



Title	Cellular function of osteocytes in normal and klotho-deficient mice
Author(s)	Amizuka, Norio; Hasegawa, Tomoka; Yamamoto, Tomomaya; Mae, Takahito; Zixuan, Qiu; Abe, Miki; Shen, Zhao; Nagai, Tomoya; Yokoyama, Ayako; Naznin, Khadiza; Haraguchi, Mai; Yamamoto, Tsuneyuki; Freitas, Paulo Henrique Luiz de; Li, Minqi
Citation	北海道歯学雑誌, 38(Special issue), 56-62
Issue Date	2017-09
Doc URL	<a href="http://hdl.handle.net/2115/67337">http://hdl.handle.net/2115/67337</a>
Type	article
File Information	08_Norio Amizuka.pdf



[Instructions for use](#)

## Cellular function of osteocytes in normal and *aklotho*-deficient mice

Norio Amizuka<sup>1)</sup>, Tomoka Hasegawa<sup>1)</sup>, Tomomaya Yamamoto<sup>7)</sup>, Takahito Mae<sup>1, 2)</sup>, Qiu Zixuan<sup>1)</sup>, Miki Abe<sup>6)</sup>, Zhao Shen<sup>1, 3)</sup>, Tomoya Nagai<sup>1, 4)</sup>, Ayako Yokoyama<sup>1, 4)</sup>, Khadiza Naznin<sup>1, 5)</sup>, Mai Haraguchi<sup>1)</sup>, Tsuneyuki Yamamoto<sup>1)</sup>, Paulo Henrique Luiz de Freitas<sup>8)</sup> and Minqi Li<sup>9)</sup>

<sup>1)</sup> Developmental Biology of Hard Tissue, <sup>2)</sup> Gerodontology, <sup>3)</sup> Oral and Maxillofacial Surgery, <sup>4)</sup> Oral Functional Prothodontics, <sup>5)</sup> Dentistry for Children and Disabled Person, Faculty of Dental Medicine and Graduate School of Dental Medicine, <sup>6)</sup> School of Dental Medicine, Hokkaido University, Sapporo, Japan, <sup>7)</sup> Self-Defense Force Hanshin Hospital, Kawanishi, Japan, <sup>8)</sup> Department of Dentistry, Federal University of Sergipe at Lagarto, Brazil, <sup>9)</sup> Division of Basic Science of Stomatology, The School of Stomatology, Shandong University, Jinan, China

**ABSTRACT** : During the last decade, osteocyte-derived factors i.e., sclerostin, dentin matrix protein-1, fibroblast growth factor 23 (FGF23) that reduces serum phosphate concentration by mediating FGF receptor 1c/*aklotho* in the kidney, have been highlighted for osteocytes' fine-tuned regulation on bone remodeling and phosphate homeostasis. Osteocytes are interconnected through gap junctions between their cytoplasmic processes, and thereby, build upon the functional syncytia, referred to as the osteocytic lacunar-canalicular system (OLCS). Osteocytes appear to communicate surrounding osteocytes and osteoblasts by means of two possible pathways of molecular transport throughout the OLCS : One is a passageway of their cytoplasmic processes, and the other is a pericellular space in the osteocytic canaliculi. The regularly-oriented OLCS in mature compact bone appears to efficiently serve for molecular transport, mechanosensing and targeted bone remodeling that would erase microdamages in bone. In a disrupted signaling state of FGF23/*aklotho*, serum concentration of phosphate would be markedly-elevated. Despite highly-elevated serum phosphate, *aklotho*-deficient mice revealed defective mineralization in bone matrix. OLCS in *aklotho*-deficient mice were irregularly-distributed and the connectivity of cytoplasmic processes of osteocytes was very poor, so that osteocytes did not seem to form functional syncytia. Therefore, osteocytes' function cooperated with other bone cells, rather than serum concentration of calcium/phosphate, and this seems to play a central role in maintaining bone mineralization. In this review, the biological function of the regularly-arranged OLCS in a normal state will be introduced, as well as dysfunctional osteocytes in *aklotho*-deficient state, using animal models.

**Key Words** : osteocyte, bone, *aklotho*, FGF23, sclerostin

### Introduction

Osteocytes establish a communication network among osteoblasts and themselves and play a central role to regulate bone metabolism, in order to adjust bone architecture against mechanical stress<sup>1)</sup>. Osteocytes exist inside osteocytic lacunae and extend fine cytoplasmic processes that run through osteocytic canaliculi in bones.

Osteocytes connect to neighboring ones and osteoblasts on the bone surfaces by their cytoplasmic processes interconnected with gap junction<sup>2)</sup>. Osteocytic cytoplasmic processes allow small molecules to pass through the gap junction from one osteocyte to the others. In addition, the pericellular space, also referred to as an annular space, in the osteocytic canaliculi may serve as an alternative transport pathway<sup>3)</sup>. By means of these two

---

### Address of Correspondence

Tomoka Hasegawa, DDS, PhD.

Developmental Biology of Hard Tissue, Faculty of Dental Medicine and Graduate School of Dental Medicine, Hokkaido University, Kita 13 Nishi 7 Kita-ku, Sapporo, 060-8586, Japan

TEL/FAX : +81-11-706-4226 ; E-mail : hasegawa@den.hokudai.ac.jp

distinct pathways, osteocytes can communicate each other and function as syncytium by molecular transport and mechanotransduction, so that the osteocytic communication system using cytoplasmic processes and their canaliculi is called osteocytic lacunar-canalicular system (OLCS)<sup>4, 5</sup>.

Proper function of the OLCS appears to depend on its anatomical arrangement and extent : The distribution of connected cytoplasmic processes of osteocytes represents the functional area of OLCS. The geometrical regularity of distribution of the cytoplasmic processes indicates the efficiency of OLCS's function, and also appears to be related to the maturity of the bone matrix. In immature woven bones, the osteocytic cytoplasmic processes are randomly-oriented and often disconnected, so that the osteocytes could not form a functional group in woven bones. In contrast, mature bones, *e.g.*, cortical bones are composed by regularly-interwoven collagen bundles and a well-mineralized matrix, in which the osteocytic cytoplasmic processes are geometrically well-arranged<sup>6</sup>. While the longitudinal axis of the osteocytes parallels the direction of the collagen fibrils in cortical bone, their cytoplasmic processes are perpendicular to them, and orderly hold the collagen bundles with a certain interval. This may enable the osteocytes to recognize the direction and degree of mechanical loading in cortical bone, by sensing bending, stretch and compression of collagen bundles.

Bone disease, on the other hand, may significantly affect the arrangement of the OLCS. In osteomalacia, haphazardly-connected, irregular OLCS are seen, and in the late stage of osteoporosis, a remarkable compromise of connectivity and regularity of that system is present<sup>5</sup>. Once bone metabolism is worsened, the anatomical regularity of osteocytic arrangement would be disrupted, leading to dysfunction of the osteocytic network. Thus, OLCS appears to fail to show proper function in pathological states of bone metabolic disease.

Recently, osteocytes-derived molecules – sclerostin, fibroblast growth factor (FGF) 23, and dentin matrix protein -1 (DMP-1) – have been highlighted for their distinctive function in bone. Among these molecules, FGF23 has been shown to regulate serum concentration of phosphate : Osteocytes secrete FGF23, which would be circulated to kidney and inhibit phosphate reabsorption in proximal renal tubules, by binding to the receptor complex of FGF receptor 1c (FGFR1c)/*aklotho*. Without *aklotho* function, it causes hyperphosphatemia

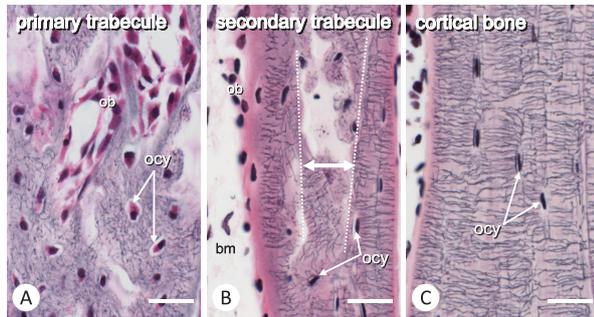
accompanied with senescence-associated phenotypes such as osteoporosis, skin atrophy, vascular calcification and so forth. Therefore, it seems of interest to examine the osteocytes' function in *aklotho*-deficient states.

In this review, from the histological viewpoint, we will introduce the cellular function of the regularly-arranged OLCS in a normal state, as well as dysfunctional osteocytes in *aklotho*-deficient state, using animal models.

## 1. Possible function on sensing mechanical stress in OLCS

One accepted concept for osteocytic function places these cells as transducers of mechanical strains into biochemical signals that affect communication among osteocytes and between osteocytes and osteoblasts<sup>3, 5, 7, 8</sup>. An intact OLCS seems crucial for molecular transport among osteocytes, and may be important for mechanosensing and bone remodeling control. Bone remodeling appears, at least, to serve for two different aspects : skeletal adaptation to the microenvironment including mechanical stress, and repairing of load-related microdamages<sup>9, 10</sup>. Bone remodeling caused by these two aspects require site-specificity in bone, the concept of which is named "targeted bone remodeling"<sup>9</sup>. The proposed cellular mechanism of targeted bone remodeling regards osteocytes as sensors for microdamages in bones<sup>11</sup>. Once microdamages take place, osteocytes and their cytoplasmic processes would be injured, triggering the resorption of the damaged region<sup>12, 13</sup>. There are many reports on osteocytic apoptosis and accumulated microdamages as important factors for initiating new site-specific remodeling<sup>14, 15</sup>. It seems likely that osteocytic apoptosis and microdamages disturb the signals carried throughout the OLCS, leading to signaling misinterpretation by osteocytes and osteoblasts and initiation of targeted bone remodeling. Recently, osteocytes have been reported to synthesize receptor activator of NF $\kappa$ B ligand (RANKL)<sup>16</sup>, and thereby, osteocytes affected by microdamages would synthesize RANKL, consequently inducing osteoclastic migration and subsequent bone resorption in the microdamaged sites, *i.e.*, targeted bone remodeling. However, it is necessary to investigate how these molecules would initiate and control targeted bone remodeling.

Osteocytes embedded in remodeled mature bones are flattened and extend their cytoplasmic processes perpendicularly to the longitudinal axis of bone surfaces (Fig. 1). Using biomechanical simulation analyses, not only



**Fig. 1** Silver impregnation for localization of osteocytic lacunar-canalicular system (OLCS)

A panel A shows an irregular distribution of osteocytes (ocy) and their lacuna-canalicular system (OLCS) in the primary trabeculae, while a panel B reveals the OLCS in the secondary trabeculae. Note an irregularly-arranged OLCS in the inner portion (encompassed by dotted lines) and well-oriented OLCS in the superficial layer of trabeculae in panel B. A panel C is a cortical bone featuring well-arranged OLCS: Osteocytes (ocy) are localized parallel to the longitudinal axis of trabeculae, while extending their cytoplasmic processes perpendicularly to the bone surface.

ob : osteoblast, bm : bone marrow  
Bar, 20  $\mu$ m

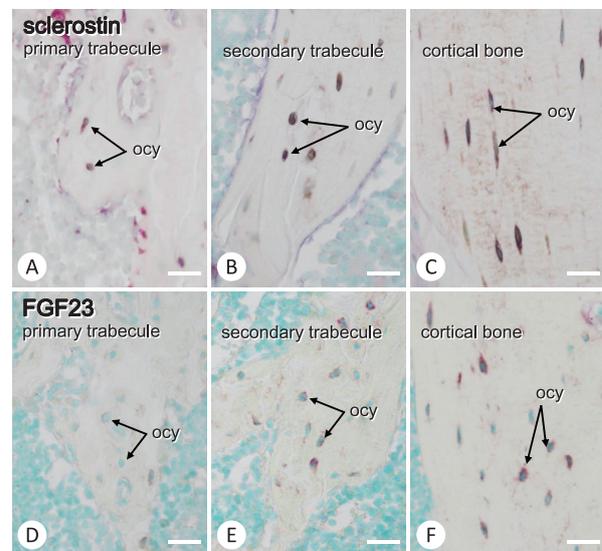
bone-lining osteoblasts, but also regularly-arranged OLCS are necessary for normal bone remodeling adapted for mechanical stress<sup>17</sup>. In cortical bone, flattened osteocytes are found among collagen bundles, which run parallel to each other and, therefore, may not disturb the seam of collagen bundles in bones. Orderly-distributed osteocytes and their cytoplasmic processes, when geometrically harmonized with the surrounding collagenous architecture, may be very effective in recognizing mechanical loading<sup>7</sup>. In addition, a regular OLCS may efficiently transport small molecules from one osteocyte to others, and to osteoblasts as well<sup>8</sup>. On the other hand, irregularly-distributed osteocytic cytoplasmic processes may not be so efficient when it comes to recognizing the direction and the intensity of mechanical loading. The notion that bone remodeling occurs as the skeleton adapts itself to its mechanical environment supports our idea that osteocytes develop a well-organized OLCS as normal bone remodeling progresses.

## 2. Osteocytes-derived factors and their involvement in bone metabolism

Osteocyte-derived factors have been recently highlighted, because these factors may reflect osteocytic function responding to mineralization in the peripheral bone matrix, regulation of bone remodeling and mineral balance in serum and so forth. DMP-1 is known as a

bone matrix protein synthesized in osteocytes, and has been assumed to play a pivotal role in mineralization of peripheral bone matrix, due to its high calcium-binding affinity<sup>18</sup>. Another osteocyte-derived molecule, sclerostin, is a glycoprotein encoded by the *sost* gene, and was reported to bind the LRP5/6 receptor, thereby antagonizing Wnt signaling and increasing  $\beta$ -catenin degradation<sup>19</sup>. Sclerostin secreted by osteocytes may pass through the osteocytic canaliculi and inhibit bone-lining osteoblasts. FGF23, which is also synthesized by osteocytes, modulates serum phosphate concentration, by co-operating with the kidney<sup>20</sup>. Thus, recently highlighted factors such as DMP-1, sclerostin and FGF23 would provide clues for better understanding for osteocytic function.

Sclerostin was reported to be abundantly synthesized in the mature bone which contains regularly-oriented OLCS, rather than in immature bone: This molecule was observed abundantly in the mature cortical bone, but not in the metaphyseal primary trabeculae beneath the growth plate of mouse femora (Fig. 2). We have examined the distribution of OLCS and sclerostin in long bones of an osteoporosis mouse model lacking osteoprotegerin



**Fig. 2** Immunolocalization of sclerostin and FGF23

Panels A-C demonstrates triple staining of sclerostin (brown color), alkaline phosphatase (blue) and tartrate-resistant acid phosphatase (red), while panels D-F show immunolocalization of FGF23 (red). Panels A, D are the primary trabeculae, B, E are obtained from secondary trabeculae, and C, F show cortical bone. Cortical bone seems to possess many osteocytes (ocy) immunoreactive for both sclerostin (C) and FGF23 (F), compared to the primary (A, D) and secondary (B, E) trabeculae.

Bar, 30  $\mu$ m

(OPG)<sup>21</sup>), which is a decoy receptor for RANKL<sup>22</sup>. *Opg*-deficient mice showed accelerated bone remodeling and an irregular distribution of OLCS. While DMP-1 was found in all osteocytes in the *opg*-deficient bone, sclerostin-immunoreactivity was significantly diminished even in the cortical bone of *opg*-deficient mice. Thus, sclerostin appears to be synthesized specifically once osteocytes possess a regular OLCS, for example in normal, mature cortical bone.

FGF23 serves as a phosphaturic agent that inhibits  $1\alpha$ -hydroxylase, *i.e.*,  $1\alpha, 25(\text{OH})_2\text{D}_3$  production, and the function of sodium/phosphate co-transporter II, which inhibits reabsorption of phosphates in the proximal renal tubules<sup>23, 24</sup>. Although *fgf23* mRNA is found in several tissues, this molecule is most abundantly expressed in osteocytes. Investigations on the biological functions of FGF23 have broadened the understanding of the systemic regulation of phosphate homeostasis, as well as the knowledge about maintaining mineralization in the bone matrix<sup>25</sup>. FGF23 appears to be synthesized mainly by osteocytes forming regularly-distributed OLCS in mature bone that had been remodeled<sup>6</sup> (Fig. 2). Alternatively, intense FGF23-immunopositivity in immature primary trabeculae with irregular OLCS could not be verified. Mature bone could, therefore, serve as an organ regulating serum phosphorus levels by secreting FGF23.

### 3. Histological abnormalities of osteocytes in *aklotho*-deficient mice

$\alpha$ Klotho was originally found to be involved in multiple aging phenotypes and age-related disorders. In a normal state, the biological functions of  $\alpha$ klotho has been reported to regulate serum calcium and inorganic phosphate homeostasis, serving as a co-factor for the FGFR1c in FGF23 signaling: Secreted FGF23 binds to the receptor complex of FGFR1c/ $\alpha$ klotho expressed in the kidney<sup>23-25</sup> (Fig. 3). The signaling linked to  $\alpha$ klotho/FGF23 is predominantly found in the proximal renal tubules, where it inhibits phosphate reabsorption and  $1\alpha$ -hydroxylase activity, thereby reducing serum phosphate and activation of  $1\alpha, 25(\text{OH})_2\text{D}_3$ . Mutations of the  $\alpha$ klotho promoter region in mice, thus called *kl/kl* mice, result in hypercalcemia and hyperphosphatemia, accompanying a syndrome that seems to considerably accelerate the multiple age-sensitive traits such as osteoporosis, skin atrophy, vascular calcification, pulmonary emphysema, gonadal dysplasia, and defective hearing in mice – all of

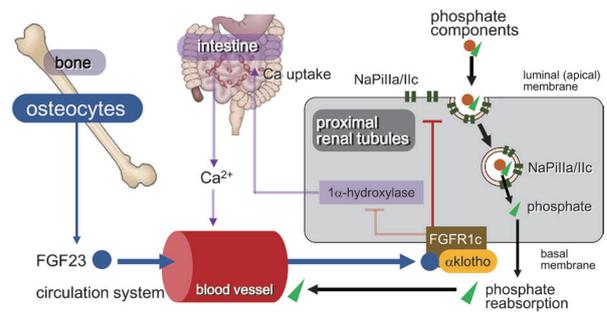


Fig. 3 Endocrine manner of FGF23 on serum concentration of phosphate

FGF23 secreted by osteocytes circulates to reach the proximal renal tubules and binds to FGFR1c/ $\alpha$ klotho receptor complex, consequently inhibiting phosphate reabsorption by NaPiIIa/IIc.

which also appear in human aging.

Hence, bone abnormalities seen in *kl/kl* mice may result from the highly elevated serum phosphate as a consequence of the defective  $\alpha$ klotho/FGF23 axis. We have histologically examined *kl/kl* mice<sup>26</sup>. Despite of high concentrations of serum calcium and phosphate, the metaphyseal trabeculae showed defective mineralization, representing an osteomalatic feature rather than osteoporosis (Fig. 4). OLCS in *kl/kl* mice was irregularly-distributed and the connectivity of cytoplasmic processes of osteocytes was very poor, so that osteocytes did not seem to form functional syncytia, which may lead to a disrupted transport of small molecules through osteocytic cytoplasmic processes and canaliculi, as well as defective sensing of mechanical loading. Electron probe microanalysis demonstrated uneven distribution of calcium and phosphorus in metaphyseal trabeculae

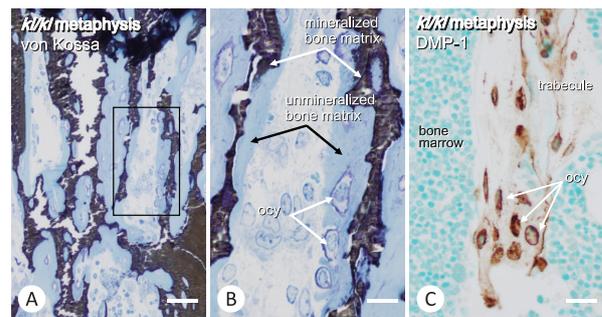


Fig. 4 Histochemical alteration of trabeculae in *kl/kl* mice. Panels A and B reveal von Kossa staining on *kl/kl* metaphyseal trabeculae. Panel B is a highly magnified image of the square of A. Many osteocytes (ocy) can be seen in broad unmineralized areas (blue color), rather than mineralized bone matrix (black) of the *kl/kl* trabeculae (B). Many osteocytes (ocy) exhibit an intense DMP-1 in *kl/kl* trabeculae. Bar, A : 50  $\mu\text{m}$ , B : 15  $\mu\text{m}$ , C : 25  $\mu\text{m}$

of *kl/kl* mice. In addition, some osteocytic lacunae were filled with a huge amount of matrix Gla proteins (MGP), DMP-1 and osteocalcin, the others had been occupied with numerous mineralized crystals, by which osteocytes seemed to be dead<sup>26</sup>. These findings suggest that osteocalcin, DMP-1 and MGP are ectopically and excessively synthesized in osteocytes as a consequence of disrupted FGF23/ $\alpha$ klotho system. It is feasible, therefore, that the elevated concentration of serum calcium and phosphate could not physicochemically accelerate mineralization in bone. This implies that bone mineralization is achieved by biological function of bone cells including osteocytes, but not physically by calcium and phosphate concentration in serum.

#### 4. Possibility on autocrine/paracrine manners of $\alpha$ klotho/FGF23 in osteocytes

There exist two kinds of klotho-deficient mice: the one is a *kl/kl* mouse induced by missense mutations in the promoter region of *aklotho* gene, and the other is a mouse with a deletion of the *aklotho* gene (*aklotho*<sup>-/-</sup> mice) generated by gene targeting technique. These two mice show the same abnormality in their bones. We raised questions which elevated serum concentration of inorganic phosphate or disrupted signaling linked to  $\alpha$ klotho/FGF23 is a predominant regulator for senescence-related degeneration in bones. In order to provide a clue for understanding this issue, we have examined histological alteration in mandibular bone of *kl/kl* mice and *aklotho*<sup>-/-</sup> mice fed with phosphate insufficient diet<sup>27</sup>.

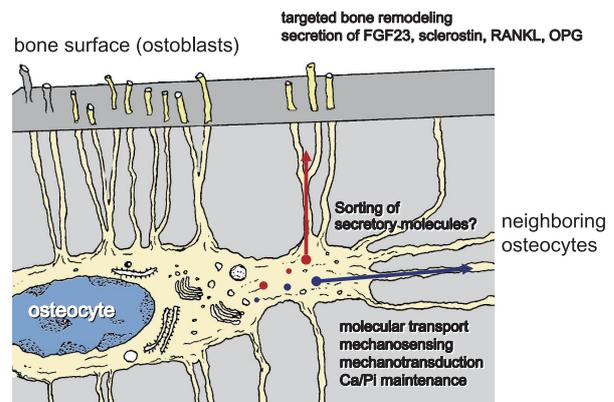
As a consequence, when fed with a low phosphate diet, *kl/kl* mice improved the osteocytic abnormalities in bone, but, *aklotho*<sup>-/-</sup> mice failed to normalize these abnormalities. When fed with low phosphate diet, the gene expression of *aklotho* was increased in kidney and bones of *kl/kl* mice but not in *aklotho*<sup>-/-</sup> mice, while the expression of FGFR1c was almost the same between *kl/kl* mice and *aklotho* mice regardless of low phosphate diet. We assume the possibility that a low phosphate concentration in serum may accelerate transcriptional activity of *aklotho* gene in *kl/kl* mice. It seems likely, therefore, that histological abnormalities of osteocytes in *kl/kl* mice have been improved by the rescued expression of *aklotho* in bones and kidney, rather than merely low concentration of serum phosphate. In contrast, since *aklotho*<sup>-/-</sup> mice have been deprived of the *aklotho* gene, a low phosphate diet did not seem to rescue the *aklotho*

gene expression.

To assess the chronological participation of FGF23 in bone metabolism, we have examined the FGF23-immunolocalization in femora at from embryonic to adult stages. As a result, FGF23 was observed mainly in osteoblasts in the embryonic and neonatal stages, but chronologically expressed in osteocytes in the young adult stages<sup>28</sup>. *Fgf23* mRNA was weakly expressed in the embryonic and neonatal stages, while *fgfr1c* and *aklotho* was strongly expressed in the age-matched femora. At the adult stages, conversely, *fgf23* expression became intense while *fgfr1c* and *aklotho* expression were reduced in the bone. Thus, it is possible that FGF23 would act in an autocrine/paracrine fashion, and therefore, the histological malformation in osteocytes and surrounding bone matrix in disrupted FGF23/ $\alpha$ klotho states appears to be, at least in part, due to not only increased concentration of phosphate, but also to defective FGF23 signaling in the bone. However, further investigation appears necessary for elucidating more detail cellular mechanism.

## Conclusion

The regularly-arranged osteocytic lacunar canalicular system, OLCS is a functional syncytia regulating molecular transport and mechanical sensing (Fig. 5). Osteocytes appears to participate in regulation of serum



**Fig. 5** Schematic design of assumed functions of an osteocyte. The regularly-arranged osteocytes build up a functional syncytia regulating molecular transport and mechanical sensing. Osteocytes appears to participate in regulation of serum concentration of phosphate by mediating the FGF23/ $\alpha$ klotho system, as well as of bone remodeling by synthesizing sclerostin, RANKL and osteoprotegerin (opg). A physiological state of osteocytes appear to be necessary for normal bone remodeling, mechanosensing, molecular transports and so forth.

concentration of phosphate by mediating the FGF23/*aklotho* system. However, in a *aklotho*-deficient state, an elevated concentration of calcium/phosphate did not result in accelerated mineralization, but induced defectively-mineralized bone matrixes. Therefore, bone cells including osteocytes, rather than serum concentration of calcium/phosphate, would regulate bone mineralization.

### Acknowledgments

This study was partially supported by grants from Japanese Society for the Promotion of Science (JSPS, Amizuka N, Hasegawa T), and Promoting International Joint Research (Bilateral Collaborations) of JSPS and National Natural Science Foundation of China (Amizuka N, Li M).

### References

- 1) Hasegawa T, Endo T, Tsuchiya E, Kudo A, Shen Z, Moritani Y, Abe M, Yamamoto T, Hongo H, Tsuboi K, Yoshida T, Nagai T, Khadiza N, Yokoyama A, de Freitas PH, Li M, Amizuka N. : Biological application of focus ion beam-scanning electron microscopy (FIB-SEM) to the imaging of cartilaginous fibrils and osteoblastic cytoplasmic processes. *J Oral Biosci* 59 : 55-62, 2016.
- 2) Shapiro F. : Variable conformation of GAP junctions linking bone cells : a transmission electron microscopic study of linear stacked linear, curvilinear, oval, and annular junctions. *Calcif Tissue Int* 61 : 285-293, 1997.
- 3) Sasaki M, Hongo H, Hasegawa T, Suzuki R, Liu Z, Freitas PHL, Oda K, Yamamoto T, Li M, Totsuka Y, Amizuka N. : Morphological aspects of the biological function of the osteocytic lacunar canalicular system and of osteocyte-derived factors. *Oral Science International* 9 : 1-8, 2012.
- 4) Burger EH, Klein-Nulend J. : Mechanotransduction in bone role of the lacuno-canalicular network. *FASEB J* 13 : 101-112, 1999.
- 5) Knothe Tate ML, Adamson JR, Tami AE, Bauer TW. : The osteocyte. *Int J Biochem Cell Biol* 36 : 1-8, 2004.
- 6) Ubaidus S, Li M, Sultana S, de Freitas PH, Oda K, Maeda T, Takagi R, Amizuka N. : FGF23 is mainly synthesized by osteocytes in the regularly distributed osteocytic lacunar canalicular system established after physiological bone remodeling. *J Electron Microsc* 58 : 381-392, 2009.
- 7) Klein-Nulend J, van der Plas A, Semeins CM, Ajubi NE, Frangos JA, Nijweide PJ, Burger EH. : Sensitivity of osteocytes to biomechanical stress in vitro. *FASEB J* 9 : 441-445, 1995.
- 8) Knothe Tate ML, Niederer P, Knothe U. : In vivo tracer transport through the lacunocanalicular system of rat bone in an environment devoid of mechanical loading. *Bone* 22 : 107-117, 1998.
- 9) Burr DB. : Targeted and nontargeted remodeling. *Bone* 30 : 2-4, 2002.
- 10) Frost HM. : Presence of microscopic cracks in vivo in bone. *Bull Henry Ford Hosp*. 8 : 25-35, 1960.
- 11) Hazenberg JG, Freeley M, Foran E, Lee TC, Taylor D. : Microdamage : A cell transducing mechanism based on ruptured osteocyte processes. *J Biomech* 39 : 2096-2103, 2006.
- 12) Verborgt O, Gibson GJ, Schaffler, MB. : Loss of osteocyte integrity in association with microdamage and bone remodeling after fatigue in vivo. *J Bone Miner Res* 15 : 60-67, 2000.
- 13) Gu G, Mulari M, Peng Z, Hentunen TA, Väänänen HK. : Death of osteocytes turns off the inhibition of osteoclasts and triggers local bone resorption. *Biochem Biophys Res Commun*. 335 : 1095-1101, 2005.
- 14) Schaffler MB, Choi K, Milgrom C. : Aging and matrix microdamage accumulation in human compact bone. *Bone* 17 : 521-525, 1995.
- 15) Mashiba T, Turner CH, Hirano T, Forwood MR, Jacob DS, Johnston CC, Burr DB. : Effects of high-dose etidronate treatment on microdamage accumulation and biomechanical properties in beagle bone before occurrence of spontaneous fractures. *Bone* 29 : 271-278, 2001.
- 16) Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, Bonewald LF, Kodama T, Wutz A, Wagner EF, Penninger JM, Takayanagi H. : Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med* 17 : 1231-1234, 2011.
- 17) Huiskes R, Ruimerman R, van Lenthe GH, Janssen JD. : Effects of mechanical forces on maintenance and adaptation of form in trabecular bone. *Nature* 405 : 704-706, 2000.
- 18) Toyosawa S, Shintani S, Fujiwara T, Ooshima T, Sato A, Ijuhin N, Komori T. : Dentin matrix protein 1 is predominantly expressed in chicken and rat osteocytes but not in osteoblasts. *J Bone Miner Res*

- 16 : 2017-2026, 2001.
- 19) Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, Shpektor D, Jonas M, Kovacevich BR, Staehling-Hampton K, Appleby M, Brunkow ME, Latham JA. : Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J* 22 : 6267-6276, 2003.
- 20) Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. : Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* 444 : 770-777, 2006.
- 21) Masuki H, Li M, Hasegawa T, Suzuki R, Ying G, Zhusheng L, Oda K, Yamamoto T, Kawanami M, Amizuka N. : Immunolocalization of DMP1 and sclerostin in the epiphyseal trabecule and diaphyseal cortical bone of osteoprotegerin deficient mice. *Biomed Res* 31 : 307-318, 2010.
- 22) Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. : Osteoprotegerin : a novel secreted protein involved in the regulation of bone density. *Cell* 89 : 309-319, 1997.
- 23) ADHR Consortium. : Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet* 26 : 345-348, 2000.
- 24) Shimada T, Mizutani S, Muto T, Yoneya T, Hino R, Takeda S, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T. : Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. *Proc Natl Acad Sci USA* 98 : 5945-5946, 2001.
- 25) Fukumoto S. : The role of bone in phosphate metabolism. *Mol Cell Endocrinol* 310 : 63-70, 2009.
- 26) Sasaki M, Hasegawa T, Yamada T, Hongo H, de Freitas PH, Suzuki R, Yamamoto T, Tabata C, Toyosawa S, Yamamoto T, Oda K, Li M, Inoue N, Amizuka N. : Altered distribution of bone matrix proteins and defective bone mineralization in klotho-deficient mice. *Bone* 57 : 206-219, 2013.
- 27) Hikone K, Hasegawa T, Tsuchiya E, Hongo H, Sasaki M, Yamamoto T, Kudo A, Oda K, Haraguchi M, de Freitas PH, Li M, Iida J, Amizuka N. : Histochemical examination on periodontal tissues of klotho-deficient mice fed with phosphate-insufficient diet. *J Histochem Cytochem* 65 : 207-221, 2017.
- 28) Sakurai A, Hasegawa T, Kudo A, Shen Z, Nagai T, Abe M, Yoshida T, Hongo H, Yamamoto T, Yamamoto T, Oda K, Freitas de PHL, Li M, Sano H, Amizuka N. : Chronological immunolocalization of sclerostin and FGF23 in metaphyseal trabeculae and cortical bone in mice. *Biomed Res in press*.