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Immunomodulation capacities of conditioned mesenchymal stromal cells: benefits and mechanisms in an experimental cell therapy approach for Myasthenia gravis.

Fundamental Myology

Miscellaneous

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Introduction

Myasthenia gravis (MG) is a rare autoimmune disease characterized by autoantibodies targeting the neuromuscular junction and precluding normal nerve-to-muscle communication. Conventional treatments are limited by hefty adverse effects mandating the set-up of novel therapies. Mesenchymal Stromal Cells (MSC) are multipotent progenitors that modulate immune and inflammatory responses. MSC conditioned (cMSC) by a co-culture step with peripheral blood mononucleated cells (PBMC) improved the clinical outcomes in a humanized MG mouse model (NSG-MG) (Sudres *et al.*, 2017, JCI Insight). To shift towards a clinical perspective we aim to characterize the phenotypic and gene expression profiles of resting (rMSC) and conditioned MSC (cMSC), as well as to validate their functional capacities *in vitro* and *in vivo*.

Results

MSC derived from adipose tissue were conditioned by PBMC, left untreated, or activated by Interferon (IFN γ , γ MSC) as a control. Gene expression profiles were analyzed by RNA-seq (Illumina). The comparison between rMSC and cMSC showed differential expression of 244 genes. Meanwhile, 2089 and 3614 genes were differentially expressed upon comparison of γ MSC with rMSC and cMSC respectively. Each conditioning involved particular pathways.

Flow cytometry phenotyping (65 antibodies tested) showed that despite close global profiles, cMSC most remarkable phenotypic traits include increased CD54, CD273 and CD49a expression and reduced HLA-DR expression, whose intensity was culture dependent. At variance, major changes in MSC phenotype were induced by IFN γ activation in agreement with literature (increased CD47, CD54, HLA molecules, reduced CD59).

Single-cell mass cytometry (CyTOF, Fluidigm, 31 antibodies) clustering confirmed proximity between cMSC and rMSC and underlined the phenotypic alterations induced by IFN γ .

In vitro immunomodulating capacities of MSC were evaluated by PBMC inhibition assay and the analysis of changes in PBMC sub-populations. cMSC supernatant was able to reduce activated PBMC proliferation by at least 50% and modulated different PBMC subpopulations.

In vivo cMSC-treated MG-NSG mice presented MG scores lowered by 50% compared to untreated mice from as early as 2 weeks post-injection.

Conclusion

This work unveiled treatment-dependent phenotypic and transcriptomic markers of MSC and demonstrated that cellular conditioning enhances immunomodulation capacities *in vitro* and *in vivo* through mechanisms distinct from that of IFN γ .