様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）A next-generation mass spectrometric/computational strategy for aging biomarker discovery

研究課題名（英文）A next-generation mass spectrometric/computational strategy for aging biomarker discovery

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研究成果の概要（和文）：今回の科研費課題では、マウスの白色脂肪組織（WAT）サンプルにプロテオミクス解析を適用し、BXD加齢コロニーに属する53のマウス系統の180のWATサンプルから、4727のタンパク質の定量化マップを作成することで、加齢に伴う肥満や代謝機能障害のバイオマーカー候補を特定した。さらに、高脂肪食と対照食を与えたマウスのWATの特定の細胞タイプに解析を拡大した結果、マクロファージの脂質代謝を制御する候補遺伝子、食餌誘発性肥満において有意に変化する細胞内リガンド・受容体ペア等を同定した。今回の結果は、脂肪組織の細胞間コミュニケーションを介した肥満の根本的なメカニズムを示す新たな証拠になると考えている。

研究成果の学術的意義や社会的意義
We have generated a comprehensive dataset that could be informative for those who study adipose tissue function and metabolic disease. We found genes that were significantly regulated by high-fat diet and could regulate lipid metabolism. These genes could be potential drug targets to treat obesity.

研究成果の概要（英文）：In this KAKENHI project, we proposed to discover biomarkers of aging and age-related metabolic diseases. We have applied the data-independent acquisition mass spectrometry (DIA-MS) method to mouse white adipose tissue (WAT) samples. We have generated a complete protein quantification map for 4727 proteins from 180 WAT samples of 53 mouse strains from the BXD aging colony. From these data, we have identified potential biomarkers for age-related obesity and metabolic dysfunction. We also expanded proteomics analysis to specific cell types from WAT tissues under high-fat and control diet. We found proteins that were significantly regulated by diet in a time-dependent manner, and candidate genes that could regulate lipid metabolism in macrophages. We have also identified cellular ligand-receptor pairs that changed significantly during diet-induced obesity. We believe our results provide novel evidence of the underlying mechanism of obesity through adipose tissue cell-cell communication.

研究分野：ゲノム生物学関連

キーワード：Proteomics　White adipose tissue　Obesity　Ligand-receptor pairs　Lipid metabolism

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

The mechanism of how genotype and environment coordinately influence phenotype remains a fundamental question in biology. Genetic reference panels, such as the BXD mice, are ideal resources to study complex diseases and gene-by-environment interactions (GXE) [1,2]. Recent advances in mass spectrometry (MS) have expanded the scope and reliability of proteomic and metabolomic measurements [3]. Previously, we have developed the Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra (SWATH-MS) technology, which enabled precise quantification of thousands of proteins across large number of cohorts [4]. I applied this integrative strategy to determine the genetic basis of changes of protein abundance and composition in liver samples from the BXD mice population [5,6]. I found a nominal correlation between transcripts and their corresponding proteins, and identified dozens of protein traits that are causative to metabolic status of the BXD mice. However, such data sets had not been generated for the white adipose tissues (WAT). In addition, cell type-specific gene expression changes at the protein level during the development of obesity remains largely unknown.

In the proposed project, I planned to applied the integrated strategy to explore proteome changes in visceral white adipose tissue from a range of BXD mouse strains and under different dietary conditions. The data from the BXD mice could be a great resource to study the genetic and environmental effects on obesity, but only bulk tissues were available for the BXD mice. Thus, I also planned to perform cell type-specific proteomics analysis for the adipose tissue, by analyzing several types of adipose tissue cells collected at different time points under high-fat diet (HFD) and control diet (CD) feeding, and identify cell type- and time-dependent proteome perturbation regulated by HFD feeding.

２．研究の目的

The goal of this project is to identify proteins and/or protein modules in adipose tissue and specific cell types that are regulated by energy excess in a time-dependent manner. At cell type level, we investigate autocrine and intercellular network at different time points of HFD feeding. We expect that this information can provide evidence to develop effective strategies for diagnosis, prevention and treatment of common diseases and health decline that are associated with obesity.

３．研究の方法

We first collected perigonadal WAT from the mice of the BXD aging colony. These include mice from 4 age groups, including ≤264, 265-419, 420-597, 598-785 days. We have applied data-independent acquisition mass spectrometry (DIA-MS) and computational analysis to precisely quantify proteins from the collected WAT.

In addition to bulk tissue analyses for the BXD mice, we have performed cell type-specific proteomic analysis for several types of cells in the visceral WAT of C57BL/6N mice under dietary intervention. Specifically, we established diet-induced obesity (DIO) mouse model and analyzed fluorescence-activated cell sorting (FACS)-sorted cells from the visceral WAT after 1, 8 and 16 weeks of HFD feeding. Samples from mice under control diet were analyzed as controls. We quantified control and HFD samples from the same type of cell at each time point using tandem mass tag (TMT) quantification strategy and then integrated data from the three time points. Furthermore, we referred to the Network Analysis Toolkit for Multicellular Interactions (NATMI) database to identify ligands and receptors quantified in our dataset and found autocrine as well as intercellular ligand-receptor pairs that were significantly regulated by diet.

４．研究成果

(1) Proteomics analyses for bulk WAT of 180 mice from the BXD aging colony

We have performed quantitative proteomics analyses for 180 WAT samples from the BXD mice. This corresponds to 53 mouse strains from the four age groups of the BXD aging colony as mentioned above. Using the DIA-MS method, we have quantified 4727 proteins across all samples. We did quantitative trait locus (QTL) analysis for all quantified peptides, and identified 1868 cis peptide QTLs, corresponding to 586 proteins. We got the data recently and we plan to do the following: 1) we will do QTL analysis for samples from HFD and CD groups of mice respectively, and identify protein QTLs that are regulated by diet; 2) we will compare the QTL results with published human genome-wide association study (GWAS) data and focus on metabolic genes. We will look for genes that have significant protein QTLs in mice, whose homologous genes are associated with metabolic traits in humans.

(2) Cell type- and time-specific proteome profiling for adipose tissue cells under HFD or CD feeding

① *Highly sensitive quantitative proteomics strategy enabled precise protein quantification from limited number of adipose tissue cells.*

The majority of effort in this project was made on analyzing FACS-sorted adipose tissue cells. As the number of FACS-sorted cells can be very low, it has not been widely used for proteomics analysis. First, we have developed DIA-MS method to quantify proteins from as low as 17,000 cells (applied elsewhere). At the same time, we have developed TMT quantification strategy to quantified FACS-sorted cells. This was the strategy that we used for adipocytes and 4 types of FACS-sorted adipose tissue cells. Specifically, we have quantified proteome of adipocytes, preadipocytes, macrophages, B cells and T cells from mouse visceral WAT (Figure 1). We collected cells from mice under 1, 8 and 16 weeks of high-fat or control diet feeding. To increase proteome coverage, we applied high-pH reversed-phase fractionation (HPRP) for the TMT-labeled peptides, and performed data-dependent acquisition mass spectrometry (DDA-MS) analysis for each fraction. We then integrated the results from all fractions from the same cell type at each time point. As a result, we generated quantitative proteomics data for 5 types of adipose tissue cells at 3 time points, which we used for further analyses.

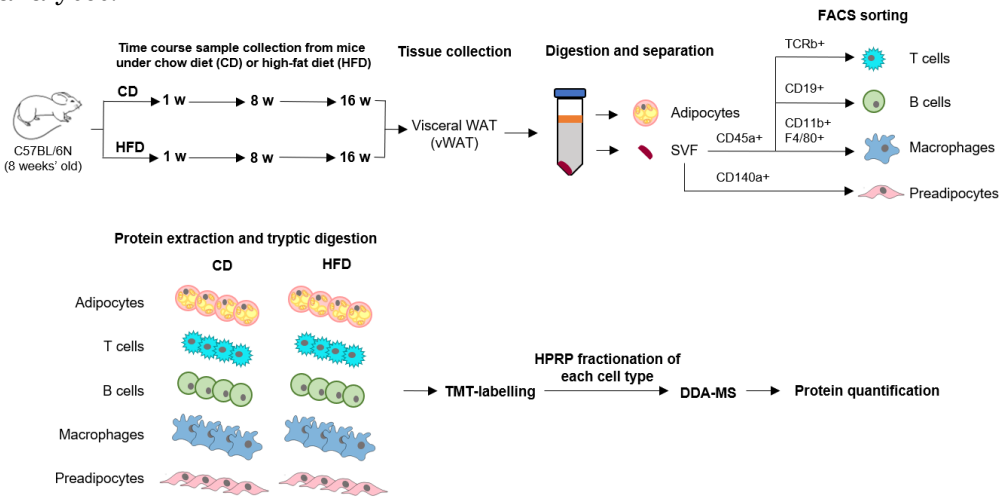

Figure 1. Proteome quantification for FACS-sorted adipose tissue cells using the tandem mass tag (TMT) mass spectrometry strategy. HFD, high-fat diet. CD, control diet. WAT, white adipose tissue. SVF, stromal vascular fraction. DDA-MS, data-dependent acquisition mass spectrometry. TMT, tandem mass tag. HPRP, high-pH reversed-phase fractionation.

② *Cell type- and time-specific proteome profiling for adipose tissue cells*

Interestingly, HFD induced obvious proteome changes from as early as 1 week. For most types of cells, the proteome changes were time-dependent, while these changes were mostly also cell type-specific (Figure 2). For example, in adipose tissue macrophages (ATMs), abundances of proteins under the gene ontology (GO) term "cell cycle" significantly increased in the HFD group compared with the CD group after 1 week feeding, indicating that adipose tissue resident macrophages may have proliferated within 1 week of HFD feeding (Figure 2b). However, expression of proteins involved in lipid metabolism, for example, lysosomal acid lipase (Lipa) and lipoprotein lipase (Lpl), didn't show significant difference between HFD and CD at 1 week, but increased after 8 or 16 weeks of HFD feeding. These data indicated that proliferation of ATMs may happen before metabolic adjustment and/or recruitment of classically activate macrophages to the adipose tissue.

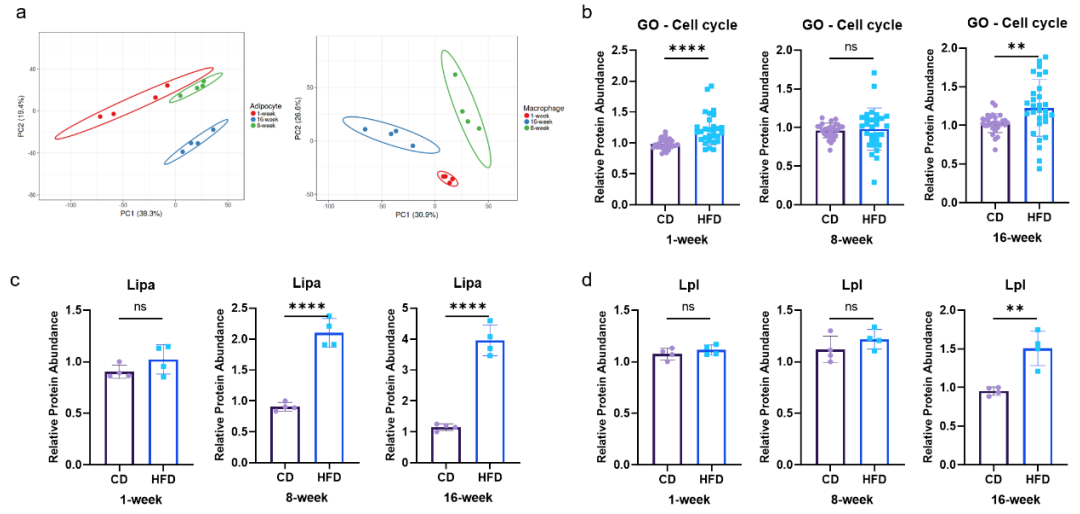

Figure 2. Cell type- and time-dependent proteome changes in adipose tissue cells following HFD feeding. a. Principal component analysis (PCA) plot for protein HFD to CD ratios in adipocytes and adipose tissue macrophages (ATMs) at 1-, 8- and 16-week of dietary intervention. b, expression of proteins involved in cell cycle in ATMs at 1-, 8- and 16-week of HFD or CD feeding as annotated by GO. c-d, protein expression of indicated genes in ATMs at 1-, 8- and 16-week of HFD or CD feeding. **, $p < 0.01$ from unpaired t-test. ****, $p < 0.0001$.

In addition, we have found a few candidate proteins that may play important roles in development of DIO. For example, protein expression of chloride intracellular channel protein 4 (Clic4), Sn1-specific diacylglycerol lipase beta (Daglb) and a cluster of V-type proton ATPase subunits (vATPases) in ATMs significantly increased after 8 weeks of HFD feeding. We have established ex vivo cell culture system using apoptotic

adipocytes-treated RAW264.7 macrophages. We are validating lipid transport and metabolism in these macrophages after knockdown of Clic4 expression with small interfering RNAs (siRNAs).

③ *Architecture of intercellular communication in adipose tissue*

To understand how different types of adipose tissue cells communicate with each other, we referred to the Network Analysis Toolkit for Multicellular Interactions (NATMI) to identify ligands and receptors quantified in each cell type [7]. NATMI uses connectomeDB2020, which consists of 2293 human ligand-receptor pairs with primary literature support. In this database, ligands and receptors are recorded only at the gene level but not at the protein level, therefore, the expression values of peptides in the filtered normalized expression matrices were summed up per gene for downstream analyses. By mapping the ligands and receptors in connectomeDB2020 to their homologous genes in mice via the NCBI HomoloGene Database [8], we have identified 197 ligands and 191 receptors out of 7044 quantified proteins combining all samples.

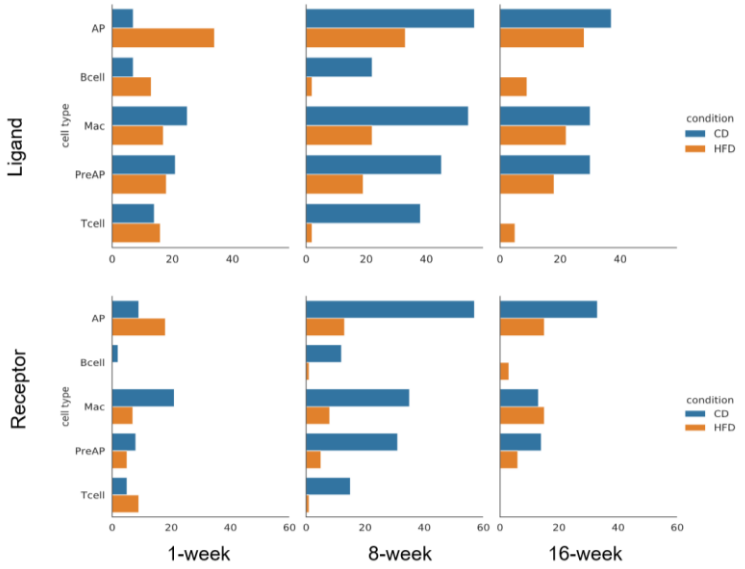

Figure 3. Number of ligands and receptors differentially expression between HFD and CD in each cell type at 1-, 8- and 16-week of feeding. Blue bar, number of ligands or receptors significantly decreased in HFD. Orange bar: number of ligands or receptors significantly increased in HFD.

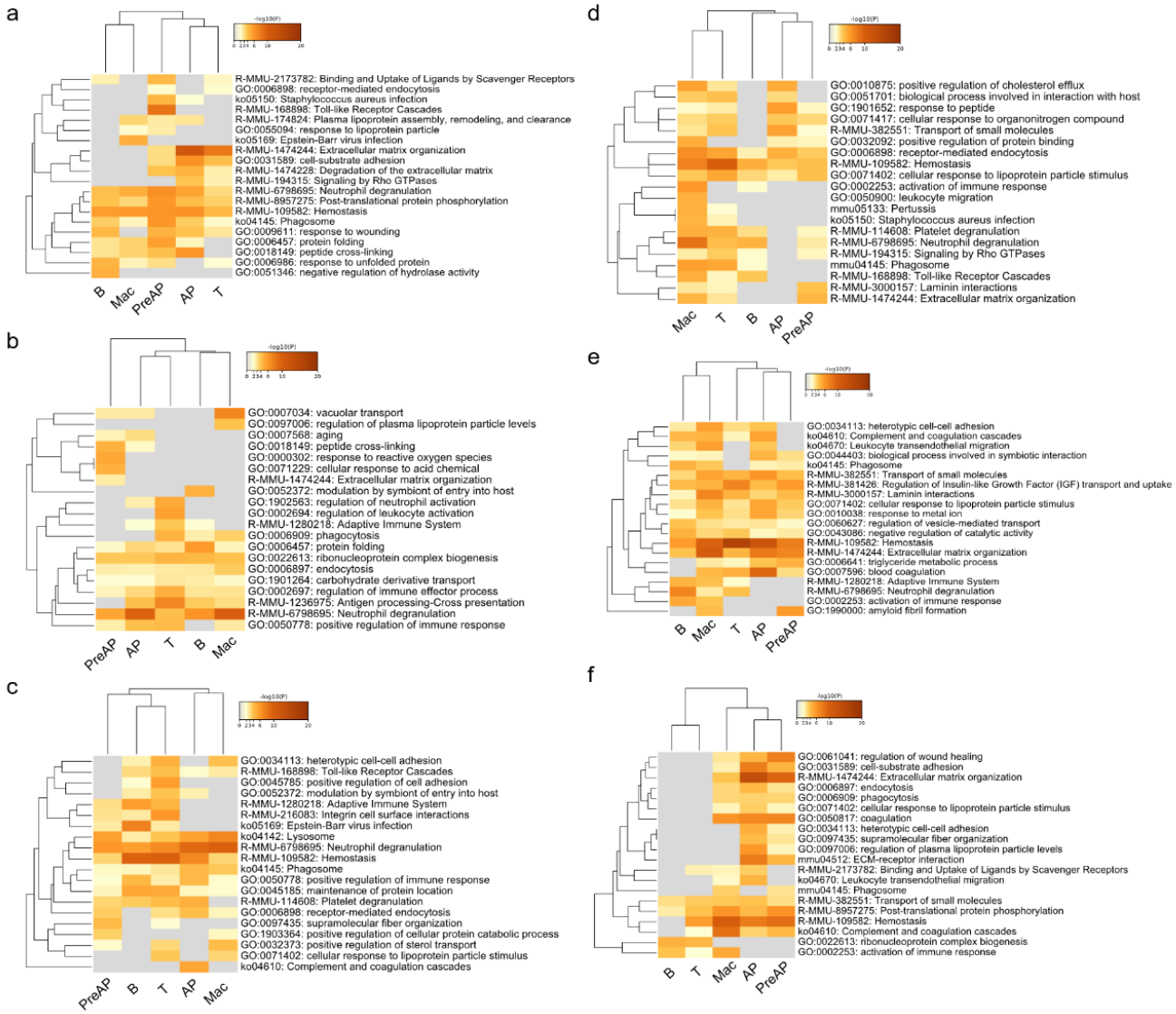

Figure 4. Heatmap of enrichment of GO terms of ligands and receptors up-regulated (a-c) or down-regulated (d-f) in HFD across five cell types in week 1, 8, and 16. Enriched terms are colored by their p-values in the corresponding cell type: a darker color represents a smaller p value, and white color indicates the lack of enrichment for that term in the corresponding cell type. AP: adipocyte. B: B cell. Mac: macrophage. PreAP: preadipocyte. T: T cell.

We next used edgeR [9] to identify differentially expressed genes between samples of two feeding regimens in each TMT batch. We observed that adipocytes, macrophages and pre-adipocytes all have up- and down-regulated ligands and receptors across three time points (Figure 3). In contrast, B cells and T cells both have 2 up-regulated ligands in the middle stage and no down-regulated ligands at 16-week. Besides, both B cells and T cells only have 1 up-regulated receptor around 8 weeks and no down-regulated receptors at 16-week. It is notable that 1-week HFD feeding has almost no impact on the expression levels of the ligands and receptors in B cell. Adipocytes, macrophages and preadipocytes all have more down-regulated ligands and receptors than up-regulated ones upon HFD feeding.

In order to understand the functions of these differentially expressed ligands and receptors, we identified all statistically enriched terms (which can be GO/KEGG terms, canonical pathways, reactome pathways etc.) of them using Metacape [10]. Using all 675 ligands and 602 receptors in mouse as the background gene set, Metascape visualized the representative terms with the best p-values of up- and down-regulated signaling factors in each cell type at each timepoint (Figure 4).

After one-week of HFD feeding, phagosome/phagocytosis process was enriched in all cell types, and the enrichment was maintained from 1 to 16 weeks of HFD feeding. Interestingly, this activation was observed in both up- and down-regulated ligands and receptors, indicating that different genes in this process were regulated in different ways. Further analysis would be required to identify the activated or inhibited genes in this process. Among the activated terms, adaptive immune system was enriched starting from 8 weeks until 16 weeks of HFD feeding, but was not enriched at 1 week (Figure 4a-c). By examining the enriched terms of down-regulated ligands and receptors (Figure 4d-f), we found that positive regulation of cholesterol efflux was enriched at 1 week but not later time points, indicating an acute response HFD feeding. These data showed that proteome changes in the analyzed types of cells upon HFD feeding are highly dynamic and cell type-specific.

In summary, our data have provided unprecedented evidence of the underlying mechanism of how different types of adipose tissue cells are modulated upon energy excess, and how these cells coordinate to adjust to microenvironmental changes in the WAT during the development of obesity.

REFERENCES

1. Andreux, P. A. *et al.* Systems genetics of metabolism: the use of the BXD murine reference panel for multiscalar integration of traits. *Cell* **150**, 1287-1299, doi:10.1016/j.cell.2012.08.012 (2012).
2. Wang, X. *et al.* Joint mouse-human phenome-wide association to test gene function and disease risk. *Nat Commun* **7**, 10464, doi:10.1038/ncomms10464 (2016).
3. Sevin, D. C., Kuehne, A., Zamboni, N. & Sauer, U. Biological insights through nontargeted metabolomics. *Curr Opin Biotechnol* **34**, 1-8, doi:10.1016/j.copbio.2014.10.001 (2015).
4. Gillet, L. C. *et al.* Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. *Mol Cell Proteomics* **11**, O111 016717, doi:10.1074/mcp.O111.016717 (2012).
5. Wu, Y. *et al.* Multilayered genetic and omics dissection of mitochondrial activity in a mouse reference population. *Cell* **158**, 1415-1430, doi:10.1016/j.cell.2014.07.039 (2014).
6. Williams, E. G. *et al.* Systems proteomics of liver mitochondria function. *Science* **352**, aad0189, doi:10.1126/science.aad0189 (2016).
7. Hou, R., Denisenko, E., Ong, H. T., Ramilowski, J. A. & Forrest, A. R. R. Predicting cell-to-cell communication networks using NATMI. *Nat Commun* **11**, 5011, doi:10.1038/s41467-020-18873-z (2020).
8. Sayers, E. W. *et al.* Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* **47**, D23-D28, doi:10.1093/nar/gky1069 (2019).
9. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139-140, doi:10.1093/bioinformatics/btp616 (2010).
10. Zhou, Y. *et al.* Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun* **10**, 1523, doi:10.1038/s41467-019-09234-6 (2019).

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　0件）

１．著者名 Shao Mengle、Hepler Chelsea、Zhang Qianbin、Shan Bo、Vishvanath Lavanya、Henry Gervaise H.、Zhao Shangang、An Yu A.、Wu Yibo、Strand Douglas W.、Gupta Rana K.	４．巻 28
２．論文標題 Pathologic HIF1α signaling drives adipose progenitor dysfunction in obesity	５．発行年 2021年
３．雑誌名 Cell Stem Cell	６．最初と最後の頁 685～701.e7
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.stem.2020.12.008	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Bo Shan, Clive S. Barker, Mengle Shao, Qianbin Zhang, Rana K. Gupta, Yibo Wu	４．巻 -
２．論文標題 Multilayered omics Reveal Sex- and Depot-Dependent Adipose Progenitor Cell Heterogeneity	５．発行年 2022年
３．雑誌名 Cell Metabolism	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　2件／うち国際学会　2件）

１．発表者名 Yibo Wu
２．発表標題 Identification of biomarkers for adipose tissue inflammation by AI
３．学会等名 The 6th RIKEN-KI-SciLifeLab Symposium: Biomedical Data for Artificial Intelligence（招待講演）（国際学会）
４．発表年 2019年

１．発表者名 Yibo Wu
２．発表標題 Rewiring of Immune Cell Network in Adipose Tissue in Obesity
３．学会等名 The 3rd RIKEN IMS-Stanford ISCBRM Joint Symposium（招待講演）（国際学会）
４．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

In collaboration with University of Texas Southwestern Medical Center, we have generated multilayered omics data showing sex- and depot-dependent adipose progenitor cell heterogeneity. We have constructed a website and made the data freely accessible at http://preadprofiler.us-east-2.elasticbeanstalk.com. Our analyses have provided unprecedented insights into adipose stromal cell heterogeneity and highlight the importance of complementary proteomics to support findings from scRNA-seq studies.

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			
米国	University of Texas Southwestern	University of Tennessee		