Extracellular vesicles derived from specific phenotype of neutrophils suppress catabolic process induced by IL-1β stimulation in chondrocytes

Keita Kitahara[1], Mohamad Alaa Terkawi[1], Taku Ebata[1], Tomohiro Onodera[1], Tsutomu Endo [1], Tomohiro Shimizu[1], Daisuke Takahashi[1], Tsuyoshi Asano[1, 2], Norimasa Iwasaki[1],

[1]Department of Orthopedic Surgery Faculty of Medicine and Graduate school of Medicine, Hokkaido University, [2]KKR Sapporo medical center

Email: keita_kitahara88-research@yahoo.co.jp.

Disclosures: Keita Kitahara: None. Mohamad Alaa Terkawi: None.Taku Ebata: None. Tsutomu Endo: None. Tomohiro Shimizu: None. Daisuke Takahashi: None. Tsuyoshi Asano: None. Norimasa Iwasaki: None.

INTRODUCTION: Osteoarthritis (OA) is the most common degenerative joint disease characterized by cartilage degradation and low-grade inflammation in synovium. Chronic inflammation results from failure of resolving function that is also known to restore/maintain tissue homeostasis. Mimicking the tactic of our body in controlling inflammation and restoring homeostasis offer an excellent approach for treatment of OA. Neutrophils and their extracellular vesicles (EVs) are known to play a key role in initiating resolving function in the tissues. In fact, depletion of neutrophils in an experimental arthritis model decreases synthesis of GAGs [1]. Components of neutrophil derived-EVs are dependent on the producing cell types and the surrounding environment [2]. Our earlier study identified a phenotype of neutrophils that protected cartilage from catabolic process of chondrocytes, thus are defined as resolving neutrophils (rPMN). Therefore, the objective of the current study is to explore beneficial effects of rPMN-EVs on catabolism process in chondrocytes induced by IL-1β stimulation.

METHODS: Human blood-derived neutrophils and C-28I2, a human chondrocyte cell line, were used in the current study. Human neutrophils were isolated from healthy donors and stimulated with different stimuli, including 50 ng/ml TNF-α and 50 ng/ml TGF-β for 30 min. One group of cells were stimulated with 50 ng/ml TGF-β plus 100 μM rosiglitazone (PPAR-γ agonist) for 30 min, in an attempt to increase the production of EVs. The cells were washed and co-cultured with C-28I2 chondrocytes cell line cultured in transwell inserts for 20 hours. C-28I2 chondrocytes were pre-stimulated with 30 ng/ml IL-1β for 4h. To confirm our findings, conditioned medium prepared by culturing mouse bone marrow-derived neutrophils stimulated with different stimuli was used to culture primary mouse chondrocytes pre-stimulated with 10 ng/ml IL-1β for 24h. EVs were isolated from human blood-derived neutrophil and subjected to C-28I2 chondrocytes at concentration of 10μg/ml. Morphologies of EVs were examined by transmission electron microscope and protein marker expression using Western blotting with antibodies to CD9, TSG101 and Annexin V. Changes in gene and protein expression of catabolic factors were evaluated using qRT-PCR, Western Blotting and bulk RNA-seq analysis. Ex vivo stimulation, mouse cartilage was collected from 6-week-old C57BL/6 mouse femoral head and incubated in medium supplemented with recombinant IL-1β with or without EVs (10 μg/ml) for 72 hours. Culture supernatant and cartilage were collected for measurement of sulfated GAG concentration and histological analysis, respectively. Statistical analysis was performed using one-way ANOVA with Bonferroni posttest, Kruskal-Wallis followed by Dunn's multiple comparison (GraphPad Software Inc.). The significant level was set at $p < 0.05$. The procedures for the animal experiments were approved by the Institute of Animal Care and Use Committee of the Hokkaido University Graduate School of Medicine.

RESULTS: EVs from PMN stimulated with TGF-β+rosiglitazone exhibited the best protective effects against IL-1β stimulation in chondrocytes (Fig.1A). These EVs displayed typical features of EVs such as size distribution, morphology, and protein contents. Proteomic analysis of EVs revealed that EVs from PMN stimulated with TGF-β+rosiglitazone have reduced number of DAMPs as compared to these in non-stimulated PMNs. Our further bulk RNA-seq demonstrated that rPMN-Evs reduced the expression of catabolic factors and inflammatory cytokines in IL-1β-stimulated chondrocytes (Fig. 2). In consistent with these results, these EVs alleviated cartilage degeneration in superficial zone of femoral head induced by IL-1β in our ex-vivo model (Fig. 3). Our results indicated that rPMN-Evs can protect cartilage from catabolic process induced by IL-1β in chondrocytes.

DISCUSSION: The results of this study demonstrated that EVs from PMN stimulated with TGF-β+rosiglitazone contain a reduced number of DAMP as compared to other stimulations and exert the best anti-catabolic effects against IL-1β stimulation. An earlier study showed that EVs from TNF-α-stimulated neutrophils had a strong anti-catabolic function in cartilage [3]. However, our data clearly showed that EVs from PMN stimulated with TGF-β+rosiglitazone exerted better effects on chondrocytes than these from TNF-α stimulated cells, indicating their potential therapeutic application. These findings support the concept that EVs of PMN promote resolving function and restore homeostatic state in tissues.

SIGNIFICANCE/CLINICAL RELEVANCE: The current study highlights potential therapeutic application of EVs from rPMN for prevention of cartilage degeneration associated with progression of OA.

ACKNOWLEDGEMENTS: This work was supported by AMED Grant Number JP22zf01270004h0002 and Grant-in-Aid for Scientific Research C (20K09471) from Japan Society for the Promotion of Science.

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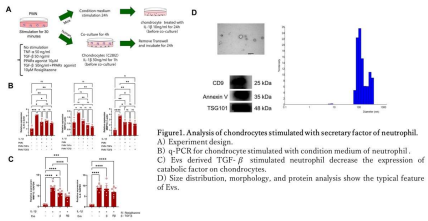

Figure1. Analysis of chondrocytes stimulated with secretary factor of neutrophil.
A) Experiment design.
B) q-PCR for chondrocyte stimulated with condition medium of neutrophil .
C) Evs derived TGF-β stimulated neutrophil decrease the expression of catabolic factor on chondrocytes.
D) Size distribution, morphology, and protein analysis show the typical feature of Evs.

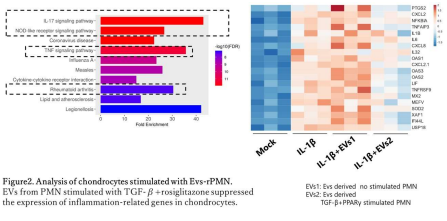

Figure2. Analysis of chondrocytes stimulated with Evs-rPMN.
EVs from PMN stimulated with TGF-β +rosiglitazone suppressed the expression of inflammation-related genes in chondrocytes.

EVs1: Evs derived no stimulated PMN
EVs2: Evs derived TGF-β+PPARγ stimulated PMN

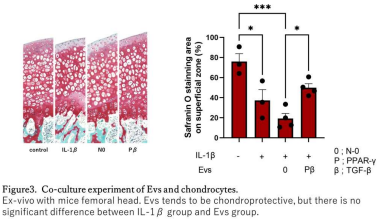

Figure3. Co-culture experiment of Evs and chondrocytes.
Ex-vivo with mice femoral head. Evs tends to be chondroprotective, but there is no significant difference between IL-1β group and Evs group.