chondrocyte during the loss of phenotype in a dedifferentiation setup. Finally, we aimed for a translational validation of our data in cartilage samples from OA patients.
Methods: Human articular chondrocytes (hACs) stimulated or not with LiCl were used to carefully map the presence or absence of DOT1L protein complexes suggested earlier in leukemia cells, using a series of specific immunoprecipitation experiments. To elucidate the transcriptional network of Dot1l, we performed a microarray of hACs from 5 non-OA fracture patients treated with a specific DOT1L inhibitor (EPZ5676) or vehicle control. The differentially expressed genes were explored with the limma package. String-DB and Panther were used for gene network analyses. Based on the microarray analysis, selected genes were confirmed by quantitative PCR. To establish the role of DOT1L in the maintenance of hACs phenotype, hACs were cultured in monolayer in the presence or absence of EPZ5676, and RNA and protein were isolated at serial passages (P0 to P5). We analyzed by quantitative PCR the gene expression of known genes important for chondrocyte biology and/or genes that appeared in the microarray of DOT1L inhibition. Activation of the Wnt canonical and non-canonical signaling cascades was analyzed by Western blot. For translational validation we analyzed the differential gene expression profile of damaged as compared to intact cartilage areas within the same joint of patients with hip OA.
Results: The presence of different DOT1L elongation complexes was confirmed in hACs. The CoIP experiments revealed that DOT1L interacts directly with ENL, AF10 and active beta-catenin (more strongly when cells were treated with LiCl to stabilize beta-catenin) and with AF4 and AF5 (only with cells were treated with LiCl). The formation of these complexes was disrupted when DOT1L was inhibited. In the microarray, 1937 genes significantly changed ($p<0.05$) in DOT1L inhibited samples compared to vehicle treated samples (1048 genes were down-regulated, 889 up-regulated). In EPZ5676 treated samples, chondrocyte differentiation-associated genes, such as ACAN, RGS5, GDF10 and LOXL2 were down-regulated; OA-related genes, such as MMP1, CCL7, CCL8 and GPNMB were up-regulated; and WNT target genes, ligands and antagonists such as LEF1, WNT5A and DKK1 were significantly up-regulated. In the de-differentiation experiment, in vitro expanded hACs showed progressive changes in gene expression. Genes involved in relevant pathways for cartilage biology such as the WNT, NOTCH and BMP pathways exhibited significant changes that were highly accentuated by DOT1L inhibition. We successfully validated these targets of interest in samples from patients with OA condition.
Conclusions: Our transcriptomic, protein and gene interaction approach provides novel insights into the DOT1L molecular network and its putative role in osteoarthritis and cartilage. These data further support an important role for DOT1L in joint homeostasis as a key regulator of WNT signaling and other growth factor cascades in the joint.

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DISCRIMINATING BETWEEN PUTATIVE EPIGENETIC OSTEOARTHRITIS DISEASE DRIVERS AND SHEER MARKERS

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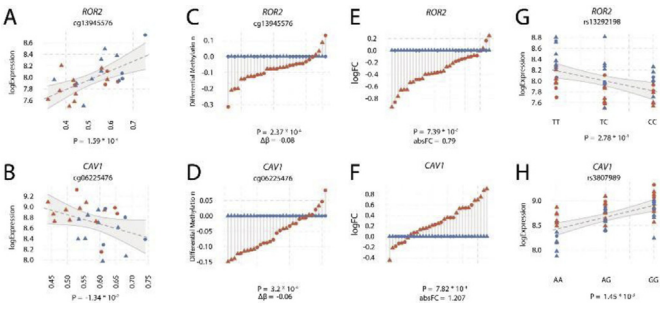
Purpose: It has become increasingly clear that DNA methylation is eminently involved in osteoarthritis (OA) pathology, as reflected by the large number of differentially methylated CpGs that have been reported between healthy, preserved and lesioned articular cartilage. However, as with the vast number of differentially expressed genes observed in OA affected articular cartilage, it remains unclear whether these observed differences are either cause or consequence of the disease. By combining genome wide methylation, expression and single nucleotide polymorphism (SNP) datasets we aim to achieve directional insight in the observed epigenetic and transcriptional changes in OA affected articular cartilage and report on putative protective and susceptibility loci.
Methods: Transcriptional activity of CpGs (t-CpGs) was assessed using genome wide gene expression and DNA methylation data of respectively 33 and 31 pairs of preserved and lesioned articular cartilage. Disease responsive t-CpGs were identified by means of differential methylation between preserved and lesioned cartilage. Additionally, we addressed proximal SNPs near the OA responsive t-CpGs. Statistical analyses were corrected for age, sex, joint and technical covariates, while a random effect was included to correct for possible correlations between paired samples.
Results: Of the 9838 transcribed genes in articular cartilage, 2324 correlated significantly with the methylation status of 3748 t-CpGs , both canonically negative (N=1741) as positive (N=2007) correlations were observed. Hypomethylation and hypermethylation (FDR<0.05, $|\Delta\beta|>0.05$) were observed for 92 and 59 t-CpGs, respectively, covering 117 unique genes. Significant enrichment for developmental and ECM maintenance pathways was observed, indicating possible reactivation of endochondral ossification. Finally, we observed 11 and 84 OA cartilage relevant genes of which, respectively, methylation and expression is additionally affected by genetic variation. Among others, we here present the potential disease driving genes ROR2 and CAV1 (figure 1), as reflected by differential expression between preserved and lesioned cartilage and, moreover, epigenetic and genetic transcriptional consequences.
Conclusions: We have shown that OA related epigenetic differences need to be integrated with other sources of molecular data, such as genomic and transcriptomic, to enhance our understanding of the pathophysiological processes of OA. Furthermore, by integration of multiple layers of genome wide data we have identified genes, such as ROR2 and CAV1, which are likely functionally involved in OA pathophysiology, as opposed to sheer consequence. Although targeting DNA methylation seems unlikely to stand at the basis for developing treatments, it serves to deepen our understanding of the complex transcriptomic changes in OA affected articular cartilage. Finally, our results comprise an important step in understanding the reported widespread epigenetic changes occurring in OA affected articular cartilage.



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PROBING THE ARTICULAR CARTILAGE TRANSCRIPTOME FOR GENETIC OSTEOARTHRITIS SUSCEPTIBLITY

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Purpose: Genome wide approaches have supplied the osteoarthritis (OA) field with robust susceptibility loci. A number of these risk alleles act by affecting transcriptional regulation of genes in cis, particularly in disease relevant tissues. For OA this has been demonstrated previously for e.g. ALDH1A2, DIO2 and GDF5 by a marked allelic imbalance (AI) in articular cartilage, a transcriptional phenomenon in which the two alleles in heterozygous carriers are expressed to unequal extent. Here, we set out to address the entire articular cartilage transcriptome by means of RNA sequencing and have assessed AI in all cartilage expressed genes.
Methods: From 21 patients (6 hips, 15 knees) preserved and lesioned cartilage were collected. Additionally, from, respectively, 21 (14 hip, 7 knee) and 5 (2 hip, 3 knee) patients, we collected preserved or lesioned cartilage only. RNA sequencing resulted in 10 million paired-end 100x2 bp long reads per sample. AI was performed on SNPs called using SNVMix2 with minimum coverage of 25. To avoid rare genetic variants, only SNPs that were observed in >9 samples were analysed. Furthermore, non-dbSNP A>G SNPs were removed as potential RNA-editing events. Differential gene expression between preserved and paired lesioned cartilage was performed using the edgeR package.
Results: We assessed AI in 15216 heterozygous coding SNPs of the articular cartilage transcriptome and observed that 1175 SNPs tagged AI of 493 unique genes (FDR<0.05, 0.1<minor/major allele ratio<0.4).