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Human Periodontal Ligament Stromal Cell's Differential Gene Expression and Protein-Protein Interaction Networks under Mechanical Tension

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Abstract

The patient will have to endure prolonged periods of high costs as well as discomfort as a result of orthodontic treatment, which is based on intricate strategies and can take years to achieve the desired therapeutic outcome. The key to successful orthodontic treatment is selecting the best settings for force intensities during the initial phase of tooth movement. It is common knowledge that the periodontal ligament transmits tensile and compressive forces to the alveolar bone, facilitating orthodontic tooth movement. The entity of molecular key players activated by tensile forces remains elusive, despite the fact that transcriptomic analysis of compressed periodontal ligament cells revealed the complex molecular network already. As a result, the purpose of this research was to examine how the initial phase of orthodontic tooth movement was simulated by applying mechanical tensile forces to the gene 543 upregulated and 793 downregulated differentially expressed genes were identified through transcriptomic analysis of tension-treated and untreated periodontal ligament stromal cells, respectively. These genes include stanniocalcin, apelin, fibroblast growth factor receptor, noggin, sulfatase, secreted frizzled-related protein, and apelin. Additionally, a significant effect size was observed in the gene expression profiles of distinct cell donors. The underlying mechanisms, which will be necessary for the creation of individualized orthodontic treatment plans, could be better understood with a deeper comprehension of the roles that the identified candidates played in the initial phase of orthodontic tooth movement.

Keywords: Dentistry; Tooth movement in orthodontics; Transcriptomics; Cellular structure; Tension in the body

Introduction

During the orthodontic tooth movement process (OTM), the periodontal ligament plays a crucial role in the biomechanical and molecular communication between mechanically loaded teeth and the alveolar bone tissue. Given the high rate of relapse, orthodontic treatment can take several months or even years to achieve the desired result [1]. Patients' primary concerns when it comes to orthodontic treatment are the lengthy treatment times and the high costs associated with them. The OTM rate peaks during the initial phase, when orthodontic forces exert their greatest influence. After an orthodontic appliance has been placed, this initial phase of OTM lasts for up to 48 hours [2]. The initial phase of OTM is an important step because the choice of force intensity is important for the patient's pain sensation and the outcome of the treatment.

OTM is driven by an interaction of tension and compression forces, as well as shear stress, which enables tooth movement through a step-by-step cascade of molecular processes. However, the biological mechanisms that underlie OTM have not been fully investigated. The primary receiver of external mechanical signals that must be transformed into biological signals is the periodontal ligament. A portion of the pathways have proactively been recognized to be engaged with this cycle, including central grip kinase-and nitric oxide-subordinate β-catenin flagging, the Rho-mDia1 flagging pathway, platelet-inferred development factor subunit B/platelet-determined development factor receptor β-initiated Janus kinase signal transducer and activator of record protein signalings, cAMP reaction component restricting protein enactment, Piezo1-interceded flagging or extracellular sign controlled kinase and yes-related protein flagging pathways. Given that OTM-mediating factors like blood flow, oxygen, and carbon dioxide levels exhibit differential behavior in response to the respective types of mechanical force, tensile or compressive mechanical forces either activate or inhibit these pathways.

The inter-individual variation in the OTM rate following the application of comparable orthodontic forces, which could presumably be explained, at least partially, by genetic factors, is another significant clinical issue in orthodontics. Until now, there has been evidence that a single molecular component, such as an interleukin-1 polymorphism or transient receptor potential cation channel, subfamily C, and member 6 activation, may be responsible for the rate of OTM [3]. However, the complicated entity of inter-individual differences in the regulatory mechanisms that are responsible for OTM has not yet been fully understood.

Because most of the research on OTM's elicited mechanisms of tensile and compressive forces comes from studies that focused on individual molecular parameters rather than on molecular networks, the knowledge of these mechanisms is limited. This restriction can be settled, undoubtedly somewhat, by the use of different innovations, for example, RNA-seq, which permits broad appraisal of transcriptomic differential quality articulation. Profiling of differentially expressed genes (DEG) on the tension side of the human periodontal ligament under conditions comparable to the initial phase during OTM is still lacking, despite initial [4]. The first attempts to determine the gene expression profiles of human periodontal ligament cells revealed that different tensile strain conditions result in different expression of mRNA and lncRNA. The gene expression profile of human periodontal ligament cells during the initial phase of OTM has not yet been examined in a setting.

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Consequently, the purpose of this study was to use RNA-seq technology to identify DEG in primary human periodontal ligament stromal cells (PDLSC) under mechanical tension that resembled the initial phase of tension forces applied during orthodontic tooth movement. To look for potential differences in gene expression profiles between individuals, primary human PDLSC from various donors were evaluated.

Materials and Procedures

Ethics and Cell Isolation Periodontal ligament tissue from extracted human wisdom teeth was manually removed without revealing any signs of inflammation or caries [5]. The removed periodontal ligament was cut into 1 mm pieces of tissue and placed in Petri dishes containing MEM Alpha Modification medium (Capricorn Scientific GmbH, Ebsdorfergrund, Germany), which was supplemented with 10% fetal calf serum, 2 mM L-glutamine (Capricorn Scientific GmbH), 100 U/ml penicillin, 100 g/ml streptomycin, and 2.5 g/ml amphotericin (anti Scientific by Thermo Fisher). Adherent cells were harvested through trypsinization, expanded, and stored in liquid nitrogen until required. Because these isolated cells meet the minimum requirements for mesenchymal stromal cells, they were given the name PDLSC. Up until passage 5, this study used human PDLSC from three male and three female donors between the ages of 18 and 45.

Prior to tooth donations, patients provided written and informed consent. The Ethics Committee of the Medical University of Vienna, Vienna, Austria, gave the study protocol its approval. The study's design adheres to the Medical University of Vienna's "Good Scientific Practice" guidelines and the Declaration of Helsinki's ethical guidelines for human subjects in medical research.

Treatment of cells under mechanical tension

A total of 300,000 cells per well were seeded onto collagen type I-coated 6-well Culture Plates for the PDLSC treatment under mechanical tension. Cells were allowed to attach overnight after being seeded [6]. Attached PDLSCs were inserted the following day to apply static mechanical tension, initially with a maximum tension intensity of 10% for the first six hours and gradually decreasing to 3% thereafter. PDLSC were subjected to mechanical tension for a total of 72 hours. Additionally, as a control group, 300,000 PDLSC per well were seeded onto regular collagen-type I-coated 6-well culture plates and incubated for 72 hours at 37 °C, 5% CO2, and 95% humidity. Live-Dead staining (Enzo Life Sciences AG, Lausen, Switzerland) was used to assess cell viability 72 hours later. The treatment group contained tension-treated PDLSC, the negative control contained untreated PDLSC, and the positive control contained both tension-treated and untreated PDLSC that had been incubated with pure DMSO for 15 minutes prior to the staining. All PDLSC samples were washed with PBS prior to staining. The staining solution was then applied for 15 minutes at 37 °C in accordance with the manufacturer's protocol. After that, Live-DyeTM-positive cells were observed at 470/40 nm excitation and 525/50 nm emission wavelengths with a fluorescence microscope at a 100-fold magnification, while PI-positive cells were observed at 560/40 nm excitation and 605/70 nm emission wavelengths. The experiment was carried out in two technical replicates, and PDLSC from three distinct donors were used for the staining.

Isolation of RNA, preparation of the library, and RNA sequencing

Total RNA was extracted using an RNeasy Mini Kit from the same three distinct PDLSC donors that were utilized for the Live-Dead staining in accordance with the manufacturer's recommendations [7]. The 260/280 absorbance ratio was used to photometrically confirm the purity of the isolated RNA. At the Biomedical Sequencing Facility of the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences in Vienna, Austria, additional assessment of fragment size and sample integrity, library preparation, RNA-seq, and fundamental bioinformatics were carried out. For differential expression profiling in tension-treated and untreated PDLSC, a poly-A-enriched mRNA-seq library was made, and transcriptome sequencing was done using an Illumina HiSeq 3000 system with a read length of 50 base pairs and a sequencing depth of 10 to 25 million reads per sample.

Using bioinformatics

The sequencing reads were aligned with the human reference genome using bioinformatics. The differential expression modeling was carried out using the Bioconductor DESeq2 R package [8]. A model-based variance stabilizing transformation was carried out, and read counts were normalized to the size of the library. To evaluate the size of the DEG effect between tension-treated and untreated PDLSC samples as well as among the various donors, log2-fold change values were calculated and reduced using the R package "ashr" on the basis of these data. Independent hypothesis weighting was used to adjust the p-values. R was used to create a volcano plot of the results. The cut-off criteria for determining which protein-coding genes were differentially expressed between all three donors were an effect size of less than 2.0 and an adjusted p-value of less than 0.05. A table provided a summary of the DEG candidates that were found, as well as their adjusted p-value and effect size. Fragments per kilobase of transcript per million mapped fragments (FPKM) values were used to calculate row-wise z-scores, or z-scores per DEG, for further comparison of tension-treated and untreated PDLSC DEG that were differentially expressed in tension-treated PDLSC. The Visplore software (Visplore GmbH, Vienna, Austria) was used to create a heatmap of the 124 DEG that was produced. In the heatmap, data clustering was done by comparing the mean z-scores of tension-treated and untreated PDLSC. Gene ontology (GO), KEGG, Reactome, and WikiPathway enrichment analyses were also carried out. Heatmaps displaying the z-scores of the combined scores from each enrichment analysis were made available with results that had an adjusted p-value of less than 0.05.

mRNA level analysis by RT-qPCR

In separate samples of tension-treated and untreated PDLSC, further mRNA level analysis by RT-qPCR DEGs related to the GO terms "bone development" or "regulation of fibroblast factor receptor signaling pathway" were examined [9]. Six distinct donors of tension-treated and untreated PDLSC were used to isolate total RNA, and each experiment consisted of two technical replicates (N = 6) as previously mentioned. Using a Thermo Fisher Scientific high-capacity cDNA reverse transcription kit, isolated RNA was transformed into cDNA. The following TaqMan gene expression assays (Thermo Fisher Scientific) were utilized in the subsequent RT-qPCR evaluation of the cDNA samples: STC1, APLN, FGFR2, NOG, SULF1, and SFRP4 GAPDH was chosen as the reference gene based on comparative CT analysis, geNorm, and Normfinder analyses of three potential reference genes. Among the samples that were tested, GAPDH exhibited the highest stability, followed by 18S and ACTB. Normalized data were used to compare tension-treated and untreated PDLSC. The CT method served as the foundation for the results' calculation. The Mann-Whitney U test was used to determine the differences between PDLSC treated with tension and those that were not. The significance level was set at p 0.05.

Protein-protein interaction network analysis

This resulted in the creation of a complete STRING network devoid of text-mining interaction sources and a minimum interaction score of 0.900. As PPI networks with network edges that indicate the kind of interaction evidence, only connected nodes were displayed.

Result and Discussion

Common issues in orthodontics include individual variations in the rate of OTM and relapses following orthodontic treatment [10]. The selection of settings for applied forces in the initial phase is critical to the success of orthodontic treatments. However, in order to select the appropriate settings, it is necessary to have knowledge of the biological processes that take place during OTM. Nevertheless, the initial biological context of OTM is still poorly understood. For studying the initial OTM phase, powerful in vitro cell culture models for modeling orthodontic forces are available. We used an "omics" technology in this study to see how tensile forces affected gene expression in periodontal ligament cells taken from different people. The findings of this study provide a solid foundation for further research into the genomic changes that occur during OTM and the creation of individualized orthodontic treatment plans.

In order to achieve our goal, we used primary human PDLSC as the basis for an in vitro model that was subjected to static tension for 72 hours, beginning with a maximum tension of 10% for the first 6 hours and gradually decreasing to 3% to resemble OTM's initial phase. RNA-sequencing was also carried out [12]. Aligner treatment uses a strategy that uses light, continuous orthodontic forces to achieve a faster OTM rate while simultaneously reducing root resorption. In experimental models, the range of orthodontic forces applied to teeth starts at 10 cN/mm2, of which only a small portion affect the periodontal ligament. In the continuous mode, the Flexcell® Tension System utilized in this study permits a maximum tension intensity of 10%, or approximately 5 cN/mm2. As a result, we chose a force that was as high as it could be for the first few hours of treatment but gradually decreased over the next 72 hours. This allowed orthodontic forces to remain low during the initial phase of orthodontic tooth movement. In an in vitro model, we were able to identify six candidate genes with potential relevance to the initial phase of OTM through transcriptomic analysis of tension-treated PDLSC. These potential genes are expressed by a variety of mesenchymal tissues, including those that can contract, and are not restricted to the periodontal ligament or any other dental tissue.

In our study, tension-treated PDLSC showed a decrease in APLN. If, as was suggested in the context of vascular calcification, APLN inhibits OPG, its decrease in mechanical tension in the periodontal ligament would encourage OPG-mediated bone formation [13]. Human periodontal ligament cells exhibit an increase in APLN and a decrease in osteoprotegerin (OPG) under hypoxic conditions. This could be a fascinating relevant connection to OTM as mechanical strain by orthodontic treatment prompts hypoxic states of the periodontal tendon, which thus invigorates bone renovating. Although our study demonstrated a significant decrease in FGFR production upon tension, it must be verified in subsequent experiments to determine whether this is a genuine biological effect or merely a cryopreservation artifact. An environment that resembled inflammatory resorption was previously linked to Fgfr2 levels in periodontal ligament fibroblasts [14]. As a result, the study's chosen force intensity—which corresponds to light orthodontic forces applied to avoid inflammation and resorption—could be a factor in the decreased FGFR2 expression. Given that NOG has an inhibitory effect on osteogenic differentiation in periodontal ligament cells mediated by hypoxia-inducible factor 1 promotor activation of NOG, our study found that the decrease in NOG and SFRP4 expression may be favorable for osteogenic differentiation on the tension side of OTM. Additionally, the periodontal ligament's epithelial growth and osteogenic differentiation were found to be inhibited by SFRP4 expression. NOG expression was found to increase upon static compression of periodontal ligament cells, suggesting that it is also important on the pressure side of OTM [15]. A similar impact of pressure was found for STC1 articulation. From the pool of significantly relevant DEG that was identified in this study, NOG was the only candidate in the PPI network analysis that is interactively involved with another protein product, bone morphogenetic protein 2. As a result, the role of NOG in OTM is relevant for subsequent research. SULF is the only candidate gene discovered by this research that has not been linked to any function of oral or dental cells. However, its relevance to the processes of bone remodeling was already established.

Conclusion

When taken as a whole, our research demonstrates that six potential genes—APLN, FGFR2, NOG, SULF1, SFRP4, and STC1—are involved in tension-based effects in human PDLSC that may be significant during OTM. Future research should look into how the mechanistic signaling network works in detail to get a complete picture of the molecular mechanisms that underlie OTM. This will make it easier to create more effective and personalized orthodontic treatment plans.

Acknowledgement

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Conflict of Interest

None

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