INVITED REVIEW ARTICLE

https://doi.org/10.5125/jkaoms.2017.43.2.63
pISSN 2234-7550 · eISSN 2234-5930

The role of nuclear factor I-C in tooth and bone development

Song Yi Roh, Joo-Cheol Park

Department of Oral Histology-Developmental Biology and Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Korea

Abstract (J Korean Assoc Oral Maxillofac Surg 2017;43:63-69)

Nuclear factor I-C (NFI-C) plays a pivotal role in various cellular processes such as odontoblast and osteoblast differentiation. *Nfic*-deficient mice showed abnormal tooth and bone formation. The transplantation of *Nfic*-expressing mouse bone marrow stromal cells rescued the impaired bone formation in *Nfic*$^{-/-}$ mice. Studies suggest that NFI-C regulate osteogenesis and dentinogenesis in concert with several factors including transforming growth factor-β1, Krüppel-like factor 4, and β-catenin. This review will focus on the function of NFI-C during tooth and bone formation and on the relevant pathways that involve NFI-C.

Key words: Nuclear factor I-C, Dentinogenesis, Osteogenesis, Osteoporosis

[paper submitted 2017. 4. 4 / accepted 2017. 4. 6]

I. Introduction

Dentin makes up the bulk of the teeth along with other tissues such as enamel, cementum, and pulp. Dental papilla cells differentiate to form dentin-pulp complex; various types of cells are found in the dental pulp including odontoblasts, fibroblasts, and immune cells such as macrophages, granulocytes, and mast cells[1]. The dental follicle can differentiate into cementoblasts, osteoblasts, and fibroblasts, which later produce cementum, alveolar bone, and periodontal ligament, respectively[2]. Strong nuclear factor I-C (*Nfic*) expression was detected in the dental papilla and dental follicle during molar development in rats from postnatal day 5 to day 16[3]. *Nfic*$^{-/-}$ mice exhibited unique phenotypes, such as defective tooth root and incisors and decreased bone density and volume[4] caused by aberrant odontoblast formation[5]. The importance of NFI-C in tooth and bone formation was confirmed; therefore, many studies have been conducted in order to elucidate the function of NFI-C and to determine how NFI-C plays a role in tooth and bone formation. This review will introduce several studies of signaling pathways and key factors correlated with the function of NFI-C in tooth and bone formation.

II. General Description of the NFI Family of Transcription Factors

The nuclear factor I (NFI) family of transcription factors plays pivotal roles in the development of the brain, lungs, muscles, and many other organs[6]. NFI protein was first isolated from HeLa cell nuclear extract and was identified as a stimulator of adenovirus DNA replication initiation[7] and a transcription factor for many viral and cellular genes[8].

There are two motifs in a consensus sequence of NFI binding site TTGGCNNNNN(N)GCCAA. Five base pair spacer region in the middle regulates the NFI binding affinity[9] and NFI proteins can bind to the TTGGC and/or GCCAA regions as a dimer or individually, with reduced binding affinity[10]. By measuring the binding specificity of individual sites, NFI was found to be identical to the TGGCA-binding protein, which binds to chicken lysozyme's enhancer region[11], and

Joo-Cheol Park
Department of Oral Histology-Developmental Biology, School of Dentistry, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Korea
TEL: +82-2-880-2338 FAX: +82-2-878-2338
E-mail: jcapark@snu.ac.kr
ORCID: http://orcid.org/0000-0002-3162-7557

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2017 The Korean Association of Oral and Maxillofacial Surgeons. All rights reserved.

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation funded by the Korean government (No. NRF-2015M3A9B6052852).

the CAAT box transcription factor, which binds to the CAAT box of the promoter region in multiple genes[12]. Subsequent studies found that NFI can also bind to the gene enhancer, promoter, and silence regions.

In vertebrates, the NFI family of transcription factors consists of four members, NFI-A, NFI-B, NFI-C, and NFI-X[8,13]. Some of the features, such as high mortality rate and delayed glial and neuronal differentiation are commonly observed in mice deficient in NFI-A, NFI-B, and NFI-X[14-16]. NFI-B deficiency also causes defects in lung maturation[17], and NFI-X deficiency leads to skeletal defects[18]. Unlike other NFI family members, NFI-C is known to play a critical role in tooth and bone formation. NFI-C knockout mice showed reduced bone density and abnormal tooth formation, but no life-threatening defects were observed[4].

III. Function of NFIC in Dentinogenesis

Odontoblasts are differentiated from the dental papilla cells that were derived from neural crest origin ectomesenchymal cells[19]. Epithelial-mesenchymal interaction induces the dental papilla cells to differentiate into odontoblasts during crown formation[20] while Hertwig's epithelial root sheath (HERS) cells drive the differentiation of the dental papilla cells into odontoblasts during root formation[21].

When *Nfic*$^{-/-}$ mice were generated by removing the second exon of the *Nfic* gene, which contains the binding and dimerization domain of the *Nfic* gene, intriguing morphologies such as the formation of brittle and short mandibular incisors and abnormal maxillary incisors along with molars with no roots were observed[4]. Markedly different morphology of the *Nfic*$^{-/-}$ mice from that of the wild type mice in the root area suggested that the failure of HERS formation may cause abnormal odontoblast differentiation in the *Nfic*$^{-/-}$ mice. However, during the tooth root formation, both groups of mice formed normal HERS by the time crown formation was completed. Aberrant, polygonal odontoblasts with no polarity were observed around the short and abnormal roots and labial crown analog of *Nfic*$^{-/-}$ mice. Since crown formation occurs during the embryonic stage and root formation takes place postnatally, it was hypothesized that NFI-C plays a crucial role in odontoblast differentiation during postnatal tooth development and dentin formation during molar development, but not in HERS[5].

Odontoblasts and dentin were observed in a more precise manner with electron microscopy to analyze the cause of defects in molars and incisors of *Nfic*$^{-/-}$ mice. Compared to those of wild type mice, many of the aberrant odontoblasts of *Nfic*-deficient mice were disorganized and round in shape. The cells were also trapped in an osteodentin-like mineralized tissue and showed no intracellular junctional complexes. These odontoblasts showed characteristics of apoptotic cells[22] which can be defined as aggregated chromatin, formation of apoptotic bodies, and swollen mitochondria[23]. Localization and expression levels of several structural proteins, such as zonula occludens-1 (ZO-1), occludin, E-cadherin (E-Cad), and connexin 43 (CX 43), which comprise the junctional complexes, were observed *in vivo* and *in vitro*. Expression of ZO-1, occludin, and E-Cad appeared to be decreased or absent, whereas that of CX 43 was observed consistently in both, wild type and *Nfic*$^{-/-}$ mice[22]. ZO-1 is a tight junction protein that can be found on the surface of cell membranes; it acts as a scaffold protein for other tight junction strand proteins[24,25]. CX 43, also called gap junction alpha-1 protein, composes gap junctions (as the name suggests), allowing gap junction intercellular communication that regulates various cellular processes[26,27]. Occludin is an integral cell-membrane protein that composes tight junctions as well. When E-Cad (a cell-adhesion glycoprotein) is downregulated, cellular adhesion strength decreases and cellular motility increases, which then may help in the invasion of adjacent tissues; this may result in cancer progression and metastasis[28,29]. This data suggested that NFI-C is involved in the formation of intercellular junctions, which occurs during the differentiation of odontoblasts from preodontoblasts[30]. Similar appearance of aberrant odontoblasts was observed in osteodentin, which is formed during dentin repair. When dentin is severely damaged and the odontoblasts are killed, the pulp cells differentiate into odontoblast-like cells to form reparative dentin[22,31].

Since *Nfic*$^{-/-}$ mice and transforming growth factor β1 (TGF-β1) transgenic mice showed similar tooth morphologies, further investigation was conducted to identify the relationship between NFI-C and TGF-β during odontoblast differentiation. The TGF-β family consists of TGF-β, activins, inhibins, and bone morphogenic proteins (BMPs)[32,33]. Members of this family of growth factors contribute to various cellular processes such as proliferation, differentiation, embryonic development, and epithelial to mesenchymal interactions[34]. One of the TGF-β family members, TGF-β1, is known to promote cell growth arrest through the Smad signaling pathway by activating the cyclin-dependent kinase inhibitor p21[35,36]. In HeLa cells, NFI contributes to the inhibition of p21[37]. After TGF-β treatment on the odontoblast-like cell line MDPC23, NFI-C degradation increased in

both, the cytosol and the nucleus. Treatment with MG132, a proteasome inhibitor, prevented the degradation of NFI-C by TGF-β1 treatment. The increased mRNA level and decreased protein level of NFI-C after the treatment with TGF-β1 suggested that TGF-β regulates NFI-C by degradation via 26S proteasome. In the primary pulp cells of *Nfic*$^{-/-}$ mice, the expression levels of TGF-β, TGF-β receptor type I (TGF-β RI), p-SMAD2/3, and p21 appeared to be increased. Overexpression of *Smad3* induced degradation of NFI-C, and silencing of *Smad3* inhibited the degradation of NFI-C by TGF-β1. When *Nfic* was overexpressed, p-SMAD3 was dephosphorylated and lost its ability to form an NFI-C degradation complex with smad4, inhibiting NFI-C degradation. Therefore, TGF-β1 signaling is suggested to be negatively regulated by NFIC during odontoblast differentiation. Smad ubiquitination regulatory factor (Smurf) is an E3 ubiquitin-protein ligase that contributes to the degradation of Smad protein, thereby negatively regulating TGF-β/BMP signaling[38-40]. Amongst several major MAPK pathways, the Jun N-terminal kinase (JNK) pathway is thought to be more likely involved in the interaction between NFI-C and Smurf 1/2[41] since JNK inhibitor decreased the interaction, whereas p38, another major MAPK pathway, inhibitor did not affect the interaction between those two[42-45]. In all, NFI-C is thought to be degraded by TGF-β1 through the Smad pathway with the aid of Smurf 1/2 during early odontoblast differentiation. In the late stage of odontoblast differentiation and mineralization, NFI-C dephosphorylates p-SMAD2/3 in both the cytoplasm and the nucleus, and is translocated into the nucleus, binding its target gene's promoter and regulating the gene expression[42].

Zinc deficiency also results in a similar tooth phenotype that is observable in *Nfic*$^{-/-}$ mice. Zinc transporter Slc39a13/Zip13 transports zinc from the Golgi apparatus to the cytosol, and therefore regulates intracellular zinc level. *Slc39a13/Zip13*-deficient mice were morphologically similar to *Nfic*-deficient mice, in terms of skeletal disorders, reduced root dentin formation in the molars, and abnormal incisors[46]. Zinc is a trace element and regulates various processes in living organisms including cellular metabolism, growth, replication, and tissue repair[47]. Maintaining the appropriate zinc level is crucial since zinc deficiency causes impairment of brain functions and immune responses, while an excessive amount of zinc can cause apoptosis and neuronal death[48,49]. Adequate zinc level is also required for odontoblast differentiation and dentin formation. After zinc treatment of the MDPC23 cell line, the nuclear translocation of p-SMAD2/3, metallothioneins (MT-1), and metal transcription factor-1 (MTF-1) were increased, whereas no marked change was observed in NFI-C localization. When TPEN, a zinc chelator, was used, translocation of NFI-C into the nucleus increased. MTF-1 is a transcription factor that contains six zinc fingers in the DNA binding domain. When zinc is present, MTF-1 binds to DNA motifs such as metal response elements (MREs) in the promoter region of MT genes and increases the transcription of MT[50-52]. NFI-C is known to bind to overlapping regions of MRE sites[53]. During the early odontoblast differentiation period, adequate amount of zinc promotes nuclear translocation of MTF-1 and phosphorylation of SMAD2/3 in the cytoplasm in a dose dependent manner. MTF-1, a transcriptional activator of MT-1, increased MT-1 transcription and induced NFI-C degradation simultaneously through the TGF-β pathway. During the late stage of odontoblast differentiation, decreased zinc levels lead to the translocation of NFI-C into the nucleus. NFI-C acts as a transcriptional repressor of MT-1 and enhancer of dentin sialophosphoprotein (DSPP) promoter, accelerating differentiation and mineralization of odontoblasts[54].

Krüppel-like factor 4 (KLF4) is a member of specificity protein/KLF (SP/KLF) transcription factor family[55]. This family of transcription factors is characterized by three zinc finger motifs in the carboxyl terminal sequences[56]. KLF4 participates in various cellular processes such as cell proliferation, differentiation, cell growth, and maintenance of normal tissue homeostasis[57]. Several members, including KLF17 and KLF4, are known to control epithelial-mesenchymal interactions[58]. KLF4 is also known to regulate odontoblast cell proliferation and differentiation via control of dentin matrix protein 1 (*Dmp1*), *Dspp*, and alkaline phosphatase (*Alp*) expression[59,60]. NFI-C is also known to control *Dspp* expression in odontoblasts; *Klf4* expression and protein level decreased significantly in the *Nfic*$^{-/-}$ mice compared to the wild type mice. Therefore, the relationship between NFI-C and KLF4 during odontoblast differentiation was investigated. The binding of NFI-C to –980 region of *Klf4* promoter was confirmed, which lead to increased expression of *Klf4*, *Dmp1*, and *Dspp*. When *Nfic* was silenced, the expression of those genes decreased significantly. Overexpressing or silencing *Klf4* affected only the expression levels of DMP1 and DSPP, but those of NFI-C was not affected. KLF4 binds to *Dmp1* promoter, and DMP1 binds to *Dspp* promoter to increase its transcription[61]; therefore, the *Nfic-Klf4-Dmp1-Dspp* pathway was suggested[62]. Although odontoblasts are cells of mesenchymal origin, they exhibit epithelial cell-like characteristics as they differentiate. When MDPC-23 cells were transfected

with *Nfic* expressing vectors and cultured in osteogenic medium, they became tightly packed like patches of epithelial cells, and actin fibers rearranged to the outer surfaces of the cells. Expression of E-Cad, a mesenchymal-epithelial transition (MET) marker, and N-cadherin, an epithelial-mesenchymal transition (EMT) marker, were observed during the differentiation. Expression of E-Cad and NFI-C increased, and N-Cad, TGF-β1, TGF-β RI decreased as differentiation progressed, which corresponded to the result of the study that investigated the relationship between NFI-C and TGF-β. Combining all the studies, it was confirmed that NFI-C controls odontoblast differentiation and mineralization through the *Nfic-Klf4-Dmp1-Dspp* pathway, and also contributes to MET induction via the *Nfic-Klf4-E-Cad* cascade[62].

IV. Function of NFIC in Osteogenesis

Osteoblasts and adipocytes differentiate from the same progenitor, bone marrow stromal cells (BMSCs). Many proteins and signaling pathways are involved in the differentiation and cell fate determination of BMSCs. Proteins such as TGF-β, BMP, hedgehog, RUNX2, osterix (OSX), and β-catenin are reported to contribute to osteogenesis. Adipogenesis is regulated by many factors such as peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT/enhancer-binding protein (C/EBP)α, and C/EBPβ. NFI-C serves as one of the critical factors for postnatal bone formation and osteogenic cell differentiation. The expression pattern of *Nfic* in mice was observed from the embryonic stage until postnatal week 60. Significant increase in *Nfic* mRNA level was observed from postnatal day 16 (P16) to 6 weeks, and then the mRNA level gradually decreased from 6 weeks until 60 weeks. *In vitro* results from mice BMSCs and MC3T3-E1, a mouse osteoblast precursor cell line, also showed a similar pattern—an increased level of *Nfic* mRNA during the early stages of osteoblast differentiation (day 4 to day 10) and decreased *Nfic* mRNA level during the late stages of osteoblast differentiation and mineralization (day 10 to day 21). Along with the delayed tooth root development, *Nfic*$^{-/-}$ mice showed reduced bone volume and thickness for both cortical and trabecular bones, and decreased bone mineral density compared with those of wild type mice. Since prenatal bone development was not affected by *Nfic* deficiency, it was suggested that NFI-C controls bone formation during the postnatal stages, and not in the prenatal stages. Osteoblast activity was determined with femora of 6-week-old mice. *Nfic*$^{-/-}$ mice showed decreased number of osteoblasts and proliferation rate, which possibly impaired osteoblast function during bone formation. *In vitro* studies suggested that the decrease was not due to the osteoclast activity, since *in vivo* results showed no difference in the number of osteoclast in *Nfic*$^{-/-}$ and wild type mice. In fact, the expression of receptor activator of nuclear factor kappa-B ligand (Rankl) was decreased in *Nfic*$^{-/-}$ mice. When Rankl binds to its receptor, Rank, osteoclast activation and differentiation is induced. Osteoprotegrin (Opg) is secreted by osteoblasts, and binds to Rank, inhibiting Rankl from binding to its receptor and therefore inhibiting osteoclast formation. *Nfic*$^{-/-}$ mice showed normal Opg but decreased Rankl expression, suggesting that decrease in the number of osteoblasts and bone formation is not caused by osteoclast activation. On the other hand, *in vitro* results with *Nfic*$^{-/-}$ BMSCs showed an increase in the number of oil red-O positive cells and increased expression of adipocyte differentiation marker, *Pparγ*. This coincides with the observation from bone sections of osteoporotic patients, which showed decreased *NFIC* expression and increased number of adipocytes. *Nfic* overexpression in *Nfic*$^{-/-}$ BMSCs showed increased ALP expression and mineralized nodule formation when cultured in odontogenic differentiation media. In adipogenic differentiation media, cells showed significantly decreased number of oil red-O positive cells along with the expression of *Pparγ*. To investigate whether NFI-C could rescue impaired bone formation, *Nfic*-overexpressing BMSCs were transplanted into femur cavities of *Nfic*$^{-/-}$ mice. Increased trabecular bone volume and number and decreased bone marrow adiposity were observed, along with the osteoblast differentiation from the transplanted BMSCs. When BMSCs overexpressing *Osx* were transplanted in the same manner, partial rescue of bone formation was observed. OSX is one of key factors that contributes to osteogenesis. BMP-2 is known to regulate osteogenic transcription factors such as runt-related transcription factor 2 (RUNX2), DLX5, MSX2, and OSX, therefore modulating osteogenesis[63-65]. Expression of *Nfic* was increased after the BMP-2 treatment. Silencing Runx2 decreased *Nfic* expression even with the BMP-2 treatment. The results confirmed that *Nfic* promoter had a binding motif for RUNX2, and RUNX2 directly bound to that site *in vitro*. An *in vivo* study showed that *Nfic* expression decreased through BMP-2 pathway in *Runx2*$^{-/-}$ mice[66]. Results from *in vivo* and *in vitro* studies demonstrated that *Runx2* acts as an upstream regulator of *Nfic*. Age-related osteoporosis patients exhibit increased bone adiposity and decreased bone formation due to impairment in osteoblast activity and differentiation. *In vitro* results using *Nfic*$^{-/-}$ mice BMSCs also showed decreased

osteoblast differentiation and increased formation of oil red-O positive cells. Since the osteoblasts and adipocytes differentiate from the same progenitor cell, BMSCs, the formation of osteoblasts and adipocytes could be inversely proportional. Finding a key factor or pathway that is capable of shifting BMSC differentiation toward the formation of osteoblasts rather than adipocytes could be a novel candidate for treatment of age-related osteoporosis[66].

V. Other Prominent Factors in Odontogenesis and Osteogenesis

Canonical Wnt signaling pathway regulates mesenchymal cell fate[67]. During adipocyte differentiation, *C/EBPβ* and *C/EBPδ* are upregulated, stimulating the expression of *C/EBPα* and *PPARγ* that in turn promote the expression of adipocyte specific genes[68]. C/EBPβ binds to the promoter region of *Wnt10b*, one of the Wnt ligands, inhibiting its expression. As a result, Wnt signaling is inhibited while adipogenesis is activated[69]. During osteogenesis, Wnt10b suppresses the expression of *C/EBPα* and *PPARγ* to shift the mesenchymal cell fate towards osteoblasts[70]. Therefore, Wnt signaling needs to be suppressed during adipogenesis, and activated during osteogenesis[71,72]. β-Catenin is a protein with a dual function; it is a cell adhesion molecule[73] and a transducer within the canonical Wnt pathway[74,75]. When Wnt ligands bind to the receptor complex of frizzled and low-density lipoprotein receptor-related protein 5/6, β-catenin gets accumulated in the cytosol and translocated into the nucleus. In the nucleus, β-catenin controls its target gene expression and cellular differentiation. Tissue specific inactivation of *β-catenin* in mice leads to the tooth phenotype that is very similar to that of *Nfic*$^{-/-}$ mice[76]. *In vitro* studies also demonstrated that *β-catenin* deficiency causes the shift of preosteoblast cell fate from osteoblast to adipocyte[67]. In *Nfic*$^{-/-}$ mice, the level of *β-catenin* expression decreased significantly, and the silencing of *β-catenin* using siRNA resulted in the decreased expression of *Nfic in vitro* (unpublished data).

A recent study suggested that NFI-C regulates adipocyte and osteocyte differentiation through the *Nfic-Wnt-Runx2-Osx* pathway[77]. However, further investigations should be conducted to explain a previous study that reported that changes in *Nfic* expression have no effect on the *Runx2* expression level[66].

The C/EBP family of transcription factors and NFI-C have the same binding motif, CCAAT[9,78]. C/EBPα, β, and δ are known to play important roles in adipocyte differentiation, and NFI-C inhibits adipogenesis; therefore, it is also possible that competitive binding between C/EBP family members and NFI-C might determine the mesenchymal cell differentiation, thereby promoting or inhibiting osteoblast and odontoblast differentiation. Further studies should be conducted on this issue.

VI. Conclusion

Several signaling pathways and proteins have been reported to regulate mineralized tissue formation. Even though NFI-C is one of the key transcription factors that induces odontoblast and osteoblast differentiation, its expression has to be precisely up- or down-regulated throughout the differentiation period in order to generate natural bone and tooth. The mechanism of maintenance of *Nfic* expression at an appropriate level throughout the differentiation period is still unknown. Further understanding of NFI-C and other factors of odontogenesis and osteogenesis will lead to the advancement of oral and maxillofacial regenerative therapies.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

ORCID

Song Yi Roh, *http://orcid.org/0000-0002-2795-8492*
Joo-Cheol Park, *http://orcid.org/0000-0002-3162-7557*

References

1. Neunzehn J, Weber MT, Wittenburg G, Lauer G, Hannig C, Wiesmann HP. Dentin-like tissue formation and biomineralization by multicellular human pulp cell spheres in vitro. Head Face Med 2014;10:25.
2. Sowmya S, Chennazhi KP, Arzate H, Jayachandran P, Nair SV, Jayakumar R. Periodontal specific differentiation of dental follicle stem cells into osteoblast, fibroblast, and cementoblast. Tissue Eng Part C Methods 2015;21:1044-58.
3. Chen X, Chen G, Feng L, Jiang Z, Guo W, Yu M, et al. Expression of Nfic during root formation in first mandibular molar of rat. J Mol Histol 2014;45:619-26.
4. Steele-Perkins G, Butz KG, Lyons GE, Zeichner-David M, Kim HJ, Cho MI, et al. Essential role for NFI-C/CTF transcription-replication factor in tooth root development. Mol Cell Biol 2003;23:1075-84.
5. Park JC, Herr Y, Kim HJ, Gronostajski RM, Cho MI. Nfic gene disruption inhibits differentiation of odontoblasts responsible for root formation and results in formation of short and abnormal roots in mice. J Periodontol 2007;78:1795-802.
6. Nagata K, Guggenheimer RA, Hurwitz J. Adenovirus DNA repli-

cation in vitro: synthesis of full-length DNA with purified proteins. Proc Natl Acad Sci U S A 1983;80:4266-70.
7. Nagata K, Guggenheimer RA, Enomoto T, Lichy JH, Hurwitz J. Adenovirus DNA replication in vitro: identification of a host factor that stimulates synthesis of the preterminal protein-dCMP complex. Proc Natl Acad Sci U S A 1982;79:6438-42.
8. Gronostajski RM. Roles of the NFI/CTF gene family in transcription and development. Gene 2000;249:31-45.
9. Gronostajski RM, Adhya S, Nagata K, Guggenheimer RA, Hurwitz J. Site-specific DNA binding of nuclear factor I: analyses of cellular binding sites. Mol Cell Biol 1985;5:964-71.
10. Meisterernst M, Gander I, Rogge L, Winnacker EL. A quantitative analysis of nuclear factor I/DNA interactions. Nucleic Acids Res 1988;16:4419-35.
11. Leegwater PA, van der Vliet PC, Rupp RA, Nowock J, Sippel AE. Functional homology between the sequence-specific DNA-binding proteins nuclear factor I from HeLa cells and the TGGCA protein from chicken liver. EMBO J 1986;5:381-6.
12. Jones KA, Kadonaga JT, Rosenfeld PJ, Kelly TJ, Tjian R. A cellular DNA-binding protein that activates eukaryotic transcription and DNA replication. Cell 1987;48:79-89.
13. Chaudhry AZ, Lyons GE, Gronostajski RM. Expression patterns of the four nuclear factor I genes during mouse embryogenesis indicate a potential role in development. Dev Dyn 1997;208:313-25.
14. Campbell CE, Piper M, Plachez C, Yeh YT, Baizer JS, Osinski JM, et al. The transcription factor Nfix is essential for normal brain development. BMC Dev Biol 2008;8:52.
15. Steele-Perkins G, Plachez C, Butz KG, Yang G, Bachurski CJ, Kinsman SL, et al. The transcription factor gene Nfib is essential for both lung maturation and brain development. Mol Cell Biol 2005;25:685-98.
16. Shu T, Butz KG, Plachez C, Gronostajski RM, Richards LJ. Abnormal development of forebrain midline glia and commissural projections in Nfia knock-out mice. J Neurosci 2003;23:203-12.
17. Gründer A, Ebel TT, Mallo M, Schwarzkopf G, Shimizu T, Sippel AE, et al. Nuclear factor I-B (Nfib) deficient mice have severe lung hypoplasia. Mech Dev 2002;112:69-77.
18. Driller K, Pagenstecher A, Uhl M, Omran H, Berlis A, Gründer A, et al. Nuclear factor I X deficiency causes brain malformation and severe skeletal defects. Mol Cell Biol 2007;27:3855-67.
19. Arana-Chavez VE, Massa LF. Odontoblasts: the cells forming and maintaining dentine. Int J Biochem Cell Biol 2004;36:1367-73.
20. Kollar EJ, Baird GR. Tissue interactions in embryonic mouse tooth germs. II. The inductive role of the dental papilla. J Embryol Exp Morphol 1970;24:173-86.
21. Thomas HF, Kollar EJ. Differentiation of odontoblasts in grafted recombinants of murine epithelial root sheath and dental mesenchyme. Arch Oral Biol 1989;34:27-35.
22. Lee TY, Lee DS, Kim HM, Ko JS, Gronostajski RM, Cho MI, et al. Disruption of Nfic causes dissociation of odontoblasts by interfering with the formation of intercellular junctions and aberrant odontoblast differentiation. J Histochem Cytochem 2009;57:469-76.
23. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 2007;35:495-516.
24. Itoh M, Nagafuchi A, Moroi S, Tsukita S. Involvement of ZO-1 in cadherin-based cell adhesion through its direct binding to alpha catenin and actin filaments. J Cell Biol 1997;138:181-92.
25. Stevenson BR, Siliciano JD, Mooseker MS, Goodenough DA. Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J Cell Biol 1986;103:755-66.
26. Kjenseth A, Fykerud TA, Sirnes S, Bruun J, Yohannes Z, Kolberg M, et al. The gap junction channel protein connexin 43 is covalently modified and regulated by SUMOylation. J Biol Chem 2012;287:15851-61.
27. Eckardt D, Theis M, Degen J, Ott T, van Rijen HV, Kirchhoff S, et al. Functional role of connexin43 gap junction channels in adult mouse heart assessed by inducible gene deletion. J Mol Cell Cardiol 2004;36:101-10.
28. Martin TA, Mansel RE, Jiang WG. Loss of occludin leads to the progression of human breast cancer. Int J Mol Med 2010;26:723-34.
29. Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, et al. Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol 1993;123:1777-88.
30. João SM, Arana-Chavez VE. Tight junctions in differentiating ameloblasts and odontoblasts differentially express ZO-1, occludin, and claudin-1 in early odontogenesis of rat molars. Anat Rec A Discov Mol Cell Evol Biol 2004;277:338-43.
31. Smith AJ, Tobias RS, Plant CG, Browne RM, Lesot H, Ruch JV. In vivo morphogenetic activity of dentine matrix proteins. J Biol Buccale 1990;18:123-9.
32. Herpin A, Lelong C, Favrel P. Transforming growth factor-beta-related proteins: an ancestral and widespread superfamily of cytokines in metazoans. Dev Comp Immunol 2004;28:461-85.
33. Burt DW. Evolutionary grouping of the transforming growth factor-beta superfamily. Biochem Biophys Res Commun 1992;184:590-5.
34. Horbelt D, Denkis A, Knaus P. A portrait of transforming growth factor β superfamily signalling: background matters. Int J Biochem Cell Biol 2012;44:469-74.
35. Liu F. Smad3 phosphorylation by cyclin-dependent kinases. Cytokine Growth Factor Rev 2006;17:9-17.
36. Siegenthaler JA, Miller MW. Transforming growth factor beta 1 promotes cell cycle exit through the cyclin-dependent kinase inhibitor p21 in the developing cerebral cortex. J Neurosci 2005;25:8627-36.
37. Ouellet S, Vigneault F, Lessard M, Leclerc S, Drouin R, Guérin SL. Transcriptional regulation of the cyclin-dependent kinase inhibitor 1A (p21) gene by NFI in proliferating human cells. Nucleic Acids Res 2006;34:6472-87.
38. Itoh S, ten Dijke P. Negative regulation of TGF-beta receptor/Smad signal transduction. Curr Opin Cell Biol 2007;19:176-84.
39. Zhang Y, Wang HR, Wrana JL. Smurf1: a link between cell polarity and ubiquitination. Cell Cycle 2004;3:391-2.
40. Ebisawa T, Fukuchi M, Murakami G, Chiba T, Tanaka K, Imamura T, et al. Smurf1 interacts with transforming growth factor-beta type I receptor through Smad7 and induces receptor degradation. J Biol Chem 2001;276:12477-80.
41. Lee DS, Park JT, Kim HM, Ko JS, Son HH, Gronostajski RM, et al. Nuclear factor I-C is essential for odontogenic cell proliferation and odontoblast differentiation during tooth root development. J Biol Chem 2009;284:17293-303.
42. Lee DS, Yoon WJ, Cho ES, Kim HJ, Gronostajski RM, Cho MI, et al. Crosstalk between nuclear factor I-C and transforming growth factor-β1 signaling regulates odontoblast differentiation and homeostasis. PLoS One 2011;6:e29160.
43. Weston CR, Davis RJ. The JNK signal transduction pathway. Curr Opin Cell Biol 2007;19:142-9.
44. Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 2001;81:807-69.
45. Nebreda AR, Porras A. p38 MAP kinases: beyond the stress response. Trends Biochem Sci 2000;25:257-60.
46. Fukada T, Civic N, Furuichi T, Shimoda S, Mishima K, Higashiyama H, et al. The zinc transporter SLC39A13/ZIP13 is required for connective tissue development; its involvement in BMP/TGF-beta signaling pathways. PLoS One 2008;3:e3642.
47. Prasad AS. Zinc: an overview. Nutrition 1995;11(1 Suppl):93-9.
48. Lobner D, Canzoniero LM, Manzerra P, Gottron F, Ying H, Knudson M, et al. Zinc-induced neuronal death in cortical neurons. Cell Mol Biol (Noisy-le-grand) 2000;46:797-806.
49. Chai F, Truong-Tran AQ, Ho LH, Zalewski PD. Regulation of caspase activation and apoptosis by cellular zinc fluxes and zinc

deprivation: a review. Immunol Cell Biol 1999;77:272-8.
50. Saydam N, Adams TK, Steiner F, Schaffner W, Freedman JH. Regulation of metallothionein transcription by the metal-responsive transcription factor MTF-1: identification of signal transduction cascades that control metal-inducible transcription. J Biol Chem 2002;277:20438-45.
51. Vallee BL, Auld DS. Zinc metallochemistry in biochemistry. EXS 1995;73:259-77.
52. Westin G, Schaffner W. A zinc-responsive factor interacts with a metal-regulated enhancer element (MRE) of the mouse metallothionein-I gene. EMBO J 1988;7:3763-70.
53. LaRochelle O, Labbé S, Harrisson JF, Simard C, Tremblay V, St-Gelais G, et al. Nuclear factor-1 and metal transcription factor-1 synergistically activate the mouse metallothionein-1 gene in response to metal ions. J Biol Chem 2008;283:8190-201.
54. Oh HJ, Lee HK, Park SJ, Cho YS, Bae HS, Cho MI, et al. Zinc balance is critical for NFI-C mediated regulation of odontoblast differentiation. J Cell Biochem 2012;113:877-87.
55. Black AR, Black JD, Azizkhan-Clifford J. Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol 2001;188:143-60.
56. Shields JM, Christy RJ, Yang VW. Identification and characterization of a gene encoding a gut-enriched Krüppel-like factor expressed during growth arrest. J Biol Chem 1996;271:20009-17.
57. Evans PM, Liu C. Roles of Krüpel-like factor 4 in normal homeostasis, cancer and stem cells. Acta Biochim Biophys Sin (Shanghai) 2008;40:554-64.
58. Gumireddy K, Li A, Gimotty PA, Klein-Szanto AJ, Showe LC, Katsaros D, et al. KLF17 is a negative regulator of epithelial-mesenchymal transition and metastasis in breast cancer. Nat Cell Biol 2009;11:1297-304.
59. Lin H, Liu H, Sun Q, Yuan G, Zhang L, Chen Z. KLF4 promoted odontoblastic differentiation of mouse dental papilla cells via regulation of DMP1. J Cell Physiol 2013;228:2076-85.
60. Lin H, Xu L, Liu H, Sun Q, Chen Z, Yuan G, et al. KLF4 promotes the odontoblastic differentiation of human dental pulp cells. J Endod 2011;37:948-54.
61. Narayanan K, Gajjeraman S, Ramachandran A, Hao J, George A. Dentin matrix protein 1 regulates dentin sialophosphoprotein gene transcription during early odontoblast differentiation. J Biol Chem 2006;281:19064-71.
62. Lee HK, Lee DS, Park SJ, Cho KH, Bae HS, Park JC. Nuclear factor I-C (NFIC) regulates dentin sialophosphoprotein (DSPP) and E-cadherin via control of Krüppel-like factor 4 (KLF4) during dentinogenesis. J Biol Chem 2014;289:28225-36.
63. Nishimura R, Hata K, Matsubara T, Wakabayashi M, Yoneda T. Regulation of bone and cartilage development by network between BMP signalling and transcription factors. J Biochem 2012;151:247-54.
64. Matsubara T, Kida K, Yamaguchi A, Hata K, Ichida F, Meguro H, et al. BMP2 regulates Osterix through Msx2 and Runx2 during osteoblast differentiation. J Biol Chem 2008;283:29119-25.
65. Lee MH, Kwon TG, Park HS, Wozney JM, Ryoo HM. BMP-2-induced Osterix expression is mediated by Dlx5 but is independent of Runx2. Biochem Biophys Res Commun 2003;309:689-94.
66. Lee DS, Choung HW, Kim HJ, Gronostajski RM, Yang YI, Ryoo HM, et al. NFI-C regulates osteoblast differentiation via control of osterix expression. Stem Cells 2014;32:2467-79.
67. Song L, Liu M, Ono N, Bringhurst FR, Kronenberg HM, Guo J. Loss of wnt/β-catenin signaling causes cell fate shift of preosteoblasts from osteoblasts to adipocytes. J Bone Miner Res 2012;27:2344-58.
68. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. Genes Dev 2000;14:1293-307.
69. Chung SS, Lee JS, Kim M, Ahn BY, Jung HS, Lee HM, et al. Regulation of Wnt/β-catenin signaling by CCAAT/enhancer binding protein β during adipogenesis. Obesity (Silver Spring) 2012;20:482-7.
70. Kang S, Bennett CN, Gerin I, Rapp LA, Hankenson KD, Macdougald OA. Wnt signaling stimulates osteoblastogenesis of mesenchymal precursors by suppressing CCAAT/enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma. J Biol Chem 2007;282:14515-24.
71. Christodoulides C, Lagathu C, Sethi JK, Vidal-Puig A. Adipogenesis and WNT signalling. Trends Endocrinol Metab 2009;20:16-24.
72. Prestwich TC, Macdougald OA. Wnt/beta-catenin signaling in adipogenesis and metabolism. Curr Opin Cell Biol 2007;19:612-7.
73. Rimm DL, Koslov ER, Kebriaei P, Cianci CD, Morrow JS. Alpha 1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. Proc Natl Acad Sci U S A 1995;92:8813-7.
74. Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG, Kemler R. Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. Mech Dev 1996;59:3-10.
75. Behrens J, von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosschedl R, et al. Functional interaction of beta-catenin with the transcription factor LEF-1. Nature 1996;382:638-42.
76. Kim TH, Bae CH, Lee JC, Ko SO, Yang X, Jiang R, et al. β-catenin is required in odontoblasts for tooth root formation. J Dent Res 2013;92:215-21.
77. Zhou J, Wang S, Qi Q, Yang X, Zhu E, Yuan H, et al. Nuclear factor I-C reciprocally regulates adipocyte and osteoblast differentiation via control of canonical Wnt signaling. FASEB J 2017. doi: 10.1096/fj.201600975RR. [Epub ahead of print]
78. Santoro C, Mermod N, Andrews PC, Tjian R. A family of human CCAAT-box-binding proteins active in transcription and DNA replication: cloning and expression of multiple cDNAs. Nature 1988;334:218-24.