DATA MINING OF SEQUENCES STORED IN PUBLIC DATABASES IS A USEFUL TOOL TO IDENTIFY GENES INVOLVED IN OA PATHOGENESIS

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Purpose: In the early days of gene expression studies, a common method was random sequencing of clones derived from cDNA libraries and annotation of the resulting expressed sequence tags (EST). As a result, more than eight million human cDNA sequences resulting from short single-pass sequencing reads from a wide variety of tissues and conditions are stored in the NCBI database. Among them, several thousands correspond to healthy (HC) or osteoarthritic (OAC) cartilage. It means a wealth of useful data largely underused. Appropriate analysis using current bio-informatic tools could help to identify genes relevant for the pathophysiology of osteoarthritis, as well as the normal homeostasis of healthy cartilage.

Methods: dbEST division of GenBank was searched for data corresponding to human normal or OA cartilage. The matching genes were identified using the Blast suite. Both data sets were compared to detect differentially expressed genes. A minimum level of expression of 0.6% (corresponding to presence of at least 3 transcripts in only one sample) was used as cutoff to assign specific expression in one of the conditions. Further analyses using Reactome and KEGG tools were used to identify biological processes and functions.

Results: 5224 and 5019 sequences were identified as unmistakably derived from HC or OA cartilage, respectively. After removing vector sequences or too short cDNAs, we selected 4722 HC and 4621 OAC EST, matching to 2384 or 2009 known sequences, respectively. Among them, 1747 (73.3%) and 1465 (72.9%) genes, respectively, were represented by a single transcript, showing great diversity and suggesting that deeper coverage of the libraries is still possible. The identified transcripts included known nuclear and mitochondrial genes, as well as previously unannotated chromosomal regions, probably corresponding to non-coding genes. The most abundant transcripts in both sets of samples included Dcn, Gpx3, Clu or Vim. Remarkably, very few collagen-derived transcripts were identified, possibly due to the fact that they are stable proteins with very low turnover rate. Selection of differentially expressed genes produced a list of 101 and 91 genes that are preferentially expressed in HC and OAC, respectively, including mitochondrial and non-coding genes. Some of them code for already known markers of cartilage damage, (Compl, Crtac1, Ctgf...) or cartilage integrity (Egr1) while some have not been previously associated with cartilage damage or protection. Among genes overexpressed in HC we found Dpp10, CsdA and the ribosomal proteins Rpl11 and Rpl41. Genes overexpressed in OAC included the candidate suppressor gene Gltsc2r, Rab3b or the ribosomal proteins Rp56 and Rp59. Pathway analysis revealed that genes involved in nicotinate and nicotinamide metabolism were overrepresented in the HC samples, while genes involved in glycolysis and aminoadamet metabolism appeared overexpressed in transcripts from OAC.

Conclusions: Data mining of sequences stored in public databases can be a useful tool to identify genes involved in biological process. We have been able to identify a series of genes differentially expressed in normal and OA cartilage. Gene expression experiments will be carried out to confirm these results in clinical samples and to identify possible interacting factors.

SALMON CALCITONIN INHIBITED CHONDROCYTE HYPERTROPHY EX VIVO


Purpose: Chondrocyte hypertrophic-differentiation, leading to cartilage calcification and sclerosis of subchondral bone, is a key event of the early OA. An ideal agent for OA treatment should be able to affect both remodeling of subchondral bone and prevention or regeneration of articular cartilage. The growing evidence indicates that hypertrophic chondrocytes represent a potential target for OA treatment. We have shown previously that salmon calcitonin could prevent chondrocyte hypertrophy which was induced by triiodo-L-thryronine (T3). In this study, we aim to investigate whether salmon calcitonin could inhibit or reverse the chondrocyte hypertrophic-differentiation and calcification of cartilage, to find the evident that calcitonin could be an ideal agent for OA treatment.

Methods: Full depth cartilage (FDC) explants (from superficial to calcified cartilage) were isolated from bovine femoral condyle and hypertrophy was induced by culture for 4 days with triiodo-L-thryronine (T3) stimulation. Non-stimulated explants were cultured as control. Doses of sCT ranged from 1nM to 100nM were added from day 4 to day 13 without continuous T3 stimulation. Explants without sCT treatment from day 4 were presented as W/O. Cartilage turnover was measured by biochemical markers of type II collagen (COL II) formation (PINP assay) and degradation (CIBM assay). A sandwich ELISA assay - AGN-X II was used for measuring aggrecan degradation. Cartilage calcification was measured as alkaline phosphatase (ALP) activity by pNPP assay. As markers of hypertrophic chondrocytes, Indian hedgehog (Ihh), COL1, matrix metalloproteinase 13 (MMP-13) and ALP were evaluated using RT-PCR.

Results: Hypertrophy was induced by 4 days stimulation with T3. Significant increased expression was found in all four hypertrophic markers (p<0.05). At day 8, sCT increased cartilage formation, and decreased cartilage degradation and calcification dose-dependently, with comparing with W/O (p<0.001). On the contrary, sCT increased aggrecan degradation slightly in a dose-dependent manner. Chondrocyte kept hypertrophic-differentiation without T3 (W/O). Dosis of sCT inhibited MMP13 and ALP expression, in which 10nM inhibited MMP13 significantly (p<0.001). In contrast, only 100nM sCT suppresses Ihh (p<0.05) and COL X expression. At day 13, sCT increased cartilage formation and decreased calcification dose-dependently, comparing with W/O (p<0.01). Interestingly, sCT decreased aggrecan degradation slightly in a dose-dependent manner at day 13. Chondrocyte continued hypertrophic-differentiation without T3 stimulation at day 13 (W/O). Dosis of sCT inhibited gene expression of all four hypertrophic molecular markers in which 10nM sCT decreased Ihh and MMP13 expression significantly (p<0.001), and the level of Ihh, COL1 and MMP13 was lower than control.

Conclusions: The outcome suggests that T3 induced hypertrophic chondrocytes at day4, and chondrocyte continued hypertrophic-differentiation after 4 days without T3. sCT inhibited expression of markers of hypertrophic chondrocytes and cartilage degradation at day 8, and could reverse chondrocyte hypertrophic-differentiation at day 13, in which 10nM sCT is the most promising. Our investigation provides useful information on the efficacy of salmon calcitonin on hypertrophic chondrocytes and promising effect for OA treatment.

CHANGES IN HTRA1 IN CARTILAGE AFTER SINGLE IMPACT LOADING

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Purpose: The serine protease HtrA1 has been demonstrated to be increased in osteoarthritic cartilage in both naturally occurring disease and animal models, however little is known of the control of HtrA1 in cartilage. In order to begin to identify key events in the control of HtrA1 in response to challenge, we have investigated the levels of HtrA1 in cartilage explants from human and horse following Single Impact Loading (SIL), a model for early OA.

Methods: Full thickness cartilage discs were cultured in 5% CO2 and 20%O2 in DMEM + 10% FCS for up to 7 days (n=16). Control samples were not loaded, experimental samples were SIL with 500g from 2.5cm (8MPs). HtrA1 distribution in cartilage samples was investigated using immunohistochemistry, total levels of HtrA1 were quantified by Western blotting and densitometry and HtrA1 RNA levels were measured using qPCR with specifically designed equine primers.

Results: HtrA1 was demonstrated to increase throughout the cartilage sections following SIL and subsequent culture, in contrast to being confined to the superficial zone in control cartilage. Surprisingly, in control cartilage, western blotting revealed an oscillatory pattern of HtrA1 protein production, with an approximate ‘cycle time’ of 72 hours. This result was demonstrable in human and horse cartilage and was repeatable in all samples studied. Moreover, after SIL this protein pattern did not change.