Novel Alterations in Chromatin Structure during Aging Reveal Cell-Specific Differential Expression of SUMO Proteins

Xiaoyin Shan, PhD, Cleresa Roberts, BS, Yemin Lam, PhD, Ivona Percec, MD, PhD

University of Pennsylvania, Philadelphia, PA, USA

PURPOSE: Human adipose derived stem cells (ASCs) are considered to be an ideal adult stem cell for regenerative medicine applications due to their wide availability, ease of harvest, multilineage differentiation potential, trophic factor secretion, and low immunogenicity and oncogenic capacity. Unfortunately, aging negatively affects the therapeutic efficacy of ASCs. Currently, the precise molecular mechanisms governing adult stem cell aging remains poorly understood. Therefore, elucidating the mechanisms of ASC aging is critical for improving treatment outcomes for human stem-cell based therapies and aging prevention.

METHODS: Subcutaneous abdominal tissue was obtained from healthy patients of various ages (25–74) undergoing abdominoplasty. ASCs were isolated via standard collagenase protocols. Dermal fibroblasts were isolated from human skin specimens obtained from healthy patients of various ages (25–64). Chromatin accessibility profiles were examined using ATAC-seq technology. Genomic nucleosome occupancy was mapped using NucleoATAC software. Pathway enrichment of genes display specific promoter nucleosome positioning was analyzed using Reactome Pathway Database. Small ubiquitin-like modifier (SUMO) protein expression levels at basal and after heat shock were assessed using the dot-blot assay.

RESULTS: We initially demonstrated that ASC genomes overall contain more peaks from Tn5 fragmentation than those of fibroblasts. Furthermore, we observed that the position of nucleosomes flanking transcription start sites (TSS) is globally well maintained in aging ASCs but not in aging fibroblasts. However, subtle age-dependent changes in nucleosome positioning were detected in the promoter region of genes involved in protein sumoylation pathways in both ASCs and fibroblasts. In ASCs, the nucleosome positions in the promoter region of these genes, especially the one at the upstream of TSS (-1 nucleosome), are more consistently narrowly distributed in the older age group. In contrast, the -1 nucleosome positioning in older fibroblasts is variable. Global SUMO protein expressions under stress and non-stress conditions indicates that SUMO expression in old ASCs is more sensitive to heat shock than in young ASCs, whereas in fibroblasts, this age-dependent sensitivity is minor.

CONCLUSIONS: Our data suggest a significant role for nucleosome positioning in sumoylation pathway regulation in response to stress during aging of adult stem cells. The distinctive difference in the chromatin structures described here between human ASCs and fibroblasts will contribute to the elucidation of mechanisms regulating gene expression during chronological aging in both stem cells and differentiated cells. The novel findings of age-dependent changes in chromatin structure and regulation of stress response in human ASCs offer significant implications for therapeutic application in regenerative medicine.

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METHODS: As part of an ongoing randomized, controlled crossover trial. Fat was harvested from the abdomen by manual liposuction, processed and injected by Coleman technique, and introduced into the macrochamber fat compartment (MAC) of 7 patients (11 feet) with an average volume of 6.9cc per heel. Patients were offloaded in a customized Darco shoe for 4wks post-operatively. Ultrasound-measured tissue thickness, pedobarograph-measured foot pressures, and Manchester Foot Pain and Disability Index (MFPDI) were obtained pre-operatively and followed for 1 year post-operatively. Outcomes were compared against a randomly selected control group (5 patients) who received standard of care offloading only.

RESULTS: Average age was 58 years. Average BMI was 30.5. No patients were active smokers or diabetic. When compared to controls, subjects who received fat grafting had significantly greater fat pad thickness at 6 and 12 months both at rest and under load (p<0.05). On pedobarograph, standing heel forces and pressures trended up across all time points while walking heel forces and pressures trended down across all time points. On the MFPDI, patients receiving fat grafting had significantly improved foot pain (p=0.015) and foot appearance (p=0.048) scores at 6 months.

CONCLUSION: Our current data suggests that fat grafting can restore foot function in patients with heel fat pad atrophy by preserving shock absorbing soft tissue and reducing pain. This has allowed many of our patients to resume previously untolerated activities. However, these findings will need to be corroborated in a larger sample and longer follow up which our ongoing trial aims to provide.

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Interrogating the Metabolic Interactions Between Adipocytes and Breast Cancer in a Patient-Derived Tissue Engineered Platform—Implications for the Safety of Autologous Fat Transfer in the Setting of Breast Ductal Carcinoma

Yoshiko Toyoda, BA, Karel-Bart Celie, BA, Justin Buro, BA, Alexandra Lin, BA, Jonathan Xu, BA, Andrew Miller, BA, John Morgan, PhD, Kristy A. Brown, PhD, Jason A. Spector, MD

Weill Cornell Medical College, New York, NY, USA

PURPOSE: Obesity is a known risk factor for the development of breast cancer (BC) and is understood to have a negative impact on prognosis. Obesity is associated with less response to BC therapy and more aggressive disease. Adipocytes have been identified as a source of exogenous lipids in many cancer cell types, and are thought to provide energy to fuel malignant survival and growth in BC. This relationship is of particular relevance in plastic surgery as the oncologic safety of autologous fat transfer, an increasingly ubiquitous adjunctive procedure for breast reconstruction after mastectomy, is largely unknown. Although clinical studies to examine this question are underway, an in vitro system is critical for elucidating the complex interplay between the cells that normally reside at the surgical recipient site. We have developed a 3D, patient-derived tissue engineered platform to directly assess the metabolic interactions among cells within the BC tumor microenvironment.

METHODS: Breast adipose tissue was acquired from patients undergoing breast reduction surgery. The tissue was enzymatically digested and sorted by differential centrifugation to retrieve adipocytes and ASCs. Polydimethylsiloxane wells were filled with type I 0.3% (w/v) collagen and seeded with varying concentrations of adipocytes labeled with the fluorescent lipid dye boron-dipyrromethene (BODIPY) and ASCs in the bulk, and fluorescently-labeled MDA-MB-231 BC cells on the surface. Cultures of BC cells in non-adipocyte containing collagen matrices served as controls. Lipid transfer and BC cell invasion into the collagen-adipocyte/ASC bulk matrices were analyzed using laser scanning confocal microscopy and image analysis.

RESULTS: As the BODIPY lipid stain was added to the adipocytes prior to seeding of the BC on the surface, any BODIPY staining seen within the BC cells must have originated from within the adipocytes. After 24 hrs of co-culture, the 3D collagen culture platform demonstrated BODIPY-stained mature adipocytes surrounded by stromal cells, akin to the native architecture in human breast tissue. At the interface of the cancer cells with the stroma, lipid transfer was observed from adipocytes to BC cells as demonstrated by the change in the morphology of BC cells in proximity to the lipid-filled adipocytes from a spindle-like shape to more round appearance, filled centrally with green fluorescent lipid droplets and the cytosol pushed to the periphery.

CONCLUSION: We have established a novel 3D platform to study BC microenvironment, including metabolic