様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Studying bone secreted molecules (osteokines) as a novel diabetes diagnostic tool

研究課題名（英文）Studying bone secreted molecules (osteokines) as a novel diabetes diagnostic tool

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：肥満と糖尿病は骨質を劣化させ、糖尿病性骨粗鬆症を引き起こす。これらは加齢と座りがちな生活習慣によって悪化する。骨は、骨因子（オステオカイン）を分泌してエネルギー代謝にも影響を及ぼす活性組織である。本研究の目的は、骨とエネルギー代謝の相互作用を解明し、糖尿病および糖尿病性骨脆弱化の早期予測・診断のための新規ターゲットとしての骨因子を検討することである。遺伝子およびタンパク質の発現、画像化技術を用いて糖尿病の進行に伴う骨について研究した。その結果、糖尿病の初期と後期で骨構造が変化することが示された。このことは、骨因子を用いた早期診断が糖尿病の病期分類に寄与し、疾患進行を抑える可能性を示唆している。

研究成果の学術的意義や社会的意義
Bone fragility is a serious complication of diabetes with substantial morbidity and increased fracture risk. Detecting the disease by developing early diagnostic tools reduces the need for operative intervention, increases patient's quality of life and alleviates diabetes- associated health burdens.

研究成果の概要（英文）：Obesity and diabetes impact bones negatively and lead to bone fragility and diabetic osteoporosis. This is exacerbated by ageing and aggravated by modern societies sedentary lifestyle. Bone is an active tissue and can affect energy metabolism by secreting bone factors (osteokines). This research focused on elucidating the interaction between the two systems (bone and energy metabolism). The purpose is to investigate bone factors as novel targets for the early prediction, and diagnosis of diabetes and diabetic bone fragility. We studied bones at multiple stages of diabetes progression using gene and protein expression and imaging techniques. Our results demonstrate that bone exhibits distinctive structural difference at the early stage versus late stage of the disease which indicates that disease progression can be intercepted if diagnosed early using osteokines (bone factors) profiled at the early stage which also acts as a proxy for diagnosing diabetes disease progression.

研究分野：Molecular Biology

キーワード：Bone　Diabetes　transcriptome　Proteome　Osteokines

１．研究開始当初の背景

(1) There exists a two-way relationship between the skeleton and energy homeostasis. First, bone has been recognized as an endocrine organ that can regulation energy homeostasis by secreting osteokines that control appetite, modulate glucose tolerance, insulin sensitivity, leptin levels and regulate energy expenditure. Second, energy perturbations (such as obesity and type 2 diabetes mellitus (T2DM)) affects the skeleton. For example, diabetic osteoporosis is a serious debilitating complication of T2DM and leads to bone fragility that can go undiagnosed for years until it manifests as bone fractures.

(2) The relationship between bone and energy metabolism is complex and there exists a gap in the knowledge of how both systems influence each other. Therefore, this project will explain the energetic needs of the skeleton and its role in maintaining whole-body energy homeostasis and determine the possibility of using osteokines as novel targets for the early prediction, and as future seeds for diagnosis and treatment of metabolic disorders such as diabetic osteoporosis using an integrated multi-omics level analysis to construct a multi-omics signature of bone fragility in T2DM. This analysis will identify potential markers and drug targets for diabetic bone fragility.

２．研究の目的

(1) This project proposes a central role for osteokines in regulating energy homeostasis and the manifestations of obesity and diabetes progression and the purpose of the research is to construct an osteokine profile of healthy, prediabetic and diabetic mice.
(2) To characterize multi-omics patterns in diabetic bones and obtain a comprehensive integrated understanding of the mechanism of bone fragility in T2DM.
(3) To characterize the effect of the skeleton on whole-body energy metabolism in transgenic mice where skeletal glucose uptake is inhibited.

３．研究の方法

(1) The methods to achieve the goals of the project included studying intermediary metabolism and bioenergetics of bone cells by creating a genetic mouse model of defective glucose uptake in osteocyte bone cells (termed Glut1ocy-/- transgenic mice). *Slc2a1*$^{tm1.1Stma}$ mice were bred with Dmp1-cre mice (Cre-lox system) and analysis of skeletal architecture and health was done via μCT immunohistochemistry.

(2) Using a multi-omics integrative approach to evaluate the mechanisms leading to the development of diabetic osteoporosis. Temporally investigation at multiple molecular levels (transcriptome and proteome) were correlated to disease progression status in a diet-induced obesity model by measure levels of osteokine in serum of healthy, prediabetic, and diabetic C57BL/6J wild type (WT).

(3) Identify transcriptional and proteomic changes in T2DM bones and compare it to age-matched controls and integrate the analysis using computational and bioinformatics tools.

４．研究成果

(1) Glucose uptake inhibition in osteocyte bone cells reduced bone volume and mass: We generated mice with glucose uptake inhibition in bone using the Cre-loxp system using the Dmp1-cre and the *Slc2a1*$^{tm1.1Stma}$ loxp mice. Figure 1 shows the genotyping results using DNA electrophoresis, yellow rectangles are mice that are transgenic and have the glut1 transporter deleted specifically in osteocytes. Figure 2 shows that transgenic mice with glut1 deletion (right) in osteocytes have reduced trabecular bone volume in the distal femur compared to control mice (left).

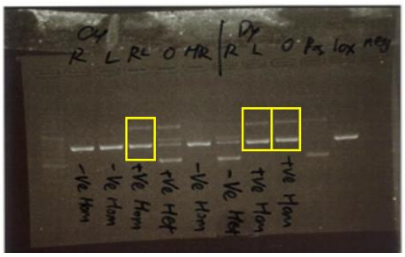

Figure 1: Genotyping results of offspring of Dmp1-Cre X glut1 loxp mice. Yellow boxes refer to Glut1ocy-/- transgenic mice.

(2) Metabolic and skeletal phenotyping of a moderately high fat high energy diet in mice: A moderately high fat high energy diet (HFD) leads to a phenotype of increased body mass fasted blood glucose levels and a decrease in glucose tolerance after 4 weeks of feeding which is exacerbated as the diet continues to 16 weeks of feeding (data not shown). Microcomputed tomography images (μCT) of the third lumbar vertebrae (Figure 3) show 3D trabecular extracts with an increase in bone density and decreased trabecular separation after 4 weeks of HF compared to normal diet (ND), however, this effect cancels out after 16 weeks of HFD where bone density decreases and trabecular separation increases, signifying a spectrum of skeletal phenotypes as disease progresses from the early to later disease stages.

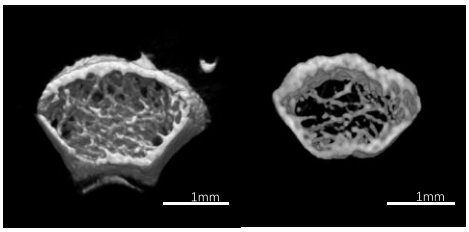

Figure 2: Glut1ocy-/- exhibit reduced trabecular bone volume in the distal femur compared to control.

(3) Omics-wide analysis of bone cells in hyperglycemic conditions:

In order to understand the mechanism leading to differential skeletal phenotypes at different diseases stages we conducted a multi-omics analysis of bone cells (osteocytes) cultured under chronic hyper glycemic conditions. To our surprise, hyperglycemia did not elicit and changes at gene transcription levels (Fig 4, A), however proteome analysis revealed differentially expressed proteins between normal and hyperglycemic osteocytes (Fig 4, B). Therefore, we expanded our analysis and found that hyperglycemia induces extensive

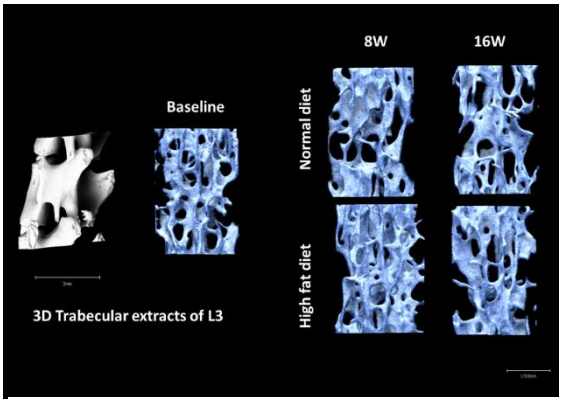

Figure 3: MicroCt images of the murine thoracolumbar vertebral column. L3 vertebrae is analyzed at 3 times points, baseline, 8 weeks and 16 weeks of a normal

alternative splicing changes in osteocytes which can explain the mechanism by which hyperglycemia diversifies the skeletal proteome (Fig 4, C). Additionally, we analyzed RNA binding protein (RBP) motif enrichments and homed in on several factors that can be driving proteome diversification (Data not shown).

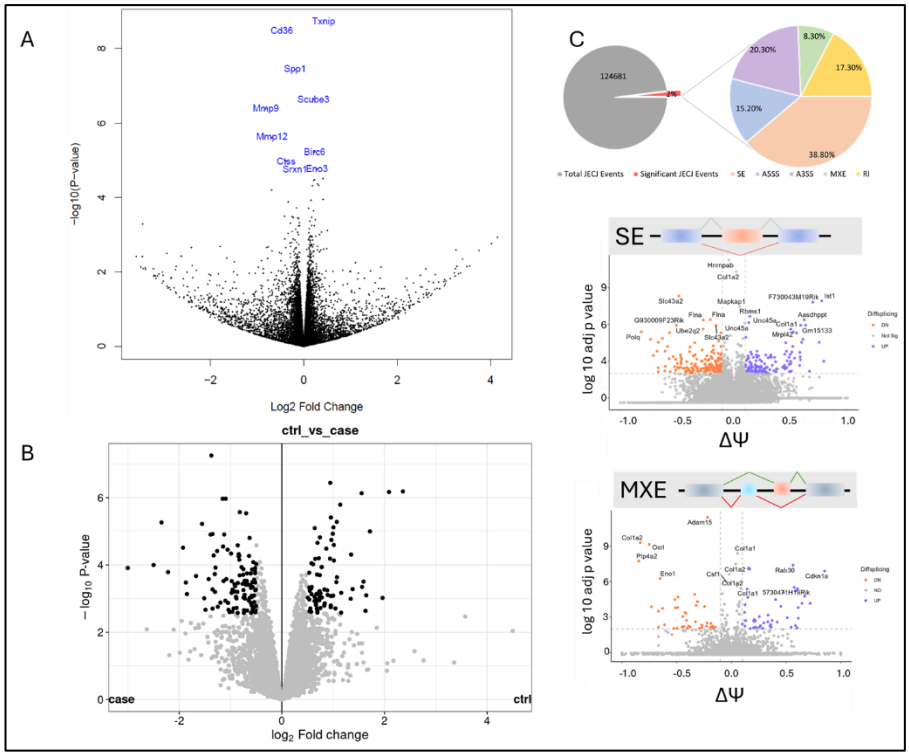

Figure 4: Hyperglycemic osteocytes exhibit significantly different proteomes in response to hyperglycemia in the absence of significant changes to their global gene expression. (A) Volcano plot comparing -log10 P value of expressed genes (B) Volcano plot of hyperglycemic osteocytes showing differentially expressed proteins between normal and hyperglycemic osteocytes. (C) Parent pie chart showing significant alternative splicing events (2%) of the total events. Volcano plots of SE, MXE, events showing inclusion level (ΔΨ) difference of gene transcripts.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Aseel Marahleh	４．巻 14
２．論文標題 The Osteocyte and its Osteoclastogenic Potential	５．発行年 2023年
３．雑誌名 Frontiers in Endocrinology	６．最初と最後の頁 1121727
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fendo.2023.1121727	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計7件（うち招待講演　2件／うち国際学会　2件）

１．発表者名 Aseel Marahleh
２．発表標題 Skeletal Regulation of Energy Metabolism
３．学会等名 2023 TI-FRIS/FRIS Symposium
４．発表年 2023年

１．発表者名 Marahleh A, Rashad S, Kitaura H, Jiayi R, Ohori F, Noguchi T, Mizoguchi I
２．発表標題 The transcriptomic landscape of hyperglycemic osteocytes.
３．学会等名 The 82nd Annual Meeting of the Japanese Orthodontic Society. TOKI MESSE Niigata Convention Center, Niigata, Japan, The Japanese Orthodontic Society.
４．発表年 2023年

１．発表者名 Marahleh A, Rashad S, Kitaura H, Jiayi R, Ohori F, Noguchi T, Mizoguchi I
２．発表標題 Hyperglycemia Induces Extensive Alternative Splicing Changes In Osteocyte Enriched Cultures With Minimal Transcriptional Alterations.
３．学会等名 ASBMR Pre-Meeting Symposium on Osteocytes in Bone Health and Disease and as Therapeutic Target Cells., Oct 12, 2024, The American Society of Bone and Mineral Research（国際学会）
４．発表年 2023年

１．発表者名
Aseel Marahleh

２．発表標題
Osteoimmunology in Orthodontics

３．学会等名
STOVIT online series, Universitas Airlangga, Dec 16, 2023（招待講演）

４．発表年
2023年

１．発表者名
Marahleh A, Rashad S, Kitaura H, Jiayi R, Ohori F, Noguchi T, Mizoguchi I

２．発表標題
High Glucose Induces Alternative Splicing Changes in Primary Osteocytes With Minimal Transcriptional Alterations.

３．学会等名
Cell Bio 2023. Boston Convention Center, Boston, MA, USA., The American Society for Cell Biology（国際学会）

４．発表年
2023年

１．発表者名
Marahleh A, Rashad S, Kitaura H, Jiayi R, Ohori F, Noguchi T, Mizoguchi I

２．発表標題
Osteocytes Exhibit Extensive Alternative Splicing Changes in Response to Hyperglycemia.

３．学会等名
The 46th Annual Meeting of the Molecular Biology Society of Japan. Online.

４．発表年
2023年

１．発表者名
Aseel Marahleh

２．発表標題
Metabolism and the Skeleton. What can our bones tell us?

３．学会等名
2022 CA+inD Winter Short-term Exchange Program Multimodal Global Leaders Development through Asian-Model Dentistry Consortium, Feb 9, 2023, Tohoku University, Graduate School of Dentistry（招待講演）

４．発表年
2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関