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REVIEW ARTICLE

IFITM5 mutations and osteogenesis imperfecta

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Abstract Interferon-induced transmembrane protein 5 (IFITM5) is an osteoblast-specific membrane protein that has been shown to be a positive regulatory factor for mineralization in vitro. However, *Ifitm5* knockout mice do not exhibit serious bone abnormalities, and thus the function of IFITM5 in vivo remains unclear. Recently, a single point mutation (c.-14C>T) in the 5′ untranslated region of *IFITM5* was identified in patients with osteogenesis imperfecta type V (OI-V). Furthermore, a single point mutation (c.119C>T) in the coding region of *IFITM5* was identified in OI patients with more severe symptoms than patients with OI-V. Although *IFITM5* is not directly involved in the formation of bone in vivo, the reason why *IFITM5* mutations cause OI remains a major mystery. In this review, the current state of knowledge of OI pathological mechanisms due to *IFITM5* mutations will be reviewed.

Keywords IFITM5 · Heterozygous mutation · Osteogenesis imperfecta type V

Introduction

IFITM5 (also known as BRIL) is a member of the interferon-induced transmembrane (IFITM) protein family. There are 5 family members in humans (IFITM1, 2, 3, 5, and 10) and 7 in mice (IFITM1, 2, 3, 5, 6, 7, and 10). IFITM1, 2, and 3 are induced by interferon and participate in a protective mechanism against viral infections [1, 2]. IFITM5 is not induced by interferon and thus does not participate in a protection mechanism against viruses [3, 4]. IFITM5's function has been investigated primarily by Moffatt's group and our group. IFITM5 was identified by high-throughput screening for cDNA encoding secretory and membrane proteins in osteoblasts [5]. Subsequently, global gene expression analysis of cultured murine osteoblasts suggested that *Ifitm5* (murine IFITM5 gene) was expressed during mineralization [6]. Other IFITM members localize to the endosome compartment [4, 7, 8], but IFITM5 localizes to the cell membrane [9]. IFITM5 was thought to have two transmembrane regions and a type III (N-out/C-out) topology [9], but recently it was shown to have one transmembrane region and a type II (N-in/C-out) topology, and to localize to the endoplasmic reticulum and cell membrane (Fig. 1) [10]. Conserved cysteine at residues 52, 53, and 86 of murine IFITM5 (C50, C51, and C84 in human IFITM5) is palmitoylated and functions as intramembrane anchors (Fig. 1) [10]. Furthermore, palmitoylation of C52 and C53 is important for targeting IFITM5 to the cell membrane and for its stability [10].

Distinct from the other IFITM members, which show ubiquitous expression, IFITM5 is expressed specifically in the initial stage of osteoblast mineralization [9, 11]. In in vitro studies, *Ifitm5* overexpression in osteoblasts promotes mineralization and, conversely, *Ifitm5* knockdown by short hairpin RNA suppresses mineralization. Thus, IFITM5 was suggested as a positive mineralization regulatory factor [9]. However, *Ifitm5* knockout mice (*Ifitm5*$^{-/-}$) did not show any major abnormalities in osteogenesis (Fig. 2a) [11]. Rarely, long bones in *Ifitm5* knockout mice exhibited bending (Fig. 2b). However, most

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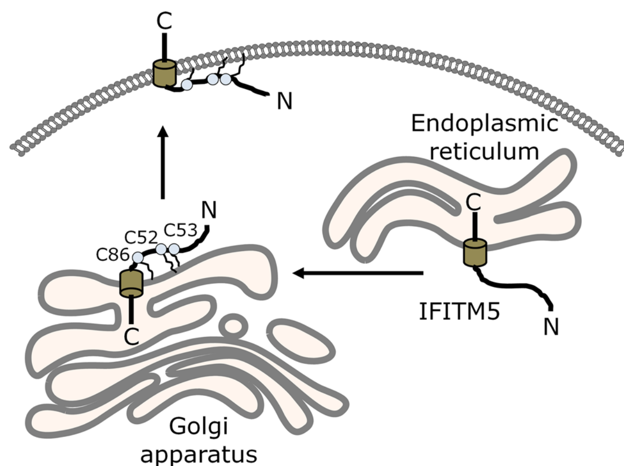

Fig. 1 Topology and localization of murine IFITM5. IFITM5 is localized to the endoplasmic reticulum and then transported to the Golgi apparatus. *S*-Palmitoylation at C52, C53, and C86 occurs either in the endoplasmic reticulum and/or the Golgi apparatus. The palmitoylated IFITM5 is finally targeted to the plasma membrane. Reproduced with permission [10]

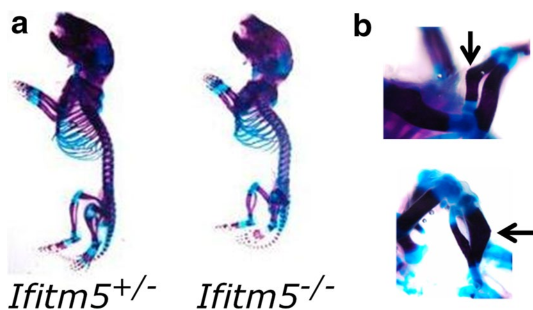

Fig. 2 Skeletal phenotype of heterozygous and homozygous *Ifitm5*-deficient newborn mice. **a** No significant change in skeletal phenotype is observed between heterozygous and homozygous *Ifitm5*-deficient newborn mice. **b** Bending of long bones is rarely observed in homozygous *Ifitm5*-deficient mice. Reproduced with permission [11]

IFITM5 knockout mice did not show these abnormalities [11]. These results collectively suggested that IFITM5 does not function as a positive regulator of mineralization in vivo.

In 2012, one year after our group published a study on *Ifitm5* knockout mice bone phenotype, it was reported that patients with osteogenesis imperfecta type V (OI-V) presented mutations in *IFITM5* (the human IFITM5 gene) [12, 13]. Furthermore, in 2014, different mutations in *IFITM5* were found in OI patients with more severe symptoms than patients with OI-V [14–16]. From these findings arose the question of how mutations in *IFITM5*, previously thought to have no function in mineralization in vivo, can induce OI.

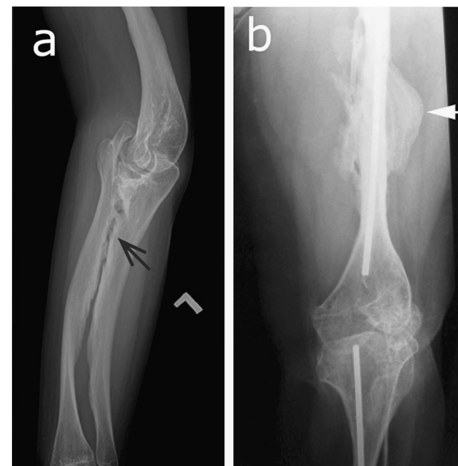

Fig. 3 Radiographs from an OI-V patient. **a** Forearm interosseous membrane calcification. **b** Hyperplastic callus in femur. Reproduced with permission [15]

The clinical phenotype of OI-V is distinct from other OI

OI is a hereditary connective tissue disorder characterized by bone fragility, and can be categorized into at least 12 subtypes [17]. In autosomal dominant OI, most cases are caused by defects in the *COLIA1* or *COLIA2* genes [18–20]. These mutations affect the basic structure or the synthesis levels of type I collagen [21–26]. On the other hand, autosomal recessive OI is caused by defects in genes that affect the stability of the structure of type I collagen (*CRTAP*, *LEPREI1*, *PPIB*, *SERPINH1*, *FKBP10*, and *BMP1*) [27–34]. In addition to these causative genes, it has been shown that mutations of *SERPINF1*, *SP7*, *TMEM38B*, and *WNT1*, whose productions are not involved in the stability of collagen structure, also cause autosomal recessive OI [35–42]. OI caused by mutations of *SP7*, *TMEM38B*, and *WNT1* are as yet unclassified [17].

OI-V is an autosomal dominant OI as defined by Glorieux et al. [43]. Less than 5 % of patients with OI are diagnosed with OI-V [44]. The OI-V clinical phenotype differs from other OI, and is characterized by forearm interosseous membrane calcification and hyperplastic callus formation (Fig. 3). However, OI-V exhibits a broad inter-patient disease severity. Forearm interosseous membrane calcification is observed in essentially all patients and it is associated with a limitation in forearm supination/pronation [12, 15, 43, 45–49]. Hyperplastic callus formation occurs during the healing period of bone fractures, but is not observed in all patients [12, 15, 43, 45–49]. Radial head dislocation is observed in many patients and is a cause of elbow deformity [12, 37, 43, 48, 49]. Furthermore, features such as a radiographically dense band of forearm metaphyses [43,

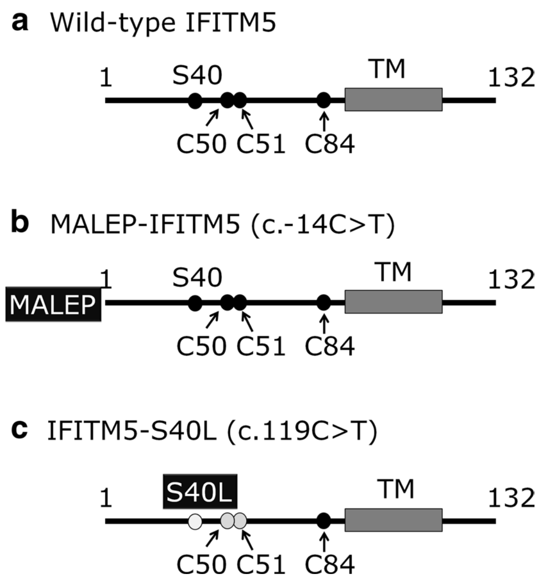

Fig. 4 Mutants of human IFITM5. **a** Wild-type IFITM5. C50, C51, and C84 are palmitoylated. **b** IFITM5 mutant produced by c.-14C>T mutation. Five amino acid residues (MALEP) are added at the N-terminus. Palmitoylation at C50, C51, and C84 normally occurs. **c** IFITM5 mutant produced by c.119C>T mutation. The 40th serine residue is substituted by a leucine residue (S40L). C50 and C51 are predicted not to be palmitoylated, resulting in localization in the endoplasmic reticulum but not the plasma membrane

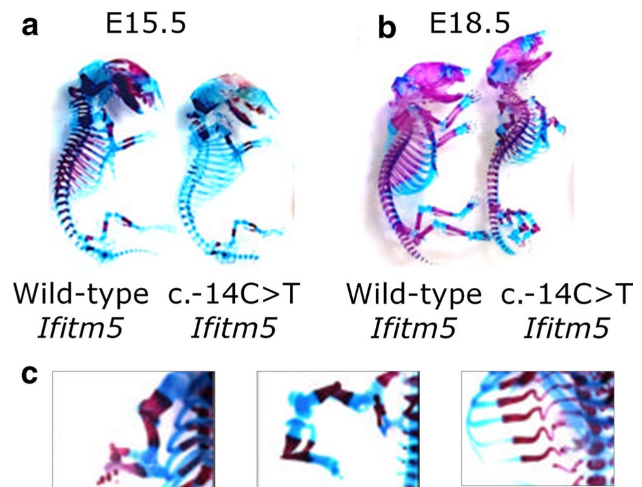

Fig. 5 Skeletal phenotype of mice with c.-14C>T mutation in *Ifitm5*. **a** Delayed bone formation in E.15.5 mutant mouse. **b** Bone fracture in E18.5 mutant mouse. **c** Abnormalities of the forelimbs, hindlimbs, and ribs. Reproduced with permission [60]

50] and mesh-like lamellation patterns on bone histology [43, 45, 49] are also OI-V characteristics. A hyper-dense metaphyseal band is first observed in infancy, interosseous membrane calcification before the age of two, radial head dislocation around age 3.5, and hyperplastic callus formation appears before 10 years of age [47, 51]. Type I collagen N-telopeptide secretion into urine is observed only during the callus formation period [43]. Blue sclera [46], short stature [45], and bone fractures at birth may be observed as rare manifestations [46]. Furthermore, OI-VI features such as vertebra compression and scoliosis have been reported to occur in OI-V [45]. Dentinogenesis imperfecta is not observed. There is a marked diversity in the frequency of bone fractures due to bone fragility.

Mutation in the UTR of *IFITM5* causes OI-V

In 2012, two groups reported the presence of a single recurrent heterozygous mutation (c.-14C>T) in the 5′ untranslated region (5′-UTR) of *IFITM5* of patients with OI-V [12, 13]. Subsequently, many groups reported *IFITM5* c.-14C>T mutation in patients with OI-V [15, 45, 46, 49, 52–57] and this mutation is now considered to be the cause of autosomal dominant OI-V. The *IFITM5* c.-14C>T mutation shifts the *IFITM5* upstream and creates a new start codon, resulting in the addition of 5 amino acids (MALEP) at the N-terminus of IFITM5 (Fig. 4a, b). The longer transcript due to this 5′-UTR mutation of *IFITM5* can be detected in bone samples from patients with OI-V [58]. To date, the mutant protein IFITM5 with 5 additional amino acids (MALEP-IFITM5) has not been directly detected in bone samples from patients with OI-V [58]. However, when *IFITM5* harboring the c.-14C>T mutation is expressed in HEK293 cells, a protein longer than the wild-type IFITM5 is synthesized [13].

The *IFITM5* c.-14C>T mutation has been found in all patients with OI-V. However, the mechanism by which this mutation causes OI-V remains unclear. *Ifitm5* knockout mice did not exhibit major defects in bone [11]. Furthermore, while *IfitmDel*$^{-/-}$ mice, lacking the whole *Ifitm* locus, did exhibit a decrease in protection against viral infections [1, 59], no gross abnormalities were observed. Two individuals with a 320- and 440-kb heterozygous genomic deletion containing *IFITM5* exhibited short stature, short phalanges, microcephaly, and clinodactyly, but did not show any bone fragility or the characteristic hyperplastic callus formation observed in OI-V [13]. These facts indicate that the cause of OI-V is related to neither the loss of function nor the haploinsufficiency of IFITM5.

Recently, a mouse model of OI-V harboring the *Ifitm5* c.-14C>T mutation was generated [60]. This OI-V mouse model showed a slow rate of mineralization in utero, and exhibited abnormal rib cage formation and long bone deformities and fractures (Fig. 5). Furthermore, growth plate expansion was also observed, as seen in infant patients with OI-V. Using cell culture of osteoblasts isolated from the calvaria of E18.5 OI-V mouse model embryos, *Sp7, Col1a1*, *Bsp*, and *Ocn* (also known as *Bglap*)

gene expression levels were shown to be reduced compared to that of osteoblasts from wild-type mice. Moreover, the degree of mineralization was reduced. These results clearly demonstrate that the *Ifitm5* c.-14C>T mutation does indeed have an effect on bone formation. On the other hand, the type I collagen of the OI-V mouse model did not show the post-translational modifications observed in other types of OI and was normal. Therefore, the decrease in mineralization rate and bone deformities observed in the OI-V mouse model is thought to be unrelated to changes in type I collagen structure. Surprisingly, the OI-V mouse model with the *Ifitm5* c.-14C>T mutation exhibited perinatal lethality. This is thought to be due to respiratory failure caused by abnormal rib cage formation in neonatal mice [60]. To date, abnormal rib cage formation has not been reported in patients with OI-V. Since the OI-V mouse model exhibits perinatal lethality, whether the characteristics of OI-V such as hyperplastic callus formation and interosseous membrane calcification occur in the post-natal phase remains unknown.

In contrast, transgenic mice overexpressing wild-type *Ifitm5* showed normal growth and pre- and post-natal development, and no skeletal defects were observed (Fig. 6) [60]. Moreover, the bone morphological parameters of these transgenic mice, obtained by micro-computed tomography, did not differ from those of wild-type mice. Therefore, it is thought that the *Ifitm5* c.-14C>T mutation does not cause a gain of normal function [60].

Mutation of *IFITM5* coding region causes OI that is similar to OI-VI, but not OI-V

In 2014, mutations in the coding region of IFITM*5* were newly identified in OI patients with more severe symptoms than patients with OI-V [14–16]. The mutation is an autosomal dominant de novo mutation (c.119C>T) and causes a serine to leucine substitution on the 40th residue (p.S40L) (Fig. 4c). Patients with this *IFITM5* heterozygous mutation (IFITM5-S40L) exhibit bending of long bones [15], short stature [14, 16], bowing of extremities [14, 16], vertebral compression [14, 15], and scoliosis [16] (Fig. 7). Furthermore, patients exhibit blue sclera [15, 16], and dentinogenesis imperfecta is also observed [16]. Regarding bone histology, broad bands of unmineralized osteoid and fish-scale patterns are seen [16]. These bone histological characteristics are similar to those observed in autosomal recessive OI-VI. However, these patients do not have mutations in *SERPINF1*, the causative gene of OI-VI [16].

SERPINF1 encodes the multifunctional secretory protein, pigment epithelium-derived factor (PEDF) [61–65]. The bones of *Serpinf1* knockout mice exhibit a reduction in the trabecular bone volume, similar to bone tissue in patients with OI-VI; the unmineralized osteoid is thick

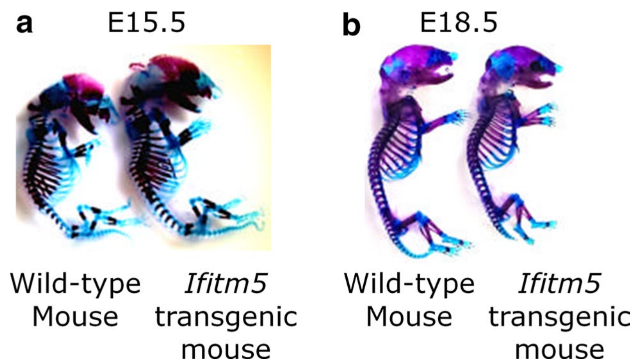

Fig. 6 Skeletal phenotype of *Ifitm5*-transgenic mice. No change in skeletal phenotype is observed between wild-type and *Ifitm5* transgenic mice at E15.5 (**a**) and E18.5 (**b**). Reproduced with permission [60]

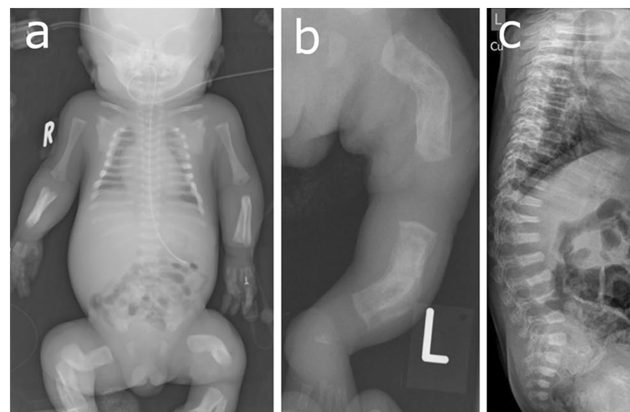

Fig. 7 Radiographs from a postnatal OI patient with c.119C>T mutation in *Ifitm5*. **a** Intrauterine fractures of the left humerus, rib deformities, and rib fractures. **b** Short and bent tibia and fibula. **c** Vertebral compression. Reproduced with permission [14]

and there is an increase in the mineral:matrix ratio [65]. The increase in the mineral:matrix ratio is thought to be due to the reduction in type I collagen brought about by the defect in PEDF [65]. The frailty in bone may be due to the increase in this ratio and, as a consequence, an inability to maintain mineralization of the matrix.

Molecular mechanisms involved in the effect of *IFITM5* mutations on the pathology of OI

The *IFITM5* c.119C>T mutation causes a severe form of OI. Its pathology differs from the OI-V caused by the *IFITM5* c.-14C>T mutation. Therefore, the onset of OI caused by the proteins IFITM5-S40L and MALEP-IFITM5 is believed to be due to different molecular mechanisms. IFITM5-S40L and MALEP-IFITM5 expression levels do not differ from wild-type IFITM5 [10, 16]. Thus, it is

unlikely that IFITM5 expression level is related to OI pathology.

It has been reported that osteoblasts expressing IFITM5-S40L and those expressing MALEP-IFITM5 show differences in *SERPINF1* expression and, accordingly, a different level of PEDF secretion [16]. In patients with OI expressing IFITM5-S40L, PEDF serum levels were normal. However, culture of isolated osteoblasts showed a low level of *SERPINF1* expression during differentiation. Consequently, the level of PEDF secretion was dramatically reduced compared to wild-type osteoblasts. OI pathology caused by IFITM5-S40L presents similarities to that of OI-VI caused by *SERPINF1* mutations. Thus, the reduction in type I collagen secretion and mineralization may originate from a localized reduction in PEDF from osteoblasts, which function in an autocrine fashion. Conversely, osteoblasts isolated from OI-V patients with the *IFITM5* c.-14C>T mutation show a significant increase in *SERPINF1* expression compared to the wild-type. As a result, the level of PEDF secretion was also increased. Exogenous PEDF is known to promote mineralization [66, 67], and thus PEDF is likely to be involved in the hyperplastic callus formation and interosseous membrane calcification observed in OI-V. Unfortunately, the OI-V mouse model exhibits perinatal lethality. Therefore, whether PEDF is involved in hyperplastic callus formation and interosseous membrane calcification after birth cannot be investigated in this mouse model. If indeed PEDF is involved in hyperplastic callus formation and interosseous membrane calcification, it means that *SERPINF1* expression is regulated, both temporally and spatially, by MALEP-IFITM5. The OI-V mouse model shows a slow rate of mineralization in utero, and osteoblasts isolated from these mice present a low expression level of *Col1a1* and a low mineralization level [60]. *Serpinf1* expression levels in osteoblasts isolated from the OI-V mouse model have not been investigated, and currently the correlation between MALEP-IFITM5 and PEDF in this mouse model remains unknown.

The IFITM5-S40L and MALEP-IFITM5 proteins have been shown to differ in their topology and localization [10]. The murine IFITM5 p.S42L mutation, which corresponds to the human IFITM5 p.S40L mutation, is not *S*-palmitoylated on C52 and C53. As a result, this mutated IFITM5 protein is not transported to the cell membrane and remains in the Golgi apparatus. On the other hand, the localization, topology, and synthesis level of MALEP-IFITM5 caused by the *Ifitm5* c.-14C>T mutation did not differ from wild-type IFITM5. Therefore, a scenario in which the mis-localization of IFITM5-S40L causes pathology similar to OI-VI is possible. However, its localization cannot be considered to be a cause of OI-V induced by MALEP-IFITM5.

A peptidyl-prolyl *cis–trans* isomerase of the FK506-binding protein (FKBP) family member, FKBP11 (also known as FKBP19), has been identified and is a partner molecule that interacts with murine IFITM5 [68]. *S*-Palmitoylation of IFITM5 C52 and C53 is essential for the interaction between FKBP11 and IFITM5 [69]. Moreover, the murine IFITM5-S42L does not interact with FKBP11 (unpublished data). When murine osteoblasts are cultured with 2-bromopalmitic acid, a palmitoylation inhibitor, diffused mineralization nodules, which differ from the normal mineralization nodules, are observed [69]. This strongly indicates that either the mis-localization of IFITM5, due to the failure of C52 and C53 *S*- palmitoylation, or the failure of interaction with FKBP11, influences OI due to IFITM5-S40L. On the other hand, murine MALEP-IFITM5 retained its interaction with FKBP11, as observed in wild-type IFITM5 (unpublished data). Mutations in FKBP65 (encoded by *Fkbp10*), from the same FKBP family as FKBP11, inhibit the hydroxylation of telopeptide lysyl residues, which are involved in the intermolecular cross-link formation of type I collagen and cause the autosomal recessive Bruck syndrome and OI-XI [70–73]. Autosomal recessive disorders caused by mutations in FKBP65 exhibit the following clinical phenotypes: bone fragility, congenital joint contracture, scoliosis, and osteoporosis [70]. *Fkbp10* knockout mice exhibit perinatal lethality. However, the calvarial collagen from these knockout mice showed instability in their intermolecular cross-link formation of telopeptide lysines [73]. This type of inhibition of hydroxylation of type I collagen telopeptide lysyl residues is not observed in the OI-V mouse model with the *Ifitm5* c.-14C>T mutation [60]. Therefore, the scenario in which MALEP-IFITM5 lowers the stability of type I collagen structure via inhibiting FKBP11 function can be excluded.

Although it appears that *S*-palmitoylation of IFITM5 C52 and C53 affects the function of IFITM5, *S*-palmitoylation is a reversible modification [74]. The peptidyl-prolyl *cis–trans* isomerase activity of FKBP12 has been reported to be involved in the depalmitoylation of the membrane protein H-Ras [75]. FKBP12 binds to *S*-palmitoylated H-Ras and depalmitoylates it by acting on proline neighboring the *S*-palmitoylated cysteine. The palmitoylation/depalmitoylation cycle is linked to the bidirectional trafficking of H-R between the Golgi apparatus and cell membranes [76–78]. The IFITM5 N-terminal region presents a lot of proline residues. A newly proposed topology for IFITM5 has shown that the N-terminal region is intracellular, not extracellular [10]. Since the peptidyl-prolyl *cis–trans* isomerase of FKBP11 is predicted to be in the extracellular region, it is unlikely that FKBP11 is involved in the depalmitoylation of IFITM5. FKBP11 also interacts with the CD81–CD9 complex, both of whose parts are *S*-palmitoylated [68]. CD81 and CD9 both have proline residues in their extracellular regions. Currently, it is unknown whether the interaction between IFITM5 and FKBP11 has an effect

on mineralization. In contrast to *Fkbp10,* the causative gene of Bruck syndrome and the OI-XI, knockout mouse phenotype, Lexicon Phenotype Analysis of *Fkbp11* knockout mice indicated an increase in bone mineral content and mineral density (http://www.informatics.jax.org/external/ko/lexicon/2549.html). Therefore, FKBP11 may be functioning as a negative regulator of bone function. Furthermore, osteopenia has been reported in CD81 and CD9 double knockout mice [79]. However, the mineralization capacity of bone marrow cells isolated from these double knockout mice did not exhibit any differences from those isolated from wild-type mice. Thus, it is thought that the effect of CD81 and CD9 on bone formation is only indirect [79]. Other than FKBP11, no other proteins interacting with IFITM5 have been reported. Proteins interacting with MALEP-IFITM5 have also not been reported. If proteins interacting specifically with the MALEP-IFITM5 protein can be identified, it will result in a better understanding of the function of IFITM5 and the causes of OI-V.

The complexity of OI-V molecular mechanisms

Although a clear positive correlation is observed between IFITM5 and mineralization in vitro, this relationship is not observed in vivo. Therefore, it is thought that there may be a different system for mineralization in vivo and that this is the main system. Furthermore, the fact that *Ifitm5* knockout mice and *Ifitm5* transgenic mice do not show major bone abnormalities indicates that, for normal bone tissue, mineralization via IFITM5 is suppressed. The hyperplastic callus formation and interosseous membrane calcification observed in OI-V may be due to removal of this suppression. Specifically, hyperplastic callus formation may be due to excess mineralization induced by the relief of IFITM5 suppression on top of the main system involved in mineralization, and interosseous membrane calcification may be due to the removal of IFITM5 suppression in locations that do not normally ossify. If this is indeed the case, in in vitro studies, where IFITM5 function is not suppressed, it will be difficult to determine whether this suppression is removed in the case of MALEP-IFITM5.

On another note, OI-V shows symptoms of bone loss and subsequent bone fragility, which contradict the other symptoms of hyperplastic callus formation and ectopic bone formation. Regarding bone fragility, major differences can be observed between patients with OI-V. Recently, in a culture of primary osteoblasts isolated from OI-V patients, it was reported that the expression level of mineralization marker genes such as *ALPL*, *IBSP*, *OPN*, and *OCN* was increased, while *COL1A1* expression was significantly decreased and the structure of secreted type I collagen was also abnormal [80]. The possibility that the abnormality of type I collagen production is thought to be involved in bone fragility in OI-V, but the mechanism by which MALEP-IFITM5 affects the level and the structure of secreted type I collagen remains unknown.

Regarding OI-V caused by the *IFITM5* c.-14C>T mutation, the bone formation mechanism of IFITM5 and the regulatory mechanism of IFITM5 function in vivo needs to be elucidated. For this to be achieved, the way in which MALEP-IFITM5 affects these mechanisms must be unraveled.

Conflict of interest The author has no conflicts of interest.

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