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研究課題名（和文）Trans-omics analysis of the difference between Cortical and Trabecular bone.

研究課題名（英文）Trans-omics analysis of the difference between Cortical and Trabecular bone.

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研究成果の概要（和文）：骨造成は複雑な生物学的プロセスであり，なかでも細胞外マトリックスは骨の構造と機能に重要な役割を果たしている．実験手法は細胞外マトリックスのタンパクプロファイルに大きく影響することから，骨のプロファイルを正確に評価するためには，タンパク質抽出方法の適正化が必要である．我々の解析結果から、初期の骨芽細胞分化中のタンパク組成において，細胞外マトリクスならびにその制御因子の総称であるMatrisomeタンパクの増加が検出された。我々の研究結果は，骨芽細胞分化における細胞外マトリックス組成の動的な変化を示しており、骨形成プロセスにおける細胞外マトリックスの重要性を示唆していた．

研究成果の学術的意義や社会的意義
Bone augmentation is a complex process with unclear mechanisms. The ECM is vital for bone structure and cellular functions, impacted by collagen crosslinks. Improved protein extraction methods are needed to study ECM profiles in bone accurately.

研究成果の概要（英文）：Bone augmentation involves a complex biological process where the extracellular matrix (ECM) plays a critical role in the structure and function of bone. However, the method used for experimentation significantly affects the profile of ECM proteins, so there is a need for better protein extraction techniques to accurately identify bone profiles. It is essential to optimize protein extraction methods to maintain the natural state of ECM proteins for precise analysis. Our data indicates that the composition of ECM changes during early osteoblast differentiation, showing an increase in Matrisome proteins. These findings suggest a dynamic shift in ECM composition, indicating its evolving role in supporting and regulating the osteogenic process.

研究分野：bone regeneration

キーワード：trans-omics　extracellular matrix　bone phenotype　bone regeneration

１．研究開始当初の背景/Background at the beginning of research

In a super-ageing society, the prevalence of chronic diseases impacting bone metabolism is increasing. During implant surgery, primary stability is a critical predictor of treatment success, heavily influenced by the quality and quantity of local cortical bone (Tanaka et al. J. Periodontal Implant Sci. 2018). For patients with insufficient cortical bone, bone augmentation is a reliable treatment option.

Bone augmentation is a complex biological process with partially understood molecular mechanisms. Current techniques can increase bone volume to some extent, but managing bone quality remains challenging. Hence, developing new strategies to improve “bone quality” would significantly benefit patients with compromised bone health. Studies have shown that the success rate of implants in the mandible is higher than in the maxilla (Tolstunov et al., J. Oral Implantol. 2007). This disparity is likely due to the mandible's thick cortical bone compared to the maxilla's trabecular bone. We propose that manipulating the bone type from trabecular to cortical bone could enhance bone quality, leading to better implant treatment outcomes.

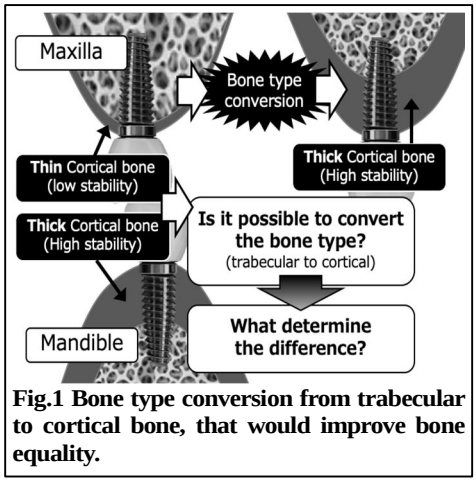

Fig.1 Bone type conversion from trabecular to cortical bone, that would improve bone equality.

Determinants of the difference between cortical and trabecular bone.

Although the structures of cortical and trabecular bones are distinct, the molecular cues differentiating these bone types remain largely unknown. Previous studies have highlighted bone type-specific responses to ovariectomy (OVX) by comparing the expression patterns of two distinct estrogen receptors (ER): ERα, which is expressed similarly in both cortical and trabecular bone, and ERβ, which is expressed at higher levels in trabecular bone (Bord et al. J. Clin. Endocrinol. Metab. 2001). Consistent with this, we observed differential sensitivity to estrogen deficiency in OVX rats. This deficiency led to a reduction in trabecular bone volume at the femur metaphysis and an increase in cortical bone volume at the femur diaphysis (Rosales Rocabado et al. J. Orthop. Surg. 2018).

Additionally, it has been reported that the expression levels of Wnt/β-catenin signaling differ between cortical and trabecular bone, with trabecular bone being more responsive to changes in this signaling pathway (Li et al. J. Orthop. Res. 2017).

Collectively, these findings strongly suggest that the differing sensitivities of bone cells to extracellular stimuli, such as estrogen and growth factors, significantly influence the maintenance and differentiation of cortical and trabecular bones. Understanding these molecular mechanisms could pave the way for new therapeutic strategies to enhance bone quality and improve outcomes for patients with compromised bone health.

The Extracellular matrix (ECM) is crucial for bone structure, providing support and anchorage for cells and forming a complex protein network that regulates cell survival and functions. Alterations in collagen

crosslinks in the ECM affect osteoblast and osteoclast differentiation (Ida et al., PLoS ONE 2018). Thus, different ECM compositions likely influence the determination of bone types, such as cortical and trabecular bone. (Ida et al. PLoS ONE 2018). Therefore, it is probable that the different ECM composition may affect the determination of bone types, i.e. cortical and trabecular bone.

Matrisome database for ECM and ECM regulators

The Matrisome database (Matrisome DB), a comprehensive resource containing over 1000 genes encoding ECM and associated proteins, offers a powerful tool to investigate compositional differences between cortical and trabecular bone. These matrisome components were identified computationally based on the presence of characteristic protein domains found in ECM molecules (Naba et al. Matrix Biol. 2016), this makes Matrisome DB ideally suited for data mining within large gene and protein datasets.

Traditionally, analyses focus on individual omics layers (genomics, transcriptomics, proteomics). However, this approach has limitations as it doesn't directly capture interactions across these layers. Integrating data from different omics platforms reveals the connections between them, providing a deeper understanding of the complex molecular networks within bone ECM. . (Yugi *et al.* Cell Syst. 2017), (Yugi *et al.* Trends Biotechnol. 2016))

２．研究の目的 / Purpose of research

Previously, it was discovered that cortical and trabecular bone molecular cues, which control the bone type-specific response to OVX, involved a differential expression pattern of distinct estrogen receptors. Additionally, extracellular matrices directly or indirectly aided in bone mineralization through a complex network of signals. The study of these networks is essential for improving bone regeneration therapies. Recently, new trans-omics approaches have been developed, allowing for the comprehensive study of ECM signaling.

In this study, we investigated sets of extracellular matrix (ECM) protein networks that regulate cortical and trabecular mineralization patterns using trans-omics RNA-sequencing and the ECM matrisome database. Our goal was to gain a better understanding of the different biological processes and interactions involved in bone mineralization and regeneration. The outcomes of this study aimed to accelerate the development of more efficient bone regeneration therapies, addressing the needs of an increasingly aged society.

３．研究の方法 / Research method

In this study, eigengenes were identified through various methods. Initially, mandibular cortical bone from C57BL/6J mice was isolated for analysis. The trans-omic analysis consisted of 1) conducting RNA-seq on cortical and cancellous bone to identify gene expression differences, 2) constructing sequence libraries for RNA-seq profiling by filtering reads and normalizing data, and 3) building a co-expression network to determine gene connectivity and module significance for further gene analysis. Additionally, the protein profile of cortical and trabecular bone samples was analyzed using LC-MS/MS and compared with the Matrisome database to select relevant proteins and genes involved in cortical bone formation. Furthermore,

a data-driven approach was applied to integrate trans-omics data and construct biochemical networks, incorporating molecular network knowledge like the Matrisome database.

４．研究成果/ Research result

Bone tissue, particularly when differentiating between trabecular and cortical bone, presents a significant challenge. The inherent heterogeneity makes interpreting gene expression data a complex task, requiring careful consideration of the biological context. Adding another layer of difficulty is the delicate process of sample collection and processing. Improper handling can easily degrade RNA, leading to misleading results. Furthermore, obtaining sufficient bone samples, especially trabecular bone was challenging and limited by sample availability. Another hurdle arose from the close physical proximity of these bone types. Isolating them for precise analysis proves difficult, potentially causing cross-contamination. This contamination significantly skewed results, making it hard to distinguish the unique signatures of each bone

In conjunction, to complement the investigation of trabecular and cortical bone differentiation, we conducted a parallel experiment to analyze the composition of the extracellular matrix (ECM) during the early stages of osteoblast differentiation. This analysis revealed a progressive increase in the proportion of Matrisome proteins over the course of the experiment. This enrichment of the Matrisome suggests a growing role for ECM components in osteoblast maturation.

In the results of the Matrisome profile, we observed a significant increase in the collagen component, with type I collagen emerging as the dominant component. The proteoglycan profile remained relatively stable. The ECM glycoprotein profile of the Matrisome exhibited a decrease in their relative abundance over the differentiation period. This suggests a potential shift in the focus of ECM composition during bone formation. This analysis of the ECM composition throughout early osteoblast differentiation provides some insights into the dynamic interplay between osteoblasts and their surrounding matrix during the bone formation process. The observed changes highlight the evolving role of the ECM in supporting and potentially regulating the osteogenic process.

5. Conclusion.

Isolating tissue exclusively from trabecular bone is challenging due to the proximity to cortical bone and the substantial tissue required, leading to overlap. The complex architecture of trabecular bone, characterized by its porous, spongy structure, complicates the separation process. Additionally, the dense, compact nature of cortical bone requires more rigorous mechanical and chemical procedures to break down and isolate proteins, increasing the risk of contamination and protein degradation. The ECM proteomic workflow significantly impacts the ECM protein profile, necessitating improved protein extraction methods to accurately determine bone profiles. Current methods often involve harsh conditions that can alter or degrade the proteins, leading to incomplete or biased profiles. Optimizing extraction techniques to preserve the native state of ECM proteins is crucial for accurate analysis. Understanding these differences enhances bone disease treatments and implant therapies, benefiting public health by advancing osteoporosis and fracture prevention strategies. By developing more refined isolation and extraction methods, researchers can achieve a clearer

understanding of the distinct molecular compositions and interactions in trabecular and cortical bone. This knowledge will lead to more targeted and effective therapies for bone-related conditions, ultimately improving patient outcomes and quality of life.

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５．主な発表論文等

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〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

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