Adipose-derived stem cells co-culture with chondrocytes secrete extracellular vesicles maintain chondrogenic phenotype of serially passaged chondrocytes

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INTRODUCTION: Autologous chondrocyte implantation (ACI) is the golden therapy for articular cartilage defects. However, clinical outcomes of ACI are not optimal for the fibrocartilage are formed in articular cartilage defect. The serially passage in monolayer makes chondrocytes lose chondrogenic phenotype is indicated as the cause of fibrocartilage formation. Studies indicate that serially passaged chondrocytes lose chondrogenic phenotype is due to dedifferentiation, senescence and hypertrophic change in these cells. Adipose-derived stem cells (ADSCs) have been studied as an alternative cell source for articular cartilage defect. Besides directing chondrogenic differentiation of ADSCs into chondrocytes, ADSCs co-cultured with chondrocytes also shows enhanced chondrogenesis and hyaline cartilage synthesis. ADSCs are known to release extracellular vesicles (EVs) for intercellular communication. We hypothesize that EVs secreted from ADSCs co-culture with chondrocytes can be used for maintenance of chondrogenic phenotype in serially passaged chondrocytes. In this study, we tested the effect EVs secreted from ADSCs, or coculture of ADSC/chondrocyte on survival, dedifferentiation, hypertrophic change and senescence in serially passaged chondrocytes.

METHODS: For induction of EVs release, ADSCs were cultured with or without chondrocytes in DMEM supplemented with 10% FBS for 48hr. After EVs were released into the conditioned medium (CM), three batches of CMs were collected for EVs isolation. Isolated EVs were characterized by Nanoparticle Tracking Analysis (NTA). For EVs treatment, the chondrocytes were serial passaged in monolayer for 5 passages, and then the cells were treated with EVs. The serially passaged chondrocytes were divided into three groups: **1. Control group:** cells without any EVs treatment, **2. ADSC-EVs group:** cells treated with ADSCs-EVs, **3. ADSC/chondrocyte-EVs group:** cells treated with ADSC/chondrocyte-EVs. At the indicated time points, the EVs treated chondrocytes were harvested for further analysis. The EVs uptake by serially passaged chondrocytes was analyzed, and then the survival of cells was further detected. To test the effect of ADSCs-EVs and ADSC/chondrocyte-EVs on chondrogenic phenotype of serially passaged chondrocyte, the chondrogenic markers (collagen type II: Col-II and sulphated glycosaminoglycan: sGAG), fibrocartilaginous matrix marker (collagen type I: Col-I), and hypertrophic marker (collagen type X: Col-X) were analyzed after ADSCs-EVs or ADSC/chondrocyte-EVs uptake. The senescence of serially passaged chondrocytes was analyzed by detecting senescence-associated β-galactosidase (SA- β -gal) staining. The data are expressed as the means ± SE from each experimental replicate. Statistical significance was evaluated by a one-way analysis of variance (ANOVA), and multiple comparisons were performed using Scheffe's method. A $p<0.05$ was considered significant.

RESULTS SECTION: EVs characterization: The ADSC-EVs and ADSC/chondrocyte-EVs were characterized by NTA. The results showed that the mean diameters of ADSC-EVs and ADSC/chondrocyte-EVs are 170 $\pm$ 17.4 nm and 136 $\pm$ 80 nm, respectively (Figure. 1A). There was no significant difference in diameter between these two EVs isolated from three batches. **Effect of EVs uptake by serially passaged chondrocytes:** Both EVs were found uptake by serially passaged chondrocytes from day 1 to 5 (Figure. 1B). **Survival of serially passaged chondrocyte after EVs uptake:** The result showed that cell survival of serially passaged chondrocytes was not altered after EVs uptake (Figure. 1B). **Effect of ADSC-EVs and ADSC/chondrocyte-EVs uptake on dedifferentiation and hypertrophic change of serially passaged chondrocytes:** The results show that serially passaged chondrocytes enhance chondrogenic markers synthesis, reduce the fibrocartilaginous matrix marker, and hypertrophic marker after uptake of ADSC-EVs of ADSC/chondrocyte-EVs. The levels of Col-II and sGAG of serially passaged chondrocyte are significantly enhanced in ADSC-EVs and ADSC/chondrocyte-EVs groups when compared with Control group (Figure.3A). The decreased the Col-I and Col-X expressions of chondrocytes was also found in ADSC-EVs and ADSC/chondrocyte-EVs groups than in Control group (Figure. 3A). Moreover, ADSC/chondrocyte-EVs showed more pronounced effect than ADSC-EVs in reducing Col-X expression of chondrocytes (Figure.3A). **Effect of ADSC-EVs and ADSC/chondrocyte-EVs uptake on senescence of serially passaged chondrocytes:** The results showed that ADSC-EVs and ADSC/chondrocyte-EVs uptake reduced senescence of serially passaged chondrocytes. The senescence marker SA-β-gal expression was decreased in ADSC-EVs and ADSC/chondrocyte-EVs groups when compared with control group (Figure.3B).

DISCUSSION: Based on these findings, we show that both ADSC-EVs and ADSC/chondrocyte-EVs can be used for enhancing chondrogenic phenotype of serially passaged chondrocytes. Moreover, ADSC/chondrocyte-EVs have more pronounce effect than ADSC-EVs on reducing hypertrophic change of serially passaged chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE: The ADSC/chondrocyte-EVs may be used as a biologic agent for more effective ACI.

Figure. 1

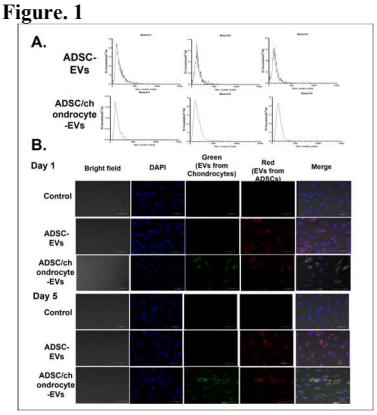

Figure. 2

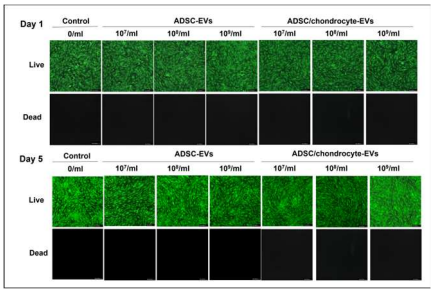

Figure. 3