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.
References:
1. Rennert RC, et al. Microfluidic single-cell transcriptional analysis rationally identifies novel surface marker profiles to enhance cell-based therapies. Nat Commun, 2016. 7:11945.

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Motor Denervation Activates Muscle Stem Cells and Leads to Muscle TGFbeta Expression

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PURPOSE: The pathophysiology underlying the irreversibility of muscle atrophy and inability to regenerate following prolonged denervation is incompletely understood. Satellite cells (SCs) are the primary resident stem cells in muscle responsible for repair after injury, and are defined by expression of the transcription factor paired box protein 7 (Pax7). Previous studies reported SC depletion after long-term denervation, possibly explaining the irreversibility of denervation atrophy, but did not quantify Pax7+ cells nor perform functional studies. It is therefore unknown to what extent SC depletion occurs or limits recovery after delayed reinnervation. This study attempts to elucidate the effect of motor denervation on SC behavior and regenerative capacity.

METHODS: The sciatic nerve of C57Bl6 mice was transected. Three, six, and 12 months following denervation, 100ug EdU was injected intraperitoneally for three consecutive days. Mice were sacrificed on day four and the tibialis anterior muscles (TAs) were processed for immunohistochemical analysis. Flow cytometry was used to select for SCs from lower leg muscles. Mitotracker staining was performed to assess mitochondrial activity in SC. SCs from three month-denervated legs or minced muscle grafts from six and twelve month-denervated TAs were transplanted into the pre-irradiated left TAs of immune-deficient/dystrophin-deficient (NSG- mdx) mice. SCs were isolated from the atrophic denervated gluteus maximus muscle of human patients with complete spinal cord injuries via flow cytometry and transplanted into irradiated left TAs of NSG mice.

RESULTS: TAs weighed 11.9±0.6mg after 3-month denervation vs 56.6.5±5.5mg (p<0.05). No significant differences between three, six, and twelve-month denervated TA weights were noted. Cross-sectional fluorescent immunohistochemical staining demonstrated a significant increase in the total number of Pax7+ cells per muscle fiber in denervated TAs three and six months following denervation compared to non-denervated TAs (p<0.05). Twelve months following denervation, the number of Pax7+ cells in denervated TAs remains unchanged relative to non-denervated TAs. EdU uptake by SCs is significantly increased three months following denervation. SCs isolated from denervated legs were larger and had higher mitotracker uptake by flow cytometry compared to uninjured legs. After transplantation into the irradiated left TA of NSG- mdx mice, SCs from 3 month-denervated leg muscles were able to engraft and produce new muscle fiber. Minced muscle grafts from six and 12 month-denervated TAs also regenerated new muscle fibers after transplantation into NSG- mdx mice. SCs isolated from denervated human gluteus maximus and transplanted into NSG mice engrafted and made human dystrophin-producing muscle fibers. Following denervation, TGFbeta expression is increased in muscle, and downstream TGFbeta signaling is significantly increased in SCs as measured by Pax7-Smad2/3 co-staining.

CONCLUSION: Contrary to previous reports, SC depletion does not occur following prolonged periods of denervation in either rodents or humans. The SC phenotype is altered from quiescence towards activation as demonstrated by their increase in number, size, EdU uptake, and mitochondrial activity post-denervation. SCs also retain robust intrinsic regenerative capacity when transplanted into a different host. The irreversibility of denervation atrophy cannot be explained by SC depletion or dysfunction. TGFbeta expression increases in muscle following denervation and may affect SC regenerative capacity.

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The Use of Paravertebral Nerve Blocks in Immediate Breast Reconstruction following Mastectomy: A Canadian Hospital-Perspective Cost Effectiveness Analysis