415

Line1 Derepression in Specific Retrotransposon Families in Aged Mice Leads to Cytosolic DNA and Increased Inflammation

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OBJECTIVES/GOALS: The major objective of this project is two points. First, is to repeat and confirm previous observations that there is elevated cytosolic Line1 DNA in the cytoplasm of cells derived from old mice compared to young. Second is to identify which Line1s in the genome are contributing to this free DNA and test if targeting them rescues the age-related phenotype. METHODS/STUDY POPULATION: This project will focus on data collected from both tissues and primary cells derived from multiple tissues. Using cellular/tissue fractionation kits, we isolate specifically from the cytoplasm. This specificity is confirmed by western blotting. Measurement of the Line1 levels is measured by quantitative PCR. Subsequently, these cytoplasmic samples are sent off for sequencing in order to quantify the length of the free DNA in the cytoplasm and to identify which Line1 genomic families the cytosolic DNA originates. Additionally, FISH is utilized to visualize Line1 DNA in the cytoplasm of aged versus young cells RESULTS/ANTICIPATED RESULTS: We anticipate this research to confirm the hypothesis that extranuclear Line1 DNA accumulates with age in both tissues and primary fibroblasts. Additionally, we expect to be able to determine which specific families of genomic Line1 is driving this extranuclear DNA, which would suggest the active retrotransopable elements that are directly involved in this aging related phenotype. Assuming successful identification of such families, we can then target and silence these specific elements to determine not only if cytoplasmic Line1 in aged mice decreases, but additionally if the healthspan and/or lifespan of these mice improves DISCUSSION/SIGNIFICANCE: Dereoressed Line1s have been shown to be involved in detrimental phenotypes, including autoimmune disease, cancer, and inflammaging. Targeting retrotransposons, either directly through degradation of transcriptional product of LINE1s or indirectly by improving function of regulators, will be crucial in ablating aging phenotypes

416

LRP1 as a modulator of hippocampal neurogenesis and neurodegeneration*

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OBJECTIVES/GOALS: This project aims to elucidate the mechanism by which LRP1 governs hippocampal neurogenesis with a particular focus on its relevance in brain aging and memory loss. We are specifically interested in further discerning the intricacies of a novel relationship we have discovered between LRP1 and CXCR4 in adult neural stem cells. METHODS/STUDY POPULATION: For the in vivo studies, we are using a triple-transgenic mouse model in which we knockout LRP1 in adult neural stem cells. This is accomplished using a nestin-driven Cre-ER system in animals with floxed LRP1 and a floxed stop codon preceding a td-tomato reporter. The reporter allows for visualization of cells with the knockout and for trafficking and differentiation assays to be easily accomplished. We study stroke recovery using the middle cerebral artery occlusion model and brain aging by inducing the knockout and allowing the mice to age. We perform behavioral batteries and histological analysis on these mice to elucidate functional changes in neurogenesis. We also incorporate in vitro studies using primary neural stem cell cultures to mechanistically test the role of LRP1 in neural stem cell function. RESULTS/ANTICIPATED RESULTS: We have discovered that neural stem cell LRP1 knockout caused a 10-fold loss of CXCR4 expression in conjunction with deficits in ischemia-stimulated migration from the subventricular zone. We also found that uninjured aged mice lacking neural stem cell LRP1 displayed spatial memory deficits at 9 months of age (6 months after knockout), suggesting dysregulated hippocampal function. Given this, we hypothesize that LRP1 regulates CXCR4 in the subgranular zone NSCs to enhance hippocampal memory function. Ongoing research is testing our hypothesis via hippocampal functional tests and in vitro trafficking/expression assays. We expect our research to elucidate a previously unknown link between three independently identified effectors of neurodegenerative disease: LRP1, CXCR4, and neurogenesis. DISCUSSION/SIGNIFICANCE: The role of LRP1 in Alzheimers disease has long eluded clarity despite its known role in trafficking many major disease players – ApoE, amyloid beta and tau. Elucidating its role in hippocampal neurogenesis, a potential disease-modifying process, could lead to novel therapeutic approaches in diseases that cause the death of 1/3 of senior citizens.

418

Activation of the Glucagon-Like Peptide-1 Pathway in Obese Pre-Diabetic Individuals Improves Endothelial Function Independently of Weight Loss

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OBJECTIVES/GOALS: We aimed to determine if GLP-1 receptor agonists exert beneficial effects on surrogate measures of cardiovascular function independently of weight loss. Our objective was to compare the outcomes between GLP-1 receptor agonist treatment versus a similar drug without cardiovascular benefit versus weight loss through diet alone. METHODS/STUDY POPULATION: We enrolled 88 individuals with obesity (BMI â‰¥ 30kg/m2) and pre-diabetes and randomized them in a 2:1:1 ratio to 14 weeks of the GLP-1 receptor agonist liraglutide, the dipeptidyl peptidase-4 inhibitor sitagliptin, or hypocaloric diet. Sitagliptin blocks degradation of endogenous GLP-1 but does not cause weight loss or lower adverse cardiovascular outcomes. Treatment was double-blinded and placebo-controlled for drug, and unblinded for diet. Primary endpoints were flow-mediated dilation (FMD) to assess endothelial vasodilatory function, and plasminogen activator inhibitor-1 (PAI-1) to assess endothelial fibrinolytic function. We used a general linear model for each outcome and included gender as a covariate for FMD. Baseline characteristics were similar. Mean age was 50, with 32% men and 13% black. RESULTS/ANTICIPATED RESULTS: At 14 weeks, diet and liraglutide caused weight loss (diet -4.3 Â± 3.2 kg, P<0.01; liraglutide -2.7 Â± 3.2, P<0.01), while sitagliptin did not (-0.7 Â± 2.0, P=0.17). Diet did not improve FMD at 14 weeks

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