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科学研究費助成事業　　研究成果報告書

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課題番号：１９Ｋ１００４４

研究課題名（和文）Investigation of chromatin remodeling in osteoclast function for therapeutic targeting of bone remodeling balance

研究課題名（英文）Investigation of chromatic remodeling in osteoclast function for therapeutic targeting of bone remodeling balance

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：エピジェネティッククロマチンリモデラーBaf155の破骨細胞における機能的役割を調査した。研究結果では、同じ年齢でも破骨細胞特異的にBaf155を欠損することにより骨密度に性差があることを明らかにした。8週齢のマウスのmicroCT解析では、オスマウスのみ骨密度が増加し、16週齢では骨密度に差がない。逆に、8週齢のメスでは、コントロールグループと比較して骨密度に差がなく、16週齢ではBaf155 cKOで有意に増加した。これらの結果から、クロマチンリモデラーであるBaf155のエピジェネティック調節が、性的二形に応じて破骨細胞から骨に異なる影響を与える可能性があることが示唆される。

研究成果の学術的意義や社会的意義
Age-related bone resorption diseases are common in women. However, there are no gender-specific clinical trials or drug guidelines. From a gender difference medical perspective, the accumulation of pathophysiological evidence is important.

研究成果の概要（英文）：This study investigated the functional role of Baf155, an epigenetic chromatin remodeler in osteoclasts. In this fiscal year, we found that the effect of Baf155 cKO mice on bone mineral density (BMD) differed between sexes, even at the same age. Interestingly, microCT analysis of 8-week-old mice showed an increase in BMD only in male mice, but there was no difference in BMD at 16 weeks of age. Conversely, in 8-week-old female mice, there was no difference in BMD compared to the control group, but at 16 weeks of age, BMD parameter significantly increased in Baaf155 cKO. Our results suggest that the epigenetic regulation of the chromatin regulator Baf155 on osteoclasts and bone differs according to sexual dimorphism.

研究分野：細胞生物-破骨細胞

キーワード：chromatin remodeler　BAF155　osteoclast　bone

１．研究開始当初の背景

Osteoclasts are the bone-resorbing cells, and also regulate bone-forming cells such as osteoblasts, thus balancer cells of bone-resorption and formation, bone remodeling in vivo. A line of our research of chemokine receptors have demonstrated that Ccr1- and Cx3Cr1-mediated signal are involved in physiological bone remodeling, therefore function as bone remodeling balancers. Our most recent study has reported that functional loss of Ccr5 in osteoclasts positively shifts the bone remodeling balance in vivo. This study further uncovered the role of Ccr5 in mature osteoclast function through regulating RANKL and integrin-mediated pathways (Lee et al., 2017 Nat Commun). These finding have prompted us to investigate functional regulation of osteoclast as a bone remodeling balancer in a way of Ccr5-mediated genome-wide regulation.

２．研究の目的

This research aims to investigate the functional role of epigenetic chromatin remodeler Baf155 in osteoclasts and bone metabolism. Our previous report demonstrated that a chemokine receptor CCR5 is required for the osteoclast function and balance of bone remodeling (Lee et al., 2017 Nat Commun). We identified chromatin remodeler Baf155 using proteomics approach were downstream targets of CCR5-mediated signal. We will expand this findings using conditional knockout (cKO) mice generated by Baf155 floxed mice with osteoclast specific Cre-recombinase mice.

３．研究の方法

(1) Generation of cKO and pathological study in vivo

BAF complexis essential gene and conventional deletion leads to embryonic lethality. Cre/LoxP technology was employed to crease cKO mice. We generated LysM-Cre; Baf155$^{fl/fl}$ mice and studied bone remodeling in adult mice.

(2) Histomorphometrical analysis was conducted on femoral bone sections obtained from adult to aged mice, and ovariectomized osteoporosis mice of cKO line. Then osteoclastic and bone resorption parameters was intensively scored.

(3) Osteoclast culture and differentiation

For in vitro assay, bone marrow cells were harvested from mouse tibiae, and then cultured with M-CSF and RANKL allowing to proliferate and differentiate into osteoclasts. Cells were carefully observed all stage of osteoclast ontogeny, which is affected by chromatin remodeling.

(4) Transcriptome analysis and validation of candidate factor from profiling

To find potent transcription factors that interact with Baf155 and function under the control of osteoclast differentiation, we applied RNA sequencing by a Next generation sequencer (illumine MiSeq) equipped in Division of Anlaytical Bio-Medicine in Ehime University. Total RNA extracts were prepared from the cultured osteoclasts obtained from Baf155$^{fl/fl}$ and LysM-Cre; Baf155$^{fl/fl}$ mice using RNeasy kit (Qiagen) according to the manufacture's instruction. Total RNA further separates into largen and small RNA enriched fractions using the miRNeasy kit (Qiagen) columns and reagents. Quantified RNA samples were sequenced and analyzed by illumine MiSeq. Data of RNA seq were analyzed using specific software (David Bioinformatics, Database for annotation, visualization, and integrated discovery etc.).

４．研究成果

(1) Bone resorption serum marker was increased in Baf155 cKO male and female mice

Serum analysis of TRAP5b, a representative activation marker of osteolasts, was performed in 8-week-old mice. The results showed a significant increase in TRAP5b level in male mice of Baf155 cKO. However, no significant difference was observed in

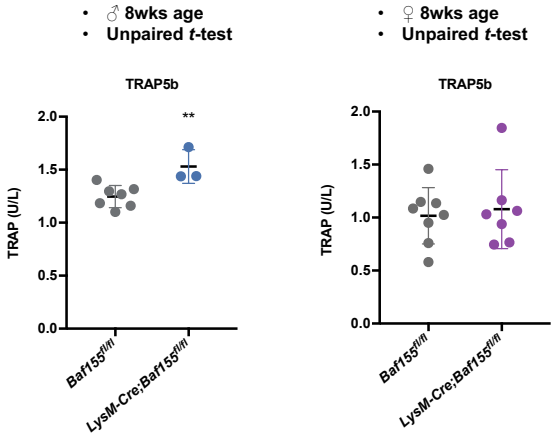

Fig.1. Serum analysis of osteoclast related markers

female mice compared to the control group.

(2) Sexual dimorphic phenotype of Baf155 cKO mice between female and male
Chromatin remodeling Baf155 floxed mice were mated with LysM-Cre recombinase mice to generate osteoclast progenitor specific deletion. We examined the bone mineral density by microCT and bone histomorphometry using femoral bone sections in adult mice of 8 and 16 weeks of female and male. We found that the effect of Baf155 cKO mice on bone mineral density (BMD) differed between sexes, even at the same age. Interestingly, microCT analysis of 8-week- old mice showed an increase in BMD only in male mice, but there was no difference in BMD at 16 weeks of age. Conversely, in 8-week-old female mice, there was no difference in BMD compared to the control group, but at 16 weeks of age, BMD parameter significantly increased in Baaf155 cKO (Fig.2 and 3). Our results suggest that the epigenetic regulation of the chromatin regulator Baf155 on osteoclasts and bone differs according to sexual dimorphism.

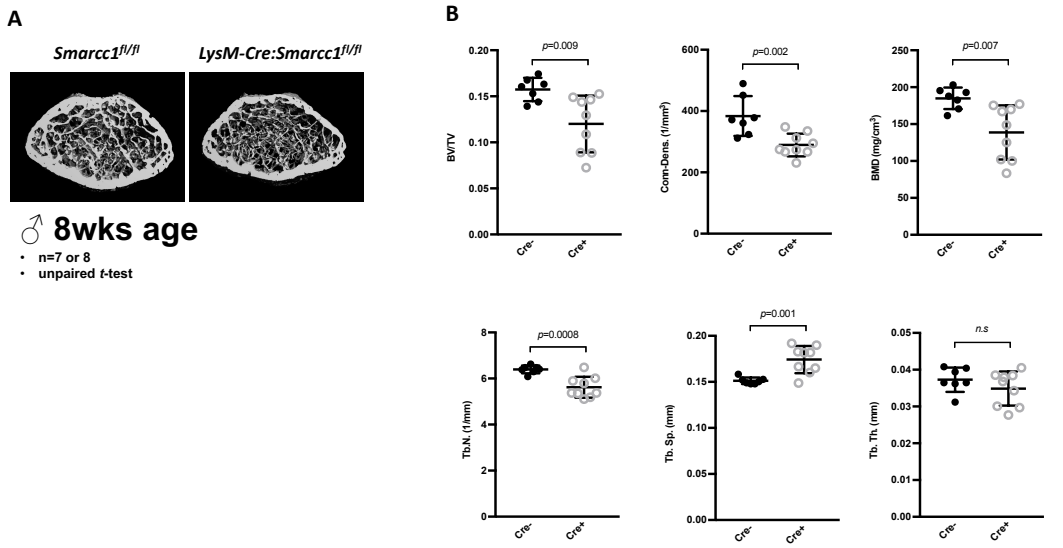

Fig.2. A) microCT images of femoral bone and B) parameters of trabecular bone analysis from Baf155 cKO (8 week-old male mice)

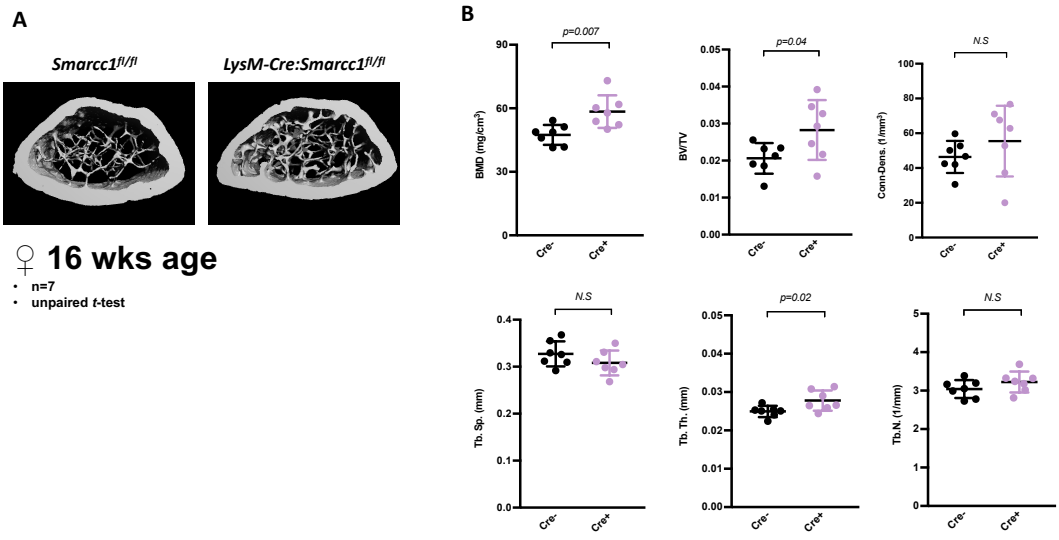

Fig.3. A) microCT images of femoral bone and B) parameters of trabecular bone analysis from Baf155 cKO (8 week-old female mice)

(3) RNA sequencing expression profiling data

To investigate the changes in the transcriptional signatures induced by CCL5 in osteoclastogensis, cultured osteoclasts at pOC stage were incubated with or without RANKL for 2 days, and then subjected for RNA-seq. Genes with a log fold-changes value of >1.0 and an adjusted P-value of <0.05 were tested for enrichment using the Gene Ontology (GO) analysis.

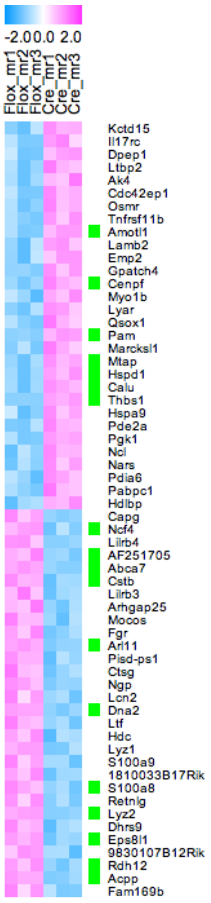

Fig. 4. Gene Ontology analysis of the genes that showed significantly altered expression levels in RNA-sequencing using cells incubated with RANKL (100 ng/mL). The significantly up- and down-regulated genes and heat maps of the obtained results are shown.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　6件／うち国際共著　6件／うちオープンアクセス　6件）

１．著者名 Xia Yuhan、Ikedo Aoi、Lee Ji-Won、Iimura Tadahiro、Inoue Kazuki、Imai Yuuki	４．巻 590
２．論文標題 Histone H3K27 demethylase, Utx, regulates osteoblast-to-osteocyte differentiation	５．発行年 2022年
３．雑誌名 Biochemical and Biophysical Research Communications	６．最初と最後の頁 132～138
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bbrc.2021.12.102	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

１．著者名 Sato Takanori、Takakura Aya、Lee Ji-Won、Tokunaga Kazuaki、Matsumori Haruka、Takao-Kawabata Ryoko、Iimura Tadahiro	４．巻 70
２．論文標題 A quantitative analysis of bone lamellarity and bone collagen linearity induced by distinct dosing and frequencies of teriparatide administration in ovariectomized rats and monkeys	５．発行年 2021年
３．雑誌名 Microscopy	６．最初と最後の頁 498～509
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/jmicro/dfab020	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

１．著者名 Lee Ji‐Won、Lee In‐Hee、Sato Takanori、Kong Sek Won、Iimura Tadahiro	４．巻 63
２．論文標題 Genetic variation analyses indicate conserved SARS‐CoV‐2 host interaction and varied genetic adaptation in immune response factors in modern human evolution	５．発行年 2021年
３．雑誌名 Development, Growth & Differentiation	６．最初と最後の頁 219～227
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/dgd.12717	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

１．著者名 Lee Ji-Won、Lee In-Hee、Iimura Tadahiro、Kong Sek Won	４．巻 9
２．論文標題 Two macrophages, osteoclasts and microglia: from development to pleiotropy	５．発行年 2021年
３．雑誌名 Bone Research	６．最初と最後の頁 11
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41413-020-00134-w	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

1．著者名 In-Hee Lee, Ji-Won Lee, Sek Won Kong	4．巻 85
2．論文標題 A survey of genetic variants in SARS-CoV-2 interacting domains of ACE2, TMPRSS2 and TLR3/7/8/ across populations	5．発行年 2020年
3．雑誌名 Infection, Genetics and Evolution	6．最初と最後の頁 104507
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.meegid.2020.104507	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

1．著者名 Tanaka Tomoya、Takao-Kawabata Ryoko、Takakura Aya、Shimazu Yukari、Nakatsugawa Momoko、Ito Akitoshi、Lee Ji-Won、Kawasaki Koh、Iimura Tadahiro	4．巻 10
2．論文標題 Teriparatide relieves ovariectomy-induced hyperalgesia in rats, suggesting the involvement of functional regulation in primary sensory neurons by PTH-mediated signaling	5．発行年 2020年
3．雑誌名 Scientific Reports	6．最初と最後の頁 5346
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41598-020-62045-4	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1件（うち招待講演　0件／うち国際学会　0件）

1．発表者名 李　智媛、In-Hee Lee, Sek Won Kong, 飯村　忠浩
2．発表標題 SARS-CoV-2の相互作用分子ACE2, TMPRSS2およびTLR3/7/8における人口集団の遺伝的変異調査
3．学会等名 日本薬理学会
4．発表年 2021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