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授与した学位	博　士
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学位論文の題目	Bioinformatic analysis of mechano-sensitive genes and pathways in osteocyte under the mechanical loadings （バイオインフォマティクス解析による骨細胞のメカノセンシティブ遺伝子と経路の解析）
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学位論文内容の要旨

Background: Review in **Chapter I** discussed the cell-cell communications among bone cells and those between the bone and other organs systems with an emphasis on the role of the energy metabolism. The intercellular network of cell-cell communications among osteocytes is mediated by gap junctions. Gap junctional intercellular communication (GJIC) is thought to play an essential role in the integration and synchronization of bone remodeling. To further understand the mechanism of bone development, it is of great importance to investigate the underlying mechanism of osteocyte differentiation-induced changes of GJIC capacity.

Methods: **Chapter II** emphasized on the mechano-sensitive genes to figure out a possible mechanism for the osteocyte differentiation-induced changes of GJIC capacity. Therefore, a bioinformatics analysis was applied to screen the key genes and pathways that are activated when different types of mechanical loading are applied to osteocytes. We retrieved the public mRNA expression datasets (series number of GSE62128 and GSE42874) from Gene Expression Omnibus database (GEO). High gravity-treated osteocytic MLO-Y4 cell-line samples from GSE62128 (Set1) and fluid flow-treated MLO-Y4 samples from GSE42874 (Set2) were employed. Functional enrichment was performed after the differentially expressed genes (DEGs) were identified. The common DEGs between Set1 and Set2 were considered as key DEGs, and then the minimal nodes from all such key DEGs were used to construct a protein-protein interaction (PPI) network that linked most of the key DEGs. Several open source software programs were employed to process and analyze the original

data. The bioinformatic results and the biological meaning were validated by *in vitro* experiments.

Results: Bioinformatic analysis in **Chapter II** demonstrated that high gravity and fluid flow induced opposite expression trends in the key DEGs. The hypoxia-related biological process and signaling pathway were the common functional enrichment terms among the DEGs from Set1, Set2, and the PPI network. The expression of almost all the key DEGs (*Pdk1*, *Ccng2*, *Eno2*, *Egln1*, *Higd1a*, *Slc5a3*, and *Mxi1*) was mechano-sensitive. Eno2, a gene related to adenosine triphosphate generation, was identified as the hub gene in the PPI network, and Eno2 knockdown resulted in expression changes of some other key DEGs (*Pdk1*, *Mxi1*, and *Higd1a*).

Conclusions: A brief review in **Chapter III** elaborated on the changes of GJIC induced by hypoxia and several hypotheses were proposed for the future studies. Our findings based on the prediction of a bioinformatics analysis indicated that the hypoxia response might have an important role in the differential responses of osteocytes to the different types of mechanical force and the osteocyte differentiation-induced changes of GJIC capacity may be subject to the regulation of hypoxia signal pathway-mediated energy metabolism.

論文審査結果の要旨

Introduction: The cell-cell communications among bone cells and the interaction between the bone and other organs systems play important roles in the energy metabolism. The intercellular network among osteocytes is mediated by gap junctions; gap junctional intercellular communication (GJIC) is thought to play an essential role in the integration and synchronization of bone remodeling. To further understand the mechanism of bone formation and repairment, it is of great importance to investigate the gene expression related with GJIC in osteocyte.
Materials and Methods: The alteration of mechano-sensitive genes were investigated in the osteocytes subjected with mechanical loadings. A bioinformatics analysis was applied to screen the key genes and pathways that are activated when different types of mechanical loading are applied to osteocytes. I retrieved the public mRNA expression datasets (series number of GSE62128 and GSE42874) from Gene Expression Omnibus database (GEO). High gravity-treated osteocytic MLO-Y4 cell-line samples from GSE62128 (Set1) and fluid flow-treated MLO-Y4 samples from GSE42874 (Set2) were employed. Functional enrichment was performed after the differentially expressed genes (DEGs) were identified. The common DEGs between Set1 and Set2 were considered as key DEGs, and then the minimal nodes from all such key DEGs were used to construct a protein-protein interaction (PPI) network that linked most of the key DEGs. Several open source software programs were employed to process and analyze the original data. The results of bioinformatic analysis were validated by *in vitro* experiments.
Results: Bioinformatic analysis demonstrated that high gravity and fluid flow induced considerably different gene expression trends in the key DEGs. However, *Pdk1*, *Ccng2*, *Eno2*, *Egln1*, *Higd1a*, *Slc5a3*, and *Mxi1, which are highly related with* the hypoxia signaling pathway, were the common functional enrichment terms in high gravity and fluid flow mechanical loadings. The expression of almost all these genes was mechano-sensitive. *Eno2*, a gene related to adenosine triphosphate generation, was identified as the hub gene and *Eno2* knockdown increased *Pdk1* expression and decreased *Mxi1* and *Higd1a expressions*.
Conclusions: My findings based on bioinformatics analysis indicated that the hypoxia response might have an important role in the differential responses of osteocytes to the different types of mechanical force and the osteocyte differentiation-induced changes of GJIC capacity may be subject to the regulation of hypoxia signal pathway-mediated energy metabolism.

The content of this doctoral dissertation covered the articles, “The temporospatial pattern of energy metabolism coordinates the interactions between the bones and other organ systems” (DOI: 10.1016/j.job.2017.11.001) and “Screening of key candidate genes and pathways for osteocytes involved in the differential response to different types of mechanical stimulation using a bioinformatics analysis” (DOI:10.1007/s00774-018-0963-7), which are published in the *Journal of Oral Biosciences* and the *Journal of Bone and Mineral Metabolism* respectively after the international peer-review.

These findings are scientifically significant, providing useful knowledge that will promote the advance in orthodontic science. Therefore, the dissertation examining committee acknowledged the value of this thesis as a doctoral dissertation.