ASC-DERIVED CONDITIONED MEDIUM (CM) STIMULATES VIABILITY OF OSTEOBLASTS AND COUNTERACTS TNFα-INDUCED INFLAMMATION IN CHONDROCYTES

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Adipose-derived Stem Cells (ASCs) are multipotent progenitors able to participate in tissue regeneration and to modulate immune response. Their regenerative and protective actions seem to be mainly mediated by the release of soluble factors together with extracellular vesicles (EVs). Recently we showed that i.v. injected ASC-derived conditioned medium (CM) counteracts neuropathic pain symptoms in two preclinical mouse models (AT Brini et al., 2017 under revision to Scientific Reports 2017 and G. Amodeo abstract SIF 2017). Here we show some preliminary results on the in vitro effects of ASC secretome on human primary cells derived from bone and cartilage.

CM was collected from ASCs derived from subcutaneous adipose tissue, cultured for 72 hours in starving conditions. The CM was then concentrated trough Amicon Ultra-15 Centrifugal Filter Unit (Merck-Millipore) of about 46±10-folds (n=26) and its trophic effect was tested on terminally differentiated cells (osteoblasts, OBs, and chondrocytes, CHs) and progenitors (ASCs). 7 days of treatment with high doses of CM (CM derived from 50 ASC per recipient cell) increased both OBs (+47) and ASCs (+93%) viability . Lower doses of CM (CM derived from 3-5 ASC per recipient cell) were still able to increase Ob viability of about 25% while CH viability was almost unaffected (+10 of increase).

In order to mimic articular inflammation in vitro, we treated CHs with 1 or 10ng/ml TNFα and then we evaluated CH proliferation up to day 9. Just the higher TNFα concentration determined a clear increase (+40% of control) in CH proliferation, suggesting the induction of a hypertrophic growth status. To determine a possible therapeutic effect of CM administration, we investigated the expression of cartilage-specific (ACAN and SOX9), inflammatory (MMP3, MMP13) and hypertrophic (type X Collagen, MMP13) markers. As expected, the inflammatory status induced by 10ng/ml TNFα was characterized by a strong increase in MMP3 and MMP13 gene expression at 24 hours (more than 50 and 10 folds the baseline values, respectively) that was considerably diminished by CM treatment (-50% and -30%, respectively). At a later time point (72 hours), the increase in both MMPs expression induced by 10ng/ml TNFα was still evident while CM exerted a positive effect on MMP3 expression only . Unexpectedly type X Collagen transcription was not particularly altered by TNFα administration. We did not observe major differences in the expression of cartilage-specific genes after treatment with TNFα in the presence or absence of CM.

Our in vitro results suggest that the trophic action of ASCs-derived CM could depend on recipient cell type and/or on its dose. They also indicate that CM exerts an anti-inflammatory potential on articular chondrocytes. All together, our data reinforce the fact that ASC secretome is a promising source of factors for future therapeutic applications.