Fibroadipogenic Progenitors Possess Endogenous Antimicrobial Properties in an Age-Dependent Fashion

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INTRODUCTION: Immunosenescence is defined as the gradual dysregulation of the immune system as a consequence of natural aging. From reduced production of T cells and B cells to the activation of chronic, hyperactive inflammatory states, older individuals are significantly more at risk for developing infections and autoimmune-related diseases than their younger counterparts [1,2]. While mesenchymal stem cells have been widely accepted as key contributors to host defense through immune modulation (e.g., secretion of antimicrobial peptides), the endogenous, antimicrobial properties of fibroadipogenic progenitors (FAPs)—resident muscle MSCs—is less defined. In this study, we thus aim to investigate their ability to inhibit bacterial growth in an age-dependent fashion, while also mechanistically exploring immune-related transcriptomic differences in young versus aged human FAP populations. We hypothesized that young FAPs would inherently possess a more potent arsenal of antibacterial properties than older FAPs.

METHODS: Healthy hamstring and deltoid muscle biopsies were obtained from young (<50) and aged (≥50) patients undergoing orthopaedic surgery after informed consent. After collection, samples were mechanically and enzymatically digested into single-cell suspensions. Fluorescence-activated cell sorting (FACS) was then utilized to isolate viable CD31-/CD45-/CD56-/CD34+/PDGFRα+/SYTOX- FAPs. After isolation, FAPs were then either directly plated into human FAP growth media for *in vitro* assays or submitted directly for single cell mRNA sequencing (scRNAseq) using 10x Genomics Chromium GEM-X Single Cell 3' Reagent Kits v4. *In vitro*, conditioned media (CM) from FAPs were generated by plating 2.1 x 10^6 cells per T75 flask with 8mL of antibiotic free media containing F-10, 10% FBS, and basic fibroblast growth factor and then incubating at 37°C in a 5% CO_2 incubator. CM from FAPs were collected 96 hours post-initial plating. For bactericidal assessment, Staphylococcus aureus bacteria were inoculated in 500uL of conditioned media in 24-well plates at 25°C. The number of viable bacteria was determined by plating log10 serial dilutions on LB agar 4 quadrant plates 3- and 24- hours post incubation. Manual counting of colony forming units (CFU) was performed after 24-hour incubation on agar plate. These results were compared with bacteria incubated in Lysogeny Broth media, control cell culture media, along with low (2ug/mL) and high (20ug/mL) doses of vancomycin. Separately, scRNAseq data was processed using CellRanger (version 9.0.2), and Seurat (version 5) was used for data integration and downstream analysis. Differential gene expression analysis was performed between young and aged samples using DESeq2, and genes with adjusted p-values < 0.05 and |log2 fold change| ≥ 0.32 were considered biologically relevant. Results were analyzed via a mixed-effects model.

RESULTS SECTION: In vitro, young FAP CM, along with low dose vancomycin, began to eliminate S. Aureus significantly at 12 hours, compared to control and aged FAP CM. At 24 hours, young FAP CM, in addition to both vancomycin conditions, fully eliminated S. Aureus. Notably, there was no significant difference between control cell culture media and aged FAP CM at any timepoint. Additionally, a total of 11,851 cells were analyzed for scRNAseq, which revealed that FAPs express antimicrobial genes (e.g. Ribonuclease A Family Member 4, Secretory Leukocyte Protease Inhibitor, Ribonuclease L, and Defensin Beta 1) and immune-modulatory genes (TLR1, S100A8, S100A9, CD55), suggesting that human FAPs possess intrinsic antimicrobial and immune-regulatory machinery (**Fig 2**). Differential expression analysis revealed 13 genes that were significantly upregulated in young versus aged FAPs; of these, we identified programmed cell death 5 (PDCD5) as a key gene that activates effector T cells and promotes the activity of IFN-gamma CD8+ T cells, $\log_2FC = 0.19$, $p \leq 0.05$ (**Fig 3**). We also identified 56 genes that were upregulated in the aged FAP cohort, 14.3% of which are involved in the maladaptive immune-mediated regulation of apoptosis.

DISCUSSION: In this study, we reveal that fibroadipogenic progenitors, isolated from young, healthy human muscle, possess antimicrobial properties, similar to the efficacy of standard antibiotic treatment in vitro. Through scRNAseq differential analysis, we identified PDCD5 as a potential mechanistic target to account for the age-dependent efficacy in immune regulation. A predominant number of pro-apoptotic genes in the aged cohort supports the inflammaging phenomenon of a deleterious, hyperactive immune state in normal, healthy human muscle.

SIGNIFICANCE/CLINICAL RELEVANCE: To our knowledge this is the first study that demonstrates the age-dependent efficacy of fibroadipogenic progenitors in clearing bacteria at levels comparable to standard antibiotic therapy, while additionally identifying key transcriptomic differences in healthy versus aged FAPs to support a mechanistic understanding of FAP's role in immunosenescence.

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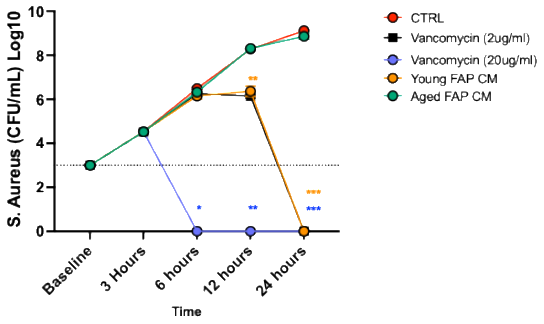

Figure 1. S. Aureus survivability in various in vitro culture conditions. *p<0.01 between high-dose vancomycin and all other conditions **p<0.005 between vancomycin and all other conditions ***p<0.001 between vancomycin and all other conditions, except young FAP CM **p<0.01 between young FAP CM and all other conditions, except low-dose vancomycin ***p<0.001 between young FAP CM and all other conditions, except vancomycin.

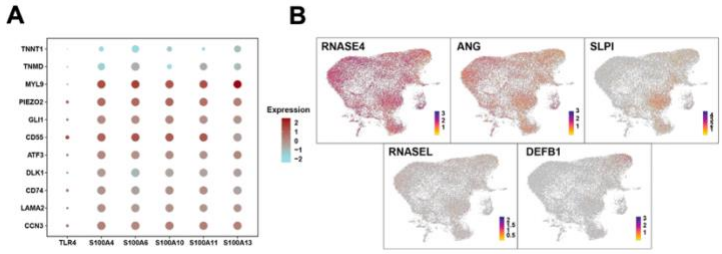

Figure 2. (A) Dotplot of genes encoding proteins that indirectly influence antimicrobial activity. (B) Feature plot of genes expressed by FAPs that encode proteins that have direct antimicrobial activity.

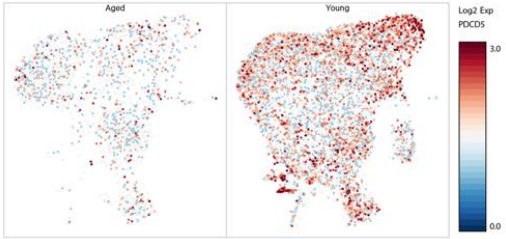

Figure 3. UMAP plot heatmaps displaying the differential expression of PDCD5 in aged versus young FAPs.