An exosome marker-enriched fibro/adipogenic progenitors (FAPs) population promotes muscle regeneration

Austin Lee[1,2], Mengyao Liu[1,2,4], Justin Lau[1,2], Brian T. Feeley[1,2] , Hubert Kim [1,2], Xuhui Liu[1,2]
1. San Francisco Veterans Affairs Health Care System 2. University of California, San Francisco, Department of Orthopaedic Surgery 3. University of California, Berkeley 4. California Northstate School of Medicine

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INTRODUCTION: Muscle interstitial fibro/adipogenic progenitors (FAPs) are non-myogenic, mesenchymal progenitors that play an important role in maintaining muscle extracellular matrix (ECM) and regulating other muscle progenitor cells, including satellite cells (SCs). FAPs actively communicate with SC and other cells, including immune cells during muscle regeneration. Though the detailed mechanisms are not fully illuminated, evidence suggests that this interaction may be mediated through extracellular vehicles (EVs), particularly exosomes [1,2]. Exosomes are nano-sized membrane EVs that are secreted by almost all cell types. Exosomes carry transmembrane markers from the tetraspanin family of proteins, including CD63, CD81, and CD9. In this study, we isolated a subpopulation of FAP with high expression level of exosome marker of CD81 and tested their role in promoting muscle regeneration in a mouse volumetric muscle loss (VML) model. We hypothesize that CD81+ FAPs promote muscle regeneration after VML.

METHODS: Human FAPs (hFAPs) were isolated from hamstring muscle from patients undergoing anterior cruciate ligament (ACL) reconstruction. Muscles were minced and further digested with collagenase. FAPs were isolated through Fluorescence-activated Cell Sorting (FACS) with their surface markers of CD39-/CD45-/CD31-/ITGA7-/PDGFRα+. CD81+ hFAP were further separated from the FAP pool with their surface marker of CD81. Unilateral volumetric muscle loss (VML) injury was created in NGS immunodeficient mice (n=13) with a defect of full thickness of muscle with a 4mm in diameter punch at proximal one third of tibialis anterior (TA) muscle. CD81+ hFAPs were then transplanted to muscle defect (1×10 6 cell in 10μl HyA Matrigel, n=4). This group was compared to unseparated (heterogenous mixture) hFAPs (n=4) and matrigel-only control groups (n=5). Mice were sacrificed at 6 weeks after transplantation and TA muscles from the injured legs were then harvested for histologic analysis for muscle atrophy and fibrosis. A separated group of human FAPs underwent single cell RNA-sequencing utilizing 10x Genomics 3' kits. The datasets were analyzed using Cellranger, an analysis pipeline for processing scRNAseq reads and Seurat, an R toolkit that allows for the exploration of scRNAseq data, to cluster cells and determine differential gene expression between different FAP subpopulations ($p = 0.05$).

RESULTS: Histological analysis showed a significant increase in muscle fiber area in the CD81+ hFAP transplanted group compared to the heterogeneous hFAP group ($p=0.02$) and the control group ($p=0.05$) (Figure 1). No significant differences in fibrosis were observed between all groups (Figure 2). scRNAseq analysis showed FAPs with high expression level of CD81 ($CD81^{high}$) has unique expression patterns. Compared to hFAPs with low CD81 expression ($CD81^{low}$), $CD81^{high}$ hFAPs have reduced expression of *SFRP1* and *MYOC* and upregulation of *MFAP5*, *TIMP1*, *CLEC3B*, and *CD55* (Figure 3).

DISCUSSION: Large-scale skeletal muscle damage and VML are extremely challenging pathologies to treat. Previous studies have attempted to address this challenge by utilizing strategies based on the transplantation of myogenic progenitor cells, namely satellite cells. In this study, we showed that transplantation of a non-myogenic cell population of CD81+ FAPs significantly improves muscle regeneration after VML. VML is a challenging model for cell transplantation due to the severity of muscle injury. Though we only observed a borderline effect of CD81+ FAPs compared to the control group, considering the small sample size, it is reasonable to believe that CD81+ FAPs indeed promote muscle regeneration. In addition, CD81+ FAPs showed a significant effect in improving muscle regeneration compared to the heterogeneous non-selected FAPs. Taken together, our data suggests that this FAP subpopulation may serve as a new cellular source for treating VML on its own or in combination with SCs. Furthermore, our work proposes that the supportive role that FAPs play in myogenesis may be mediated through exosomes. Exosomes carry and transfer a variety of cell-signaling molecules including protein and RNA [1]. Myoblast and myotube-derived EVs carry growth factors and miRNAs (MyoMirs) that have been implicated in controlling myogenesis [2, 3]. Future work is needed to define the role of CD81+ FAP-derived exosomes in muscle regeneration.

SIGNIFICANCE: Exosome marker-enriched CD81+ FAPs may be used as a cellular source for treating volumetric muscle loss and other muscle injuries.

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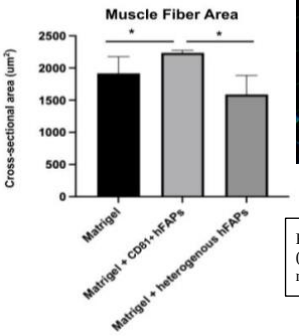


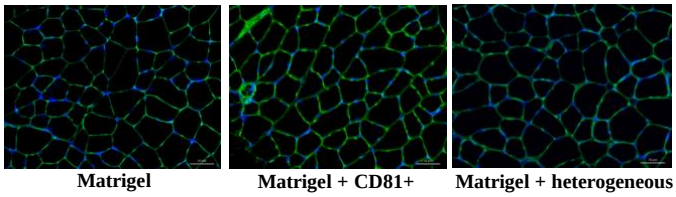
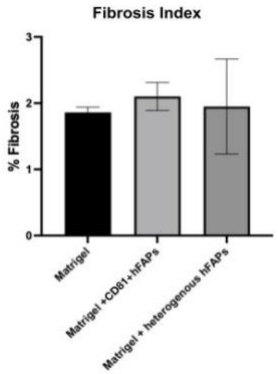


Figure 1. Representative images and correlated graphs (SEM bars) for muscle fiber cross-sectional area (CSA). Significant differences in muscle fiber area found between CD81+ hFAP transplanted group and matrigel-only control group and heterogeneous hFAP group.



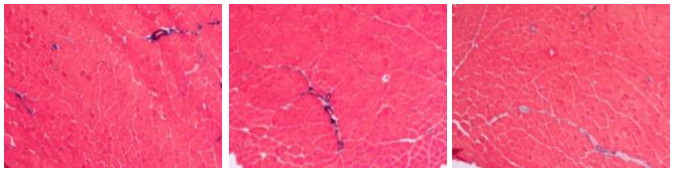
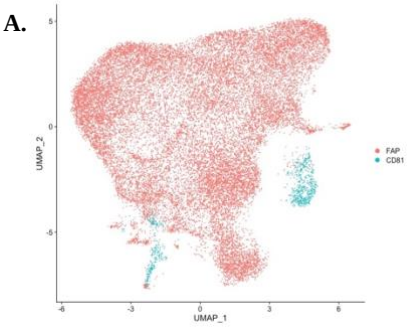


Figure 2 Representative images and correlated graphs (SEM error bars) for fibrosis index percentage. No significant differences found between any groups.



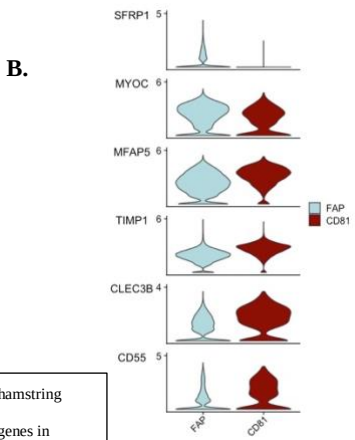


Figure 3. A) UMAP plot identifying the CD81+ FAP subpopulation from human hamstring muscle samples.
B) Downregulation of *SFRP1* and upregulation of *MFAP5*, *TIMP1* and *CLEC3B* genes in CD81+ hFAPs suggest these cells may create an environment conducive to muscle regeneration and wound healing after injury. Downregulation of *MYOC* and upregulation of *CD55* and *ACTA2* suggest that CD81+ hFAPs favor fibrogenesis over adipogenesis.