



Transcriptomic study on the impact of temporomandibular joint internal derangement in the condylar cartilage of rabbits



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ABSTRACT

Internal derangement (ID) in the temporomandibular joint (TMJ) compromises a group of clinical problems, and holds a relative high prevalence in populations. However, the temporal genomic change in gene expression of condylar cartilage during continuous ID remains unclear. Here we reported the differentially expressed gene pattern in condylar cartilage of rabbits with ID from 1 to 8 weeks by microarray analysis. The whole genome project was deposited at GenBank under the accession PRJNA278127. The microarray analysis showed that 6478 genes have more than two-fold changes among all the tested transcripts. Many inflammation gene increased rapidly in the early stage while decrease later. On the contrary, the bone construction related genes showed a low level at first and increased at later period in the ID progression. Besides, the current study found some genes such as HLA2G, which had never been reported, might be relevant with ID.

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Specifications	
Organism/cell line/tissue	Rabbit temporomandibular condylar cartilage
Sex	Male
Sequencer or array type	Agilent-020908 <i>Oryctolagus cuniculus</i> (Rabbit) Oligo Microarray (Probe Name version)
Data format	Raw data
Experimental factors	Group 1 [1 week temporomandibular joint internal derangement (TMJ ID)] vs. normal Group 2 [2 week TMJ ID] vs. normal Group 3 [4 week TMJ ID] vs. normal Group 4 [8 week TMJ ID] vs. normal
Experimental features	The gene expression mode of condylar cartilage cell under TMJ ID progression was tested. The expression levels of selected genes were confirmed by RT-PCR.
Consent	Allow for reuse citing original author
Sample source location	Hangzhou, Zhejiang, China

1. Direct link to deposited data

The microarray raw data has been uploaded in GEO database under the accession GSE66864 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66864>).

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The whole genome project was deposited at GenBank under the accession PRJNA278127 (<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA278127>).

2. Experimental design, materials and methods

2.1. Animal mode establish

20 male rabbits were randomly distributed into 5 groups (4 treatment groups and 1 control group) with 4 rabbits in each. All the rabbits were free to tap water and a standard diet in the normal lab environment. After one week fed, the internal derangement surgery was done on 16 rabbits which were distributed into treatment groups. The surgery outcomes have been reported before [1]. The condylar cartilages of rabbits were then took out immediately (control group), after 1 week, 2 weeks, 4 weeks and 8 weeks respectively. The gene chips were prepared for RNA microarray analysis.

2.2. RNA extraction and microarray analysis

All the groups (control group, Group 1, Group 2, Group 3, and Group 4) were prepared for RNA microarray analysis. TRIzol Reagent (Invitrogen) was used for RNA total extraction and RN easy Mini kit (QAGEN) was used for RNA purification, all the procedures followed the manufacturer's instructions [2]. The whole RNA was amplified, labelled, and purified by GeneChip 3' IVT Express Kit in accordance with

the instructions to obtain biotin labelled cRNA. The slides were scanned by Spike GeneChip scanner 3000 and Command Console Software 3.1 with the default settings, then an Affymetrix Genechip Operating System was used to analyse the scanned image and obtain the scale quantitative information [3]. All the data was standardized by quantile method with the software of Gene spring version 12.5. The differentially expressed genes in normal and treatment groups were selected by unpaired T-test with significance level of $P \leq 0.05$. Then, the genes with fold change less than 0.5 upregulated or greater than 2.0 downregulated between any two groups were selected to analysis.

2.3. Statistical analysis

Unpaired T-test (two-tailed, unequal variance) was used to analyse and determine the significance of changes. The data of test groups (*Group 1*, *Group 2*, *Group 3*, and *Group 4*) were compared with control group respectively. P value less than 0.05 in the comparison of control group to each treatment group was regarded as statistically significant.

3. Results

In the comparison between control group and treatment groups, there were 6478 genes that showed more than two fold change. The inflammation gene and acute phase response were shown to upregulate at the first two weeks, MMP12 was one of the representative gene which increased more than two fold change significantly in differential gene expression [4]. Besides, the inflammation acute reaction can be proved by significantly expressed gene of COL, G-protein, MAPK, HIA1 α and so on.

After 1 week of recovery, the inflammation genes decreased whereas some TMJ ID related gene expressed significantly compared to the first two weeks, such as IL1 β , NOTCH, 4FGFR1, PLA2G, and Sox9. Among them, IL1 β hold a twofold increasing expression at 1 week and 8 weeks. Among those differentially expressed genes, there were bond reconstruction genes such as Sox 9 [5], and tissue reconstruction genes such as VEGFC [6].

4. Discussion

In recent years, microarray analysis on gene expression level change has been applied and welcomed by studiers in many fields [2,3,7]. It is one of the new and powerful technologies in various pathological genetics researches and can measure the expression levels of large numbers of genes accurately [8,9]. This technique can provide different genomic maps of various samples in a single experiment and has become the most popular approach to study gene changes, pathway dissection, drug identification and clinical samples' classification [3,10]. In 2012, rtPCR experiment showed that the expression of HAS3 mRNA with the addition of interleukin-1 beta (IL β) is related to TMJ ID [2]. Some other studies revealed that matrix metalloproteinases-3 (MMP-3), MMP-7 and MMP-9 upregulated in discs of human with TMJ ID [11,12]. Besides, the greater condylar movement pathway and mitochondrial pathway might contribute to TMJ ID [13]. However, less study tested time-dependent gene expression of rabbit, especially for the temporomandibular analysis. In this study, integrating differential

genomic expression in the condylar cartilage of growing rabbits was observed to improve our understanding of ID pathology at different stages.

5. Conclusions

In summary, 6478 transcripts showed twofold express change. The inflammation genes such as ACE and IL1 β expressed high level at first and decreased in the next 8 weeks. The bond reproduction genes related to TMJ ID, such as MMP12, and Sox 9 showed an increased value during 8 weeks of TMJ ID development. Some new genes may help to illustrate the disease, such as HIF1 α , FGF, VEGFC, and PLA2G that need to be analysed in the further study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jgdata.2015.06.034>.

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