**The crosstalk of post-injury events: A driver for posttraumatic osteoarthritis?**

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INTRODUCTION: Posttraumatic osteoarthritis (PTOA) is a common consequence of knee injuries, such as anterior cruciate ligament rupture. Early pathological events after ACLR include injury-induced cell damage, leading to programmed cell death (apoptosis) and perpetuation of an unresolved inflammatory response to trauma. Blocking either inflammation or apoptosis alone cannot fully prevent the vicious cycle that perpetuates the joint damage responsible for PTOA, indicating the possible existence of a crosstalk between these events.
The objective of this study is to investigate the crosstalk between apoptotic articular chondrocytes (AC) from cartilage and the inflammatory response of fibroblast-like synoviocytes (FLS) from the synovial tissue.

METHODS: **Apoptosis fosters apoptosis in AC and FLS.** We induced apoptosis in human AC by exposure to different concentrations of staurosporine (25, 50,100 and 200nM) or the vehicle (DMSO) for 1 hour; and then shifted to fresh media for 1 day. Supernatants (SN) were collected and spun at 3000 rpm to eliminate cells. We added the SNs to healthy AC and FLS for 24 h. After incubation, we assayed the cells for TUNEL to detect DNA fragmentation (APO-BrdU TUNEL Assay Kit). All experiments were repeated three times in different runs.
**Pro-inflammatory signaling from FLS aggravates apoptosis in AC.** We induced apoptosis in AC (50 nM ST for 1 h) and incubated them with the SNs from FLS pre-treated 24 h with or without IL-1β. We used a 2-way ANOVA with an interaction term to test the association of both ST and IL-1β signals in apoptosis progression. All experiments were repeated six times in different runs.
**The crosstalk between apoptotic AC and inflammatory FLS aggravates the expression of inflammatory and catabolic markers.** To evaluate the effects of a crosstalk between apoptotic AC and inflammatory FLS on cell homeostasis, we determined the mRNA levels of inflammatory (IL-6, COX-2) and catabolic markers (MMP-13) by using RT-PCR. For the first set of samples, we pre-treated FLS with IL-1β or vehicle for 24 h, and then incubated the FLS with the SNs from AC pre-treated with 0 or 50 nM ST. For the second set, we pre-treated AC with or without ST (50 nM, 1 h) and incubated for 24 h with the SNs from FLS pre-treated with or without IL-1β. The mRNA levels of IL-6, COX-2, and MMP-13 were normalized to 18S RNA and relative expression calculated to the control cells (without any stimuli). We used a 2-way ANOVA with an interaction term to test the association of both ST and IL-1β signals in the expression of the markers. Data was obtained from duplicate PCRs using RNA from 5 different cultures.

RESULTS SECTION: **Apoptosis fosters apoptosis in AC and FLS.** Fraction of TUNEL positive cells increased significantly in both AC and FLS after incubation with SNs from apoptotic AC [SN(AC+ST)] (Fig.1), indicating that the SN from apoptotic AC can induce apoptosis in both healthy AC and FLS.
**Pro-inflammatory signaling from FLS aggravates apoptosis in AC.** Figure 2 shows the fraction of TUNEL positive cell in % for all four combinations of ST and IL-1β. Our results show that the SN of FLS pre-treated with IL-1β did not induce apoptosis in healthy AC (no ST, $ST^-$). However, the same SN of FLS pre-treated with IL-1β more than doubled the fraction of apoptotic AC treated with ST ($ST^+$), indicating the presence of a crosstalk since both stimuli synergize to foster the progression of apoptosis (2-way ANOVA with interaction $p<0.01$). **The crosstalk between apoptotic AC and inflammatory FLS aggravates the expression of inflammatory and catabolic markers.** Figure 3, top shows how FLS homeostasis changed with the presence of an inflammatory environment and apoptotic signaling from AC. The transcriptional response of FLS was modulated by both stimuli with a clear synergistic crosstalk leading to overproduction of inflammatory and catabolic markers (2-way ANOVA with interaction $p<0.01$). FLS in an inflammatory medium (IL-1β) had increased gene expression in all genes (2.0-fold IL-6, 4.8-fold COX-2, 38.7-fold in MMP-13) when exposed to the SN of AC treated with ST. Figure 3, bottom shows how AC homeostasis is affected by an apoptotic environment and inflammatory signals from FLS. AC treated with ST showed a >4-fold expression in all genes when exposed to the inflammatory SN from FLS (4.7-fold IL-6, 4.7-fold COX-2, 5.3-fold MMP-13, $p<0.01$ 2-way ANOVA with interaction).

DISCUSSION: There is a synergistic crosstalk between apoptotic AC and the inflammatory response of FLS. This crosstalk aggravates apoptosis and increases pro-inflammatory signaling, creating a devastating hyperproduction of pro-inflammatory and catabolic markers.

SIGNIFICANCE/CLINICAL RELEVANCE: The synergistic crosstalk between apoptotic AC and the inflammatory response of FLS is a potential driver to PTOA and would explain why injuries progress to PTOA even after optimal reconstitution; and therefore, it represents a potential therapeutic target to reinstate joint homeostasis after injury and prevent or delay PTOA onset.

**Figure 1**

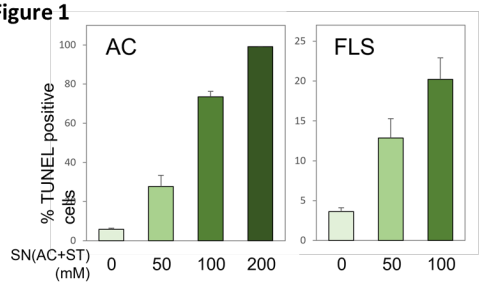


**Figure 2**

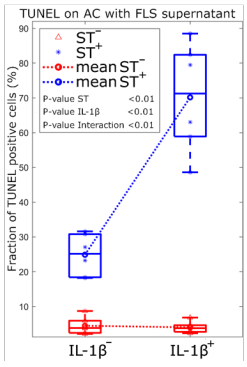


**Figure 3**