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研究課題名（和文）Comprehensive analysis and biomarker identification of osteoarthritis

研究課題名（英文）Comprehensive analysis and biomarker identification of osteoarthritis

研究代表者

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研究成果の概要（和文）：このプロジェクトの目的は、軟骨と軟骨下骨の両方における変形性関節症の疾患の進行中にメチル化の変化を同定することでした。
メチル化プロファイリングは、軟骨下骨と軟骨の両方からの96データポイントの合計12人の患者で行いました。私たちは、メチル化pattersは、これら2つの組織で異なっていたことがわかりました。データは、軟骨下骨におけるメチル化の変化は、軟骨の前に起こることを示唆しました。遺伝子発現の複雑な調節を示す遺伝子発現の変化をもたらしていないすべてのメチル化の変化。我々はまた、そのような強く疾患の後期段階中の修理の再生を指示するホメオボックス遺伝子などの多くの転写因子を同定します。

研究成果の概要（英文）：The aim of this project was to identify methylation changes during osteoarthritis disease progression in both the cartilage and subchondral bone.
Methylation profiling was performed on 12 patients with total of 96 data points from both subchondral bone and cartilage. We found that the methylation patters were different in these two tissue. Data suggested that methylation changes in the subchondral bone occurs before the cartilage. Not all the methylation changes led to changes in gene expression, indicating the complex regulation of gene expression. We also identify many transcription factors, such as the homeobox genes strongly indicating the regeneration of repair during the late stages of the disease.

研究分野：　osteoarthritis

キーワード：　osteoarthritis　methylation　epigenetics

１．研究開始当初の背景

Osteoarthritis (OA) is a large and growing global health burden that represents one of the most common causes of disability in the Western World. As the population continues to age and struggles with obesity, the incidence and prevalence of OA will continue to grow. OA has a strong heritable component that is polygenic in nature. However, the identified genetic factors so far account less than 20% of the heritability in OA. The low heritabilities of these disease progression measures relative to the heritabilities of OA suggest that additional factors, beyond the inherited genome, might be involved.

DNA methylation of CpG dinucleotides in differentiated cells is associated with regulation of key physiological processes. Additionally, 20-23bp cytoplasmic miRNAs are involved in the post-translational modification of gene expression by binding to the 3' untranslated regions of a gene and thereby facilitating non-sense mediated decay of the messenger transcript. This epigenetic on/off switching of the transcriptional machinery is not only regulated temporally during development but also during a lifespan because epigenetic modifications are labile in response to environmental influence and may change with age. Epigenetic regulation also plays a crucial role during cellular development and acts in a tissue specific manner. Therefore, to understand how predisposing epimutations and environmentally induced epigenetic changes (such as induced by obesity or aging) drive OA development, one must profile the primary site of disease etiology, the articular cartilage.

２．研究の目的

Osteoarthritis (OA) is the most prevalent form of arthritis world-wide. Despite this global impact there are currently no approved treatments to slow disease development or progression. Applying the powerful new tool of epigenetics and miRNA to the study of normal and diseased OA knees, we will identify and characterize fundamental mechanisms leading to the development of OA to enable us to develop new OA biomarkers and targets for drug discovery.

３．研究の方法

Human OA knee joints were collected from 12 patients who underwent joint replacement surgery due to the primary knee OA (10 females and 2 males; mean age (SD) = 74.5(8.7), range = 55~83). The diagnosis of OA was based on the criteria of the American College of Rheumatology, and all the knees were medially involved in the disease. The specimens were obtained from the National Hospital Organization Sagamihara Hospital (Kanagawa, Japan), the National Center for Global Health and Medicine Center Hospital (Tokyo, Japan) and the Tokyo Yamate Medical Center (Tokyo, Japan). All the knee joint tissues collected were immediately stored in liquid nitrogen. This study was approved by all participating institutions. Informed consent was obtained from each patient enrolled in this study.

Cartilage from three regions with different levels of cartilage degeneration was collected as previously described. Briefly, three regions were selected for this study: (a) outer region of the lateral tibial plateau (oLT) with a visibly smooth cartilage surface; (b) the inner region of lateral tibial plateau (iLT) with sufficient cartilage to detect visible fissures on tissue cross-section; (c) the inner region of medial tibial plateau (iMT) with visible loss of articular cartilage. All the samples used in this study had the same pattern of compartmental involvement of knee OA described above. Our previous work showed that these regions could encompass a full range of histological severity in knee OA cartilage . These regions were then sectioned and powdered using a high speed drill in liquid nitrogen. Approximately 100 mg of powder was obtained for each region.

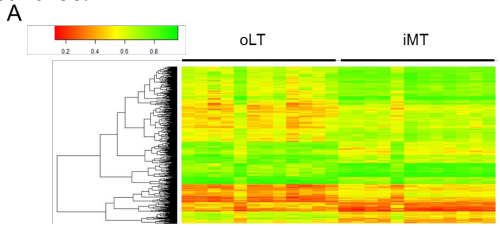
DNA extraction and methylation profiling

Genomic DNA was extracted from the isolated powder using QIAGEN's QIAamp DNA Mini Kit. DNA quality and quantity was examined by agarose gel electrophoresis Spectrophotometer (SpectraMax Plate Reader, Molecular Devices, Sunnyvale, CA). DNA (2 µg) was then bisulfited using EZ DNA methylation kit (Zymo Research, Irvine, CA) before assessing the methylation status using Infinium HumanMethylation450 BeadChips (Illumina, San Diego, CA, USA).

Data analysis wascarried out using R (version 3.2.1; http://www.r-project.org/). Geneontology was performed using DAVID tools and pathway analysis was performed using IPA to identify functional annotations and to predict potential biological interactions.

４．研究成果

A total of 519 significant DMPs covering 224 genes were identified in the iMT/oLT group (Figure 1), of which 354 DMPs (68.2%) corresponding to 166 genes were hypo-methylated and 165 DMPs (31.8%) corresponding to 58 genes were hyper-methylated. The top 20 CpGs showing largest differences in methylation levels were listed in Table 1. Examples of methylation β value plot for several probes are shown in Figure 1C. Many of the DM genes, such as GDF6, BMP6, ABI3BP, FZD1, AXIN2, MSX2, IBSP, CHSY1, and MATN2, have been implicated in previous studies.



The identified DMPs were first analyzed by GO. The DM genes were enriched in skeletal system morphogenesis and development, transcription regulation, and metabolic and biosynthetic process of DNA, RNA and macromolecules. A large proportion of the DMPs were enriched in the transcription activation and regulation. In particular, of the 35 transcription regulator genes identified, 18 belonged to the homeobox transcription factor. Several of these showed multi-site methylation alteration. For instance, 13 CpGs were hyper-methylated in HOXB3, 9 of which were found in 5'UTR or 1st exon. Seven CpGs were hypomethylated in HOXD9, 5 of which were found in the TSS1500 region. Intriguingly, 4 DMPs in EMX2, and 19 DMPs in its opposite strand EMX2OS which encodes lncRNA, were identified. Other transcription factors also showed multiple DMPs. Fourteen CpGs were found to be hypo-methylated in GATA2, which encodes a transcription factor highly expressed in hematopoietic progenitor cells and embryonic stem cells. TBX15, which is required in the development of skeletal system, was found harboring 4 DMPs in TSS1500.

IPA of DM genes

IPA was performed to predict potential biological pathways, upstream regulators and associated networks of the DM genes. The three most enriched canonical pathways predicted by IPA were G protein coupled receptor (GPCR) signaling ($p=4.71\times10^{-3}$), tRNA splicing ($p=5.29\times10^{-3}$) and cAMP mediated signaling ($p=7.12\times10^{-3}$) (Supplementary figure 5). Upstream regulators which were clustered on microRNAs were identified. miR-130a-3p ($p=5.11\times10^{-9}$) was the first ranked upstream regulator. The target genes included OA-related genes, for instance, AXIN2, BMP6, CSMD1, and NRP2. miR-128 (1.95×10^{-6}), which was reported to be an upstream regulator in hip OA, was also identified in this study. Fourteen networks were found associated among the identified DM genes by IPA. The top two networks were associated with skeletal and muscular system development and function. Network 1 was enriched in transcription factors, both homeobox genes and non-homeobox genes, and centered on histone deacetylases (HDACs), which can regulate the transcription of matrix metalloproteinase (MMPs), and the signaling pathway centered on NF-κB complex. Network 2 was found enriched of growth factors like GDF6 and BMP6, extracellular matrix like MATN2 and IBSP, and cell signaling centered on ERK1/2. The DM extracellular molecules are either targets of Wnt signaling pathway (IBSP) or the TGF/BMP signaling pathway (MATN2, BMP6, GDF6, and ITGA11).

５．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　1　件）

1. Yanfei Zhang, Naoshi Fukui, Mitsunori Yahata, Yozo Katsuragawa, Toshiyuki Tashiro, Shiro Ikegawa, Ming Ta Michael Lee. Genome-wide DNA methylation profile implicates potential cartilage regeneration at the late stage of knee osteoarthritis. Osteoarthritis and Cartilage. 2016 May;24(5):835-43 **査読有**
2.

〔学会発表〕（計　2　件）

1. Y.Zhang, N. Fukui, M. Yahata, M. Lee. Genomewide DNA methylation profiling of osteoarthritic articular cartilage. 2015 OARSI World Congress. April30-May 3, 2015. Seattle, USA
2. Y.Zhang, N. Fukui, M. Yahata, M. Lee. Genomewide DNA methylation

profiling of osteoarthritic subchondral bone. 2015 OARSI World Congress. April30-May 3, 2015. Seattle, USA

〔図書〕（計 0 件）

〔産業財産権〕
○出願状況（計 0 件）

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発明者：
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国内外の別：

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名称：
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〔その他〕
ホームページ等

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