324
SET7/9 REPRESSES SIRT1 TO INDUCE COLLAGEN TYPE II EXPRESSION IN 3D CULTURED HUMAN CHODROCYTES

H. Oppenheimer[†], H. Meir[†], A. Zini[‡], A. Haze[‡], L. Kandel[‡], Y. Mattan[‡], M. Leibergall[‡], M. Dvir-Ginzberg[†]. [†]*Hebrew Univ. of Jerusalem, Jerusalem, Israel;* [‡]*Hebrew Univ. of Jerusalem-Hadassah Med. Center, Jerusalem, Israel*

Abstract
Objective: Collagen type II (Col-II) plays a significant role in skeletal development and cartilage homeostasis and its loss with age is associated with degenerative joint diseases such as osteoarthritis (OA). Previously findings establish that the protein deacetylase SirT1 is required for enhanced expression of Col-IIα1, a major cartilage structural component of Col-II. Here we profile the mechanism by which SirT1 activates promoter-driven Col-IIα1 expression.
Methods: Chondrocytes derived from human osteoarthritic knee joints were encapsulated in three-dimensional alginate hydrogel microbeads (3D) and compared to paired monolayer (2D, passage 2) cultures derived from the same donor tissue. 3D-cultured cells displayed augmented expression of Col-IIα1 and were subject to serial chromatin immunoprecipitation (ChIP) and ChIP reChIP analyses to characterize epigenetic variations as compared to 2D cultures.
Results: ChIP analyses exhibited enrichment for SirT1 and the histone methyl transferase Set7/9, which formed a complex in 3D cultures wherein Col-IIα1 was augmented in expression. Consistent with enriched Set7/9 levels on the Col-IIα1 promoter, ChIP data revealed enriched levels of trimethylation on histone 3 lysine 4 (3MeH3K4). Acetylated H3K9/14 and H4K16 on Col-IIα1 promoters of 3D-cultured chondrocytes were also elevated indicating that SirT1 is enzymatically repressed by Set7/9 on the transactivated Col-IIα1 promoter. Elevated marks of the Histone acetyl transferases GCN5 and P300 also supported the local enrichment of their acetylated histone marks (i.e. H3K9/14,H4K5 and H4K16).
Conclusions: The data support that Set7/9 is capable of locally repressing SirT1 enzymatic activity on the promoter of Col-IIα1, resulting in its augmented expression dependent on cell morphology.

325
NEW INSIGHTS INTO THE MOLECULAR BASIS OF THE METABOLIC ALTERATIONS IN THE OSTEOARTHRITIS (OA) DISEASE

M.E. Vázquez-Mosquera[†], I. Rego-Pérez[†], A. Soto-Hermida[†], M. Fernández-Moreno[†], J. Fernández-Tajes[†], E. Cortés-Pereira[†], S. Relaño-Fernández[†], N. Oreiro-Villar[†], C. Fernández-López[†], F.J. Blanco[†,‡]. [†]*INIBIC-Hosp. A Coruña. Rheumatology Div. Genomic Group, A Coruña, Spain;* [‡]*CIBER-BBN-ISCIII, Madrid, Spain*

Purpose: Metabolic alterations take place in osteoarthritis (OA). Thus, the aim of this work is to analyze the gene expression of glucose and lipid metabolism related genes such as Facilitated glucose transporter members (GLUT1, GLUT3 and GLUT5), Hexokinase isoforms 1 and 2 (HK1, HK2), Insulin Receptor (INSR), Mitochondrial uncoupling protein 2 (UCP2) and Oxidized low density lipoprotein receptor 1 (OLR-1) in articular cartilage samples from OA patients and normal (N) controls.
Methods: Total RNA from 12 OA and 8 N cartilage samples was isolated using RNeasy Kit (Qiagen) following manufacturer's instructions. RNA was checked for integrity and purity by means of both NanoDrop spectrophotometer (Thermo Scientific) and agilent 2100 bioanalyzer (Agilent). The cDNA was obtained by reverse transcription using Superscript VILO cDNA Synthesis kit (Invitrogen), and amplified by qRT-PCR with LightCycler 480 II system (Roche). The Hypoxantine phosphoribosyltransferase-1 (HPRT1) was used as constitutive gene. Data analysis was performed with qBase plus software (Biogazelle) and SPSS software (v19).
Results: We analyzed the expression levels of GLUT1, GLUT3, GLUT5, HK1, HK2, INSR, UCP2 and OLR-1 in OA and N cartilage samples. The OA/N expression ratio of GLUT1 (1.42 ± 0.6), GLUT3 (1.26 ± 0.06), GLUT5 (0.7 ± 0.2), HK1 (1.04 ± 0.05), HK2 (1.6 ± 0.21) and INSR (0.99 ± 0.08) did not show significant differences in any case ($p > 0.05$). However, OA cartilage samples showed a significantly increased expression ratio for UCP2 (3.86 ± 0.12) ($p < 0.05$); moreover, in the case of normal cartilage, a positive correlation between the expression of UCP2 and GLUT3 was also detected (Pearson correlation coefficient 0.917, $p < 0.05$), whereas this correlation is lost in the case of OA. Only OA cartilage samples expressed OLR-1, and even significant differences in gene expression between two subgroups of OA samples were detected (fold change = 38.6; $p < 0.0001$).
Conclusions: Overexpression of UCP2 in OA may be important in causing tissue damage in addition to its function as uncoupling of the respiratory chain. The increased OLR-1 expression in a subgroup of OAs could be a factor relevant to the origin of the OA in certain individuals, and could support the possible involvement of oxidized low density lipoprotein (ox-LDL) in the impairment of both the ox-LDL metabolism and the inflammatory process.

326
AN EPIGENOME-WIDE ASSOCIATION STUDY OF OSTEOARTHRITIS

G. Zhai, Z. Rabie, E. Aref Eshghi, R. Green, A. Furey, G. Martin, G. Sun, P. Rahman. *Mem. Univ. of Newfoundland, St. John's, NL, Canada*

Osteoarthritis (OA) represents the most common form of arthritis and has a substantive clinical and economic impact. Our recent studies have successfully identified numerous genetic loci associated with OA. Despite this, a substantial proportion of the causality for OA remains unexplained. We hypothesize that epigenetic mechanism is an important factor that accounts for the missing heritability in OA and we tested our hypothesis using an epigenome-wide association approach.
Methods and Patients: Human cartilage tissue samples were collected from six total hip joint replacement patients, two of which had primary OA and four controls with hip fracture that exhibited no evidence of OA. DNA was extracted from the cartilage samples using DNeasy Blood and Tissue kit. Genome-wide DNA methylation profiling was performed on these six cartilage DNA samples plus additional 18 blood DNA samples from 18 OA patients. The profiling was done using Illumina HumanMethylation450k Beadchip, which measures up ~480,000 different CpG sites per sample, covers 96% of RefSeq genes, and provides comprehensive gene region coverage, targeting multiple sites with promoter, 5' UTR, 1st exon, gene body, and 3' UTR. The methylation level at each CpG site was measured by β values varying from 0 (no methylation) to 1 (100% methylation).
Results: A series of QC steps were applied to the initial methylation data on 485,577 CpG sites. After QC, we had data on 383,136 autosomal CpG sites, which were included in the subsequent analysis. We found that the distributions of the global methylation levels for the three groups (cartilage DNA for OA, and controls, and blood DNA for OA patients) are bimodal, which is consistent with previous report. However, blood samples had a significantly higher number of methylated regions than cartilage samples of both OA cases and controls. We found that three CpG sites in the MMP3 gene and one CpG site in MMP13 were demethylated in OA cases compared to controls (all $p < 0.05$), consistent with previous reports. Two CpG sites in MMP9 were associated with OA (all $p < 0.05$), but the effect size was opposite between these two CpG sites. We didn't find any association with ADAMTS4 but found two CpG sites located in the 1st exon of ADAMTS5 were significantly methylated in OA cases (all $p < 0.01$). We found one CpG site in IL-1β was methylated and one CpG site in GDF5 was demethylated in OA. Epigenome-wide association analysis didn't find any signals that reach genome-wide significance level ($p < 10\text{-}7$) due to small sample size but found there are many potential OA genes with methylation changes that haven't been reported previously.
Conclusion: Our preliminary result demonstrate that global DNA methylation pattern in human cartilage tissue is different from that in blood and we confirmed many of previously reported candidate gene methylation associations with OA. Our study also demonstrated that epigenome-wide approach is feasible to investigate the epigenetic factors in OA.