Exploring the Immunoregulatory Potential of Adipose-Derived Stem Cells (ASCs) in an Indirect Co-Culture System with Arthritic Chondrocytes

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INTRODUCTION: Osteoarthritis (OA) is a leading cause of chronic disability in the world and the current methods for its treatment are limited. In early stage of OA, breakdown of articular cartilage by matrix metalloproteinases (MMPs) is thought to be driven by the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and the downstream hypoxia inducible factor 2 alpha (HIF-2α encoded by EPAS-1).[1] Pro-inflammatory cytokines, especially tumor necrosis factor alpha (TNF-α) which affect both the quantity and quality of the cartilage extracellular matrix (ECM), are important in the pathogenesis of OA.[2] TNF-α stimulate the release of MMP-13 and downregulates expression of type II collagen and aggrecan in chondrocytes. The mechanism of cartilage degradation in OA is primarily driven by the production of catabolic cytokines and oxidative stress.[3] These factors lead to the degradation of the extracellular matrix and the chondrocytes death, primarily through catabolic molecular cascades within the cartilage. Moreover, the lack of vascularization in articular cartilage limits access to progenitor cells at chondral lesion sites resulting in a reduced capacity for self-repair. Therefore, the prospect of regenerative medicine therapies involving reparative cells like stem cells (SCs) for the treatment of osteoarthritic lesions holds promise in the healing of osteoarthritic lesions.[4] In this study, we investigated the anti-inflammatory ability of adipose-derived mesenchymal stem cells (ASCs) and their recovery mechanism using an ASC-chondrocyte co-culture system.

METHODS: Cell isolation and culture (Chondrocytes and ASCs): Articular cartilage samples were taken from the knees of cadaver pigs and chondrocytes were isolated and were cultured at 37°C in a humidified atmosphere at 5% CO2 and the medium was changed every 2-3 days. The subcutaneous adipose tissue was isolated from 3-4 months old healthy pigs according to the protocol and ethical guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee Health Science Center (UTHSC). The adipose tissue was digested with 200 µg/mL collagenase II solution (Sigma) to yield ASCs that were cultured in Dulbecco's Modified Eagle Medium-High Glucose (DMEM-HG) (Gibco) supplemented with antibiotics at 37°C and 5% CO2. The second passage (P2) ASCs were used in all experiments. ASCs-Chondrocyte Co-Culture: In this study, we used a TNF-α stimulated chondrocytes model to evaluate ASCs anti-inflammatory efficacy. The ASCs were cultured in trans-well culture plates over chondrocytes to determine the release and effect of their paracrine factors. Briefly, chondrocytes were stimulated with TNF-α then co-cultured with ASCs. We used the following experimental groups of co-culture system (indirect contact): Chondrocytes and ASCs were cultured in 6-well plates and in trans-well, respectively at a density (1:1) of $1x10^5$ cells per well. Mono-cultured chondrocytes treated with TNF-α will serve as the positive control. Co-cultures will be incubated for 3 days and total RNA will be extracted from the mono-cultured and co-cultured chondrocytes for RT-qPCR analysis. Gene expression analysis: The qPCR was done at the end of treatment using TaqMan™ Gene Expression Assays (Thermo Fisher Scientific). qPCR has been done to measure the inflammatory and oxidative stress-related genes including NFkB, NOS2, and IL-1b. β-Actin served as an internal control. PGE2, LDH, Total NO measurement: Assay kits (PGE2, LDH, Total NO) from Cayman Chemicals were used to determine the concentration of the pro-inflammatory prostaglandin PGE2, LDH, and total NO in the supernatant of the cell cultures. Supernatant fluid was diluted to be in range of the standard, developed and read. Statistical analysis: Student's t-test was used to determine statistical significance for $p < 0.05$. Data is represented as mean±SD.

RESULTS: Cytotoxicity of TNF-α treatment: Lactate dehydrogenase (LDH) release into the medium, an indication of cellular toxicity, was analyzed. Normal chondrocytes released low levels of LDH (18.1 ± 1.04%). TNF-α treatment significantly increased LDH release compared to normal untreated chondrocytes (Figure 1A). Total nitric oxide (NO) level and inflammatory changes in cultured chondrocytes after TNF-α stimulation: TNF-α treatment significantly increased total NO levels by 4.3-fold compared to normal chondrocytes, indicating that TNF-α induces oxidative stress in chondrocytes (Figure 1B). Additionally, PGE2 production increased by 4.5-fold in the culture media after TNF-α stimulation. PGE2 is a marker of inflammation and a downstream mediator of the anti-proliferative effects of NO (Figure 1C). We also found that the TNFα treatment led to a significant upregulation of catabolic genes such as EPA1 and MMP-13, while simultaneously downregulating anabolic genes SOX9 and aggrecan, compared to normal untreated chondrocytes (Figure 2). Immunomodulation effect of ASCs co-culture: Co-culture of chondrocytes with ASCs significantly reduced gene expression levels of NF-κB, IL-1β, and NOS2, demonstrating that ASCs reduced chondrocyte inflammation through paracrine secretion (Figure 3). Inflammatory mediators such as NF-κB and IL-1β, and free radical-related genes such as NOS2, were significantly downregulated within 72 hours. These data demonstrate the significant potential for ASC-based therapy in OA.

DISCUSSION: Our findings demonstrate that ASCs effectively reduce the expression of key mediators (NFκB, NOS2, IL-1β) associated with OA development and progression in response to TNF-α. This suggests that ASC transplantation at the articular surface holds potential as an on-site therapeutic approach for OA. ASCs appear to impede OA progression by mitigating catabolic processes. Furthermore, the observed reduction in inflammatory and oxidative stress mediators, coupled with the promotion of anti-inflammatory gene expression via the NFκB pathway through ASC-secreted paracrine factors, highlights the multifaceted impact of ASCs. Notably, NFκB plays a pivotal role in both apoptosis and inflammation regulation. Future studies exploring ASCs' role in the context of the NFκB pathway hold promise for further insights. Additionally, future prospects of our study includes the potential for the transplantation of healthy ASCs in animal models of OA, offering a path for continued research into ASC-based interventions for OA management.

SIGNIFICANCE: The study presented here clearly showed that treatment of stem cells makes chondrocytes resistant against inflammatory reactions and oxidative stress. ASCs can suppress chondrocyte production in vitro of catabolic genes such as NFkB, IL-1b, and NOS2 against TNFα-induced stress. Thus stem cell therapy can be used to suppress catabolism during the early stages of OA.

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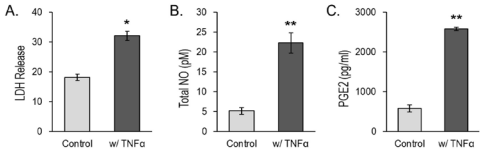

Figure 1. Biochemical assay in cultured chondrocytes after TNF-α stimulation. LDH assay (A), Total NO (B), level of PGE2 (C). Data is represented as mean ± SD.*$p < 0.05$, **$p < 0.01$ as compared to TNF-α treatment group

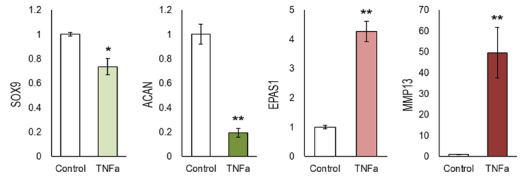

Figure 2. Gene expression in chondrocytes with TNFa-induced inflammation. In the control group, chondrocytes were incubated with medium alone. In another group, the chondrocytes were treated with TNFa (10ng/mL) for 24 h. *$P< 0.05$, **$p < 0.01$ significant difference compared to the Control group.

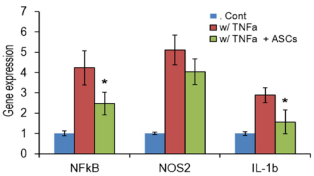

Figure 3. Gene expression in co-culture ASCs-chondrocytes with TNFa-induced inflammation. In the control group, chondrocytes were incubated with medium alone. In all other groups, the chondrocytes were treated with TNFa (10ng/mL) for 24 h, then the TNFa containing medium was removed and replaced with fresh medium (TNFa group) or added ASCs on trans well for a further 72 h . *$P< 0.05$, significant difference compared to the Control group.