RESULTS: We successfully isolated and cultured LGR6+ ESCs from GFP expressing SD rats. We confirmed their multipotency via separate inductions towards osteoblast, chondrocyte and adipocyte lineages visible under light microscopy. The adipo-, osteo- and chondro-inductions were observed at days 12, 26 and 29 respectively. Uninduced and induced cells were shown on confocal microscopy and SEM to grow, adhere, survive and maintain their respective non-stressed or osteogenic morphology on the surface of TCPS and CCS at days 7, 14, 21, and 28. The MTT assay demonstrated that cells seeded on CCS and TCPS have no significant variation in proliferation rate between coverslip materials for either the induced or uninduced conditions.

CONCLUSION: We successfully isolated LGR6+ ESCs and demonstrated the multipotency of the isolated ESCs. We determined that LGR6+ cells grown on CCS have similar proliferation rates and morphology to cells grown on the industry standard TCPS for both induced and uninduced conditions. However, we anticipate in further studies that CCS grown cells will have an increased expression of osteogenic markers compared to cells seeded on TCPS. Post-completion of this study, we will evaluate the in-vivo potential of LGR6+ ESCs in augmenting fracture healing in non-stressed (1mm), atrophic non-union (3mm) and hypertrophic (6mm) non-union fracture models.

DNA Damage Signaling and Cell Senescence in BRCA1 Mutated Adipose Stem Cells Leads to Breast Cancer Progression

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INTRODUCTION: The tumor microenvironment can influence the progression of breast cancer. Targeting active pathways within the tumor microenvironment may be especially beneficial for triple negative breast cancer (TNBC). Our study focuses on the phenotype of human adipose-derived stem cells (ASCs) with the BRCA1 mutation and the resulting effects on breast cancer cells. We hypothesize that the mutation of BRCA1, a DNA damage response protein, induces cell senescence in ASCs and promotes the secretion of inflammatory cytokines, which leads to increased tumor invasion and metastasis.

METHODS: CRISPR/Cas9 was used to generate de novo BRCA1-knockdown in human ASCs (BRCA1-KD). We have previously generated a human breast ASC cell line repository from mastectomy specimens. Breast ASCs from patients who have BRCA1 mutation (BRCA1 -/-) and normal BRCA1 expression (WT) were also evaluated. Tumor proliferation was examined by co-culturing ASCs and luciferase-labeled TNBC cell line MDA-MB-231 in 5% FBS media over 8 days. Tumor invasion was evaluated using transwell assays, in which the CRISPR/Cas9 control and BRCA1-KD ASCs served as feeders. To investigate DNA damage in ASCs, immunofluorescence staining was performed for phosphorylated ATM, an established marker for DNA double strand breaks. The level of cell senescence was tested using beta-galactosidase assays. Finally, conditioned media was obtained from ASC lines after 48hr incubation and the inflammatory cytokine levels were evaluated using quantitative ELISA.

RESULTS: We demonstrated 79.1% knockdown expression of the BRCA1 gene in our CRISPR/Cas9-mediated knockdown; there are 52.1% and 94.9% decreased BRCA1 expressions in the two patients with BRCA1 mutation, respectively. Tumor growth in vitro was significantly faster when co-cultured with BRCA1-KD ASCs (7.43% vs 5.16% increase at day4 comparing to day0, \( P = 0.0074 \); 10.17% vs 6.57% at day6, \( P = 0.036 \); 13.39% vs 8.02% at day8, \( P = 0.045 \)). Serving as feeders, BRCA1-KD ASCs induced significantly higher level of tumor cell migration (151 ± 17.39 vs 66 ± 13.17 cells migrated. \( P = 0.0171 \)). Immunofluorescence staining revealed higher level of phosphorylated ATM activation in BRCA1-KD cells compared to controls (62.73 ± 3.12 vs 45.4 ± 2.91%. \( P = 0.0049 \)). The beta-galactosidase assay demonstrated more cell senescence in both BRCA1-KD and patients with BRCA1 mutation (7.9 ± 0.25% vs 0.17 ± 0.17%, \( P < 0.0001 \) in BRCA1-KD; 41.7 ± 2.30% vs 18.4 ± 2.34%, \( P < 0.0001 \) in patient samples). By performing ELISA assay on supernatant of BRCA1-KD and control ASCs, we found significantly higher level of IL-8 inflammatory cytokine (2.57 ± 0.32 fold change, \( P = 0.0049 \)).
CONCLUSION: We show here for the first time that BRCA1 mutation in adipose-derived stem cells leads to increased inflammatory cytokine production via a DNA damage-mediated cell senescence pathway. The effect from ASCs increases tumor proliferation and invasion. This interaction between ASCs and breast cancer cells can be targeted for more effective anticancer therapies in the setting of high-risk breast cancer.

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Treatment Resistant Stem Cell Subpopulation Depletion in Obesity and Diabetes Mellitus

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PURPOSE: There is intense interest in adipose-derived stem cells in recent years due to their importance in normal tissue homeostasis and their potential in fat grafting, stem cell therapies and tissue engineering. In parallel, the incidence of both morbid obesity and diabetes mellitus has increased worldwide but relatively little is known about how these conditions affect stem cell health and function. Recently we identified a novel mechanism through which obesity and diabetes affect health by depletion of specific subpopulations of stem cells within adipose tissue.1 Here we examine the impact of obesity and diabetes mellitus on adipose-derived stem cell health and function in humans and to what degree such impairments can be rectified through conventional therapies such as diet, exercise and bariatric surgery.

METHODS: Adipose tissue samples were taken from over 100 patients during elective surgery (bariatric, post-bariatric and controls) with appropriate institutional ethics and consent. Patients were sampled serially when possible. In parallel, the effects of exercise and diet on the recruitment of stem cells and subpopulations within adipose tissue were examined in human and murine studies respectively. Blood samples were taken pre- and post-exercise in 12 patients and then again following a 4-week exercise program in half the group with the remainder forming untrained controls. The effect of diet was studied using a diet induced obesity model (60% fat in diet) with cross-over to normal chow across both short term (6 months) and long-term (18 months) periods with all experiments duplicated to confirm the results. The stromal vascular fraction of subcutaneous fat was isolated by fluorescence cytometry (CD45-CD31-CD34+), then single cell microfluidics was performed with hierarchical cluster-based analysis to examine for subpopulation changes between groups. Subpopulations where validated at a protein level by mass cytometry (CYTOF), including markers for stemness (Sox-2, Nanog, Oct 3/4) and relevant subpopulation surface markers (CD26, CD55). Subpopulations were further validated through additional cluster-based analyses including ViSNE, SPADE and CITRUS algorithms.

RESULTS: Obesity and diabetes mellitus are both associated with a significant reduction in a specific mesenchymal stem cell subpopulation that is potently pro-angiogenic. Subpopulation depletion is strongly associated with impaired wound healing (p<0.05, One-way ANOVA). Despite correction of diabetic status and massive weight loss, subpopulation depletion is not reversed in post-bariatric patients (p<0.0001, ANOVA). Exercise did not increase recruitment of these stem cell subpopulations and diet was unable to restore stem cell subpopulations to pre-morbid levels.

CONCLUSION: Stem cell subpopulation depletion offers a novel basis for the increased morbidity and mortality seen in morbid obesity and diabetes mellitus and for the resistance of subpopulation depletion to treatment using existing therapies (bariatric surgery, diet and exercise). These data offer a new paradigm to our understanding of stem cell health and function in obesity and diabetes mellitus, prompting the need for novel therapies to address this unmet clinical need.