# Infrapatellar Fat Pad Mesenchymal Stem/Stromal Cell Derived Exosomes for Substance P (SP) and Calcitonin Gene Related Peptide (CGRP) Inhibition: Potential Implications for Inflammation/Pain Reversal

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**INTRODUCTION:** During the progression of knee osteoarthritis (OA) the synovium and infrapatellar fat pad (IFP) can serve as source for Substance P (SP) and calcitonin gene-related peptide (CGRP), two important pain-transmitting, immune and inflammation modulating neuropeptides [1, 2]. Regulation of SP and CGRP activity is achieved partly by the cell membrane-bound neutral endopeptidase CD10 (neprilysin) [3] which multiple mesenchymal stem/stromal cell (MSC) types express [4]. Our previous work demonstrated that human IFP-MSCs acquire a potent immunomodulatory phenotype and actively degrade SP via CD10 *in vitro* and *in vivo* [5, 6]. Notably, recent preliminary data indicated that enzymatically-active CD10 is present in MSC-derived exosomes (EXOs). The purpose of this project was to explore genetic manipulation of CD10 high IFP-MSCs so that their yielded EXOs could target both SP and CGRP activities yielding robust anti-inflammatory and analgesic effects *in vivo*.

**METHODS:** Human IFP-MSC cultures were transfected with an adeno associated virus (AAV) vector carrying a GFP-labelled gene for the CGRP antagonist peptide (aCGRP). The GFP positive aCGRP IFP-MSCs were selected by fluorescence-activated cell sorting and their cultures' secretome was used to isolate EXOs. CD10 high/aCGRP IFP-MSCs EXOs miRNA profile was assessed using a pre-designed human MSC exosome 166 miRNA qPCR arrays (GeneCopoeia) and putative miRNA interactomes were generated using a miRNet centric network visual analytics platform (https://www.mirnet.ca/). The miRDB online database (http://mirdb.org) for prediction of functional microRNA targets has been used to correlate highly expressed target genes of interest with specific miRNAs identified by miRNA profiling. The CD10 high/aCGRP IFP-MSCs EXOs protein profile was assessed using inflammation protein arrays (Raybiotech). Putative protein interactomes were generated by Search Tool for Retrieval of Interacting Genes/Proteins (STRING 11.0; available from: http://string-db.org).

**RESULTS:** Purified CD10 high/aCGRP IFP-MSC cultures yielded EXOs with cargo of 147 distinct MSC-related miRNAs. Reactome analysis of detected miRNAs revealed their strong involvement in the regulation of six gene groups related to the: gene expression, immune system, TGF-β/Wnt/FGFR pathways, cell cycle, NGF and Toll-like receptor pathways, and cellular responses to stress. An nalysis for functional correlation of identified miRNAs in CD10 high/aCGRP IFP-MSCs EXOs revealed that 19 highly present miRNA cargo regulate known target genes involved in pathways that control pain, inflammation and cartilage homeostasis. Protein array of the EXOs cargo noted the high presence of nine immunomodulatory proteins (ICAM-1, IL-6, IL-8, MCP-1, TIMP-2, MIP-1β, S TNF RI, and IP-10). All detected proteins were involved in multiple biological processes and pathway regulation in KEGG reactome analysis.

**DISCUSSION:** Our study identified distinct miRNA and protein signatures as cargos within CD10 high/aCGRP IFP-MSCs EXOs. In silico analysis demonstrated that detected miRNAs and proteins regulate multiple pathways and biological processes involved in the control of pain, inflammation and cartilage homeostasis. Therefore, our preliminary data suggest that yielded EXOs can putatively target both SP and CGRP, two signaling molecules involved in inflammation and pain *in vivo*

**SIGNIFICANCE/CLINICAL RELEVANCE:** To date, no treatments result in arrest or mitigation of OA progression. EXOs derived from genetically manipulated IFP-MSCs targeting Substance P and CGRP contain a plethora of anti-inflammatory and analgesic cargo suggesting the promise for novel EXO-based therapeutic approaches to diseases such as OA.

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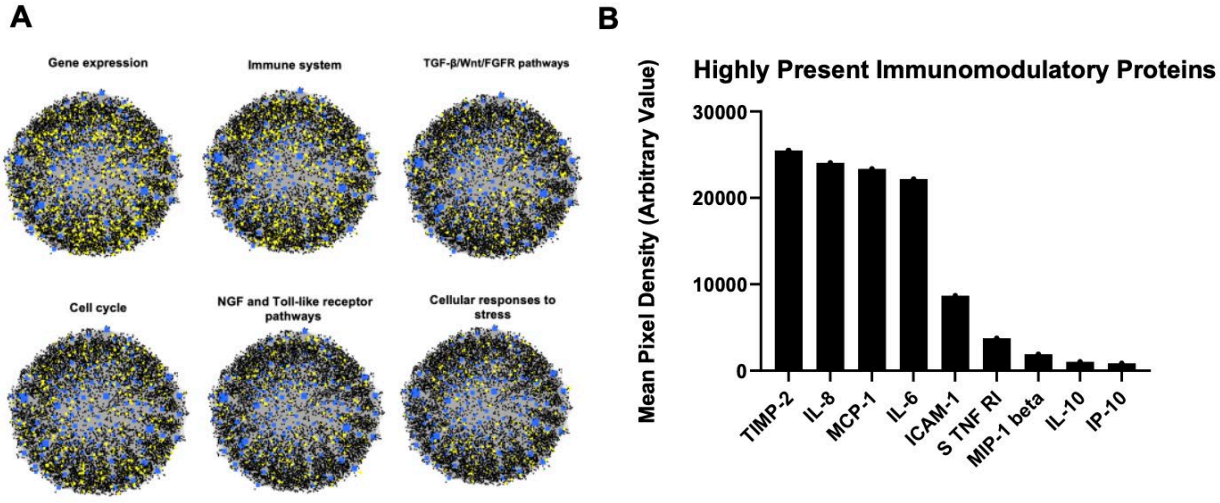


**Figure 1.** (A) Reactome analysis of detected miRNAs in CD10 high/aCGRP IFP-MSCs EXOs showed their strong involvement in the regulation of six gene groups. (B) Protein Array of CD10 high/aCGRP IFP-MSCs EXOs noted the high presence of nine immunomodulatory proteins.

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