Runx1 is Required for Oleanolic Acid Induced Mesenchymal Stem Cell Chondrogenesis

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INTRODUCTION: Oleanolic acid (Ola) is a naturally occurring triterpenoid with anti-inflammatory properties, recently shown to exhibit a wide range of potential therapeutic applications. However, its effects on chondrogenesis and the underlying mechanism remain unknown. Growing evidence indicates that transcription factor Runx1is a critical mediator in the development of cartilage, while no previous studies have addressed a functional relationship between Runx1 and Ola during Mesenchymal Stromal Cell (MSC) differentiation towards chondrogenesis. This study aimed to explore whether Ola enhances chondrogenesis by regulation of Runx1.

METHODS: Mouse limb bud derived MSCs were isolated from embryos extracted from CD1 pregnant mice at stage E11.5 with the approval from LSU Health Sciences Center Animal Use review board. MSC stemness phenotype was evaluated by flow cytometry. Cells were seeded in micro-mass at a high density of 1×10^5 cells per 10 ul of media in 12-well plates. Cells were maintained in culture for a time-course of 3, 6, and 9 days treated with or without Ola, before harvesting for Alcian blue staining and RNA extractions. shRNA lentiviruses were used to knock down Runx1 expression in MSCs cultured either in monolayers or in micro-mass. Protein and total RNA were extracted on day 9 after Ola treatment and lentivirus infection for further Western blot and PCR analysis.

RESULTS: The flow cytometry results indicated that mouse stromal cell markers Sca-1. CD29, CD44 and proliferation marker Ki-67 were highly expressed in these MSCs which are isolated from limb buds. In MSC chondrogenic micro-mass cultures, these cells exhibit chondrocyte phenotypic features because they show Alcian blue staining of their matrix and start forming mineralized nodules in vitro after 3 days in culture. More importantly, Ola treatment significantly enhanced the MSC chondrogenic differentiation by showing stronger Alcian blue staining and forming larger nodules in vitro after 9 days in culture when compared to untreated control cells. Consistent to the Alcin blue staining, expression of early chondrogenic marker Type II collagen was gradually increased by Ola from day 3 to day 6 and reached to the peak expression at day 9 with 10-fold increase in comparison with the level at day 3. On the other hand, later stage chondrogenic marker type X collagen expression is only slightly upregulated to reach a 2-fold increase on day 9 in comparison with its low level on day 3. Interestingly, Runx1 transcripts are rapidly upregulated between 3 and 6 days of culture with Ola treatment, to be thereafter slow increase at day 9. More importantly, knockout expression of Runx1 in MSCs not only inhibited control MSC chondrogenesis, but also significantly blocked Ola-induced chondrogenesis in MSC micro-mass cultures (Figure 1).

DISCUSSION: Our data demonstrated that spontaneous chondrogenic nodule formation in high density micro-mass limb bud MSC cultures is a useful tool to validate Ola chondrogenic effect *in vivo*. Treatment of limb bud MSC micro-mass cultures with Ola resulted in rapid chondrogenic differentiation and early induction of type II collagen. Furthermore, shRNA knockout experiments clearly showed that transcription factor Runx1 is required for Oleanolic acid induced MSC Chondrogenesis. These data strongly suggest that Ola enhances chondrogenesis via regulation of transcription factor Runx1, which plays a critical role in the initial steps of mesenchymal cell commitment toward the chondrogenic lineage.

SIGNIFICANCE/CLINICAL RELEVANCE: Taken together, our findings highlight an important role for Runx1 in Oleanolic acid-mediated MSC chondrogenic differentiation. These data further support that Oleanolic acid is a potential effective therapeutic agent in the treatment of cartilage injury, or osteoarthritis, a joint disease with no effective disease-modifying therapy in clinic.

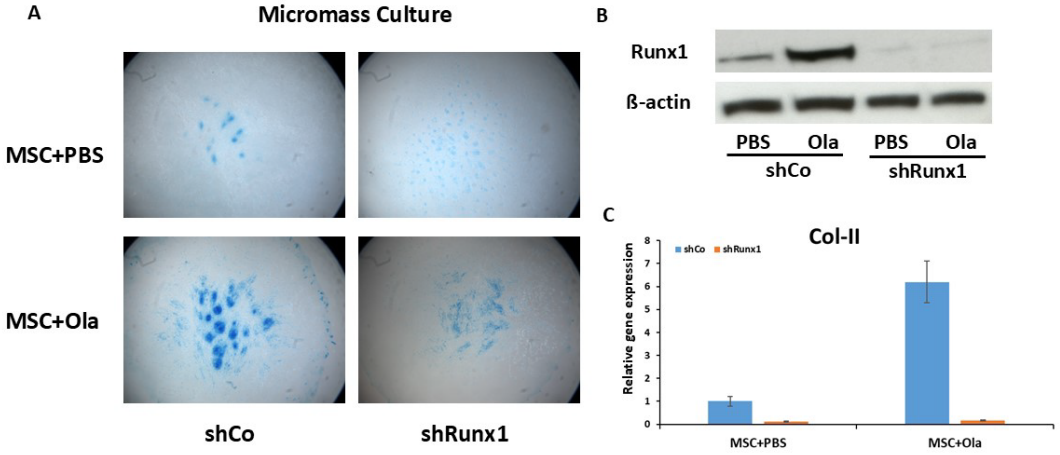

Figure 1: Alcian blue staining of Ola treated- and Runx1 shRNA lentivirus infected limb bud-derived mesenchymal cells cultured at high density in micromass for 9 days. Total protein and RNA was extracted from these micromass cultures for western blot and RT-PCR analysis. (A) Knock down Runx1 in mesenchymal cells totally blocked Ola-induced chondrogenesis. (B) Runx1 protein levels are significantly increased by Ola treatment and shRNA lentivirus targeting Runx1 efficiently knocked down the protein level in MSCs. Empty lentivirus was used as control(shCo). (C) Real time PCR data shows Ola significantly induced type II collagen expression in control MSCs, but not in Runx1 shRNA lentivirus infected cells.