trials was modest (mean Jadad score = 2.08). All studies reported a positive association between 2 to 5 weeks of Herbal Medicated-Bath Therapy and improved clinical pain symptoms compared with non-steroidal anti-inflammatory analgesics or hyaluronate injection controls. Mild adverse events were reported in both groups. Discordant trial designs, lack of reported sufficient qualitative outcome measures and other methodological limitations precluded a pooled meta-analysis.
Conclusions: These studies suggest that Chinese Herbal Medicated Bath Therapy may be helpful in the treatment of pain symptoms in patients with KOA; however, most studies are modest in quality. Further rigorously designed and well-controlled RCTs are warranted.

Proteomics & Metabolomics

764
THE MAMMALIAN TARGET OF RAPAMYCIN REGULATES LIPID METABOLISM IN OSTEOARTHRITIC HUMAN ARTICULAR CARTILAGE

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Purpose: There is increasing evidence indicating that lipid accumulation increases with aging and OA, perhaps contributing to the chondrocyte dysfunction. The mammalian target of rapamycin (mTOR) is a conserved serine-threonine kinase that regulates cell growth, cellular proliferation and metabolism in response to nutrient signals. In fact, it has been demonstrated that mTOR mediates in the onset and progression of diabetes, cancer and ageing process. However, the specific involvement of mTOR in the regulation of lipid metabolism in articular cartilage is poorly understood. The objective of this study is to determine the effect of mTOR inhibition in the regulation of the lipid metabolism in human articular cartilage.
Methods: OA cartilage slices were treated with mTORC1 selective inhibitor Rapamycin (Rapa, 10 μM), mTORC1 and mTORC2 inhibitor Torin 1 (75 nM), as well as with a limited medium under tissue starvation conditions (Hank's Balance Salt solution, HBSS) for 24 hours. Oil Red and Nile blue stainings were performed to analyze the effect of mTOR inhibitors on lipidic species present in OA articular cartilage. Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI) was used to study the differential lipidomic profiles under all conditions. Samples were sectioned in triplicate in 10 μm thick sections. 2,5-dihydroxybenzoic acid (DHB) matrix at 20 mg/mL in 20 mg/mL MeOH:AcN (50:50) was deposited by a vibrational sprayer system. A Synapt HDMS MALDI-Q-TOF instrument was used to perform the imaging-MS and MS/MS experiments with a spatial raster size of 150 μm. Principal Component Analysis (PCA) and Discriminant Analysis (DA) were used to build regional molecular classifiers. Biomap software was used to visualize molecular distributions. The Lipid Maps database was used for lipid identification.
Results: Lipid histological staining demonstrate that the inhibition of mTOR dramatically decreases the lipid pool positive staining in OA articular cartilage, indicating a direct regulation of lipid dynamics by both pharmacological and physiological regulation of mTOR. However, the histological analysis was insufficient to detect and identify specific lipid species. We employed a MALDI-MSI analysis to extend our understanding of these processes. PCA and DA revealed different lipidomic profiles among control, rapamycin, torin and starvation conditions. Firstly, we analyzed the four conditions in a single dataset where the first discriminant function (DF1) clearly separated the nutrient depleted tissue from the other groups. DF2 predominantly distinguished between control and rapamycin treated tissue. DA revealed that Palmitoleamide and Arachidonic acid were increased in the control condition whereas Phosphocholine and Sphingomyelin related peaks were specific from rapamycin condition (Table 1). In addition, rapamycin fragments were found in the treated samples of this drug such as m/z 582.3 and m/z 936.5 demonstrating the capability of the technique to study the lipid metabolism in addition to drug metabolism in the cartilage.
Conclusions: The MSI analysis data indicates that the mTOR regulates the lipid profile of human OA cartilage. The palmitoleamide and arachidonic acid, the lipid species identified on control condition had been implicated in the aging process and in the regulation of inflammatory mediators, such as leukotrienes and prostaglandins, respectively. Interestingly, rapamycin treatment modified the lipid profile to choline, phosphocholine or sphingomyelin, all implicated to maintain the cellular membrane integrity. In summary, these results demonstrate that MALDI-MSI analysis is a useful tool in the study of lipid and elemental composition changes associated with rheumatic pathologies and exhibit outstanding diagnostic potential.

Table 1
Lipidomic profiles of OA articular cartilage in response to mTOR regulation

m/z peak	Lipid assignment	Designation	Condition
104	Choline	[M+H]+	Rapamycin
184	Phosphocholine	[M+H]+	Rapamycin
2	Palmitoleamide	[M+H]+	Control
285	Stearic acid	[M+H]+	Rapamycin
304,2	Arachidonic acid	[M+H]+	Control
522,6	Phosphocholine (18:1)	[M+H]+	Rapamycin
582,3	Rapamycin		Rapamycin
666,4	From m/z Sphingomyelin	[M+Na]+	Rapamycin
725,4	Sphingomyelin	[M+Na]+	Rapamycin
936,5	Rapamycin	[M+Na]+	Rapamycin

765
QUANTITATIVE PROTEOMIC ANALYSIS REVEALED DIFFERENTIALLY EXTRACELLULAR PROTEIN PROFILES OF OSTEOARTHRITIC AND NORMAL MESENCHYMAL STEM CELLS UNDERGOING CHONDROGENESIS

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Purpose: Chondrogenesis occurs as a result of mesenchymal stem cells (MSCs) condensation and chondroprogenitor cell differentiation. Following chondrogenesis, the chondrocytes remain as resting cells to form the articular cartilage. A major challenge for the osteoarthitic (OA) cartilage reparation by stem cell-based approaches is the understanding of this multi-step process. In this work, we have analyzed the extracellular protein expression profile of human bone marrow MSCs (hBMSCs) of osteoarthritic patients and control donors undergoing chondrogenesis, in order to compare the mechanisms involved in the cartilage extracellular matrix (ECM) remodeling that occurs during the chondrogenic differentiation process.
Methods: hBMSCs isolated from 3 OA patients and 3 healthy controls were grown with different isotope variants of lysine and arginine (Arg6, Lys4 for the control population and Arg10, Lys8 for the OA population) during 4-6 weeks, until achievement of full protein labeling. The labeled populations were then subjected to differentiation in 3D cultures (micromasses) supplemented with chondrogenic inducers for 14 days. Proteins in the conditioned media from the two cell populations were combined, separated by 1D-SDS-PAGE and subjected to in-gel trypsin digestion using an automatic digestor. The resulting peptide mixtures were analyzed by nanoLC coupled on-line to an LTQ-Orbitrap XL mass spectrometer and quantified using the MaxQuant software and the Perseus tool.
Results: Real-Time PCR assays showed a relevant difference in the gene expression of collagen type II in the normal donors when compared to the OA patients. Moreover, the chondrocyte phenotype was confirmed in both cases by the proteglycan immunostainings such as aggrecan and chondroitin-6-sulfate after 14 days in chondrogenesis. Using the proteomic approach, we compared the extracellular protein profiles of OA and normal hBMSC at the same time of differentiation. Among the 531 proteins, 56 had significantly altered levels. 35 proteins displayed consistently higher levels in the OA samples compared to normal

donors. Many of these proteins are cartilage specific proteoglycans such as hyaluronan and proteoglycan link protein 1, aggrecan core protein or lumican as well as some proteins with a well-known role in the pathogenesis of OA like COMP or MMP3. On the other hand, 21 proteins exhibited a significantly reduced abundance in OA patients when compared to controls. Interestingly, we detected several proteins which belong to the tenascin protein family, like tenascin-X, which accelerates collagen fibril formation. We also found WISP2 decreased at day 14, suggesting a lower activity of the Wnt signaling pathway in OA cells.
Conclusions: The identification and quantification of these secreted proteins enhance our knowledge on the extracellular regulation of the chondrogenesis and allow the identification of extracellular markers of this process. Moreover, the lower expression of some of them in OA patients (like tenascin-X or WISP2), suggest their putative usefulness for the molecular monitorization of the chondrogenesis in cell therapy-based approaches for cartilage repair.

766
A LIPIDOMIC STUDY OF SPHINGOLIPID SPECIES IN HUMAN SYNOVIAL FLUID

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Purpose: Articular synovial fluid (SF) is a complex mixture of components either derived from plasma or locally synthesized by synovial tissues, which are involved in nutrition, communication, shock absorption, and lubrication. Alterations in its composition can be associated with increased friction, leading to articular cartilage damage, or with changes in inflammatory status of synovial joints. We already quantified 130 phospholipid species in human osteoarthritis (OA) and rheumatoid arthritis (RA) SF. The presented lipidomic investigation aims to quantify for the first time, the composition of sphingolipids [sphingomyelins (SM), ceramides (Cer), hexosylceramides (HexCer) and dihexosylceramides (Hex2Cer) species] in the SF of knee joints from unaffected controls, patients with early (eOA) and late (lOA) stages of OA and from RA patients, and to further elucidate whether the concentrations of individual lipid species are associated with the stage of OA disease.
Methods: According to predefined inclusion and exclusion criteria patients were included in the study. The use of human SF for this study was approved by the local ethics committee of our university, and all participants provided written informed consent. Lipids were extracted from SF without cells and cellular debris from 9 postmortem donors (control), 18 RA, and 30 OA patients. OA group was subcategorized as early ($n = 17$) and late ($n = 13$) using Outerbridge score. RA patients were classified according to the criteria of the American College of Rheumatology. Lipids were quantified using electrospray ionization tandem mass spectrometry - directly or coupled with hydrophilic interaction liquid chromatography. The quantitative values of all lipid species were corrected by the method described by Kraus et al. (2002). The Kruskal-Wallis test adjusted with a false discovery rate (FDR) of 10% and the Wilcoxon rank sum test were applied to determine statistically significant differences. P values < 0.05 were considered to be statistically significant.
Results: We provide a novel, detailed overview of sphingolipid species in human SF. The analytical set-up used in the present study allowed us to quantitate 35 different sphingolipid species. We identified 19 SM species, based on the length and saturation of the attached fatty acids. SM 34:1 was the predominant species among SMs. In addition, versus control SF, all species were elevated in eOA, lOA and RA SF. Remarkably, all SM species rose approximately 2-fold in SF from eOA to lOA and approximately 1.5-fold in SF from eOA to RA. However, we found no significant different levels of SM species in lOA compared with RA SF. Also, 6 Cer, 5 HexCer and 5 Hex2Cer were identified in human SF. Cer d18:0/24 was the predominant species. Moreover, most Cer species contained saturated fatty acids in all cohorts. Compared to control SF, the levels of most Cer species were 4.5-fold higher in lOA and 5.8-fold in RA SF. Moreover, all Cer species rose approximately 1.7-fold in SF from eOA to RA, and nearly 1.3-fold in SF from lOA to RA.
Conclusions: This lipidomic investigation present for the first time a detailed overview of sphingolipid species in human SF. Compared to control SF the concentrations of sphingolipid species such as SM and Cer were found to be elevated in eOA, lOA and RA SF. Since, disease and stage-dependent differences in the composition and concentrations of lipid species might result not only in an impaired lubrication but also in an altered inflammatory status of joints, sphingolipids appear to be associated with the pathogenesis of OA and RA. Our study lays the foundation for addressing specific questions regarding the biosynthesis and function of sphingolipid species in SF.

767
IDENTIFICATION OF AUTOANTIBODIES IN SERUM FROM OSTEOARTHRITIS PATIENTS USING MICROARRAYS

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Purpose: Osteoarthritis (OA) is characterized by the loss of structural components from the extracelullar matrix (ECM) of articular cartilage. The progressive release of proteins from the tissue and an abnormal metabolic activity can be specifically detected by the immune system, leading to a humoral immune response producing immunoglobulins against these proteins (autoantibodies). Autoantibodies are stable circulating proteins, easily measurable in serum, and may be the signature before clinical manifestations of the disease. Protein microarrays have emerged providing a tool for the identification of disease immunosignatures. The aim of this study was to detect the presence of autoantibodies in OA serum samples and compare these results with those obtained from healthy (CTRL) and rheumatoid arthritis (RA) sera.
Methods: Antibodies were detected using an specific Nucleic Acid Protein Programmable Array (NAPPA) designed and constructed as previously described by Ramachandran et al. 2008, containing 80 sequence-verified full-length human genes obtained from the Center for Personalized Diagnostics at the Arizona State University (www.dnasu.org). Once protein was displayed by in situ protein expression system, NAPPA arrays were incubated in optimized conditions with 20 OA, 20 RA and 18 CTRL serum samples. The autoantibodies were detected by anti-human IgG-HRP and fluorescence dye. Array images were obtained and processed by Genepix4000B and GenePix Software 6.0. For data analysis, background correction was performed by subtraction of the first quartile of non-DNA spots intensity and then divided the excess intensity by the median of spots without DNA across arrays.
Results: Significantly ($p < 0.05$) 4 different expression of autoantibodies against four different proteins has been observed between OA and CTRL serum samples. Of note, 2 of these proteins are related to the metabolism of ECM; the others are associated to cell adhesion (1) and bone mineralization (1). Most interestingly, this approach allowed the differential classification of RA and OA patients by the detection of 3 specific autoantibodies against proteins involved in cell proliferation and bone mineralization processes; one of these proteins also distinguishing between CTRL and RA subjects.
Conclusions: We have identified the presence of specific autoantibodies in OA allowing to distinguish between OA and CTRL patients and most interestingly, between OA and RA patients. These autoantibodies released to the serum might have a biomarker value to more accurate early diagnosis and prognosis of OA patients in clinical routine.

768
PEPTIDES PROFILING OF CARTILAGE SECRETOME: A PROMISING ALTERNATIVE FOR OA BIOMARKER DISCOVERY

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Purpose: The low-molecular-weight subset of the cartilage proteome (also termed cartilage peptidome) may have a diagnostic potential in osteoarthritis (OA) research, not yet fully exploited. The aim of this study is to investigate the cartilage secretome by means of peptidomic analysis and to provide a novel source for OA biomarker discovery.
Methods: Tissue explants were obtained from the dissection of human OA cartilages, both from the unwounded zone (UZ) and the wounded zone (WZ). The study was approved by the local ethical committee. Cartilage shavings from each zone were cut into 6 mm discs and five