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学位論文題目 Genome-wide Accessible Chromatin Mapping for Articular Knee Cartilage Reveals Abnormal Endochondral Ossification in the Primary Osteoarthritis
（関節膝軟骨のゲノムワイドクロマチン解析による原発性膝変形性関節症における異常軟骨内骨化の同定）

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Abstract of thesis

In this research, the author employed an epigenomic method, Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq), to characterize the genome-wide chromatin alterations associated with osteoarthritis (OA). A novel protocol to generate high-quality ATAC-seq data from fresh hard tissue, human cartilage was first established using collagenase II to study human OA disease.

In chapter 1, the author introduced the background of this research, including human knee OA (i.e. current statistics, risk factors, diagnosis and treatments), tissue features of cartilage and subchondral bone in OA and non-OA knee joint, the importance of epigenetics in disease and a method to study the chromatin structure (i.e. ATAC-seq). It was pointed out that OA is a chronic disease affecting the aging society and the limitation of epigenetically studying OA clinical samples. The research objectives were proposed in this chapter using an epigenetic tool to investigate the chromatin accessible regulatory elements to gain further insight into the pathogenesis of OA.

In chapter 2, the author introduced how to optimize a protocol to generate high-quality epigenomic profiling data at chromatin level comparing with fresh (n=8) and flash frozen (n=4) human knee cartilage tissue. To investigate the variations of chromatin accessibility in cartilage associated with OA, the author applied previously published modeling system representing damaged (iMT) and intact (oLT) cartilage region in the knee. The results showed using fresh human cartilage is able to generate high-quality ATAC-seq data using collagenase II to study human OA disease. Quality check qPCR method can be used before sequencing to test the signal to the noise ratio. It can be concluded that the ATAC-seq libraries generated from fresh cartilage tissue had good and indistinguishable quality between iMT and oLT regions.

In chapter 3, the author introduced NGS data processing (i.e. peak calling and quality assessment), bioinformatic methods for ATAC-seq data downstream analysis and integration with other publicly available data (i.e. GWAS, DNA methylation and RNA-seq). The author defined a set of robust accessible chromatin regions (n=109,215) from 16 libraries generated from the fresh (n=8) human knee cartilage tissue, of which 71.1% were annotated as enhancers and 16.9% were annotated as promoters according to Roadmap DHS annotation dataset. Integrating the epigenomic data of clinically relevant tissues with the publicly available genetic and transcriptomic data the author identified loci may contribute to OA pathogenesis. Most of these annotated accessible enhancers were linked to their putative target genes using public datasets. With the enhancer-gene map in chondrocyte, the author identified the linked genes of the previously identified OA GWAS

(Single Nucleotide Polymorphisms) SNPs or OA differential methylated loci located lie outside of the coding regions. The differentially accessible promoters (7.6%) or enhancers (85.3%) relevant to OA were identified. Further analyses for differential accessible enhancer regions showed bone-related enhancers are more likely to be dysregulated in OA diseased tissue. Consistently, 371 protein-coding genes that are dysregulated both at the transcriptomic (RNA-seq) and epigenomic (ATAC-seq) levels in OA with the concordant direction of change. These genes are enriched for pathways regulating chondrogenesis, ossification, and mesenchymal stem cell differentiation. Moreover, integrating ATAC-seq data with public available database also allowed to identify altered cis-regulatory elements as well as transcription factors binding. Taken together, abnormal self-healing in OA knee cartilage was observed in this study, suggesting induced endochondral ossification-like cartilage-to-bone conversion is a characteristics of OA progression.

In chapter 4, the author summarized the conclusion. This study identified altered promoters and enhancers of genes that might be involved in the pathogenesis of OA. The analyses suggest aberrant enhancers associated with MSC differentiation and chondrogenesis in OA, and the genes that involved in the endochondral ossification signals pathway should be critical for OA development and progression. The single-cell transcriptomics or single-cell ATAC-seq experiment were proposed as the future research to investigate the osteoblast-like signal or ossification signal is from a chondrocyte undergoing terminal differentiation, or from MSC direct differentiation.

Abstract of assessment result

This study demonstrated genome-wide investigations of accessible chromatin regions is powerful in probing changes of regulatory genomic elements in clinical samples relevant to a disease. The chromatin accessibility map in cartilage will be a resource for future GWAS and DNA methylation studies in OA and other musculoskeletal diseases. The findings are of very high interest to the field, as this extensive analysis on ATAC-seq datasets on clinical samples of OA patients suggests aberrant enhancers associated with MSC differentiation and chondrogenesis in OA and it could expound the possible pathogenesis mechanism, leading to the identification of potential targets of biomarkers or therapies. Understanding these molecular bases of OA is necessary for future therapeutic intervention.

The final examination committee conducted a meeting as a final examination on 20th November 2018. The applicant provided an overview of the dissertation, addressed questions and comments raised during the Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination. Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Biological Science.