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Inorganic polyphosphates stimulate FGF23 expression through the FGFR pathway

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ABSTRACT

Polyphosphate (polyP) is composed of linear polymers of orthophosphate residues linked by high-energy phosphoanhydride bonds. It has been reported to improve osteoblastic differentiation, stimulate periodontal tissue regeneration, and accelerate bone repair. The aim of this study was to evaluate the effect of polyP on the expression of FGF23, a hormone secreted mostly be mature osteoblasts and osteocytes. In this study, different types of polyP were synthesized and co-cultured with osteoblast-like UMR-106 cells. Real-time PCR and western blot were used to analyze the gene and protein expression of FGF23. We found that 1 mM polyP was able to increase FGF23 expression after 4 h, reaching a peak after 12–24 h, with expression decreasing by 48 h. We also found that polyP could activate the FGFR pathway, as evidenced by increased phosphorylation of FGFR, FRS2, and Erk1/2. When FGFR signaling was inhibited by the specific inhibitor SU5402, the effect of polyP on FGF23 expression was significantly reduced. Our results indicate that polyP is able to stimulate osteoblastic FGF23 expression and that this effect is associated with activation of the FGFR pathway. These findings provide support for the clinical use of polyP by indicating a mechanism for polyP in bone regeneration.

1. Introduction

Inorganic polyphosphate (polyP), a material found abundantly in nature, is a linear polymer consisting of many orthophosphate residues linked by energy-rich phosphoanhydride bonds [1,2]. PolyP has been found in the brain, liver, peripheral blood mononuclear cells, blood plasma, erythrocytes, gingival fibroblasts and osteoblasts [3–6]. Interestingly, the levels of polyP in human osteoblasts have been shown to be relatively high [4,5].

We and others have previously reported that polyP is a biomaterial useful in the acceleration of bone defect repair and periodontal tissue regeneration [7–9], and it can be used as a stimulant of bone formation [10,11]. Recent studies have shown that treatment with polyP can improve the proliferation, differentiation and mineralization of osteoblasts andmesenchymal stem cells through the facilitation of FGFR signaling [2,12–14].

FGF23 is a hormone secreted mostly by bone [15,16] that plays a key role in mineral ion homeostasis [17,18]. Altered FGF23 function leads to human disorders associated with improper phosphate wasting or retention, such as rickets or ectopic calcification [18–21]. In addition to its function in regulating mineral ion metabolism, FGF23 is also recognized as an inhibitor of mineralization. Wang et al. [22] showed that adenoviral overexpression of FGF23 in rat calvarial cells inhibits bone mineralization independent of its systemic effects on phosphate homeostasis. This observation was supported by later reports, which showed that FGF23 treatment of osteoblasts led to an inhibition of mineralization [23,24]. Moreover, genetically altered mice in which FGF23 activity is lost exhibit severe skeletal defects [25,26].

Most recently, studies have shown that FGF23 is a specific target gene of FGFR signaling [27,28]. As polyP is able to regulate the FGFR pathway [14,29], we hypothesized that polyP is able to stimulate FGF23 expression through the FGFR pathway. To test our hypothesis, we treated UMR-106 osteoblast cells with different types of polyP and analyzed the expression of FGF23 using real-time PCR and western blot. The effect of polyP on the FGFR pathway and its relationship to FGF23 expression were also evaluated.

2. Materials and methods

2.1. Materials

All types of polyP were obtained from Regenetiss Inc. (Tokyo, Japan). Sodium salt polyPs with an average chain length of 15

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(short length, polyP-S), 60 (medium length, polyP-M) and 110 (long length, polyP-L) phosphate residues were used. The concentration of polyP was calculated in terms of phosphate residues. $NaPO_4$ buffer, adjusted to pH 7.0 by mixing equal concentrations of Na_2HPO_4 and NaH_2PO_4 solutions, was used as a control. Osteoblast-like cells (UMR-106) were purchased from ATCC (Manassas, VA). Eagle's minimum essential medium alpha modification (α-MEM) and fetal bovine serum (FBS) were obtained from Gibco (Gaithersburg, MD, USA). Penicillin–Streptomycin mix was purchased from Invitrogen Life Technologies (Baltimore, MD, USA). Antibodies for western blot were obtained from Cell Signaling (Danvers, MA), with the exceptions ofanti-FRS2 (Sigma, St. Louis, MO) andanti-FGF23 (Santa Cruz, CA, USA). FGF9 and SU5402 were obtained from Prospec (East Brunswick, NJ, USA) and Biovision (Mountain View, CA, USA), respectively.

2.2. Cell proliferation assay

UMR-106 osteoblast-like cells were seeded on 96-well culture plates (Falcon) at a density of 3000 cells/well and incubated in α-MEM supplemented with 10% FBS and 1% Penicillin–Streptomycin. After approximately 8 h to allow for initial attachment, the culture medium was aspirated and the cells were treated with fresh culture medium containing 1 mM polyP of different chain-lengths or 1 mM $NaPO_4$ for 48 h. The relative number of viable cells in each well was determined using Cell Proliferation Reagent WST-1 (Roche, Indianapolis, IN, USA). Briefly, 10 μl of reagent was added to each well, including three wells containing only medium for background subtraction. After incubation at 37 °C for 1 h, the absorbance at 450 nm was measured using a microplate reader (HTS 7000p1us, PerkinElmer).

2.3. RNA extraction and quantitative real-time RT-PCR

Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) strictly according to the manufacturer's instructions. The concentration of freshly isolated RNA was determined by measurement of absorbance at 260 nm, and the purity was calculated using A260/A280. First-strand cDNA was synthesized from 2 μg of RNA using a SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, Baltimore, MD, USA) and used for quantitative real-time PCR. Expression of FGF23 was quantified with an ABI Prism 7300 Sequence Detection System and TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The cycler was programmed to run at 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The relative expression levels of genes were analyzed using the $2^{-\Delta\Delta Ct}$ method and normalized using GAPDH as a control, with results presented in fold increase relative to the control.

2.4. Western blot

Cells were grown in 100-mm dishes to 80% confluence and then treated with polyP for 24 h. The total protein was isolated using a RIPA buffer (Pierce Biotechnology, Rockford, IL, USA) supplemented with protease andphosphatase inhibitors (Roche Applied Science, Penzberg, Germany). Samples were heated at 95 °C for 5 min in sample buffer containing 2% SDS and 1% 2-mercaptoethanol and were subjected to 10% SDS–polyacrylamide gel electrophoresis. The separated proteins were transferred from the gel *via* electrophoresis to nitrocellulose membranes. After incubation in blocking solution, the membranes were treated with diluted rabbit FGF23 antibody (1:2000), rabbit phospho-ERK1/2 (p-ERK1/2) antibody (1:1000), total ERK1/2 antibody (1:1000; Assay Biotechnology), phospho-FRS2antibody (1:1000; Assay Biotechnology), total FRS-2antibody (1:1000; Assay Biotechnology), or β-actin antibody (1:2000) overnight. Horseradish peroxidase-conjugated IgG was used as the secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), and signals were detected using SuperSignal West PicoChemiluminescent Substrate System (Pierce, Rockford, IL, USA).

2.5. Statistics

Statistically significant differences were assessed by ANOVA followed by Tukey's multiple comparisons. All values were expressed as the mean ± SD. A p value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. PolyP treatment stimulates FGF23 expression

To test the effects of polyP on FGF23 expression, we first treated UMR-106 cells with 1 mM polyP-S, polyP-M, or polyP-L for 48 h. As shown in Fig. 1A, polyP-S, polyP-M and polyP-L each induced increases in FGF23 expression of 4.01 ± 0.73, 8.16 ± 1.32 and 35.75 ± 2.58-fold, respectively, while $NaPO_4$ failed to upregulate the expression of FGF23. From these results, we conclude that the rise in FGF23 expression levels correlates with an increase in phosphate chain length.

We then evaluated the proliferation of UMR-106 cells and found that there was no significant change in growth according to OD readings after $NaPO_4$ or polyP treatment with the exception of polyP-L, which caused a decrease in proliferation. This finding suggests that 1 mM polyP-L is toxic to the cells; therefore, we selected polyP-M for use in further experiments.

We next tested the effects of different concentrations of polyP on the expression of FGF23. UMR-106 cells were treated with 0.1 mM, 1 mM or 5 mM polyP-M for 24 h. As shown in Fig. 2A and B, all three concentrations of polyP-M significantly increased the expression of FGF23. Among these concentrations, 1 mM polyP-M induced the highest increase in FGF23 expression at 23.62 ± 3.08-fold over the control.

We further compared the expression of FGF23 at different time points. As shown in Fig. 2C and D, FGF23 expression was significantly elevated after 4 h of treatment, reached its peak value after 12–24 h, and then dropped off at 48 h.

3.2. PolyP treatment activates FGFR signaling

To determine if polyP increases FGF23 expression through FGFR signaling, cells were treated with 1 mM polyP for 24 h, and then whole proteins were collected for western blot analysis. As shown in Fig. 3, polyP, but not phosphate, markedly increased the phosphorylation of the FGF receptor (FGFR). We further investigated the changes in signaling downstream of FGFR and found that phosphorylation of FRS2 and ERK1/2 was also elevated, indicating that polyP is able to activate FGFR signaling.

3.3. Inhibition of FGFR signaling blunts the effect of polyP treatment on FGF23 expression

To inhibit FGFR1 signaling, cells were incubated with the FGFR1-specific inhibitor SU5402 at a concentration of 20 μM overnight before polyP treatment. As shown in Fig. 4A, SU5402 alone significantly reduced the expression of FGF23 in UMR-106 cells. Moreover, the effect of polyP on FGF23 expression was largely blunted in the presence of SU5402. Expression was more than 10-fold lower than that observed with polyP treatment alone, indicating that FGFR signaling is a key pathway by which polyP

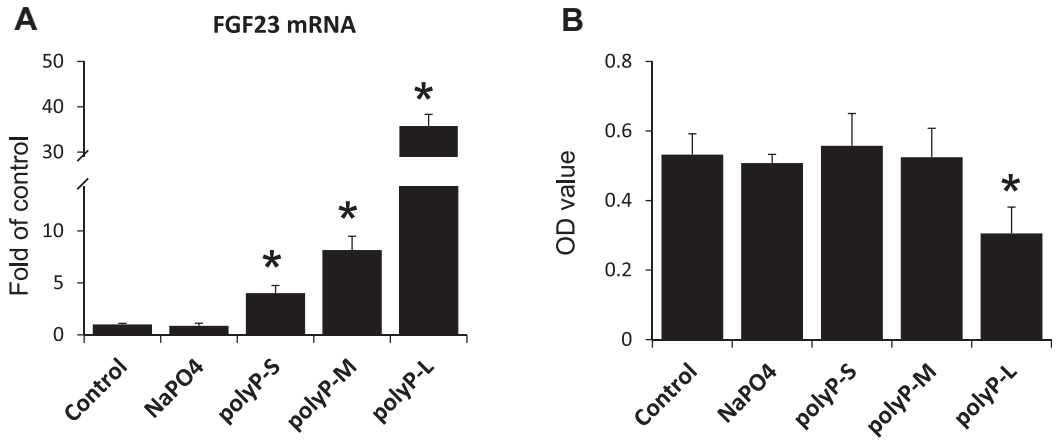

Fig. 1. The effects of different phosphate chain lengths of polyP on FGF23 expression and proliferation. (A) Gene expression of FGF23 after polyP treatment for 48 h. (B) OD values of osteoblasts after polyP treatment for 48 h. $^{*}p < 0.05$.

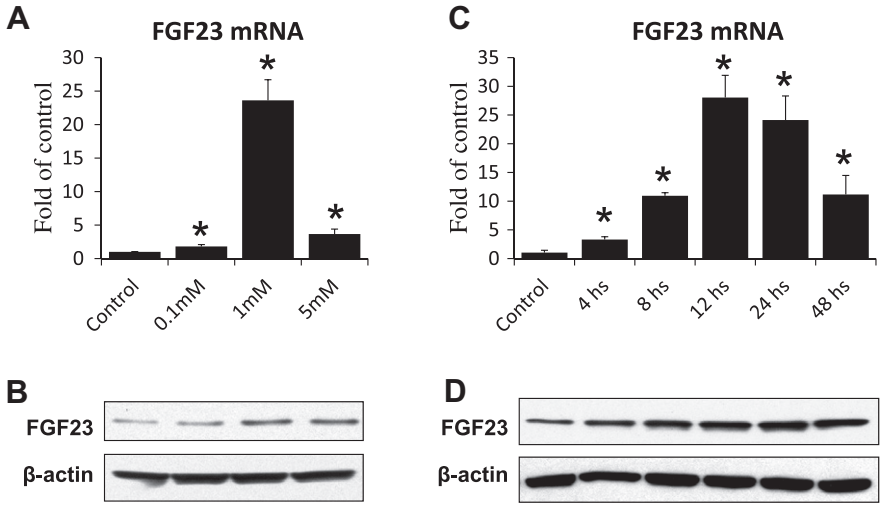

Fig. 2. Gene and protein expression of FGF23. (A, B) The effects of different concentrations of polyP on FGF23 expression. (C, D) The effect of polyP on FGF23 expression at different time points. $^{*}p < 0.05$.

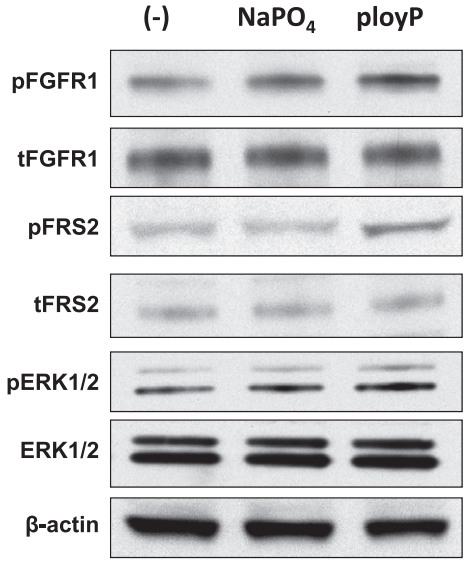

Fig. 3. Activation of FGFR signaling by polyP.

regulates FGF23 expression. We also used FGF9 as a positive control and observed similar results. These observations were further confirmed by western blot analysis (Fig. 4B).

4. Discussion

In this study, we showed that treatment of UMR-106 cells with polyP stimulates an increase in FGF23 expression and activates the FGFR pathway. Moreover, blocking the FGFR pathway significantly inhibited polyP-induced FGF23 expression, indicating that the effect of polyP is accomplished at least partially through FGFR signaling. Although the results show that polyP can activate FGFR signaling and induce FGF23 expression, the mechanism of how FGFR is activated by polyP is still unclear. One possibility is that polyP enhances the activity of growth factors present either in the culture medium or released from the cultured cells themselves. Shiba et al. [29] reported that polyP modulates FGF activity by stabilizing FGFs and facilitating their binding to FGFR. Our previous study also demonstrated that combined use of polyP and FGF2 improved osteoblastic proliferation, differentiation and mineralization *in vitro* and accelerated bone regeneration *in vivo* [8];

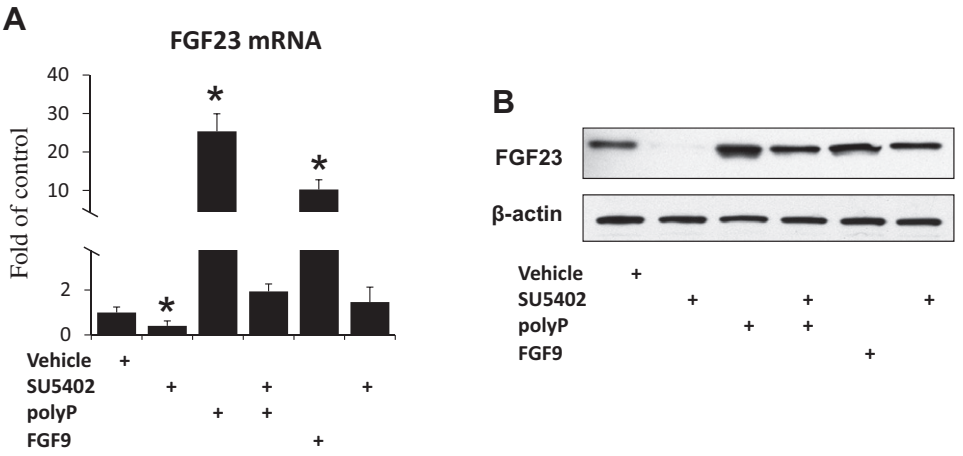

Fig. 4. Blocking FGFR signaling blunts FGF23 expression. (A) Gene expression and (B) protein expression. $^{*}p < 0.05$.

however, the involvement of other signaling pathways cannot be excluded.

FGF23 is a key regulator of mineral ion homeostasis [17,18]. PolyP treatment increases FGF23 secretion, suggesting that polyP may be involved in mineral ion metabolism. PolyP accumulates, especially in osteoblasts, and hydrolyzes locally at distinct sites on the plasma membrane, resulting in a release of phosphate [4]. Moreover, a very recent study showed that polyP could modulate the intracellular calcium levels in osteoblasts [30]. FGF23 also plays an important role in inhibiting osteoblastic mineralization [22–24]; the expression of FGF23 increases during the process of mineralization. In contrast, polyP accelerates the deposit of calcium and phosphate and exhibits a very strong effect on the induction of mineralization [2]. Therefore, the observed increase in FGF23 expression may be the result of a negative feedback loop to inhibit polyP-induced mineralization.

Previous studies have shown that bone is the main source of FGF23 [15,16] and that mature osteoblasts and osteocytes are the major producers of secreted FGF23. Prior to this study, we screened the endogenous mRNA levels of FGF23 in several osteoblastic linages, including primary calvarial osteoblasts, MC3T3-E1, UMR-106, ROS17/2.8, MG-63 cells and an osteocyte-like cell line, MLO-Y4. The FGF23 mRNA levels in these cells were very low to undetectable with the exception of UMR-106 cells; therefore, we chose UMR-106 cells for our experiments. The UMR-106 cell line is a clonal derivative of a transplantable rat osteogenic sarcoma. The cells retain many of the characteristics of osteoblasts and are widely used in the field of bone research.

In this study, we used three types of polyP. The average phosphate residue chain lengths were 15 (short length, polyP-S), 60 (medium length, polyP-M) and 110 (long length, polyP-L). Our results showed that all three types of polyP were effective in inducing FGF23 expression and that this effect becomes more pronounced as the chain length increases. However, polyP-L caused significant cell death after co-culture for 2 days; thus, we selected polyP-M for further experiments. Additionally, the average phosphate chain length of polyP-M is closest to the phosphate chain length of the polyP found in humans [31,32].

In summary, we successfully demonstrated that polyP, an inorganic biomaterial used for bone regeneration, is able to stimulate FGF23 expression through activation of the FGFR pathway, suggesting a role for polyP in modulating mineral ion metabolism. These findings provide support for the clinical use of polyP, indicating a mechanism for polyP in bone regeneration. However, the direct molecular interaction between polyP and FGFR will require further investigation.

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References

[1] M.R. Brown, A. Kornberg, Inorganic polyphosphate in the origin and survival of species, Proc. Natl. Acad. Sci. USA 101 (2004) 16085–16087.
[2] Y. Kawazoe, T. Shiba, R. Nakamura, A. Mizuno, K. Tsutsumi, T. Uematsu, M. Yamaoka, M. Shindoh, T. Kohgo, Induction of calcification in MC3T3-E1 cells by inorganic polyphosphate, J. Dent. Res. 83 (2004) 613–618.
[3] K.D. Kumble, A. Kornberg, Inorganic polyphosphate in mammalian cells and tissues, J. Biol. Chem. 270 (1995) 5818–5822.
[4] G. Leyhausen, B. Lorenz, H. Zhu, W. Geurtsen, R. Bohnensack, W.E. Muller, H.C. Schroder, Inorganic polyphosphate in human osteoblast-like cells, J. Bone Miner. Res. 13 (1998) 803–812.
[5] H.C. Schroder, L. Kurz, W.E. Muller, B. Lorenz, Polyphosphate in bone, Biochemistry (Mosc.) 65 (2000) 296–303.
[6] H.C. Schroder, B. Lorenz, L. Kurz, W.E. Muller, Inorganic polyphosphate in eukaryotes: enzymes, metabolism and function, Prog. Mol. Subcell Biol. 23 (1999) 45–81.
[7] K. Morita, K. Doi, T. Kubo, R. Takeshita, S. Kato, T. Shiba, Y. Akagawa, Enhanced initial bone regeneration with inorganic polyphosphate-adsorbed hydroxyapatite, Acta Biomater. 6 (2010) 2808–2815.
[8] Q. Yuan, T. Kubo, K. Doi, K. Morita, R. Takeshita, S. Katoh, T. Shiba, P. Gong, Y. Akagawa, Effect of combined application of bFGF and inorganic polyphosphate on bioactivities of osteoblasts and initial bone regeneration, Acta Biomater. 5 (2009) 1716–1724.
[9] M. Yamaoka, T. Uematsu, T. Shiba, T. Matsuura, Y. Ono, M. Ishizuka, H. Naramoto, M. Takahashi, M. Sugiura-Tomita, K. Iguchi, S. Yamashita, K. Furusawa, Effect of inorganic polyphosphate in periodontitis in the elderly, Gerodontology 25 (2008) 10–17.
[10] Y. Hacchou, T. Uematsu, O. Ueda, Y. Usui, S. Uematsu, M. Takahashi, T. Uchihashi, Y. Kawazoe, T. Shiba, S. Kurihara, M. Yamaoka, K. Furusawa, Inorganic polyphosphate: a possible stimulant of bone formation, J. Dent. Res. 86 (2007) 893–897.
[11] X Wang, H.C. Schroder, M. Wiens, H. Ushijima, W.E. Muller, Bio-silica and bio-polyphosphate: applications in biomedicine (bone formation), Curr. Opin. Biotechnol. (2012).
[12] W.E. Muller, X. Wang, B. Diehl-Seifert, K. Kropf, U. Schlossmacher, I. Lieberwirth, G. Glasser, M. Wiens, H.C. Schroder, Inorganic polymeric phosphate/polyphosphate as an inducer of alkaline phosphatase and a modulator of intracellular Ca^{2+} level in osteoblasts (SaOS-2 cells) *in vitro*, Acta Biomater. 7 (2011) 2661–2671.
[13] D. Morimoto, T. Tomita, S. Kuroda, C. Higuchi, S. Kato, T. Shiba, H. Nakagami, R. Morishita, H. Yoshikawa, Inorganic polyphosphate differentiates human mesenchymal stem cells into osteoblastic cells, J. Bone Miner. Metab. 28 (2010) 418–423.
[14] Y. Kawazoe, S. Katoh, Y. Onodera, T. Kohgo, M. Shindoh, T. Shiba, Activation of the FGF signaling pathway and subsequent induction of mesenchymal stem cell differentiation by inorganic polyphosphate, Int. J. Biol. Sci. 4 (2008) 37–47.
[15] Y. Yoshiko, H. Wang, T. Minamizaki, C. Ijuin, R. Yamamoto, S. Suemune, K. Kozai, K. Tanne, J.E. Aubin, N. Maeda, Mineralized tissue cells are a principal source of FGF23, Bone 40 (2007) 1565–1573.

[16] M. Mirams, B.G. Robinson, R.S. Mason, A.E. Nelson, Bone as a source of FGF23: regulation by phosphate?, Bone 35 (2004) 1192–1199
[17] C. Bergwitz, H. Juppner, Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23, Annu. Rev. Med. 61 (2010) 91–104.
[18] ADHR Consortium, Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23, Nat. Genet. 26 (2000) 345–348.
[19] K. Goji, K. Ozaki, A.H. Sadewa, H. Nishio, M. Matsuo, Somatic and germlinemosaicism for a mutation of the PHEX gene can lead to genetic transmission of X-linked hypophosphatemic rickets that mimics an autosomal dominant trait., J. Clin. Endocrinol. Metab. (2005).
[20] J.Q. Feng, L.M. Ward, S. Liu, Y. Lu, Y. Xie, B. Yuan, X. Yu, F. Rauch, S.I. Davis, S. Zhang, H. Rios, M.K. Drezner, L.D. Quarles, L.F. Bonewald, K.E. White, Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism, Nat. Genet. 38 (2006) 1310–1315.
[21] O. Topaz, D.L. Shurman, R. Bergman, M. Indelman, P. Ratajczak, M. Mizrachi, Z. Khamaysi, D. Behar, D. Petronius, V. Friedman, I. Zelikovic, S. Raimer, A. Metzker, G. Richard, E. Sprecher, Mutations in GALNT3, encoding a protein involved in O-linked glycosylation, cause familial tumoralcalcinosis, Nat. Genet. 36 (2004) 579–581.
[22] H. Wang, Y. Yoshiko, R. Yamamoto, T. Minamizaki, K. Kozai, K. Tanne, J.E. Aubin, N. Maeda, Overexpression of fibroblast growth factor 23 suppresses osteoblast differentiation and matrix mineralization *in vitro*, J. Bone Miner. Res. 23 (2008) 939–948.
[23] D. Sitara, S. Kim, M.S. Razzaque, C. Bergwitz, T. Taguchi, C. Schuler, R.G. Erben, B. Lanske, Genetic evidence of serum phosphate-independent functions of FGF-23 on bone, PLoS Genet. 4 (2008) e1000154.
[24] V. Shalhoub, S.C. Ward, B. Sun, J. Stevens, L. Renshaw, N. Hawkins, W.G. Richards, Fibroblast growth factor 23 (FGF23) and alpha-klotho stimulate osteoblastic MC3T3.E1 cell proliferation and inhibit mineralization, Calcif. Tissue Int. 89 (2011) 140–150.
[25] Q. Yuan, D. Sitara, T. Sato, M. Densmore, H. Saito, C. Schuler, R.G. Erben, B. Lanske, PTH ablation ameliorates the anomalies of FGF23-deficient mice by suppressing the elevated vitamin D and calcium levels, Endocrinology 152 (2011) 4053–4061.
[26] D. Sitara, M.S. Razzaque, M. Hesse, S. Yoganathan, T. Taguchi, R.G. Erben, H. Juppner, B. Lanske, Homozygous ablation of fibroblast growth factor-23 results in hyperphosphatemia and impaired skeletogenesis, and reverses hypophosphatemia in Phex-deficient mice, Matrix Biol. 23 (2004) 421–432.
[27] S. Wohrle, O. Bonny, N. Beluch, S. Gaulis, C. Stamm, M. Scheibler, M. Muller, B. Kinzel, A. Thuery, J. Brueggen, N.E. Hynes, W.R. Sellers, F. Hofmann, D. Graus-Porta, FGF receptors control vitamin D and phosphate homeostasis by mediating renal FGF-23 signaling and regulating FGF-23 expression in bone, J. Bone Miner. Res. 26 (2011) 2486–2497.
[28] A. Martin, S. Liu, V. David, H. Li, A. Karydis, J.Q. Feng, L.D. Quarles, Bone proteins PHEX and DMP1 regulate fibroblastic growth factor FGF23 expression in osteocytes through a common pathway involving FGF receptor (FGFR) signaling, FASEB J. 25 (2011) 2551–2562.
[29] T. Shiba, D. Nishimura, Y. Kawazoe, Y. Onodera, K. Tsutsumi, R. Nakamura, M. Ohshiro, Modulation of mitogenic activity of fibroblast growth factors by inorganic polyphosphate, J. Biol. Chem. 278 (2003) 26788–26792.
[30] W.E. Muller, X. Wang, B. Diehl-Seifert, K. Kropf, U. Schlossmacher, I. Lieberwirth, G. Glasser, M. Wiens, H.C. Schroder, Inorganic polymeric phosphate/polyphosphate as an inducer of alkaline phosphatase and a modulator of intracellular Ca^{2+} level in osteoblasts (SaOS-2 cells) *in vitro*, Acta Biomater. 7, 2011, 2661–2671.
[31] F. Muller, N.J. Mutch, W.A. Schenk, S.A. Smith, L. Esterl, H.M. Spronk, S. Schmidbauer, W.A. Gahl, J.H. Morrissey, T. Renne, Platelet polyphosphates are proinflammatory and procoagulant mediators *in vivo*, Cell 139 (2009) 1143–1156.
[32] F.A. Ruiz, C.R. Lea, E. Oldfield, R. Docampo, Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes, J. Biol. Chem. 279 (2004) 44250–44257.