Enhanced Immunomodulatory Capacity of Rejuvenated Mesenchymal Stem/Stromal Cells

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INTRODUCTION: Aging contributes to the diminishing regenerative capabilities and functional attributes of mesenchymal stem/stromal cells (MSCs), which are vital for maintaining skeletal homeostasis and facilitating tissue repair. Additionally, the therapeutic efficacy of harvested MSCs is intrinsically linked to the age-related state of the cells. Our research spotlights GATA binding protein 6 (GATA6) as a pivotal orchestrator of MSC aging; inhibiting GATA6 rejuvenates MSC characteristics, offering insights into engineering robust MSCs with heightened therapeutic potential. Nonetheless, the role of the GATA6-mediated mechanism we have identified in governing the immunomodulatory prowess of MSCs remains elusive. In line with our ongoing explorations in this domain, our current study was undertaken to unveil the involvement and underlying mechanisms by which GATA6 governs the immunomodulatory capacity of MSCs.

METHODS: Human synovial fluid-derived MSCs from three donors (ages 40 to 55) and "rejuvenated" MSCs reprogrammed from these synovial fluid-MSCs were previously generated and characterized, as described in our published report. Three independent cell lines were prepared for the study. These rejuvenated MSCs, referred to as "rMSCs," demonstrated enhanced phenotypes compared to their parental synovial fluid-derived MSCs, designated as "pMSCs" hereafter. Both pMSC and rMSC cultures were maintained in MSC complete growth medium, with regular passaging conducted between passages 6 and 8 for the experiments. Mouse periosteal skeletal stem cells (SSCs) were extracted from $Gata6^{fl/fl}$/Prx1-Cre^{ERT2} mice following ethical approval from IACUC at UW-Madison. Sorted periosteal SSCs were cultured and utilized between passages 1-3. Loss-of-function assays utilized Silencer Select siRNA targeting GATA6, IL6, SOCS3, and scrambled siRNA. After transfection into MSCs, RNA was extracted 48 hours later for analysis. RNA isolation, cDNA synthesis, and quantitative RT-PCR were performed using standard protocols. The relative expression of target molecules compared to housekeeping genes was calculated using the $2^{-\Delta Ct}$ method. For cell contact-mediated immunomodulation assays, human peripheral blood mononuclear cells (PBMCs) were cultured and co-cultured with pMSCs or rMSCs, labeled, and analyzed for T cell proliferation using flow cytometry. Secreted factor-mediated immunomodulation assays employed MSC conditioned medium (CM) to culture PBMCs and assess T cell proliferation. Expression of PDL1 was detected using immunoflow cytometry. Statistical analysis was carried out using GraphPad Prism 9 software, with t-tests and one-way analysis of variance followed by Tukey test for comparisons. Results are presented as mean ± standard deviation, with p-values below 0.05 considered significant.

RESULTS: Comparing isogenic pMSCs and rMSCs from humans, we observed that rMSCs effectively suppressed proliferation of CD3+, CD4+, and CD8+ T cells in comparison to pMSCs (**Fig. 1A**). We further investigated the role of GATA6 in enhancing the immunomodulatory capacity, discovering that the mechanism involves cell-cell contact rather than the influence of soluble factors (**Fig. 1B-D**). Subsequently, utilizing our previously collected RNA-seq data and knockdown assays, we identified the key molecules, IL6 and SOCS3, regulated by GATA6, which play pivotal roles in differentially modulating this immunomodulatory potential (**Fig. 2**). Furthermore, knockdown of *GATA6*, *IL6*, and *SOCS3* resulted in increased *PDL1* expression (**Fig. 3A**). The consistency of these in vitro findings was supported by results from a *Gata6*-knockout mouse model, where periosteal SSCs displayed consistent expression of *Socs3* and *Il6* despite *Gata6* knockout (**Fig. 3B**). Collectively, we elucidated the GATA6/SOCS3/PDL1 pathway as a central orchestrator of MSC immunomodulation.

DISCUSSION: Building on our previous investigation into GATA6's influence on MSC aging, our current study seeks to unravel how GATA6 governs MSC immunomodulation, specifically through the mediation of SOCS3 and PDL1. Existing research has underscored the pivotal role of SOCS3 in inflammation regulation. Triggered by the IL6/JAK/STAT3 pathway, SOCS3 primarily functions as a negative feedback regulator within this pathway. Despite these insights, the precise role of SOCS3 in MSCs remains uncharted. Our study unveils GATA6's modulation of SOCS3, which in turn directly regulates IL6 expression in rMSCs. Notably, downregulation of SOCS3 corresponds to increased PDL1 expression. These observations align with prior studies highlighting a negative correlation between SOCS3 and PDL1 expression in human T cells. Moreover, our findings demonstrate that rMSCs exhibit heightened T cell inhibition capacity through PDL1.

SIGNIFICANCE/CLINICAL RELEVANCE: The importance of our study lies in unveiling a crucial mechanism driving the immunomodulatory capacity of rMSCs. These findings offer valuable insights for the development of effective strategies to enhance the immunomodulatory prowess of MSCs.

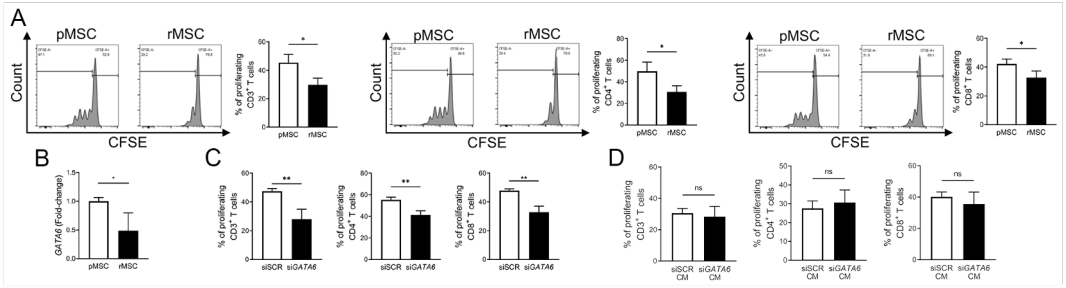

Figure 1. Augmented immunomodulation of rMSCs by GATA6 facilitated through cell-cell interactions, rather than the induction of soluble factors in the medium.

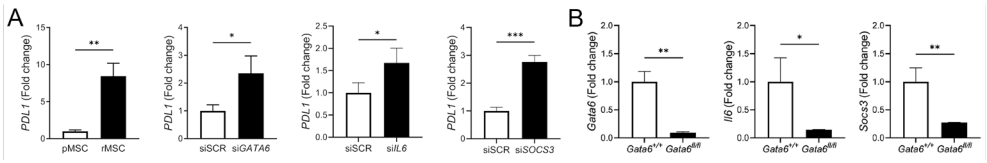

Figure 3. (A) Increased PDL1 expression in rMSCs following GATA6, IL6, or SOCS3 silencing. (B) Consistent findings elucidating Gata6/Il6/Socs3 regulation observed in SSCs of a mouse model.

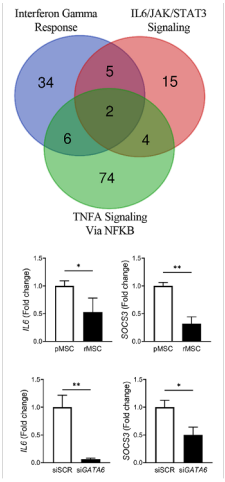

Figure 2. GATA6 governing activity of IL6 and SOCS3.