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FRI0529 SPREAD OF SENESCENCE AND JOINT INFLAMMATION VIA CONNEXIN43-POSITIVE EXOSOMES RELEASED BY OSTEOARTHRITIC CHONDROCYTES

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Background: Chondrocytes (CHs) in articular cartilage undergo phenotypic changes and senescence, restricting cartilage regeneration and favoring osteoarthritis (OA) progression. CHs and synovial cells from OA patients show a chronic increase in the channel protein connexin43 (Cx43), which regulates signal transduction. Extracellular vesicles (EVs), including exosomes, have been shown to play important roles in many biological functions and harbour Cx channels that allow the formation of gap junctions (GJs) between the exosome and the target cell, but the role of these EVs and exosomal-Cx43 in OA progression has not been studied yet.
Objectives: The objective of this study was to investigate the role of EVs released by OA chondrocytes (OACs) in cellular plasticity, inflammation and senescence of surrounding joint tissues.
Methods: CHs, bone and synovial cells were isolated from healthy and OA donors. EVs were obtained by ultracentrifugation and their protein content was analysed by LC-MS/MS. Protein levels were evaluated by western blot, immunofluorescence and flow cytometry. RNA expression was evaluated by RT-qPCR. Senescence and GJ intercellular communication was studied by flow cytometry and scrape loading assay, respectively.
Results: OACs showed increased levels of Cx43 within their EVs in comparison to the EVs isolated from healthy donors. Overexpression of Cx43 in CHs increased senescence and exosomal Cx43 levels. Interestingly, the treatment of CHs, bone cells and synoviocytes (target cells) with Cx43-EVs released by OACs, led to a significant increase in both Cx43 mRNA and protein levels in the recipient cells. The increase of Cx43 in target cells acted as a positive regulator of the reversion to a less differentiated state via EMT by activation of Twist-1, associated with increased levels of the mesenchymal markers, as CD105/CD166. The phenotypic changes detected in OACs lead to a decrease in Col2A1 and aggrecan expression in CHs, and increased the levels of cellular senescence and the senescence associated secretory phenotype (SASP) in the target cells in target cells via p53/p16 and NF-kß. These results were corroborated by analysing the protein cargo of these Cx43 positive EVs by LC-MS/MS, finding enrichment in proteins related with catabolic, senescence and wound-healing pathways.
Conclusion: Our results indicate that Cx43-positive exosomes released by OACs may be involved in the spread of cellular senescence and inflammation involved in wound healing failure. Further understanding of the role of exosomal Cx43 in OA will help to halt the disease spread and progression.
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FRI0530 ADIPOSE DERIVED STEM CELLS TRANSPLANTATION AMELIORATES OSTEOARTHRITIS THROUGH AUTOPHAGY INDUCING VIA PI3K/AKT/MTOR SIGNALING

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Background: Osteoarthritis is characterized by joint inflammation and cartilage degradation. Adipose derived stem cells (ADSCs), as a source of adult mesenchymal stem cells, display similar multiple-linage differentiating potentials to bone marrow MSCs, only are much more abundant, much easier to isolate and expand, have been suggested for suppressing inflammatory responses and repairing cartilage damage. Autophagy are known to be take part in the pathogenesis of OA. Aging related changes of chondrocytes were related to increased mammalian target of rapamycin (mTOR) signaling and defective autophagy (1). We aim to determine the effect of ADSC on autophagy and its underlying mechanism.
Objectives: To investigate the effect of ADSCs on autophagy in a rat osteoarthritis model and IL-1-induced chondrocytes, and to ascertain whether it regulates autophagy via PI3K/AKT/mTOR signaling.
Methods: ADSCs and chondrocytes were isolated from SD rats. Flow cytometry was performed for ADSC phenotypic characterization. ADSCs and chondrocytes coculture were established in the presence of IL-1β. A rat anterior cruciate ligament transection (ACLT) OA model were established. ADSCs or irrelevant cell lines of similar number were injected in the osteoarthritis-affected joints. Autophagic activation was determined by Western blotting for LC3-II, p62, MDC (monodansylcadaverine) staining and GFP-LC3 fluorescence microscopy. Autophagy inhibition was mediated by siRNA knockdown of ATG5. Relevant proteins in the PI3K/AKT/mTOR signaling pathway were detected by western blotting. IL-1β, IL-6, TNFα, IFN-γ, Col2, MMP-1, 3 and -13 were measured. Histology of the target joints were evaluated.
Results: Application of ADSCs resulted in downregulation of MMP13 and upregulation of Col2 in OA model cartilage and IL-1β induced chondrocytes. ADMSCs decreased pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in rat OA and IL-1β-induced chondrocytes. Autophagy was inhibited in OA model and IL-1β induced chondrocytes. ADSCs increased cell viability and autophagy-related proteins levels in vitro and in vivo. In IL-1β induced chondrocytes with ATG5 knockdown by siRNA, the effect of ADSCs on autophagy activation and its function as suppression of IL-1β induced pro-inflammatory cytokines was undermined. Furthermore, ADSCs remarkably decreased the expressions of phosphorylated (p)-PI3K, p-AKT and p-mTOR in IL-1β induced chondrocytes.
Conclusion: ADSCs inhibited the progression of cartilage degeneration in a rat OA model and provide an effective approach to decrease the pro-inflammatory cytokines secretion. ADSCs' anti-inflammatory effect was associated with cell autophagy and these roles of ADSCs may be associated with PI3K/AKT/mTOR signaling pathway.

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FRI0531 THE ARTICULAR PROTECTION EFFECT OF ISORHAMNETIN IN THE RATS OF MONOSODIUM IODOACETATE-INDUCED OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is a degenerative joint disease with damage to the articular cartilage. Active production of inflammatory cytokine/chemokine and matrix metalloproteinases may be found during the progression of OA. Isorhamnetin had the effects of anti-inflammatory, antioxidant, anti-ischemia, anti-atherosclerotic hepatoprotective and anticancer activities.
Objectives: Our study was focused on the effects of isorhamnetin treatment in OA.
Methods: We used monosodium iodoacetate (MIA)-induced OA rats to evaluate the effects of isorhamnetin related anti-inflammatory process. The rats in all groups were sacrificed on four weeks post-MIA injection. The measurements of knee joint swelling, histological analysis, serum inflammatory biomarkers and Western blot were evaluated.
Results: We found that isorhamnetin may reduce MIA-induced knee swelling by significantly reduction of articular cartilage damage in rats. The severity of OA lesion was graded using the modified Mankin scoring system, and we found that the overall modified Mankin's scores were significantly decreased after isorhamnetin treatment compared with a MIA-treated group. The production of IL-1β, IL-6, and TNF-α decreased in isorhamnetin treatment group compared with MIA alone group.

Isorhamnetin inhibited the production of NO and PGE2, and the expression of iNOS and COX-2. The production of COMP and CTX-II were also inhibited in MIA-induced OA rats.
Conclusion: Isorhamnetin may modulate the inflammatory progression of OA in MIA-induced OA rats. The prevention of cartilage damage was found in OA after adequate isorhamnetin treatment. Isorhamnetin may serve as a potential agent for the management of OA.

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FRI0532 SONIC HEDGEHOG PROMOTES PROLIFERATION, MIGRATION AND INVASION OF FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS VIA P38-MAPK SIGNALING

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Background: Abnormal activation of Sonic hedgehog (Shh) signaling has been found in synovium from patients with rheumatoid arthritis and inhibition of Shh signaling pathway suppresses the proliferation and migration of fibroblast-like synoviocytes in rheumatoid arthritis (RA-FLS). However, the mechanism how Shh signaling promotes tumor-like behavior of RA-FLS is not well elucidated.
Objectives: In this study, we aimed to investigate the effect of Shh on p38 MAP kinase signaling and hypothesized that Shh promotes proliferation, migration and invasion of RA-FLS via p38-MAPK signaling.
Methods: Cultured RA-FLSs were treated with Smoothened agonist (SAG) or IL-1β combined with Smoothened antagonist (Cyclopamine). The levels of phosphorylation of p38, its upstream kinases including TGFβ activated kinase 1 (TAK1), MKK3, MKK6 and downstream target MAPKAPK2 (MK-2) were determined by western blot. The functional state of p38 was determined using in vitro kinase assay. Cell proliferation was evaluated by Cell Counting Kit -8 assay. Cell migration and invasion were performed by Transwell assay. The expression of matrix metalloproteinase proteins (MMPs), IL-6 and IL-8 was examined by real-time PCR.
Results: SAG rapidly increased phosphorylation of p38, TAK1, MKK3, MKK6 and MK2 in RA-FLS and combination of SAG and p38 inhibitor significantly decreased the phosphorylation of these kinases (P<0.01). Inhibition of Shh significantly decreased the levels of phosphorylation of p38, TAK1, MKK3, MKK6 and MK2 in IL-1β stimulated RA-FLS (P<0.05). In vitro kinase assay showed that stimulation of SAG significantly increased the kinase activity, and the kinase activity was inhibited by p38 inhibitor. Furthermore, SAG increased cell proliferation, migration, invasion and production of MMP1, MMP3, MMP13, IL-6 and IL-8. However, these enhanced activities of RA-FLS were inhibited in the presence of p38 inhibitor.
Conclusion: The study indicates that Shh is associated with activation of p38 MAPK signaling in RA-FLS and Shh may promote the tumor-like behavior of RA-FLS via p38 signaling.
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Paediatric rheumatology

FRI0533 ETHNICITY AND NEONATAL LUPUSRISK IN A LARGE MULTI-ETHNIC COHORT

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Background: Neonatal Lupus (NL) is an acquired autoimmune disorder of newborns secondary to the transplacental passage of maternal anti-Ro and/or anti-La. Approximately 2% of children exposed to these antibodies develop NL. Prior studies have suggested that babies of non-European ancestry have a higher proportion of cardiac NL when compared to babies of European ancestry. This finding has not been consistently replicated.
Objectives: To examine the association between ethnicity and clinical manifestations of NL in our multi-ethnic population.
Methods: We conducted a cohort study of our large, multi-ethnic NL clinic population. The Neonatal Lupus clinic at the Hospital for Sick Children, Toronto, Canada was established in 1986. Children born to anti-Ro and/or anti-La antibody positive mothers are referred to the NL clinic. Antenatal testing is completed due to maternal rheumatologic diagnosis, a prior child born with NL and/or a history of symptoms that prompted physician testing for these antibodies. Beginning in 2011, families routinely reported ethnicity (Canadian census categories). We divided our NL patient cohort in European and non-European groups; the non-European group includes patients of African, Latin American, East Asian, South Asian and Mixed non-European ancestry (i.e. combination of two or more of non-European ethnicities). We included children assessed in the NL clinic ≤ 1 year of age between January 2011 to April 2018. There were 59 children censored for this analysis (7 missing ethnicity and 52 Mixed European-Non European ancestry). We analyzed prospectively collected data from our NL database, on specific NL manifestations: cardiac (heart block, myocarditis, endocardial fibroelastosis), dermatologic (typical rash of NL), hematologic (cytopenias), hepatic (transaminitis) and neurologic (macrocephaly). The frequency of NL clinical manifestations was compared among ethnicity groups (Fisher's exact test). We tested the association between ethnicity and NL clinical manifestations in logistic models.
Results: Our study included 301 children, 149 (50%) female and 164 (55%) with NL (Table 1). The median follow-up period was 12.2 months (IQR: 4.8, 28.8 months). Ethnicity data was available for 294 (98%) of the children. The non-European group (40%) was comprised of East Asian (14%), South Asian (13%), African (10%), Latin American (2%) and Mixed Non-European ancestry (17%). We did not observe a difference between European and Non-European babies in the proportion with NL, nor any difference in the frequency of specific NL manifestations (p-values > 0.3).
Conclusion: In our multiethnic NL cohort of children born to mothers with positive anti-Ro and/or anti-La antibodies, there was no association between ethnicity and NL, nor specific NL manifestations. Future analyses will examine the effect of maternal ethnicity and rheumatic disease status on the risk of NL and specific NL manifestations.

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Table 1. Neonatal Lupus Clinical Manifestations in European and non-European children.

	European n = 99 (%)	Non-European n = 143 (%)	p-value
Female	45 (45%)	72 (50%)	0.51
NL	48 (48)	80 (56)	0.29
Cardiac	10 (21)	11 (14)	0.32
Dermatologic	11 (23)	19 (24)	1.00
Hematologic	18 (38)	33 (41)	0.71
Hepatic	26 (54)	42 (53)	1.00
Neurologic	6 (13)	4 (5)	0.17

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FRI0534 ORAL GLUCOCORTICOIDS AND NEWLY TREATED DIABETES MELLITUS, HYPERTENSION, AND THROMBOSIS IN CHILDREN WITH CHRONIC DISEASES

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Background: Systemic glucocorticoid use is associated with a well-known spectrum of toxicities. Nonetheless, the risks of cardiometabolic complications in children are poorly understood.