by chondrocytes under natural tissue culture conditions and elicit an anti-inflammatory effect on the chondrocytes. Arthritis is an inflammatory disease so the anti-inflammatory effects of EV show potential as a preventative treatment for arthritis however a regenerative effect was not observed. This highlights the potential of MSC EV as a preventative therapy for arthritis.

Mesenchymal stem cell therapy for inflammatory arthritis: from the cell to its extracellular vesicles

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Introduction: Novel biological therapies have revolutionised the management of Rheumatoid Arthritis (RA) but no cure currently exists. Mesenchymal stem cells (MSCs) immunomodulate inflammatory responses through paracrine signalling via growth factors, cytokines, chemokines and extracellular vesicles (EVs) in the cell secretome. Previously, our group has demonstrated the immunomodulatory capacity of both MSCs and their conditioned medium to reduce inflammation in a murine antigen-induced arthritis (AIA) model. In this project, we tested the therapeutic potential of EVs in the AIA model of inflammatory arthritis.
Materials and Methods: EVs isolated from bone marrow MSCs in normal (21% O2, 5% CO2) or hypoxic (2% O2, 5% CO2) culture or from MSCs pre-conditioned with a pro-inflammatory cytokine cocktail were applied into the AIA model. Disease pathology was assessed 3 days post arthritis induction through histopathological analysis of knee joints. Spleens and lymph nodes were collected and assessed for T cell polarisation within the immune response to AIA. Activated naïve CD4+ T cells from spleens of healthy mice were cultured with EVs to assess deactivation capabilities.
Results: AIA is an acute model of inflammatory arthritis that typically exhibits peak joint swelling at 24 hours post induction with clinical symptoms and histopathological signs that resemble rheumatoid arthritis. All EV treatments prompted amelioration of clinical symptoms of AIA with increased effect seen in reduction of joint swelling when treated with EV-2%O2 or EV-Pro-Inflam. Histopathological analysis of joint damage which is reported as arthritis index (AI) showed significantly reduced overall joint damage in mice treated with EV-NormO2 and EV-Pro-Inflam compared to EV-depleted medium control. Whilst EV-2%O2 overall showed a tendency to reduce total AI scores compared to controls, this was not statistically significant. Polarisation of T cells towards CD4+ helper cells expressing IL17a (Th17) was reduced when EV treatments from MSCs cultured in hypoxia or pro-inflammatory priming conditions were applied.
Discussion: Our results suggests the reduction in Th17 cells, that led to restoration of the Treg:Th17 ratio which is typically unbalanced in inflammatory arthritis, as the main therapeutic mode of action of MSC-derived EVs.

Development of novel sample preparation protocols to increase identification and coverage of ECM proteins using mass spectrometry

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Introduction: Proteomic methods tailored to specifically study the extracellular matrix (ECM) have powerfully revealed ECM signatures and biomarkers associated with diseases including cancer and fibrosis. However, because the ECM is a complex meshwork of proteins with a wide range of biochemical properties and a broad dynamic range of expression, some ECM proteins remained understudied using existing methods. Furthermore, the varying degrees of post-translational modifications and isoforms resulting from alternative splicing generate numerous proteoforms and create a diversity that also cannot be fully captured with existing proteomic methods. Here, we present a novel approach based on differential proteolytic digestion of ECM samples aimed at increasing the diversity of peptides obtained from any given sample to enhance identification and sequence coverage of ECM proteins in complex samples.
Materials and Methods: ECM produced in vitro by mouse embryonic fibroblasts were decellularized. ECM proteins obtained were uniformly reduced, de-glycosylated, and initially digested with Lys-C, and then differentially digested with trypsin for 0.5 hour, 2, 4, 18 or 20 hours, the last time point serving as a comparison to more classical tryptic digestion protocols. The resulting peptide solutions were analysed using tandem mass spectrometry.
Results: Our preliminary data suggests that a short 30-minute digestion is sufficient to identify most proteins also identified after an over-night digestion. Moreover, combining the proteins identified from the 4 timepoints increases the number