Normal and Osteoarthritic Human Synovial Fluid Regulation of Articular Cartilage Proteoglycan-4 Secretion: Role of O₂, TGF-β, and IL-1

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Disclosures:

Introduction: Synovial fluid (SF) interacts with articular cartilage in synovial joints by providing biomechanical, metabolic, and regulatory functions. PRG-4 is a mucinous glycoprotein in SF that normally lubricates articular cartilage but is at variable concentrations in health and disease. A critical determinant of PRG-4 concentration is its rate of synthesis by chondrocytes from the superficial zone of cartilage. While chondrocyte PRG-4 secretion in vitro can be regulated by a number of factors, including TGF-β, IL-1α, a potent stimulus, and IL-1α, a potent inhibitor, the active regulators of chondrocyte PRG-4 secretion in SF in vivo are unclear. It is unknown if SF regulates PRG-4 secretion, as it does with some articular cartilage responses. The regulatory functions of TGF-β and IL-1α can be inhibited by LY2157299 and IRAP, respectively, blocking TGFβ receptor I (TGFβR1) kinase signaling and IL-1 binding. In addition, the hypoxic environment of the joint, ranging from ~1-10% O₂ in states of health and disease, may also regulate chondrocyte functions. This study tested the hypothesis that human SF (hSF) from normal (NL) and osteoarthritic (OA) joints regulate PRG-4 secretion through both TGF-β and IL-1 activity. The aims were to determine if (1) NL-hSF and OA-hSF, at various O₂ concentrations, regulate cartilage explant secretion of PRG-4, and (2) if such regulatory responses could be abolished by inhibition of TGF-β and IL-1 signaling.

Methods: Synovial Fluids. Following IRB-approved human subjects protocols, hSF was aspirated from knee joints of cadaveric donors (n=7; age mean±SD of 71±13 yr, range 55-90 yr) and OA patients (n=7; 61±6 yr, range 49-65 yr). The former were considered NL-hSF, as they were collected within 48-72 h of death, and knee joints were excluded if osteophytes were present or subchondral bone was exposed. OA-hSF was obtained from subjects undergoing total knee arthroplasty who had granted informed consent. SF samples were clarified of cells and debris by centrifugation, and the resultant samples stored at -70°C before use in experiments.

Cartilage Explants. A total of 574 cartilage disks, 2 mm diameter x 0.5 mm thick, were harvested from the femoral condyles of immature (n=8, 1-3 week old) bovine stifle joints so that the articular surface was contained intact.

Culture Conditions. Cartilage explants were incubated for 6 days at 37°C and 5% CO₂ in DMEM with 25 μg/ml ascorbic acid, 0.01% BSA, and 0.1% DMSO, alone as a negative control, with 10 ng/ml rhTGF-β1 (Peprotech) and/ or 10 ng/ml IL-1α (In Vitrogen) as positive controls, with one or more of the following stimuli at the standard concentration as well as the range indicated for dosimetry experiments: (1) 10% (5-20%) O₂, (2) 5% (0.05-20%) NL-hSF, OA-hSF, or human serum (HS) (Gemini Bio-Products), (3) 1 μM (0.01-10 μM) LY2157299 (Selleckchem), (4) 100 ng/ml (1-100 ng/ml) IRAP (Amgen). Conditioned medium was collected and replaced with fresh medium every 2 days.

PRG-4 Protein Secretion. PRG-4 secreted into conditioned medium from day 4-6 (at which time regulatory effects have stabilized) was quantified by a bovine-specific ELISA and bovine PRG-4 standards. PRG-4 secretion is expressed normalized to cartilage surface area and duration of incubation (μg/[cm²·day]).

Statistics. Data are expressed as mean±SEM, n=# of cartilage disks, and m=# of donors per condition. PRG-4 secretion data were log-transformed prior to statistical analysis. The effects of various stimuli on PRG-4 secretion was assessed by ANOVA with human SF donor as a random factor and stimulus as a repeated factor, with comparisons to basal conditions and/or with/without inhibitor by t-test with Bonferroni correction as appropriate.

Results: O₂ and OA-hSF regulate cartilage PRG-4 secretion. Cartilage PRG-4 secretion was modulated by O₂ (p < 0.01) and OA-hSF (p < 0.01) (Fig. 1). At 5% O₂, PRG-4 secretion was different compared to at 10% O₂ (p < 0.05) and 20% O₂ (p < 0.01), but secretion at 10% O₂ and 20% O₂ was similar (p = 0.96). At 10% O₂, explants secreted higher PRG-4 (p < 0.001) with 2.5% OA-hSF compared to without (6.6 ± 3.9 vs. 0 μg/[cm²·day]), but was similar (p = 0.63-0.82) to 5-20% (6.7 ± 2.3, 6.8 ± 3.7, 5.4 ± 2.6 μg/[cm²·day]) at 5%, 10%, 20%, respectively. Thus, 10% O₂ and hSF up to 5% were used in subsequent experiments.

OA-hSF, but not HS, stimulates cartilage PRG-4 secretion. Cartilage PRG-4 secretion was stimulated by OA-hSF (p < 0.001), with a trend for NL-hSF (p = 0.14) and no effect of HS (p = 0.49) (Fig. 2). Explants incubated with 5% NL-hSF, OA-hSF, or HS secreted PRG-4 at levels intermediate that of explants incubated in medium alone (2.3 ± 0.6 μg/[cm²·day]) and those in medium with 10 ng/ml TGF-β1 (21.5 ± 3.1 μg/[cm²·day]). Explants in 5% OA-hSF secreted substantially higher (p < 0.01) PRG-4 compared to basal, while explants incubated in 5% NL-hSF (p = 0.26) and 5% HS (p = 0.99) did not.

LY2157299 inhibition of TGF-β1-stimulated cartilage PRG-4 secretion is dose-dependent. The TGF-β1-stimulated secretion of
PRG-4 was inhibited in a dose-dependent manner by LY2157299 (Fig. 3). LY2157299 at both 1 and 10 μM lowered PRG-4 secretion from stimulated levels (each, p < 0.05) to basal levels (p = 0.35-0.61). Thus, LY2157299 at 1 μM was used in subsequent studies.

**LY2157299 inhibits TGF-β1-stimulated cartilage PRG4-secretion in the presence of hSF.** Cartilage PRG4 secretion was modulated by hSF (p < 0.05), TGF-β1 (p < 0.01), and LY2157299 (p < 0.01) (Fig. 4). TGF-β1-stimulated PRG4 secretion by explants was lowered by 1 μM LY2157299, whether in medium alone (p < 0.001), NL-hSF (p < 0.01), or OA-hSF (p < 0.001), back to non-stimulated levels (p = 0.21-0.75).

**hSF regulation of cartilage PRG-4 secretion involves IL-1 signaling.** Cartilage PRG-4 secretion stimulated by OA-hSF was not substantially altered by the additional supplementation of LY2157299 (Fig. 5). In the presence of 1 ng/ml IRAP, OA-hSF stimulated PRG4 secretion was higher compared to without inhibitor (5.1 ± 1.3 vs. 2.0 ± 0.5 μg/[cm²·day]).

**Discussion:** These results indicate that SF and O₂, which regulate some cartilage and chondrocyte responses, also modulate cartilage PRG-4 secretion. SF is a plasma ultrafiltrate with additional modifications from cells from the synovial joint. The differences in PRG-4 secretion response to OA-hSF compared to HS suggest that the regulatory factors mediating OA-hSF stimulation of PRG-4 secretion may be unique to SF, possibly secreted by cells into the synovial joint. These regulators in SF of chondrocyte PRG-4 secretion likely depend on the individual effects of O₂ and IL-1, and possibly other cytokines and growth factors.

**Significance:** Human SF regulates cartilage PRG-4 secretion through both stimulatory and inhibitor factors, including IL-1. Modulation of these regulatory factors in SF may affect PRG-4 concentrations in SF.

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**References:**
17. Grimshaw+, *Osteoarthritis Cartilage*
Figure 2: Dose-response of normal and osteoarthritis synovial fluids, and human serum, on PRG-4 secretion. PRG-4 in conditioned medium from days 4–6 of culture of calf explants (n=4–6 disks per condition) incubated in medium alone, with 10 ng/ml TGF-β1, or with increasing concentrations (0.05%, 0.5%, 5%) of hSF, either from m=4 normal (NL-hSF, in red) or m=4 osteoarthritis (OA-hSF, in blue) donors, or normal human serum. Pooled human serum was tested on disks from 3 calf knees. Mean±SEM. **p < 0.01 compared to basal condition.

Figure 3: Dose-response of TGFβR1 inhibitor LY2157299 on TGF-β1-stimulated PRG-4 secretion. PRG-4 in conditioned medium from days 4–6 of culture of calf explants (n=2–6 disks per condition per knee from 3 calf knees) incubated in medium alone, or with 10 ng/ml TGF-β1, and increasing concentrations (0, 0.01, 0.1, 1, 10 μM) of LY2157299. Mean±SEM. *p < 0.05 compared to TGF-β1 stimulated group without inhibitor.
Figure 4: Effects of TGF-β1, LY2157299, and hSF on cartilage PRG4 secretion. PRG4 in conditioned medium from days 4–6 of culture of calf explants (n=2–6 disks per condition per knee from 7 calf knees) incubated in medium ± 10 ng/ml TGF-β1 ± 1 μM LY2157299 ± 5% NL-hSF (m=7 donors or OA-hSF (m=7 donors). Mean±SEM.

Figure 5: Role of TGF-β and IL-1 signaling on hSF-stimulated PRG-4 secretion. PRG4 in conditioned medium from days 4–6 of culture of calf explants (n=4–6 disks per condition) incubated in medium with 5% OA-hSF, with or without 100 ng/ml IRAP and 1 μM LY2157299, with explants incubated in medium with IL-1α or TGF-β1 as controls. Mean±SEM.

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