Optimized approach combining laser capture micro dissection and micro array analysis for regional gene expression profiles of mandibular condylar cartilage

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Mandibular Condylar Cartilage (MCC) is a fibrocartilage that lines the mandibular condyle of the TMJ. Trauma and/or diseases can cause permanent tissue loss and disability; moreover, clinical management via cell-based regenerative therapies is limited due to the paucity of accurate molecular and genetic data. The advent of Micro Array Analysis (MAA) has enabled us to analyze the expression of thousands of genes in a single experiment. However, MAA reliability relies on procuring pure cell populations. Combining Laser-Capture Micro dissection (LCM), which allows precise cells isolation from heterogeneous tissues, and MAA technologies, enables accurate large-scale studies.

Objective: of this study is to optimize a method combining LCM and MAA to perform zone-specific gene expression analysis for MCC using 5-week-old rats.

Materials and Methods: Two MCC and two Femoral Condylar Cartilages (FCC) specimens were harvested from 5-week-old male SD rat, and then the LCM protocol previously described was applied to collect RNA from FCC (Control) and MCC zones; fibrous (FZ), proliferative (PZ), mature (MZ), and hypertrophic (HZ) zones individually. An optimized approach was established to subject the LCM-RNA samples to two-cycle linear amplification, Biotin-labeling and fragmentation, and then sent to a specialized center to perform microarray hybridization using Affymetrix GeneChip Rat Genome 230 2.0 Array.

Results: All quality control measurements at three points; LCM-RNA integrity (before amplification), purity and RNA integrity after amplification, and after fragmentation, revealed high quality that fulfills requirements for the subsequent procedures. Specificity (background) and sensitivity (percentage of genes detected) of the hybridization process were 63.9-74.5 and 54.6-60.1% respectively, showing higher values than Affymetrix and Arcturus protocols. Likewise, 3′:5′ ratio of mRNA, was 5.5-16.2 for GAPDH, and 11.8-32.9 for β-Actin, indicating the better quality of our samples when compared with other reports.

Conclusions: An approach for zonal gene expression analysis of the MCC from 5-week-old rats using LCM and MAA was successfully performed, and a well-supported hypothesis was formulated to distinguish the genes of MCC cells/zones from each other and from articular chondrocytes. Resolving zonal gene expression differences among the cell populations in MCC will enhance basic understanding of fibrocartilage biology and contribute to the future cell-based therapies.

Biography

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