CCL2 promotes macrophage chemotaxis and induces osteogenesis during the acute phase of inflammation

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Disclosures: All authors have no potential conflict of interest

INTRODUCTION: Local cell therapy has recently gained attention for refractory joint diseases such as non-traumatic osteonecrosis of the femoral head (ONFH). One reported finding in ONFH is the inhibition of differentiation of mesenchymal stem cells (MSCs) into osteoblasts. Therefore, MSCs are a potential target for the treatment of ONFH. MSCs are not only involved in osteogenesis, but they also possess immune-modulatory functions including the induction of macrophage migration during bone regeneration via macrophage crosstalk [1]. C-C motif chemokine ligand 2 (CCL2), a known inflammatory mediator, is associated with the migration of macrophages and MSCs during inflammation. We hypothesize that CCL2 is a mediator that regulates cellular crosstalk between MSCs and macrophages. The aim of this study was to investigate the potential utility of CCL2 as a therapeutic target for local cell therapy.

METHODS: Bone marrow MSCs from New Zealand White Rabbits were used for this study (Cyagen, USA). Genetically modified CCL2-overexpressing MSCs (rCCL2$^+$MSCs) were generated using rabbit CCL2-secreting pCDH-CMV-rCCL2-copRFP-expressing lentiviral vectors (Figure 1a). Viral infection was confirmed by positive RFP on fluorescence microscopy 3 days later (Figure 1b); CCL2 expression was compared to CCL2 mRNA levels in unmodified rabbit MSCs and in rCCL2$^+$MSCs. Cell proliferation assays were performed to evaluate cytotoxicity caused by genetic modification and CCL2. To evaluate the function of genetically modified CCL2, a scratch test cell migration assay was performed in indirect co-culture with macrophages. For the cell migration assay, the distance and area of macrophages after scratching were evaluated using QuPath. The results of each treatment were compared at 0, 6, 24, and 72 hours after scratching among 5 groups (1) macrophages-only; (2) temporary stimulation (single addition of 10 ng/ml at 24 hour) with recombinant CCL2 protein; and co-culture with (3) MSCs; (4) empty vector MSCs (virus$^+$MSCs); and (5) rCCL2$^+$MSCs. Osteogenesis was assessed in monocultures and co-cultures with macrophages using alkaline phosphatase (ALP) and alizarin staining as osteogenic differentiation assays. These results were compared among (1) unmodified MSCs, (2) virus$^+$ MSCs, (3) MSCs temporarily stimulated with recombinant CCL2 and (4) rCCL2$^+$MSCs groups. Statistical analysis was performed using the Mann-Whitney U test or the Kruskal-Walli's test for multiple comparisons. *P* values less than 0.05 were considered significant.

RESULTS: rCCL2$^+$MSCs had significantly higher levels of CCL2 mRNA expression compared to unmodified MSCs ($p < 0.05$) (Figure 1C). There were no significant differences among the groups with respect to cell proliferation. Regarding the cell migration assay, distance and area were significantly reduced in the groups with temporary addition of CCL2 protein and rCCL2$^+$MSCs group after 24 hours and were disappeared by 72 hours (Figure 2). No significant difference in osteogenesis was observed between the groups in monoculture. However, in co-culture with macrophages, the percentage of ALP staining was significantly higher in the group with recombinant CCL2 temporarily added to MSCs and the group with rCCL2+MSCs (Figure 3). The group in which recombinant CCL2 was temporarily added to MSCs had a greater alizarin red positive area than the other groups ($p < 0.05$).

DISCUSSION: We examined the therapeutic potential of CCL2-mediated local cell therapy using genetically modified rCCL2+ MSCs and recombinant CCL2 protein. CCL2 did not affect osteogenesis under monoculture conditions but promoted migration capacity and bone formation when co-cultured with macrophages. This suggests that CCL2 induces macrophage chemotaxis and enhances crosstalk between MSCs and macrophages, which may promote bone formation during the acute inflammatory phase of bone healing. However, as shown in our previous research, continuous CCL2 stimulation may lead to suppression of bone formation by facilitating the pro-inflammatory state and chronic inflammation due to continued macrophage migration and polarization [2].

SIGNIFICANCE/CLINICAL RELEVANCE: Local, short-term delivery of CCL2 in the acute phase of inflammation promotes osteogenesis by facilitating chemotaxis of macrophages and MSCs and may be a strategy for local cell therapy in refractory diseases such as ONFH.

REFERENCES:
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[2] Toya et al. CCL2 promotes osteogenesis by facilitating macrophage migration during acute inflammation. *Front Cell Dev Biol.*2023; 11:1213641.

ACKNOWLEDGEMENTS: This work was supported in part by the ON Pilot Grant Hip 2022 (project number 22-160) and the Ellenburg Professorship of Surgery at Stanford University.

IMAGES AND TABLES:

Figure 1

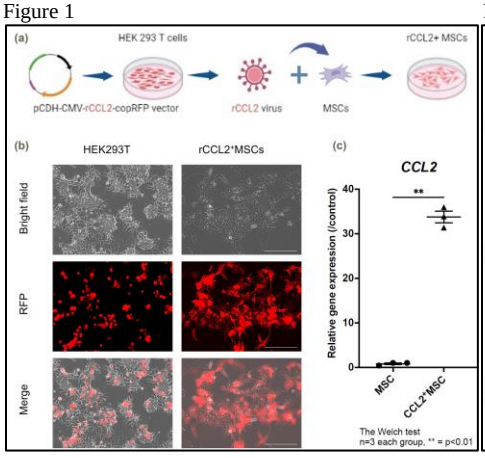

Figure 2

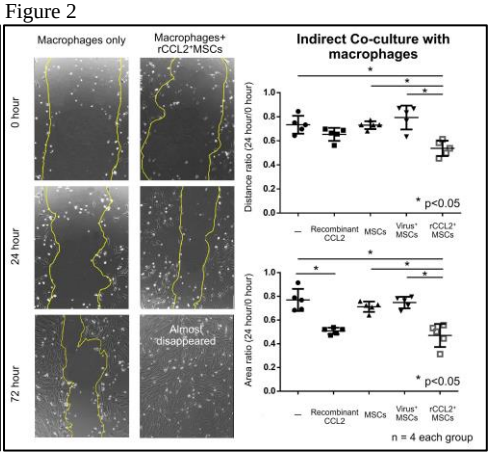

Figure 3