot ulcers, thereby reducing the need for limb amputation and mortality rate of diabetic patients. We anticipate that the diabetes research and regenerative medicine communities will (1) gain a platform for targeted delivery of growth factors, (2) understand the dependence of vascularization within 3D collagen constructs on VEGFp densities and VEGF receptor activation in controlling the degree of angiogenesis, and (3) gain the benefits of controlled angiogenesis in ischemic diabetic wound healing.

2262

The impact of alcohol dysbiosis on host defense against pneumonia

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OBJECTIVES/SPECIFIC AIMS: Alcohol consumption perturbs the normal intestinal microbial communities (alcohol dysbiosis). To begin to investigate the relationship between alcohol-mediated dysbiosis and host defense we developed an alcohol dysbiosis fecal adoptive transfer model, which allows us to isolate the host immune response to a pathogenic challenge at a distal organ (ie, the lung). This model system allowed us to determine whether the host immune responses to *Klebsiella pneumoniae* are altered by ethanol-associated dysbiosis, independent of alcohol use. We hypothesized that alcohol-induced changes in intestinal microbial communities would impair pulmonary host defenses against *K. pneumoniae*. METHODS/STUDY POPULATION: Mice were treated with a cocktail of antibiotics daily for 2 weeks. Microbiota-depleted mice were then recolonized by gavage for 3-days with intestinal microbiota from ethanol-fed or pair-fed animals. Following recolonization groups of mice were sacrificed prior to and 48 hours post respiratory infection with *K. pneumoniae*. We then assessed susceptibility to Klebsiella infection by determining colony counts for pathogen burden in the lungs. We also determined lung and intestinal immunology, intestinal permeability, as well as, liver damage and inflammation. RESULTS/ANTICIPATED RESULTS: We found

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that increased susceptibility to *K. pneumoniae* is, in part, mediated by the intestinal microbiota, as animals recolonized with an alcohol-induced dysbiotic intestinal microbial community have significantly higher lung burdens of *K. pneumoniae* (5 × 104 CFU vs. 1 × 103 CFU) independent of EtOH. We also found that increased susceptibility in alcohol-dysbiosis recolonized animals was associated with a decrease in the recruitment and/or proliferation of CD4+ and CD8+ T-cells (1.5 × 109 cells vs. 2.5 × 109 cells) in the lung following Klebsiella infection. However, there were increased numbers of T-cells in the intestinal tract following Klebsiella infection, which may suggest that T cells are being sequestered in the intestinal tract to the detriment of host defense in the lung. Interestingly, mice recolonized with an alcohol-dysbiotic microbiota had increased intestinal permeability as measured by increased levels of serum intestinal fatty acid binding protein (55 vs. 30 ng/mL). Alcohol-dysbiotic microbiota also increased liver steatosis (Oil Red-O staining) and liver inflammation (>2-fold expression of IL-17 and IL-23). DISCUSSION/SIGNIFICANCE OF IMPACT: Our findings suggest that the commensal intestinal microbiota support mucosal host defenses against infectious agents by facilitating normal immune responses to pulmonary pathogens. Our data also suggest that increased intestinal permeability coupled with increased liver inflammation may impair the recruitment/proliferation of immune cells in the respiratory tract following infection. The role of the microbiota during host defense will be important areas of future research directed at understanding the effects of microbial dysbiosis in patients with AUDs.

2275

Essential amino acid supplementation improves lipid metabolism in older adults with elevated triglycerides

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OBJECTIVES/SPECIFIC AIMS: This study will assess the effect of essential amino acid (EAA) supplementation on plasma triglyceride (TG) in elderly adults. We will also explore the mechanisms mediating EAA mediated changes in fat metabolism and to suggest promising routes to refine therapy of hypertriglyceridemia. METHODS/STUDY POPULATION: In total, 7 nondiabetic male and female subjects ages 50–75 years with elevated plasma TG levels (130–500 mg/dL) were recruited to participate in an acute (5 h) and long-term (8 wk) EAA supplementation study. We measured changes in regional and whole body fat metabolism, including changes in body composition, plasma TG levels, whole body fat metabolic rates, tissue mitochondrial respiratory capacity, and metabolomic profiles before and after supplementation. RESULTS/ANTICIPATED RESULTS: Long-term EAA supplementation decreased fasted plasma TG levels by 19% ($p < 0.01$). Metabolomics of skeletal muscle found acute EAA supplementation resulted in increased EAA metabolic products while long-term supplementation resulted in increased anaplerosis [flux into the tricarboxylic acid cycle (TCA) intermediate pool] and anaplerotic substrates [propionyl ($p < 0.01$) and succinyl ($p < 0.01$) carnitine] and intermediates of long-chain fatty acid metabolism [stearoyl ($p < 0.01$) and myristoyl ($p < 0.05$) carnitine]. However, tissue level respiratory capacity appeared to be unaffected by EAA supplementation. DISCUSSION/SIGNIFICANCE OF IMPACT: EAA supplementation has potential to improve lipid metabolism and plasma TG levels in non-diabetic older adults. Mitochondrial metabolomics suggest that insufficient TCA pool size may limit tissue fatty acid oxidation and may provide an additional route for nutritional therapy.

2290

Control of atherosclerosis regression by LXRα S198 phosphorylation

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OBJECTIVES/SPECIFIC AIMS: Accumulation of cholesterol-laden macrophages in arterial walls leads to atherosclerosis. LXRs induce expression of genes that are atheroprotective in macrophages including CCR7, a chemokine receptor that promotes their emigration from the plaque. CCR7 expression has been shown to be negatively regulated by phosphorylation of LXRα at S198 and is reduced in diabetic mice that show impaired plaque regression. I hypothesized that LXRα phosphorylation at S198 diminishes macrophage emigration from atherosclerotic plaque and contributes to impaired regression in diabetes. METHODS/STUDY POPULATION: Inducible LXRα S198A phosphorylation deficient knock in mouse were used as donors for bone marrow transplantation into mice prone to develop atherosclerosis. Plaques were developed by placing mice on western diet; and regression was induced by lowering their lipid levels. Aortic plaques were then analyzed by using morphometric, histological, and molecular analyses in control and diabetic mice expressing either LXRα WT or LXRα S198A during regression. RESULTS/ANTICIPATED RESULTS: Surprisingly, lack of phosphorylation increased plaque macrophage content and impaired regression under normoglycemic condition; however, it did not exacerbate diabetic regression. Plaques in diabetic mice were associated with increased LXRα S198 phosphorylation. Consistent with this, LXRα phosphorylation is enhanced in macrophages cultured under hyperglycemic conditions indicating glucose-dependent regulation of LXRα phosphorylation. Monocyte trafficking studies reveal that lack of phosphorylation and diabetes independently increase recruitment of monocytes in the plaque that might contribute to increased macrophage content. Importantly, I found that diabetes also increases macrophage retention in the plaque, which is reversed in the absence of phosphorylation. We predict that this increased retention results from inhibition of emigration of plaque macrophages through enhanced phosphorylation in diabetes. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings suggest that inhibiting LXRα phosphorylation could be beneficial in diabetic atherosclerosis to reverse the accumulation of macrophages in the plaque. This study imparts insight on regulation of plaque macrophage trafficking through LXRα S198 phosphorylation.

2295

A novel in vivo zebrafish model of hematopoietic stem cell-driven regeneration of blood

Samima Sultana Habbsa, Mia McKinstry, Sara Payne, Christian Mosimann and Teresa Bowman

OBJECTIVES/SPECIFIC AIMS: Hematopoietic stem and progenitor cells (HSPCs) function to maintain steady state production of new blood cells and to rapidly respond to blood cell loss. Little is known regarding how HSPCs develop the ability to sense and respond to blood cell loss during embryogenesis. Gaining insight into the robust ability of HSPCs to regenerate blood during early development may allow us to develop therapies to rejuvenate this capacity at any stage. METHODS/STUDY POPULATION: We generated a new hematopoietic-specific and inducible cell ablation zebrafish model to uncover the origins of regenerative capacity in HSPCs during development. These transgenic zebrafish express a cyan fluorescent protein (CFP)-nitroreductase (NTR) fusion construct under the control of the draculin (drl) promoter (drl:CFP-NTR), which restricts NTR expression to blood cells. Co-expression analyses of drl:CFP-NTR with known markers of other blood types including HSPCs (runx1 + 23:mCherry), erythroid cells (gata1:dsRed), and lymphoid cells (rag2:RFP), revealed drl:CFP-NTR was restricted to HSPCs and erythrocytes. To delineate the regeneration potential of embryonic HSPCs, we exposed drl:CFP-NTR transgenic zebrafish embryos to Metronidazole (MTZ), which results in selective ablation of only NTR-expressing blood cells. Embryos were treated from 2 to 3 days postfertilization and recovery of drl+ and gata1+ cells was evaluated over a 7-day recovery period. RESULTS/ANTICIPATED RESULTS: Following MTZ exposure, the nadir of drl+ cell ablation occurs at 2 days post MTZ (dpM) treatment. During the renewal phase of blood regeneration, we first observe recovery of drl+ cells by about 4 dpM, while more mature blood cells like gata1+ erythrocytes show a delayed recovery at about 6 dpM. Our results suggest that HSPCs can respond to injury as early as 2 days of life and that the HSPC-driven regeneration of embryonic blood cells occurs in a hierarchical fashion, similar to regeneration of the adult blood system. DISCUSSION/SIGNIFICANCE OF IMPACT: We have established a quantitative method for in vivo real-time monitoring of embryonic and larval blood regeneration. A significant advantage of our system is that we can use these insights to guide an in-vivo drug screen for factors that accelerate HSPC-driven blood regeneration in a complex biological environment.

2300

E-prescribing research participation: Feasibility of recruiting research participants using an EMR-integrated health information technology

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OBJECTIVES/SPECIFIC AIMS: To study the rate of recruitment to the Pulmonary Research Registry (PRR) at the University of Chicago using HealtheRx recruitment Versus usual practice. METHODS/STUDY POPULATION: CommunityRx is a health information technology, integrated with electronic medical record (EMR) platforms, that generates personalized referrals ("HealtheRxs") for community-based programs and services that

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