Analgesic proteins in injectable bone marrow aspirate concentrate and adipose tissues

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Disclosures: The authors have nothing to disclose.

INTRODUCTION: Bone marrow aspirate concentrate (BMAC) and minimally manipulated adipose-derived tissue preparations (FAT) have been marketed as a rich source of mesenchymal stem cells (MSCs), growth factors, and anti-inflammatory cytokines. They have been used interchangeably to treat a variety of orthopedic conditions including osteoarthritis, tendinitis, and joint pain. Studies have suggested that both tissue sources may improve pain in these patients, but the mechanism-of-action is unknown and may be independent of stem cells. Therefore, the purpose of this study was to identify and compare proteins with known analgesic properties in BMAC and FAT, and to investigate which BMAC and FAT cells synthesize these proteins.

METHODS: Ready-to-inject BMAC and FAT were prepared from iliac bone marrow aspirates and subcutaneous adipose tissue collections from patients either undergoing knee or hip injection, or during total hip arthroplasty (12 male, 57.3 ± 19.9 years old, body mass index 28.3 ± 4.3 kg m^{-2}; 9 female, 64.2 ± 10.4 years, body mass index 25.2 ± 2.7 kg m^{-2}). Label-free mass spectrometry was performed on 15 BMAC and 13 FAT samples, 8 of which were paired. All detected proteins (abundance > 0) were included. In parallel, a literature search was performed to curate a list of proteins with known inhibiting or facilitating function on pain, which was then compared with mass spectrometry results. Finally, transcripts of detected proteins were identified in a single cell transcriptional atlas of the exact same tissues, to identify the cell types and their numbers transcribing and thus potentially secreting these proteins of interest. This study was approved by the local institutional review board.

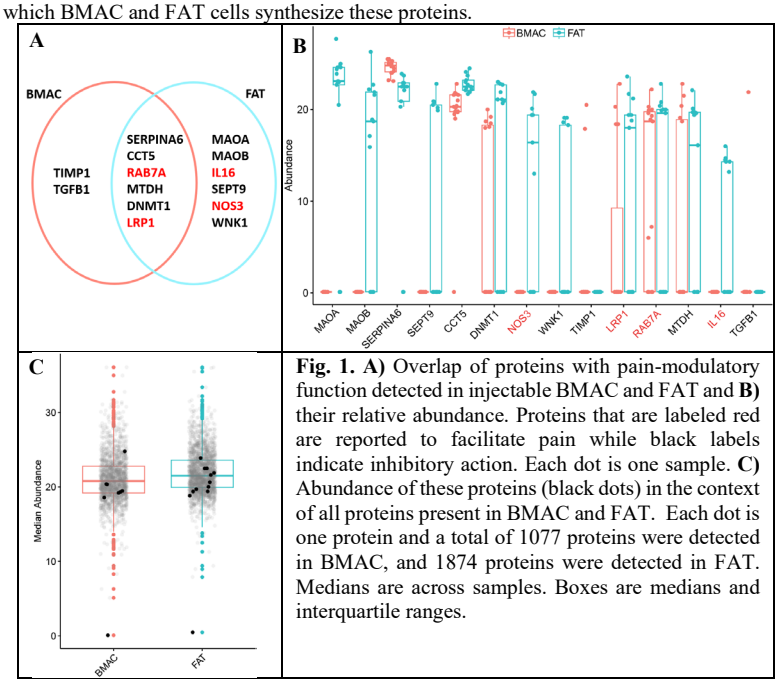

Fig. 1. A) Overlap of proteins with pain-modulatory function detected in injectable BMAC and FAT and **B)** their relative abundance. Proteins that are labeled red are reported to facilitate pain while black labels indicate inhibitory action. Each dot is one sample. **C)** Abundance of these proteins (black dots) in the context of all proteins present in BMAC and FAT. Each dot is one protein and a total of 1077 proteins were detected in BMAC, and 1874 proteins were detected in FAT. Medians are across samples. Boxes are medians and interquartile ranges.

RESULTS SECTION: A list of 100 proteins with pain modulatory function was composed from the literature; 52 of which with documented pain-inhibiting action, 29 with pain-facilitating action, and 19 with variable effects on pain. Two of these proteins were detected in BMAC only (TIMP, TGFB1), six were detected in FAT only (MAOA/B, IL16, SEPT9, NOS3, WNK1), and another six were detected in both tissues (SERPINA6, CCT5, RAB7A, MTDH, DNMT1, LRP1). Importantly, RAB7A, LRP1, IL16, and NOS3 are reported to facilitate, not inhibit pain (Fig. 1). 92 of these proteins were detected in a single-cell transcriptional atlas of BMAC, and 92 were detected in FAT. SERPINA6, a corticosteroid-binding protein was the protein most consistently found in BMAC and FAT, but none of the resident cells synthesize it (data not shown). The top two most abundant pain-modulatory proteins in BMAC (CCT, RAB7A) are predominantly synthesized by monocytes, erythroblasts, and immune cells. In FAT, the top two were MAOA and CCT5 which appear to be synthesized by fibroblasts and endothelial cells (Fig. 2).

DISCUSSION: While this study contributes to the identification of potentially active ingredients in BMAC and FAT injections, it also highlights the complexity given the abundance of pain-facilitating proteins, and their variable concentrations. The cell type for which these injections are typically advertised (MSCs) do not appear to play a key role with regards to synthesis of pain-modulatory proteins. A limitation is the false negative rate for low abundance proteins associated with this mass spectrometry technique. If these biologics are considered in future, studies should investigate whether more sustained pain relief is related to the concentration of cells which synthesize the proteins of interest.

SIGNIFICANCE/CLINICAL RELEVANCE: Based solely on the ratio of pain-inhibiting to -facilitating proteins present, BMAC may be superior (3:1 vs. 2:1 in FAT). However, individual protein concentrations (and cells secreting them) may have to be considered more carefully to help explain the variable outcomes observed clinically.

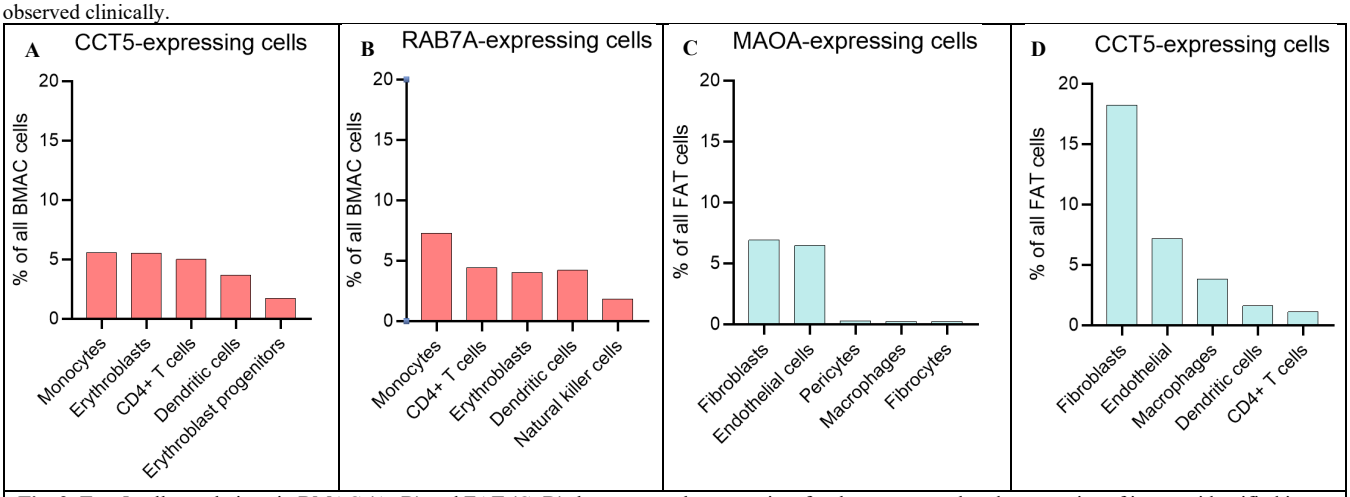

Fig. 2. Top 5 cell populations in BMAC (A+B) and FAT (C+D) that express the transcripts for the two most abundant proteins of interest identified in Fig. 1. These are potentially the cell populations secreting the proteins *in vivo*. Pooled single-cell RNA sequencing datasets from 13 BMAC and 11 FAT samples encompassing 50,836 and 40,129 cells, respectively.