**FAM20A is Dispensable for Dentinogenesis and Osteogenesis**

Chunxiao Ran, Yiding Shi, Nan Li, Chao Liu and Jing Xiao

Department of Oral Pathology, College of Stomatolgy, Dalian Medical University, Dalian, Liaoning, China

(Accepted for publication, May 11, 2021)

Abstract: Family with sequence similarity 20, member A (Fam20a) encodes a pseudokinase which is regarded to facilitate the role of Fam20c in phosphorylating secreted proteins. Fam20c deficiency causes Raine Syndrome in human and impaired the amelogenesis, dentinogenesis and osteogenesis in mice. Mutations in Fam20a are associated with Amelogenesis Imperfecta and Enamel-Renal Syndrome in human. Similarly, abrogation of Fam20a in ectoderm caused Amelogenesis Imperfecta in mice, however, the global knock-out of Fam20a in mice showed few anomaly in dentin and bone. In this study, the Fam20a<sup>+/+</sup> mice showed that at the E17.5, the LacZ staining was located in the osteogenic lining of calvarium and mandibular bone, odontoblasts, ameloblasts, the gingival and subcutaneous fibroblasts. During the postnatal life, the LacZ staining was detected in the osteogenic and gingival cells in mandibular bone, as well as the osteogenic and the marrow cells in long bone, but excluded from the joint cartilage. Both the LacZ staining in the mandibular and long bone became faint with the life increased. To address if Fam20a is required for the role of Fam20c during the dentinogenesis and osteogenesis, we first examined the mandibular bone and femur of the Wnt1-cre;Fam20a<sup>f/f</sup>, Osr2-cre;Fam20a<sup>f/f</sup> and Coll1-cre; Fam20a<sup>f/f</sup> mice. The gross views and X-ray plain images showed no difference in the tissue morphology and mineralization density between these conditional knock-out mice and their controls. Our findings suggested that Fam20a was not required by Fam20c during dentinogenesis and osteogenesis.

Key words: Dentinogenesis, Fam20a, Fam20c, Mineralization, Osteogenesis

**Introduction**

FAM20 refers to Family with Sequence Similarity 20, in which the genes encode a series of kinases in secretory pathway<sup>4,5</sup>. There are three members in Fam20 family: Fam20a, Fam20b and Fam20c. Among all three members, Fam20c get the intensive concerns because it is identified as the kinase phosphorylating the secreted proteins<sup>7,8</sup>. As a Golgi apparatus associated kinase<sup>9</sup>, Fam20c recognizes and phosphorylates the S-X-E/S motif in the secreted proteins, such as extracellular matrices, growth factors, or even the cell surface molecules<sup>9</sup>. Moreover, the metabolism of phosphorus in the Fam20c deficient human and mice are also impacted<sup>9</sup>. Fam20c has been proved the causative gene for human Raine Syndrome manifested by lethal sclerosis bone dysplasia or hypophosphatemia rickets<sup>10-12</sup>. Inactivation of Fam20c in ectoderm leads to Amelogenesis Imperfecta<sup>13,14</sup>, while abrogation of Fam20c in mesenchyme results in Dentinogenesis Imperfecta<sup>4,15</sup> and osteoporosis<sup>4,15</sup>. Fam20c deficiency in mice not only reduces the expression and phosphorylation of the extracellular matrices in enamel, dentin and bone<sup>16-18</sup>, but also elongates the half-time of serum FGF23<sup>19,20</sup>, which enhances phosphorus excretion and decreases serum phosphorus. Therefore, Fam20c can regulate the mineralization of enamel, dentin and bone through both the local and systemic manner.

Fam20b is a xylose kinase responsible for the synthesis of glycosaminoglycan (GAG) chains of proteoglycans<sup>21</sup>. Fam20b specifically phosphorylates the xylose in the initial tetrasaccharides, which is essential for the maintenance of the tetrasaccharide linker and the subsequent elongation of GAG chains<sup>21,22</sup>. As a highly conserved molecule in evolution, there is no Fam20b mutation confirmed in human diseases to date, implicating the lethality of Fam20b deficiency to early embryo genesis. Such embryonic lethality was detected in the conventional Fam20b knock-out mice that die at E12.5-13.5<sup>23</sup>. Fam20b deficient zebrafish and the conditional deletion of Fam20b by Osr2-cre in mouse suffer from the malformations in cartilage and bone<sup>24-29</sup>. Interestingly, latest study showed the inactivation of Fam20b in ectoderm resulted in supernumerary incisors by regulating FGF and Wnt signaling pathways<sup>26,27</sup>. Genetic and cell biological studies have showed that HSPGs can regulate morphogen activities at various steps including control of morpogenesis movement, signaling, and intracellular trafficking<sup>28</sup>

Fam20a is considered as a pseudokinase facilitating the function of Fam20c<sup>29,30</sup>. Although binds ATP, Fam20a is incapable of catalyzing phosphorylation due to the lack of the key residues typically required for kinase activity<sup>30,31</sup>. Fam20a was predicted to form the homodimer and then, bind the homodimer of Fam20c to form tetramers<sup>30</sup>. Recent studies reported that mutations in Fam20a in human were associated with Amelogenesis Imperfecta (AI) and Enamel-Renal Syndrome (ERS), which manifestations included hypoplastic enamel, unerupted teeth, gingival fibromatosis and nephrocalcinosis<sup>32,33</sup>. However, it is still unknown how Fam20a regulates the function of Fam20c. Conditional Fam20a knock-out mice exhibit the typical Amelogenesis Imperfecta<sup>32,34</sup>, while the latest study showed that the global knock-out of Fam20a in mice also only suffered from the Amelogenesis Imperfecta

---

Correspondence to: Dr. Jing Xiao, Department of Oral Pathology, College of Stomatolgy, Dalian Medical University, Dalian, Liaoning, China; Tel: 86-411-86110396; E-mail: dalianxiaojing@163.com

Dr. Chao Liu, Department of Oral Pathology, College of Stomatolgy, Dalian Medical University, Dalian, Liaoning, China; Tel: 86-411-86110396; E-mail: 1506217121@qq.com
without Dentinogenesis Imperfecta and osteoporosis, suggesting Fam20a was not required for dentinogenesis and osteogenesis. To address if Fam20a is essential for the role of Fam20c in dentinogenesis and osteogenesis, Fam20a mice are applied to clarify the expression pattern in craniofacial bone, teeth, joint and long bone. Furthermore, Wnt1-cre;Fam20a and Col1-cre; Fam20a mice are investigated to examine the role of Fam20a during amelogenesis, dentinogenesis and osteogenesis.

Materials and Methods

Mouse lines and sample collection

The Fam20a and Fam20d mice were generated and gifted by Dr. Pin Zhang at School of Stomatology, Harbin Medical University. The Wnt1-cre, Osr2-cre and 2.3Kb Collal-cre mice were obtained from the lab of Dr. Yiping Chen at Tulane University, New Orleans, USA. All the mice were fed and mated in the SPF facility of the Institute of Genome Engineered Animal Models for Human Diseases at Dalian Medical University. To get the Wnt1-cre; Farn20a, Osr2-cre; Fam20a and 2.3Kb Collal-cre; Fam20a mice, the Fam20a male was first crossed to Wnt1-cre, Osr2-cre or 2.3Kb Collal-cre females to obtain Wnt1-cre; Fam20a, Osr2-cre; Fam20a and 2.3Kb Collal-cre; Fam20a mice. Then, the Wnt1-cre; Fam20d, Osr2-cre; Fam20d and 2.3Kb Collal-cre; Fam20d mice mated the Fam20a mice to get Wnt1-cre; Fam20d, Osr2-cre; Fam20d and 2.3Kb Collal-cre; Fam20d mice for further analyses. To get the Fam20a embryos, the Fam20a female mated WT females and the morning found vaginal plug was recorded as E0.5. The timed pregnant female mice or the postnatal mice were euthanized by carbon dioxide inhalation and then, cervical dislocation to collect the embryos or bones. All the procedures for euthanization and embryo collection were approved by the Animal Care and Use Committee at Dalian Medical University (Protocol NO. AEE17038).

Cryostat section for X-gal/LacZ staining

The heads dissected from the E17.5 Fam20a mouse embryos were fixed in the ice-cold 2% paraformaldehyde mixed with 15% sucrose for 3 hours and then, in 2% paraformaldehyde with 30% sucrose solution overnight. The fixed embryonic heads were embedded with O.C.T. compound (Tissue-Tek) for 10 μm serial cryostat sections in a cryostat microtome. The cryostat sections were incubated in X-gal solution (Gold Biotechnology, St Louis, MO, USA) for 24 hours at 37 °C in darkness, then, dehydrated in 70% alcohol. For the gross views of the mandibular bone and femurs, the bright field images were taken by a stereomicroscope of Olympus SZX16 with the digital camera of Olympus DP72. For the plain X-ray images, a Faxitron X-ray Radiography System (MX-20; Faxitron X-ray, Wheeling, IL, USA) in Department of Stomatology, the Second Affiliated Hospital of Harbin Medical University was applied at a voltage of 26 kVp and an exposure time of 8.5 s.

Results

The expression pattern of Fam20a in the embryonic craniofacial tissues

To address the expression pattern of the Fam20a in the embryonic craniofacial tissues, the E17.5 Fam20a mouse embryos were applied to cryostat section and X-gal/LacZ staining. The LacZ staining was detected in the calvarium and facial bones (Fig. 1A), the mandibular bone (Fig. 1B), and the ameloblasts and odontoblasts in the molars (Fig. 1C). The magnified scope showed that in the calvarium, the LacZ staining was concentrated in the osteogenic lining covering calvarial surface, but distributed sporadically in the osteocytes inside the calvarium (Fig. 1D). In contrast, in the mandibular bone, although also robust in the osteogenic lining, the LacZ staining was almost excluded from the osteocytes (Fig. 1E). In the molars, the entire layers of the ameloblasts and odontoblasts were found LacZ staining positive, but the satellite reticulum and the dental papilla were devoid of the positive staining (Fig. 1F).

The expression pattern of Fam20a in the postnatal mandible

To explore the expression pattern of Fam20a in the postnatal craniofacial tissues, the mandibles from the P5 Fam20a mice were fixed and stained with X-gal/LacZ staining for histological sections. In the P5 mandibles, the LacZ staining was sustained in the ameloblasts, but faded in the odontoblasts (Fig. 2A). However, the gingival layer above the erupting tooth exhibited LacZ staining (Fig. 2B). Similarly, the cells in the subcutaneous tissue surrounding the mandibular bone also exhibited a weaker LacZ staining (Fig. 2C).

To convince the expression of Fam20a in the postnatal mandibular bone, the soft tissues in the mandibles were peeled off and the mandibular bones were stained by X-gal and then, decalcified for histological section. The gross view of the mandibular bones showed that at P5, the intensive LacZ staining was distributed throughout the mandibular bone, except the condylar and coronal cartilages (Fig. 2D). At P2W, the LacZ staining was weaker than that at P5, especially in the alveolar bone of molars (Fig. 2E), and such fading became much more remarkable at P4W when the LacZ staining was almost faint in the mandibular bone (Fig. 2F).

The histological section revealed that the osteocytes in the mandibular bones gave rise to a robust LacZ staining, while the dental papilla of the molars and incisors, and the coronal cartilage were devoid of the staining (Fig. 2G). At P2W, The LacZ staining faded in the mandibular bone, while became robust in the gingiva covering the erupting molars (Fig. 2H). At P4W, the LacZ staining was only sporadically detected in the mandibular bone, but disappeared in the dental pulp and gingiva (Fig. 2I).

The abrogation of Fam20a in mandibular mesenchyme causes no malformation

Since Fam20a mice displayed the staining in the osteocytes, osteoblasts, ameloblasts and odontoblasts in the embryonic and postnatal craniofacial region, 2.3Kb Collal-cre, Osr2-cre and Wnt1-cre were utilized to inactivate the conditional Fam20a allele, respectively. Surpris-
ingly, all the 23 Kb Col1a1-cre; Fam20a<sup>−/−</sup>, Osr2-cre; Fam20a<sup>−/−</sup> and Wnt1-cre; Fam20a<sup>−/−</sup> mice are viable and fertile without any craniofacial malformation. Compared with the WT littermates, there was no deform-

Figure 1. The pattern of Fam20a-LacZ in the craniofacial region. (A) The crosse section of the E17.5 Fam20aLacZ/+ mouse cranium with X-gal/LacZ staining. (B) The crosse section of the E17.5 Fam20aLacZ/+ mouse mandible with X-gal/LacZ staining. (C) The crosse section of the E17.5 Fam20aLacZ/+ mouse oral cavity with X-gal/LacZ staining. (D) The magnitude view of the red box area in (A), red arrow indicated the LacZ positive cells in the calvarium. (E) The magnitude view of the red box area in (B), red arrow indicated the LacZ positive cells in the mandibular bone. (F) The magnitude view of the red box area in (C), red arrow indicated the LacZ positive odontoblasts and yellow arrow indicated the LacZ positive ameloblasts in molar. (Scale bar of A = 500 μm, Scale bar of B = 100 μm, Scale bar of C = 200 μm, Scale bar of D = 100 μm, Scale bar of E and F = 50 μm)

Figure 2. The pattern of Fam20a-LacZ in the postnatal mandibular bones. (A) The sagittal view of the subcutaneous layer in the P5 mandible. The green box contained the magnitud view in the red box area. (B) The sagital view of the P5 mandibles. Arrow indicated the LacZ positive gingiva. (C) The sagital view of the P5 molar containing the LacZ positive odontoblasts. The green box showe the magnitude view of red box. (D) The gross view of the P5 Fam20a<sup>−/−</sup> mandibular bone with LacZ staining. (E) The gross view of the P2W Fam20a<sup>−/−</sup> mandibular bone with LacZ staining. (F) The gross view of the P4W Fam20a<sup>−/−</sup> mandibular bone with LacZ staining. (G) The sagittal section of the P5 Fam20a<sup>−/−</sup> mandibular bone with LacZ staining, the section in the box is enlarged and displayed in the left corner of the image. (H) The sagittal section of the P2W Fam20a<sup>−/−</sup> mandibular bone with LacZ staining, the section in the box is enlarged and displayed in the left corner of the image. (I) The sagittal section of the P4W Fam20a<sup>−/−</sup> mandibular bone with LacZ staining, the section in the box is enlarged and displayed in the left corner of the image. (Scale bar of A = 200 μm, Scale bar of B and C = 100 μm, Scale bar of G-I = 500 μm)
ference in the mineral density of teeth and mandibular bones between the WT littermate and the conditional knock-out mice (Fig. 3A'-F').

**The expression pattern of Fam20a in the long bone and joint**

The knee joints and femur heads from Fam20a<sup>−/−</sup> mice were used for X-gal/LacZ staining to clarify the expression pattern of Fam20a in the long bones. In the knee joints from the P1W and P2W Fam20a<sup>−/−</sup> mice, LacZ staining was distributed dominantly in the epiphysis, sporadically in the shafts of the femurs and tibias, but excluded from the joint cartilage (Fig. 4A, B). Similar to that in mandibular bone, the staining became faint in the epiphysis and shafts of the P4W Fam20a<sup>−/−</sup> femurs and tibias (Fig. 4C). In the femur heads of P1W Fam20a<sup>−/−</sup> mice, LacZ staining seemed to be concentrated in the bone shafts and excluded from the chondrogenic heads (Fig. 4D). At P2W, the LacZ staining was weaker in the shaft and still absent from the femur heads (Fig. 4E). At P4W, the LacZ staining almost disappeared in the femur shaft and shrunk to the epiphyses under the femur heads (Fig. 4F). Histological sections disclosed the locations of the LacZ staining in the femur and knee joint.
The deletions of Fam20a in osteogenic mesenchyme lead to no deformity

To address if the deletion of Fam20a from the osteogenic mesenchyme could impact the development and mineralization of long bone, the P4W femurs and tibias from the conditional Fam20a knock-out mice were examined. Similar to the craniofacial region, there was no discrepancy found in the gross view (Fig. 4A, B) and X-ray images (Fig. 4A’, B’) between the wildtype and Col1a1-cre; Fam20a<sup>f/f</sup> mice. The gross views of the P6W femur and tibia from the WT (C) and Osr2-cre; Fam20a<sup>f/f</sup> mice (D). The plain X-ray images of the P6W femur and tibia from the WT (E) and Wnt1-cre; Fam20a<sup>f/f</sup> mice (F). The plain X-ray images of the P6W femur and tibia from the WT (E’) and Wnt1-cre; Fam20a<sup>f/f</sup> mice (F’).

The deletions of Fam20a in osteogenic mesenchyme lead to no deformity

To address if the deletion of Fam20a from the osteogenic mesenchyme could impact the development and mineralization of long bone, the P4W femurs and tibias from the conditional Fam20a knock-out mice were examined. Similar to the craniofacial region, there was no discrepancy found in the gross view (Fig. 5A, B) and X-ray images (Fig. 5A’, B’) between the wildtype and Col1a1-cre; Fam20a<sup>f/f</sup> mice. The gross views of the P6W femur and tibia from the WT (C) and Osr2-cre; Fam20a<sup>f/f</sup> mice (D). The plain X-ray images of the P6W femur and tibia from the WT (E) and Wnt1-cre; Fam20a<sup>f/f</sup> mice (F). The plain X-ray images of the P6W femur and tibia from the WT (E’) and Wnt1-cre; Fam20a<sup>f/f</sup> mice (F’).

Recent studies reported that the a series of Fam20a mutations were associated with the congenital AI and ERS in human newborns<sup>22,33</sup>. Our previous study indicated that the ectoderm-specific inactivation of Fam20a caused enamel and gingival malformation<sup>39</sup>. The latest study by utilizing Sox2-cre to inactivate Fam20a in the entire embryo at early gestation also revealed the impact of Fam20a deficiency on amelogenesis, as opposed of dentinogenesis<sup>90</sup>. These findings implicated that although activated in the differentiating odontoblasts<sup>46,57</sup>, Fam20a was contributed little to dentinogenesis.

In our study, taking the advantage of the Fam20a<sup>LacZ</sup> allele which provided the timely tracing of the Fam20a expression, we found the activation of Fam20a not only in the differentiating ameloblasts, odontoblasts and the gingival cells, but also in the osteocytes, osteoblasts and the subcutaneous cells in the craniofacial region. Moreover, Fam20a was suggested to be activated in the cells located in the bone marrow and the epiphymes of long bones during postnatal stage. However, the fanning LacZ staining in the craniofacial and long bones suggested the decreasing intensity of Fam20a expression with the age increasing.

The hypoplastic enamel and gingival fibromatosis resulting from the Fam20a mutations in human and mouse were attributed to the disrupted Fam20a expression in ameloblasts and gingiva. Thus, Fam20a was suggested to play a role in the differentiation or maturation of the ectoderm-derived enamel and gingiva. To explore the contribution of Fam20a expression to the development of the mesenchyme-derived tissues, the conditional Fam20a<sup>LacZ</sup> allele was deleted by 2.3Kb Col1a1-cre in the differentiating osteogenic progenitors and odontoblasts in the E13.5 mouse embryos. However, the normal phenotype of the 2.3Kb Col1a1-cre; Fam20a<sup>LacZ</sup> mice implicated that the expression of Fam20a in the odontoblasts, the osteoblasts and osteocytes of the craniofacial and long bones was not required for the differentiation, maturation and mineralization of odontoblasts and osteoblasts. Then, the Osr2-cre which was activated at E11.5 in the mesenchyme presumptive for palatal shelves, dental papilla and joint cartilage, was crossed to the conditional Fam20a<sup>LacZ</sup> allele to examine if the earlier inactivation of Fam20a could impact the development of the mesenchyme-derived tissues. As expected, the joints and cartilages in the Osr2-cre; Fam20a<sup>LacZ</sup> mice were normal because Fam20a was not activated during chondrogenesis. The palatogenesis and odontogenesis were normal in the Osr2-cre; Fam20a<sup>LacZ</sup> mice, though Fam20a expression was detected in the palatal bone and odontoblasts, suggesting that Fam20a was dispensable for osteogenesis and dentino genesis. Even when the Fam20a was abrogated from the neural crest-derived mesenchyme by Wnt1-cre at E9.5, the morphology and mineralization of the teeth and mandibular bone in the Wnt1-cre; Fam20a<sup>LacZ</sup> mice showed no difference from the WT littermates. Since the craniofacial bone and dental papilla were originated from the neural crest-derived mesenchyme, the normal phenotype of the Wnt1-cre; Fam20a<sup>LacZ</sup> mice confirmed that the Fam20a was not required for the craniofacial osteogenesis and dentinogenesis.

Fam20a was regarded as a pseudokines facilitating the kinase activity of Fam20c. Fam20a homodimer was predicted to form a tetramer with the Fam20c homodimer, which was essential for the kinase activity of Fam20c<sup>22,30</sup>. However, two Fam20c homodimers was also predicted to form a tetramer, which was adequate for the kinase activity<sup>46,51</sup>. Combined with the previous results and our findings, it implicated that in the ectoderm-derived tissues, Fam20c might be required to form the complex with Fam20a to exert the kinase activity, while in the mesenchyme-derived tissues, Fam20c could form the homotetramer which was adequate to the kinase activity. However, the mechanism controlling...
the formation of the Fam20a-Fam20c tetramer and Fam20c homotrimer is still unknown. Furthermore, the nephrocalcinosis in human Fam20a deficiency suggested that Fam20a was essential for the development and homeostasis of certain mesenchyme-derived tissues and organs, because kindney was also developed from mesenchyme. Thus, we can conclude that Fam20a is dispensable for osteogenesis and dentinogenesis.

Acknowledgement
This work is supported by Natural Science Foundation of China Grant (81771055 to C.L.).

Conflict of Interest
The authors declare that they have no conflicts of interest.

References
1. Zhang H, Zhu Q, Cui J, Wang Y, Chen MJ, Guo X, Tagliabracci VS, Dixon JE and Xiao J. Structure and evolution of the Fam20 kinases. Nat Commun 9: 12-18, 2018
2. Nalbant D, Youn H, Nalbant SI, Sharma S, Cobos E, Beale EG, Du Y and Williams SC. FAMILY: an evolutionarily conserved family of secreted proteins expressed in hematopoietic cells. BMC Genomics 6: 11, 2005
3. Koike T, Izumikawa T, Tamura J and Kitagawa H. FAM20B is a kinase that phosphorylates xylose in the glycosaminoglycan-protein linkage region. The Biochem J 421: 158-162, 2009
4. Tagliabracci VS, Engel JL, Wen J, Wiley SE, Worby CA, Kinch LN, Xiao J, Grishin NV and Dixon JE. Secreted kinase phosphorylates extracellular proteins that regulate biomineralization. Science 366: 1150-1153, 2019
5. Nadanaka S and Kitagawa H. EXTL2 controls liver regeneration and homeostasis of certain mesenchyme-derived tissues and organs. BMC Genomics 16: 99, 2015
6. Couchman JR and Pataki CA. An introduction to proteoglycans and their localization. J Histochim Cytochem 60: 885-897, 2012
7. Xiao J, Tagliabracci VS, Wen J, Kim SA and Dixon JE. Crystal structure of the Golgi casein kinase. Proc Natl Acad Sci USA 110: 10574-10579, 2013
8. Esko JD and Selleck SB. Order out of chaos: assembly of ligand binding sites in heparan sulfate. Annu Rev Biochem 71: 435-571, 2002
9. Hui Z, Qin Y, Jixin C, Yuxin W, Jack E and Junyu X. Structure and evolution of the Fam20 kinases. Nat Commun 9: 12-18, 2018
10. Simpson MA, Hsu R, Keir LS, Hao J, Sivapalan G, Ernst LM, Zackai EH, Al-Gazali L, Hulskamp G, Kingston HM, Prescott TE, Ion A, Patton MA, Murday V, George A and Crosby AH. Mutations in Fam20C are associated with lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. Am J Hum Genet 81: 906-912, 2007
11. Wang X, Wang S, Li C, Gao T, Liu Y, Rangiani A, Sun Y, Hao J, George A, Lu Y, Groppe J, Yuan B, Feng Q and Qin C. Inactivation of a novel FGF23 regulator, FAM20C, leads to hypophosphatemic rickets in mice. PLoS Genet 8: e1002708, 2012
12. Sugahara K and Kitagawa H. Recent advances in the study of the biosynthesis and functions of sulfated glycosaminoglycans. Curr Opin Struct Biol 10: 518-527, 2000
13. Corradetti B, Taraballi F, Giretti I, Bauza G, Pistillo RS, Banche Niclot F, Pandolfi L, Demarchi D and Tasciotti E. Heparan sulfate: A potential candidate for the development of biomimetic immuno-modulatory membranes. Front Bioeng Biotechnol 5: 54, 2017
14. Du EX, Wang XF, Yang WC, Kaback D, Yee SP, Qin CL, George A and Hao JJ. Characterization of Fam20C expression in odontogenesis and osteogenesis using transgenic mice. Int J Oral Sci 7: 89-94, 2015
15. Wang X, Wang J, Liu Y, Yuan B, Ruest LB, Feng Q and Qin C. The specific role of FAM20C in dentinogenesis. J Dent Res 94: 330-336, 2015
16. Wang X, Hao J, Xie Y, Sun Y, Hernandez B, Yamoka AK, Prasad M, Zhu Q, Feng Q and Qin C. Expression of FAM20C in the osteogenesis and odontogenesis of mouse. J Histochem Cytochem 58: 957-967, 2010
17. Ishikawa HO, Xu A, Ogura E, Manning G and Irvine KD. The Raine syndrome protein FAM20C is a Golgi kinase that phosphorylates bio-mineralization proteins. PLoS ONE 7: e42988, 2012
18. Christensen B, Schytte GN, Savenius C, Enghild JJ, McKee MD and Sorensen ES. FAM20C-mediated phosphorylation of MEPE and its acidic serine- and aspartate-rich motif. JMBR Plus 4: e10378, 2020
19. Wang X, Wang S, Lu Y, Gibson MP, Liu Y, Yuan B, Feng Q and Qin C. FAM20C plays an essential role in the formation of murine teeth. J Biol Chem 287: 35934-35942, 2012
20. Kinoshita Y, Hori M, Taguchi M and Fukumoto S. Functional analysis of mutant FAM20C in Raine syndrome with FGF23-related hypophosphatemia. Bone 67: 145-151, 2014
21. Koike T, Izumikawa T, Tamura J and Kitagawa H. FAM20B is a kinase that phosphorylates xylose in the glycosaminoglycan-protein linkage region. Biochem 421: 157-162, 2009
22. Wen J, Xiao J, Rahdar M, Choudhury BP, Cui J, Taylor GS, Esko JD and Dixon JE. Xylose phosphorylation functions as a molecular switch to regulate proteoglycan biosynthesis. Proc Natl Acad Sci U S A 111: 15723-15728, 2014
23. Vogel P, Hansen GM, Read RW, Vance RB, Thiel M, Liu J, Wronski TJ, Smith DD, Jeter-Jones S and Brommage R. Amelogenesis imperfecta and other biomineralization defects in Fam20a and Fam20c null mice. Veterinary Pathology 49: 998-1017, 2012
24. Saiyin W, Li L, Zhang H, Lu Y and Qin C. Inactivation of FAM20B causes cell fate changes in annulus fibrosus of mouse intervertebral disc and disc defects via the alterations of TGF-β and MAPK signaling pathways. Biochim Biophys Acta Mol Basis Dis 1865: 165555, 2019
25. Ma P, Yan W, Tian Y, Wang J, Feng Q, Qin C, Cheng YS and Wang X. Inactivation of Fam20B in Joint Cartilage Leads to Chondrosarcoma and Postnatal Ossification Defects. Sci Rep 6: 29814, 2016
26. Tian Y, Ma P, Liu C, Yang X, Crawford DM, Yan W, Bai D, Qin C and Wang X. Inactivation of Fam20B in the dental epithelium of mice leads to supernumerary incisors. Eur J Oral Sci 123: 396-402, 2015
27. Capurro M, Izumikawa T, Suarez P, Shi W, Cydzik M, Kaneiwa T, Zackai EH, Al-Gazali LI, Hulskamp G, Kingston HM, Prescott TE, Ion A, Patton MA, Murday V, George A and Crosby AH. Mutations in Fam20C are associated with lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. Am J Hum Genet 81: 906-912, 2007
28. Wang X, Wang S, Li C, Gao T, Liu Y, Rangiani A, Sun Y, Hao J, George A, Lu Y, Groppe J, Yuan B, Feng Q and Qin C. Inactivation of a novel FGF23 regulator, FAM20C, leads to hypophosphatemic rickets in mice. PLoS Genet 8: e1002708, 2012
29. Sugahara K and Kitagawa H. Recent advances in the study of the biosynthesis and functions of sulfated glycosaminoglycans. Curr Opin Struct Biol 10: 518-527, 2000
30. Ohyama Y, Lin JH, Govitvattana N, Lin IP, Venkitapathi S, Alamoudi A, Husein D, An C, Hotta H, Kaku M and Mochida Y. FAM20A
binds to and regulates FAM20C localization. Sci Rep 6: 27784, 2016
31. Pêgo SPB, Coletta RD, Dumitriu S, Iancu D, Albanyan S, Kleta R, Auricchio MT, Santos LA, Rocha B and Martelli-Júnior H. Enamel-renal syndrome in 2 patients with a mutation in FAM20 A and atypical hypertrichosis and hearing loss phenotypes. Oral Surg Oral Med Oral Pathol Oral Radiol 123: 229-234, 2017
32. O’Sullivan J, Bitu CC, Daly SB, Urquhart JE, Barron MJ, Bhaskar SS, Martelli-Júnior H, dos Santos Neto PE, Mansilla MA, Murray JC, Coletta RD, Black GC and Dixon MJ. Whole-exome sequencing identifies FAM20A mutations as a cause of amelogenesis imperfecta and gingival hyperplasia syndrome. Am J Hum Genet 88: 616-620, 2011
33. Kantaputra PN, Kaewgahya M, Khemaleelakul U, Dejkhamron P, Sutthimethakorn S, Thongboonkerd V and Iamaroon A. Enamel-renal-gingival syndrome and FAM20A mutations. Am J Med Genet A 164A: 1-9, 2014
34. Azuma K, Casey SC, Urano T, Horie-Inoue K, Ouchi Y, Blumberg B and Inoue S. Pregnan X receptor knockout mice display aging-dependent wearing of articular cartilage. PLoS One 10: e0119177, 2015
35. Li L, Saiyin W, Zhang H, Wang S, Xu Q, Qin C and Lu Y. FAM20A is essential for amelogenesis, but is dispensable for dentinogenesis. J Mol Histol 50: 581-591, 2019
36. Hassib NF, Shooib MA, ElSadek HA, Wali ME, Mostafa MI and Abdel-Hamid MS. Two new families with enamel renal syndrome: A novel FAM20A gene mutation and review of literature. Eur J Med Genet 63: 104045, 2020
37. Nitayavardhana I, Theerapanon T, Srichomthong C, Piwluang S, Wichadakul D, Poontaveetus T and Shotelersuk V. Four novel mutations of FAM20A in amelogenesis imperfecta type IG and review of literature for its genotype and phenotype spectra. Mol Genet Genomics 295: 923-931, 2020
38. Cui J, Zhu Q, Zhang H, Cianfrocco MA, Leschziner AE, Dixon JE and Xiao J. Structure of Fam20A reveals a pseudokinase featuring a unique disulfide pattern and inverted ATP-binding. eLife 6: 0422, 2017
39. Dourado MR, Dos Santos CRR, Dumitriu S, Iancu D, Albanyan S, Kleta R, Coletta RD and Marques Mesquita AT. Enamel renal syndrome: A novel homozygous FAM20A founder mutation in 5 new Brazilian families. Eur J Med Genet 62: 103561, 2019
40. de la Dure-Molla M, Quentric M, Yamaguti PM, Acevedo AC, Mighell AJ, Vikkula M, Huckert M, Berdal A and Bloch-Zupan A. Pathognomonic oral profile of Enamel Renal Syndrome (ERS) caused by recessive FAM20A mutations. Orphanet J Rare Dis 9: 84, 2014
