Fellowship ID: P17124

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Form 7／様式 7

外国人特別研究員作成／By Fellow

2019, 04, 24

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Signature (Handwritten only) : _____

Research Report (by Fellow)
(Cover Page)

I hereby submit the research report of my fellowship.

1. Name (Print) : Md Abdulla Al Masud Khan
2. Nationality : Bangladesh
3. Host Institution : Tokyo Medical and Dental University
4. Host Researcher : Kazuhiro Aoki
5. Title of Research in Japan : The therapeutic application of RANKL working as a bone-formation-~~stimulation~~ stimulating-receptor
6. Fellowship Tenure : From 2017/ 04/ 01 To 2019/ 03/ 31
   (YYYY) (MM) (DD) (YYYY) (MM) (DD)

***Notes for writing the Research Report**
***Type this form except the date and the signature.**
Please prepare your Research Report in English or Japanese within three to ten pages including this page. The contents should include:

7. Background of Research

W9 peptide (W9) was originally designed to mimic a critical binding site of TNF- α on TNF type 1 receptor (TNFR1) and antagonized the TNF-α and TNF receptor interaction. Subsequently, W9 has been proven to bind with RANKL by BIAcore and molecular modeling (J Clin Invest. 2006; 116 (6): 1525–15349), blocking the RANKL-induced bone resorption activity and osteoclastogenesis.

WP9QY peptide (W9), which is an inhibitor of bone resorption, was found to simulate bone formation. Subcutaneous injections of W9 promoted the bone mineral density (BMD) and mineral apposition rate of femur in mice. Furthermore, we found that subcutaneous injections of W9 (10 mg/kg) accelerated BMP-2-induced ectopic bone formation (J Biol Chem, 2013; 288(8): 5562–5571). When W9 was applied locally with BMP-2, I found that the W9 accelerated ectopic bone formation. In order to clarify the stimulatory mechanism of W9 on ectopic bone formation, I

used TNF-α deficient and TNFR1-deficient mice since W9 binds to TNF-α and was designed from the binding site on TNFR1. We found that the local administration of W9 enhances BMP-2-induced ectopic bone formation even in the TNF-α deficient and the TNFR1-deficient mice to the same extent as in wild-type mice. These results suggest that the stimulatory mechanism of W9 on bone formation is regulated by a mechanism other than antagonizing TNF-α action (Khan et al. J Oral Biosci 2013; 55, 47-54). Since W9 is proven to bind to RANKL as well as TNF-α, the stimulatory mechanism of W9 might be involved in the RANKL-dependent signals.

Some TNF superfamily members are known to act as bidirectional signaling molecules that generate intracellular reverse signaling (Nat. Neurosci 2013; 16, 865–873). The RANKL-binding peptides W9 can stimulate osteogenic activity in osteoblasts (BioEssays 2016; 38, 717–725). Therefore, osteoblastic RANKL potentially acts as a physiological osteogenic signal acceptor. RANK, is a transmembrane protein that is expressed in the osteoclastic lineage. Recent reports suggest that osteoblasts that are not in direct contact with osteoclasts at the resorption pit contribute to new bone synthesis during bone remodeling. A lack of cell–cell interactions between osteoblasts and osteoclasts during the bone remodeling process is also implied by the minor contribution of osteoblasts to physiological RANKL provision. In addition, osteoclast-derived semaphorin 4D inhibits osteoblast migration to the resorption pit, suggesting that osteoclasts cannot stimulate RANKL reverse signaling in osteoblasts through direct interactions. However, maturing osteoclasts (maturing osteoclasts) secrete small extracellular vesicles (SEVs) that contain RANK. Therefore, it is possible that RANKL reverse signaling in osteoblasts is activated by SEVs, which is secreted from osteoclasts.

In our present study, we tested RANKL reverse signaling in osteoblasts is activated by vesicular RANK, which is secreted from osteoclasts.

8. Research methodology

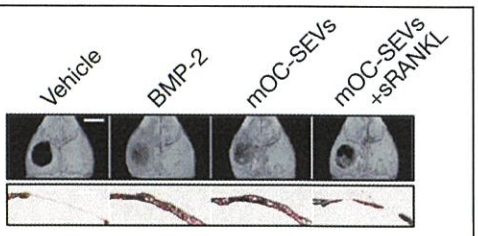
The osteogenic potency of mOC-SEV was evaluated using a mouse calvarial defect model according to previously described methods (Endocrinology 2010; 151: 4626–4634). In brief, recombinant human BMP-2 (1 μg, Bioventus) or mOC-SEVs (50 μg) were added to gelatin hydrogel sheets (4 mm diameter). A calvarial defect was generated on the left parietal bone of 5-week-old male C57BL/6 mice (Clea Japan) using a biopsy punch (Kai Industries; 3.5 mm); a gelatin hydrogel sheet was placed on the defect. To measure bone formation parameters, calcein (Sigma-Aldrich; 40 mg/kg) and demeclocycline (Sigma-Aldrich; 20 mg/kg) were administered on days 18 and 25 after implantation, respectively, and mice were killed on day 28. Calvariae were fixed in phosphate-buffered glutaraldehyde (0.25%)-formaldehyde (4%) fixative (pH 7.4) for 3 days at 4 °C. Soft X-ray images of calvariae were acquired using a cabinet X-ray apparatus (SRO-M50, Sofron Co. Ltd, Tokyo, Japan). Calvarial bone mineral density (BMD) and bone mineral content (BMC) were measured by dual-energy X-ray absorptiometry (DXA, DCS-600R, ALOKA). Standard bone histomorphometric analyses were performed using an image analysing

system (KS400, Carl Zeiss) as described previously (J. Bone Miner 2013; 28, 2–17).

9. Results/impacts

Summery of Results:

The presence of mOC-SEVs increased osteoblastic mineralization; this effect was inhibited by RANK-masking pre-treatment of the mOC-SEVs. We examined the effect of mOC-SEVs on bone formation *in vivo*. We performed trepanation on mouse calvaria, and placed a gelatin hydrogel sheet impregnated with mOC-SEVs on the injury. Four weeks after the procedure, mice treated with mOC-SEVs exhibited marked healing of the skull injury, whereas mice treated with the control gelatin hydrogel showed little or no healing. RANK-masking pre-treatment prevented this stimulatory effect. Histomorphometric analyses measuring bone formation rate and mineralizing surface, confirmed the upregulation of bone formation in the presence of mOC-SEVs.



Modified from Nature 2018; 561, 195-200

10. Research Presentations during the period of the fellowship (Name of the conference, title, place, date)

None

11. A list of paper published during or after the period of the fellowship, and the names of the journals in which they appeared (Please fill in the format below). Attach a copy of each article if available.

Author(s)	Title	Name of Journal	Volume	Page	Date	Note
Eri Sone, Daisuke Noshiro, Yuki Ikebuchi , Mami Nakagawa, **Masud Khan**, Yukihiko Tamura, Masaomi Ikeda, Meiko Oki , Ramachandran Murali, Toshihiko Fujimori, Tetsuya Yoda, Masashi Honma, Hiroshi Suzuki, Toshio Ando, Kazuhiro Aoki.	The induction of RANKL molecule clustering could stimulate early osteoblast differentiation.	Biochem Biophys Res Commun	509 (2)	435-440	Feb 5, 2019	

Yuki ikebuchi1, Shigeki Aoki1, Masashi Honma1, Madoka Hayashi, Yasutaka Sugamori, **Masud Khan**, Yoshiaki Kariya, Genki Kato, Yasuhiko tabata, Josef M. Penninger, Nobuyuki Udagawa, Kazuhiro Aoki & Hiroshi Suzuki.	Coupling of Bone Resorption And Formation by RANKL Reverse Signaling.	Nature	561	195–200	Sep 13, 2019	Press released
Lia Kartika Wulansari, Boosana Kaboosaya, Masud Khan, Mariko Takahashi, Shinji Kuroda, KazuhiroAoki, ShoheiKasugai.	Beneficial effects of fasting regimens on periodontal tissues in experimental periodontitis mice model.	Journal of International Dental and Medical Research	11(2)	362-369	May 30, 2018	
Yasuhiro Shimizu, Khan Masud, Genki Kato, kazuhiro Aoki, Takashi Ono	Occlusal Disharmony-Induced Stress Causes Osteopenia of the Lumbar Vertebrae And Long Bones In Mice.	Scientific Reports	8 (173)	1-9	Jan 9, 2018	

12. Awards during the period of the fellowship (Name of the award, Institution, date etc.)

None