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Original Article

Identification of important genes for human periodontal ligament cells in response to mechanical force based on WGCNA

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Abstract

To identify the differentially important genes of human periodontal ligament cells (PDLC) in response to different types of force, the dataset with regard to human PDLC in response to force was retrieved from the GEO. Differentially expressed genes (DEG) analysis between mechanical force (MF) and the control group was conducted. The gene set enrichment analysis (GSEA) was applied to identify the functional enrichment in different MF groups. Weighted gene co-expression network analysis (WGCNA) of transcriptomic data was performed to identify the highly correlated genes in human PDLC in response to MF. The Lasso regression model was applied to screen the key genes. Results showed A total of 2861 DEGs were identified between the MF group and control group, including 1470 up-regulated DEGs and 1391 down-regulated DEGs. Different biological processes were enriched between the static group and the intermittent group. The Myc targets, TGF-β signaling pathway and PI3K/AKT/MTOR signaling pathway were enriched in intermittent-MF and static-MF groups. The turquoise module (including 386 hub genes) in WGCNA was highly correlated with intermittent traits and the black module (including 33 hub genes) was positively correlated with static traits. The lasso analysis result showed that the CLIC4, NPLOC4 and PRDX6 had the greatest impact on the human PDLC with mechanic stimuli with good predictive efficiency. In conclusion, we developed important genes for human PDLC in response to MF, which might be potential markers for orthodontic tooth movement.

Keywords: Periodontal ligament, Mechanical force, Orthodontic tooth movement, Mechanosensing.

1. Introduction

Periodontal ligament (Periodontal fiber), usually abbreviated as PDL, is a group of specialized connective tissue fibers that originally connect the tooth to the alveolar bone in which it is located. It enters the root cement from one side and the alveolar bone from the other side [1,2].

Periodontal tissue remodeling is the physiological basis for orthodontic tooth movement (OTM) during appropriate mechanical stimulation. Periodontal ligament cell (PDLC), the major cell type in periodontal tissue, regulates the osteoblastic differentiation, activity and even life span(1). The PDLC functions as force-sensitive and force-conducting cells. When the orthodontic force was applied to the tooth, the periodontal ligament (PDL) transferred the external load to the alveolar bone and led to bone remodeling. Simultaneously, the PDLC began to differentiate into compression-associated osteoclasts and tension-associated osteoblasts to engage in periodontal tissue remodeling(2). Periodontal tissue in the compressed area was characterized by disturbance of blood flow, cell death and bone resorption, while bone formation in the tension area(3). Many reports have focused on the molecular mechanism of osteogenic differentiation of PDL during stretching force stimuli(4, 5). However, how PDL transfers mechanical signals into biochemical events and regulates cellular metabolism remains inconclusive(3). In this study, we aimed to investigate the alteration of transcriptome and potential function in human PDLC in response to different types of mechanical stimulations based on a bioinformatics analysis.

2. Materials and Methods

2.1. Data collection

The dataset regarding human PDLC in response to force was retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). Only 1 dataset (GES112122) was enrolled in this study, which included 4 groups (static group (n=3), static-control group (n=3), intermittent group (n=3) and intermittent-control group (n=3)). After normalization and standardization, the gene expression profile was applied for further difference analysis.

2.2. Differentially expressed genes (DEGs) between mechanical force (MF) and control group

Differential gene expression analysis between mechanical force (MF) and control group was conducted based on the negative binomial distribution with R package (DESeq2)(6) To further analyze the effect of different types of MF on DEGs, differential gene expression analyses (between the static and control group, the intermittent and

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control group) were performed respectively. The DEGs with a false discovery rate (FDR) < 0.05 and |fold change (FC)| >1.5 were considered statistically significant. In addition, the common DEGs among the above 3 pairs of analyses were depicted with Venn plot (Supplementary Fig 1).

2.3. Identification of function enrichment based on the gene set enrichment analysis (GSEA)

Gene set enrichment analysis (GSEA, https://www.gsea-msigdb.org/gsea/index.jsp)(7) was used to annotate the functional enrichment of the above gene expression profile. The GSE112122 dataset was divided into four groups (static group, static-control group, intermittent group and intermittent-control group). Simultaneously, the annotated dataset (h.all.v7.4.symbols.gmt) was downloaded from the Molecular Signatures Database(8). The enriched pathways with P< 0.05 were considered statistically significant.

2.4. Weighted gene co-expression network analysis (WGCNA) of transcriptomic data

To identify highly correlated genes in human PDLC in response to MF, the gene expression profile was applied to perform a WGCNA analysis after normalization with DESeq2. The optimal soft threshold was calculated to obtain a scale-free topology. The samples in GSE112122 were clustered using hierarchical clustering. In addition, the correlation of all identified modules was conducted. To investigate the physiological significance of the clustered modules, the phenotypes in GSE112122 were correlated with modules. In the following, the centrally intramodular hub genes in the (static and intermittent) traits-related modules were selected with |module membership|>0.8 and |gene significance|>0.2.

2.5. Lasso regression model for key gene selection

To further discard genes of little impact on the target vector, the least absolute shrinkage and selection operator (LASSO) analysis was performed to screen the key genes that had the greatest impact on PLDC in response to MF. The hub genes detected from WGCNA and DEGs were intersected. This dataset was divided into the training set and the test set. The regularization penalty (λ) was included for nonzero coefficients to overcome the over-fitting problem. To calculate the weight of the LASSO penalty (labeled as lambda), a ten-fold cross-validation was performed on the training set. The area under the curve (AUC) was evaluated on the test set to assess the discriminative ability. The LASSO algorithm was conducted using the R package (glmnet).

3. Results

3.1. Identified DEGs between MF and control group

There were 2861 identified DEGs between the force (intermittent and static) group and control group, including 1470 up-regulated DEGs and 1391 down-regulated DEGs (Fig 1A and 1D). In addition, a total of 3812 DEGs were identified between the intermittent group and control group, including 1915 up-regulated DEGs and 1897 down-regulated DEGs (Fig 1B and 1E). However, only 939 DEGs were identified between the static group and control group, including 542 up-regulated DEGs and 397 down-regulated DEGs (Fig 1C and 1F). There were 821 common DEGs among the above 3 groups. Furthermore, the static group and intermittent group shared 823 common DEGs (Fig 1G).

3.2 Identified functional enrichments between MF and control group based on GSEA analysis

Different biological processes were enriched between static group and intermittent group. For the intermittent MF treatment, the interferon (IFN)-γ response, IFN-α response, PI3K/AKT/MTOR signaling, peroxisome, Myc targets, xenobiotic metabolism and transforming growth factor beta (TGF-β) signaling were enriched from the hallmark gene datasets. Interestingly, lipid metabolism including fatty acid metabolism and bile acid metabolism-related signaling pathways were also collected (Fig 2A).

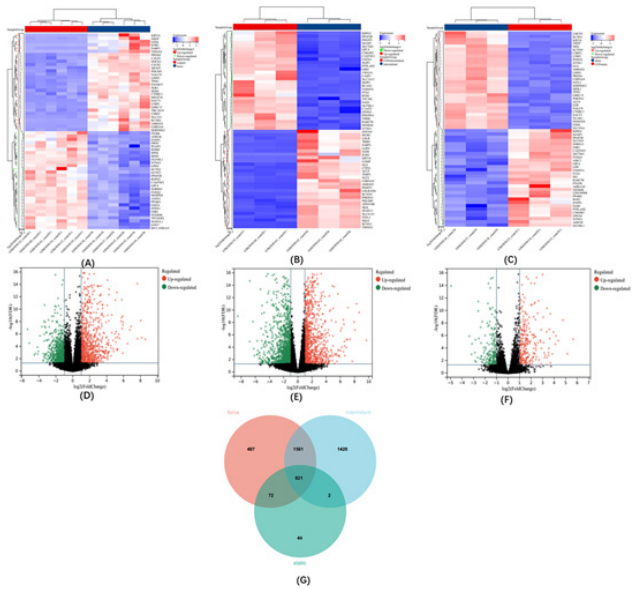

Fig. 1. Differentially expressed genes (DEGs) between the MF and control group. (A)Heatmap of top 30 DEGs between the force (static and intermittent) and control group. (B)Heatmap of top 30 DEGs between the static and control group. (C)Heatmap of top 30 DEGs between the intermittent and control groups. (D)Volcano plot of DEGs between the force (static and intermittent) and control group. (E)Volcano plot of DEGs between the static and control group. (F)Volcano plot of DEGs between the intermittent and control groups. (G)Venn plot among the force group, static group and intermittent group.

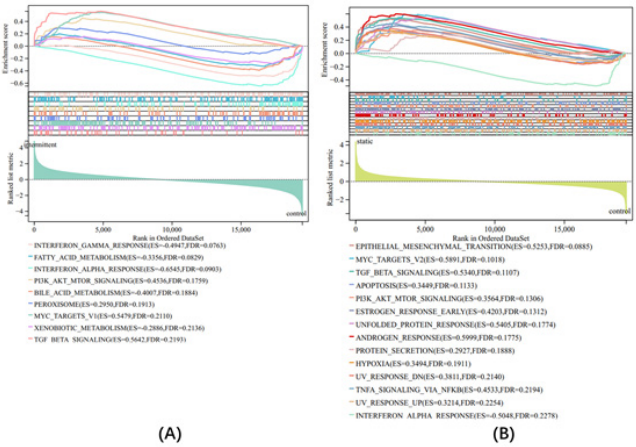

Fig. 2. Gene set enrichment analysis (GSEA) between MF and the control group. (A)GSEA analysis between the intermittent group and control group. (B)GSEA analysis between the static and control groups. ES, enrichment score; FDR, false discovery rate.

For the static MF treatment, the androgen response, protein secretion, epithelial-mesenchymal transition (EMT), apoptosis, Myc targets, TGF-β signaling via receptor activator of nuclear factor (NF)-κB, PI3K/AKT/MTOR signaling, MTORC1 signaling, hedgehog signaling, cholesterol homeostasis, glycolysis and UV response related signaling pathways were aggregated in HPDLC with static MF treatment (Fig 2B).

As shown in Fig 3A, the network exhibited a scale-free topology (mean connectivity of 95) with a soft threshold of 16. The hierarchical clustering in Fig 3B showed sample correlations. A total of 12 modules were identified and depicted in Fig 3C and Fig 3D. The turquoise module (including 722 genes) was highly correlated with the intermittent trait (r= 0.827, P<0.01) and the black module (including 72 genes) module was positively correlated with static trait (r= 0.336, P>0.05) (FigA). A total of 386 hub genes which were highly positively correlated with intermittent traits in the turquoise module were identified (Fig 4B). In addition, a total of 33 hub genes in the black module were identified which were positively correlated with static traits (Fig 4C).

3.3. Lasso analysis for key gene selection

Total 248 differentially expressed hub genes were identified (Fig 5A). To further identify the key genes which have the greatest impact on human PDLC with mechanical stimuli, LASSO analysis was performed to shrink the gene collection. The lasso analysis result found that the CLIC4, NPLOC4 and PRDX6 had the greatest impact on the human PDLC with mechanical stimuli (Fig 5B and 5C). In addition, the ROC curves revealed a good predictive efficiency for the lasso regression model (Fig 5D).

4. Discussion

Mechanical force regulates PDLC behaviors. The application of different types of force could lead to different responses of the PDLC. In oral cavity, the PDLCs suffer from different directions, magnitude and frequency of forces from normal mastication and OTM. During OTM, the compression force loading promoted bone resorption, while the traction force loading induced bone formation(9). Periodontal ligament cells induced by a continuously compressed force led to apoptosis(10). Recent research identified the tumor necrosis factor (TNF) ligand and receptor family, the Bcl-2 family and the caspase family as force-sensing genes for the apoptotic PDL(11). However, other studies found that cyclic stretching force also caused apoptosis through caspase family, including caspase-9(4), caspase-3 and caspase-5(12).

Interestingly, glucolipid metabolism played an important role in the PDLC response to MF. According to our study, the cholesterol homeostasis, fatty acid metabolism and bile acid metabolism were enriched to interpret the biological process of MF-driven PDLC. Fatty acids, as an essential component of cells, have critical impacts on the survival, differentiation and cellular function of PDLC(5). Glucose, the main energy source of PDLC, is essential for osteogenic differentiation and reprogramming(13). A recent study suggested that the undifferentiated PDLC prefers to metabolize glucose through glycolysis, rather than oxygen phosphorylation(14). Compared with oxygen phosphorylation, glycolysis enables the production of energy rapidly in compressed PDL with a hypoxic microenvironment. Another study indicated that short-term hypoxia

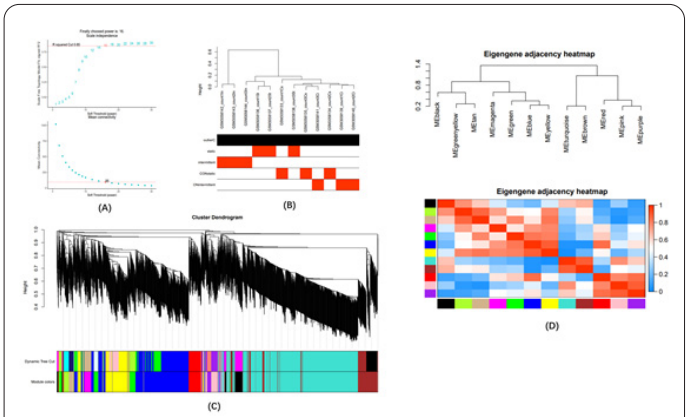

Fig. 3. Weighted gene co-expression network analysis (WGCNA) of human PDLC in response to MF. (A)Detection of soft power. (B) Sample dendrogram with trait heatmap. (C) Gene dendrogram and module colors. (D)Eigengene adjacency heatmap.

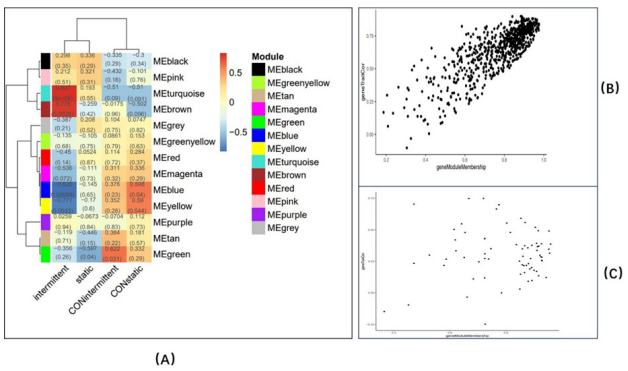

Fig. 4. Interesting modules and hub gene selection based on WGCNA. (A)Correlation plot between modules and traits attribute. (B) Scatter plot between gene significance of intermittence and module membership in the turquoise module. (C)Scatter plot between gene significance of static and module membership in the black module.

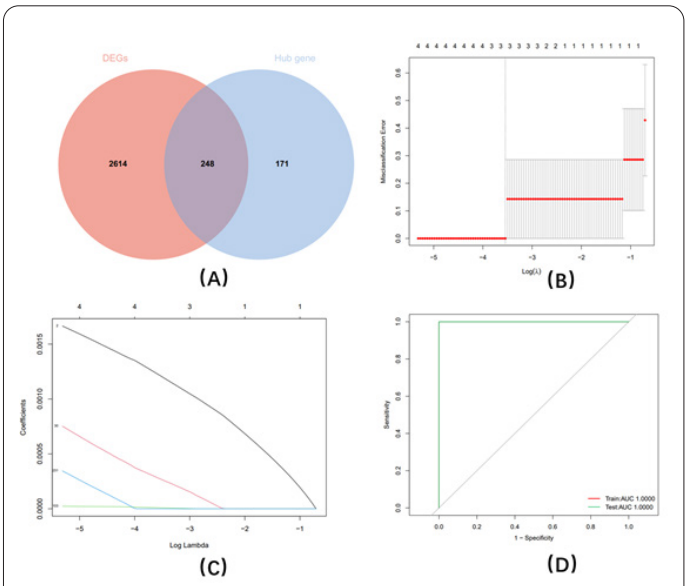

Fig. 5. LASSO regression model for human PDLC in response to MF. A. Venn plot of the intersection between DEGs and hub genes. B. Cross-validation for the penalty (labeled as lambda) selection. C. LASSO regression of the MF-related genes in human PDLC. D. ROC curves for the lasso regression model in the training set and test set.

might affect the proliferation, differentiation, migration and apoptosis, even the immunomodulatory functions in periodontal ligament stem cells(15). Hypoxia could inhibit the intermittent MF-induced insulin-like growth factor-1 (IGF-1) in huma PDLC through TGF-β1(16).

Similarly, not only intermittent MF but also static MF shared the biological signaling pathways, which included Myc targets, TGF-β signaling and PI3K/AKT/MTOR signaling. TGF-β signaling pathway in human PDLC is essential for the development of periodontal tissue, which was involved in various cellular processes. Interestingly, increased expression of TGF-β expression was detected on the compression and tension sides during the OTM(17). Both positive and negative effects on osteoclastogenesis in periodontal tissues were observed in TGF-β signaling(18). Some studies found that TGF-β signaling periodontal tissues suppress osteoclastic activity. There was evidence suggesting that TGF-β receptor inhibitor inhibits MF-induced bone mineralization in vitro(18). Furthermore, the intermittent compressive force could promote the osteogenic differentiation of human PDLC by TGF-β signaling pathway(19), which increased the expression of insulin-like growth factor-1(16). However, other researchers suggested that the bone resorption induced by TGF-β depends on the dose of exposure and the cell types involved(18, 20).A recent study found that TGF-β1 stimuli facilitated ligament-fibroblastic differentiation and inhibited the cementoblastic differentiation of PDLC through activating β-catenin(21).In addition, TGF-β accelerated the epithelial-mesenchymal transition by activating the PI3K-Akt-mTOR pathway(22).

Mechanosensing occurs when periodontal tissue is subjected to MF and leads to orthodontic bone remodeling. Although several ion channels (including Piezo1 and the transient receptor potential cation channel subfamily V member 4) have been described in osteocytes and PDL fibroblasts have been described, which function as mechanosensors when subjected to mechanical loading(18), CLIC4 was seldom mentioned in periodontal tissues. CLIC4, a chloride intracellular channel, was involved in the regulation of the cell cycle. Compared with bone marrow stem cells, CLIC4 was highly expressed in periodontal ligament stem cells and dental pulp stem cells(23). A recent study indicated that CLIC4, regulated by TGF-β signaling, Hedgehog signaling, and Wnt-3a signaling, enabled to promotion of fibrosis in dermal fibroblasts(24). In addition, the CLIC4 could activate NLRP3 inflammasome and leads to bone diseases by accelerating the Cl^- outflow(25). NPLOC4 involved in the misfolded proteins from the endoplasmic reticulum to the cytoplasm. Furthermore, the ternary complex, consist of UFD1, VCP and NPLOC4, negatively regulated the production of type I interferon by binding ubiquitinated proteins(26). However, few studies described the association between periodontal tissue and NPLOC4. Peroxiredoxin 6 (PRDX6), a downstream target of nuclear factor erythropoietin 2-related factor 2 (NRF2), plays an important role in maintaining the homeostasis of reactive oxygen species (ROS) homeostasis(27). In addition, PRDX6 functions as a ferroptosis regulator of ferroptosis(28), which reduced the lipopolysaccharide-induced inflammation and ferroptosis in gingival tissues with periodontitis(29).

5. Conclusion

Our study revealed differential biological processes in different types MF induced human PDLC and developed the important genes for human PDLC in response to MF, which might be potential markers for OTM.

Conflict of interest
The author has no conflicts of interest to report for this study.

Consent for publications
The author has to write this sentence that they read and approved the final manuscript for publication.

Availability of data and materials
The datasets involved can be accessed from the GEO database(http://www.ncbi.nih.gov/geo)

Author contributions
The authors confirm their contribution to the paper: study conception and design; data collection; analysis and interpretation of results; draft manuscript preparation and writing.

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Ethics Approval
This study does not involve ethical issues.

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