

A Specific Osteogenic Transcription Factor - Activating Transcription Factor 4(ATF4)

Yi Liu, Dapeng Ren, Xuemin Zeng, Dongxu Liu, Chunling Wang

Department of Orthodontics & Shandong Provincial Key Laboratory of Oral Tissue Regeneration, School and Hospital of Stomatology, Shandong University, Jinan, Shandong 250012, P.R. China

Abstract

As we all known, skeleton remodeling exists in all process of tooth movement which is highly related with the orthodontics treatment. During the past several years, we have witnessed significant progresses in skeleton biology. The mechanisms and therapy of molecular, cellular and genetic is crucial to make a good understanding of complicated intracellular and extracellular signals involved in bone formation and homeostasis. Various transcription factors have been identified to play important roles during the skeleton remodeling, such as inducing the expression of osteogenic genes at the transcriptional level, promoting the differentiation of osteoblasts, osteoclasts and chondrocytes, and assisting these cells to accomplish their normal functions. The role of activating transcription factor 4 (ATF4) as a specific osteogenic transcription factor has come under attention since the year 2004. It has also been demonstrated there are more and more upstream and downstream factors of ATF4 in the intracellular and extracellular signal pathways regulating the expression of osteogenic genes. However, there exist other many nuclear accessory factors that interact with ATF4 to promote or prohibit its role of transactivation of the osteogenic genes, which is indispensable in the strictly regulated network to control the osteogenic cells differentiation and bone remodeling. In this review, we distill the factor functions about ATF4 into four types. Each type is illustrated with examples to demonstrate the functional complexity.

Key Words: Osteogenic, Transcription factor, ATF

Introduction

Bone is a dynamic tissue that is constantly remodeled, including the formation and resorption of bone, both of which are necessary for the skeletal homeostasis. Clear understanding about skeleton remodeling will definitely be beneficial to orthodontics treatment. Ducky.P and colleagues show that three types of cell, osteoblasts, osteoclasts and chondrocytes, which are critical for the growth and maintenance of the skeleton [1], however, transcription factors exert important functions in the control of the proliferation and differentiation of the cells we mentioned above. As early as 1997, runt related transcription factor(RUNX2) was found to be a vital specific osteogenic transcription factor [2]. Subsequently, another important bone specific transcription factor, Osterix, was discovered in 2002 and proved to act downstream of RUNX2 [3]. ATF4 was first considered to be able to control the differentiation of mature osteoblasts in 2004 [4]. Here, we will present an overview of ATF4 involvement regulatory mechanism that are requisite for differentiation, proliferation of osteoblasts, osteoclasts and chondrocytes, as well as for expression of osteogenic genes to sustain skeletal homeostasis. We will also review some other factors that can interact with ATF4 in maintaining bone homeostasis. This may deepen our understanding about the successive steps and molecular mechanisms involved in bone diseases, such as osteopetrosis and osteopenia.

Factors that act upstream of ATF4

It has been reported that ATF4 was regulated by a number of hormones, cytokines, kinases and specific signaling receptors on transcriptional level, translational level and post-translational level. We will list some well-studied factors here that can affect the differentiation of osteoblasts and expression of bone related genes in ATF4-dependant pathways.

Parathyroid hormone (PTH)

PTH is a main regulator of skeleton homeostasis and osteoblast activity. PTH functions through binding to its receptor PTH1R, a G protein coupled receptor that is expressed in osteoblasts [5-7], and then can activate multiple pathways. Some study showed that PTH rapidly stimulates OCN gene expression in mouse MC3T3-E1 pre-osteoblastic cells and primary bone marrow stromal cells, and this effect is due to the increasing binding affinity of ATF4 to the promoter of OCN induced by treatment of these cells with PTH [8]. Some study has shown that treating mouse MC3T3-E1 subclone 4 (MC-4) cells with increasing concentration of PTH can dose-dependently elevate the level of both ATF4 mRNA and protein, and further using the transcription inhibitor ActD completely abolished the PTH-stimulated ATF4 mRNA induction, while the protein synthesis inhibitor CHX had no effect on the PTH-stimulated ATF4 protein induction. So the studiers presumed that PTH induce the expression of ATF4 on transcriptional level, not on translational level [9]. Recently, another study has proved that intermittent injection of PTH on mice can raise the bone mass on three aspects: promoting the proliferation of osteoblasts/preosteoblasts, stimulating differentiation of osteoblasts, and inhibiting the apoptotic of osteoblasts/osteocytes. Comparing with the wild mice, the PTH-induced increase in differentiation and proliferation of osteoblasts were severely reduced or completely abolished in ATF4-deficient mice, while the inhibition on apoptotic of osteoblasts had little change. Thus, these results suggest that ATF4 mediate the anabolic action of PTH on bone mass through the first two aspects [10].

Kinase

It is known that polypeptide is translated from messenger RNA initially, and then need further post-translational regulation set by some other factors to fulfill its functions. Among all kinds of post-translational regulations, phosphorylation is the most

Corresponding author: Chunling Wang, Department of Orthodontics & Shandong Provincial Key Laboratory of Oral Tissue Regeneration, School and Hospital of Stomatology, Shandong University, Jinan, Shandong 250012, P.R. China, Tel: +86-531-88382070, e-mail: wangchl@sdu.edu.cn

common which is accomplished by upstream kinases. Now there were two well known kinases that can phosphorylate ATF4 protein: RSK2 and PKA. RSK2 (ribosomal kinase 2) belongs to a family of ribosomal serine/threonine kinases that contain two distinct kinase domains, one at the C-terminal and one at the N-terminal [11-13]. RSK2 could be phosphorylated by some kinase upstream of itself on C-terminal domain, this results in the activation of the N-terminal domain, which can then phosphorylate its substrates [14,15]. RSK2 can phosphorylate ATF4 protein, and this interaction happens on serine 251 and 254 in mouse and human ATF4 respectively. DNA cotransfection assays showed that RSK2 phosphorylation modulates ATF4 transactivation function, thus increase the expression of osteoblast maker genes OCN and BSP. In RSK2-deficient mice, ATF4 phosphorylation was hindered and cannot transactivate its target genes [4]. RSK2 may also affect the level of ATF4 expression provided by the fact that both transient and stable knock-down of RSK2 lead to the decrease of ATF4 expression [16]. This may partially explain the reason why RSK2-deficient mice has severely low bone mass.

PKA (protein kinase A) is another kinase that also phosphorylates human ATF4 in serine 254, and phosphorylated ATF4 by PKA can induce the expression of RANKL and differentiation of osteoclasts. Overexpression of PKA in osteoblasts enhanced ATF4 transactivation function, while point mutation in serine 254 completely obstruct ATF4 phosphorylation by PKA [17].

Signaling pathways

Mitogen-activated protein kinase MAPK can integrate a wide range of extracellular signals and transform them into intracellular signals that stimulate cellular proliferation, differentiation and apoptosis [18]. It includes three pathways: ERK1/2 (extracellular signal related kinase 1/2), JNK (c-jun NH2-terminal kinase) and p38MAPK. JNK was reported to be related with the expression of ATF4 and treatment of MC3T3-E1 and primary osteoblasts with JNK inhibitor decreased the ATF4 expression in these cells [19]. Even though the action of JNK on ATF4 is not direct, JNK can phosphorylate c-jun and thus promote the formation of AP-1 complex which is composed of c-jun and c-fos. This complex can bind to the ATF4 promoter in the sequence of 1081-1088, so this mechanism can explain the decreased expression of ATF4 after using the JNK inhibitor.

PERK (Double stranded RNA activated protein kinase (PKR)-like endoplasmic reticulum kinase) belongs to the type endoplasmic reticulum transmembrane proteins. Endoplasmic reticulum stress (ERS) can activate the PERK protein, which leads to the phosphorylation of eIF2 α , the α subunit of the eukaryotic initiation factor 2. This process resulted in the translational obstruction of most proteins but ATF4 protein, because ATF4 has upstream open reading frames (ORFs) in its 5'-untranslated region. These ORFs prevent translation of ATF4 under normal levels, but can be bypassed once eIF2 α is phosphorylated, thus ATF4 translation occurs [20,21]. PERK deficient mice has an osteopenia phenotype similar with that of ATF4 deficient mice, and in the skeletal tissue of PERK deficient mice, ATF4 protein decreased notably together with osteogenic factors, while

ATF4 mRNA remained normally [22]. So the PERK-eIF2 α pathway may be involved in the translational regulation of ATF4 protein, and therefore leads to the skeletal formation and osteoblast differentiation.

Fibroblast growth factor 2FGF2

FGF2 is expressed by osteoblasts and stored in extracellular matrix. It can stimulate the osteoblast differentiation and bone formation probably through increasing the expression of ATF4 mRNA and protein, and FGF2 deficiency resulted in decreased ATF4 expression together with decreased ALP activity and marked reduction of mineralized nodules [23]. The stimulatory effect of PTH on ATF4 was also attenuated in FGF2 deficiency mice compared to wild type mice, this suggests that FGF2 acts on the upstream of ATF4 and mediate the regulation of PTH on ATF4 and other bone-related genes [24].

Nf1

Nf1 is a gene encoding RasGAP (GTPase activating protein) neurofibromin. Histomorphometric analysis of the Nf1^{-/-} mice revealed two abnormalities: increase in osteoid volume and increase in bone resorption. Further study discovered that in Nf1^{-/-} mice osteoblasts, RSK2 and PKA phosphorylation were increased, and this led to the transactivation of ATF4 [25]. Phosphorylation of ATF4 by RSK2 and PKA can stimulate the expression of osteogenic genes and osteoclast differentiation factor RANKL respectively, and this helps explain the phenotypes in Nf1^{-/-} mice [4,17]. So, ATF4 is also involved in the NF1-dependent pathway controlling bone remodeling, and NF1 can regulate ATF4 transactivation negatively.

Gpr48

Gpr48 is a member of GPCRs (G-protein-coupled receptors), which are integral membrane proteins that involved in the transmission of signals from the extracellular environment to the cytoplasm. Gpr48 is expressed in bone lineage cells and is highly related with bone remodeling and formation. Gpr48^{-/-} mice has a lower expression of ATF4 mRNA and protein, while overexpression of Gpr48 led to a significant increase in ATF4 expression [26]. Further study found that Gpr48 could promote the activation of cAMP and subsequently the phosphorylation of PKA and CREB, a substrate of PKA. There is a CRE sequence in atf4 promoter, and phosphorylated CREB can bind to the atf4 promoter to activate the ATF4 expression on the transcriptional level. Hence, the cAMP-PKA-CREB pathway might be involved in the Gpr48-related regulation of ATF4 and skeletal remodeling [21,26].

TGF β

Transforming growth factor β (TGF β) is one of the most abundant cytokines stored in bone matrix and can promote osteoblast proliferation and inhibit osteoblast differentiation. TGF β could suppress ATF4 transcriptional activity via an indirect mechanism, not by directly decreasing the level of ATF4. Further study reported that this mechanism may involve the PI3K-Akt-mTOR signaling and vimentin. Firstly, TGF β up-regulates vimentin production at post-transcriptional

level, via the PI3K-Akt-mTOR signaling. Through the protein interaction between vimentin and ATF4, and subsequent obstruction of ATF4 binding to its target genes, TGF β then prevent ATF4 from transactivating its downstream genes, such as osteocalcin. So, aside from the TGF β -Smad3-Runx2 signaling, the TGF β -PI3K-Akt-mTOR-vimentin-ATF4 pathway may be another mechanism underlying the TGF-dependent regulation of osteocalcin and inhibition of osteoblast differentiation.

Factors that act downstream of ATF4

Since ATF4 is a bone specific transcription factor and regulate the bone remodeling, a lot of factors have been found to be positively correlated with the ATF4 expression during the differentiation of osteoblasts, osteoclasts and chondrocytes. Some of these factors are directly secreted by those bone lineage cells as marked genes of the mature differentiation, while others are also some transcription factors that control the bone formation and resorption.

Osteoblastic specific genes

Osteocalcin(OCN) is the most osteoblast-specific structural gene identified to date and is the latest molecular marker gene of the osteoblast phenotype. The mouse OCN gene promoter contains two OSE2 sequences and one OSE1 sequence [27]. ATF4 can directly bind to the OSE1 and increase the activity of OCN gene promoter, thus induce the expression of OCN [28]. In the skeletal tissue of ATF4-deficient embryos and newborn mice, the expression of OCN was dramatically decreased in comparing to normal mice [4]. Inhibition of ATF4 degradation in non-osteoblastic cells, such as C2C12 myoblasts and NIH3T3 fibroblasts, leads to the accumulation of ATF4 protein in these cells and also the detectable expression of OCN [29].

Bone sialoprotein(BSP)is a bone matrix glycoprotein and works as a nucleator of hydroxyapatite crystals through its glutamic acid rich clusters [30]. Overexpression of ATF4 in periodontal ligament cells stimulate the expression of OCN, as well as BSP [31]. Compared to normal mice, the expression of BSP in skulls and long bones was also much weaker in ATF4-deficient mice [4].

Osteriox (OSX)

Osteriox is another osteoblastic specific transcription factor that can control the expression of osteogenes. RUNX2 is known to be acting upstream of osteriox and there is a RUNX2 binding site existing on the osteriox promoter [32]. Recently, some study found a putative ATF4 binding sequence (CTTCCTCA) on the mice osteriox promoter and mutation of this sequence can abolish the ATF4-activated osteriox expression [10]. Thus, ATF4 is also required for the expression of osteriox and ATF4 could cooperate with RUNX2 to activate the maximum expression of osteriox.

Cyclin D1

Cyclin D1 is a key sensor and integrator of extracellular signals of cells and plays a critical role in cell cycle progression and proliferation. Expression of Cyclin D1 is highly dependent upon the presence of ATF4, as demonstrated by the fact that Cyclin D1mRNA and protein was dramatically

reduced in ATF4 $^{-/-}$ BMSCs while overexpression of ATF4 in ATF4 $^{-/-}$ BMSCs rescued the decreased Cyclin D1 expression at both the mRNA and protein level [33]. In ATF4 $^{-/-}$ BMSCs, shrinking cytoplasm, chromatin condensation and DNA fragmentation were all increased. These evidences showed that ATF4 promote cell proliferation through its regulation of Cyclin D1 and cell circle progression [33]. Mechanisms whereby ATF4 increase expression of Cyclin D1 is probably as follows: There is a CRE/ATF sequence on the promoter of Cyclin D1, and ATF4 can directly bind to this sequence or interact with CREB and AP-1 to stimulate the expression of Cyclin D1, and ATF4 could also stabilize the Cyclin D1mRNA at post-transcriptional level [33].

Osteoclast differentiation factors

RANKL is a member of TNF superfamily and can bind to RANK on osteoclast precursors, resulting in the activation of multiple signaling and osteoclast differentiation. ATF4 can bind to the CRE sequence that is present on the RANKL promoter and regulate the expression of RANKL, and this action of ATF4 required the phosphorylation by PKA rather by RSK2 [17]. NFATc1 is another critical regulator of osteoclast differentiation that acts downstream of RANKL. The fact that expression of NFATc1mRNA was greatly decreased in ATF4 $^{-/-}$ BMSCs showed that ATF4 is a regulator of NFATc1. ATF4 can either directly activated the -847/+66 Nfatc1 P1 promoter to stimulate the NFATc1 expression, or increase the expression of RANKL at first and promote the NFATc1 expression further. So, ATF4 can control the expression of NFATc1 through these two ways mentioned above [34].

Indian hedgehog (Ihh)

Endochondral ossification is one of the two types of ossification that relies on chondrocyte proliferation and differentiation and is tightly regulated by systemic factors, locally secreted factors and transcription factors. Indian hedgehog (Ihh) belongs to the hedgehog (Hh) family and is one of the aforementioned locally secreted factors required for mammalian endochondral ossification. Ihh $^{-/-}$ mice exhibit a reduction in chondrocytic proliferation, impaired chondrocyte maturation and absence of osteoblasts [35,36]. Some experiment found proliferative chondrocyte disorganization and delayed hypertrophic mineralization in ATF4 $^{-/-}$ mice, and this is resulted from the decreased expression of Ihh in ATF4 $^{-/-}$ mice. Nine putative binding site(A1-19) in Ihh promoter was tested, and A9TGGCAACA was the one that mediates ATF4 transcriptional regulation of Ihh, so ATF4 can control the proliferation and differentiation of chondrocytes via directly binding to the Ihh promoter and increasing the expression of Ihh [37]. ATF4 can also promote the differentiation of bone marrow stromal cells into osteoblasts via Ihh in chondrocytes. Through creating Atf4 $^{-/-}$;Col2a1-Atf4 mice in which Atf4 was overexpressed specifically in chondrocytes in an Atf4 $^{-/-}$ genetic background, other study found that expression of Ihh, OCN, BSP, BV/TV(a ratio of bone volume over total tissue volume) and BFR/BS (a ratio of bone formation rate over bone surface) were evidently increased in Atf4 $^{-/-}$;Col2a1-Atf4 mice compared to Atf4 $^{-/-}$ mice [38]. So, Ihh is an important factor that acts downstream

of ATF4 in chondrocytes through which ATF4 exert its transcriptional activity in chondrocytic differentiation, proliferation and osteoblast differentiation.

DDR2

The discoidin domain receptor (DDR) proteins are members of the receptor tyrosine kinase superfamily that contain DDR1 and DDR2. DDR2 is a key regulator for the expression of osteoblast-specific genes, and knockout of DDR2 in mice leads to dwarfism, manifested by shortening of long bones [39]. Another study showed that mutation of the human DDR2 gene is responsible for spondylometaepiphyseal dysplasia, which is manifested by short limbs with abnormal calcification [40]. Some study showed that ablation of ATF4 caused a delayed induction of DDR2 expression during osteoblast differentiation. Although a C/EBP binding site at 1150 bp was found in the DDR2 promoter and mutation of this site abrogated the binding of ATF4 to DDR2 promoter, ATF4 could not bind to the DDR2 promoter alone since the transactivation of DDR2 by ATF4 required the interaction of ATF4 with C/EBP [41].

-catenin

β -catenin acts as a downstream component of the WNT signaling, which is important for controlling bone mass in humans and vertebrates. Some study manifested that the expression of β -catenin is critical for osteoblast differentiation because mesenchymal stem cells lacking β -catenin can not differentiate into osteoblasts. The level of β -catenin protein was markedly decreased in ATF4-deficient mice, while the level of β -catenin mRNA was not. Similar to the in vivo experiment, shRNA knockdown of Atf4 expression reduces and overexpression of ATF4 increases β -catenin protein levels in MC-4 cells without affecting the mRNA level of β -catenin. The exact mechanism of this phenomenon is not clear right now, but the same investigators don't think of ATF4 as an upstream factor of the WNT/ β -catenin pathways and the ATF4-induced β -catenin protein expression may be more related with a post-translational regulation based on the proof that ATF4 and β -catenin can form a protein-protein complex.

Factors that modify ATF4 protein stability on the post-translational level

ATF4 mRNA is expressed ubiquitously in almost all cell types, while ATF4 protein is only accumulated strictly in some specific tissues such as skeleton, liver, lung and intestine [29]. ATF4 protein is very unstable and can be degraded rapidly in non-osteoblasts. Some factors participate in the regulation of ATF4 protein stability and thus control ATF4 protein on the post-translational level.

(1) β TrCP1 is a component of ubiquitin ligase. It can interact with ATF4 protein to trigger ATF4 ubiquitination, and this leads to ATF4 proteasomal degradation in HeLa cells [42]. The absence of β TrCP1 expression could not affect its interaction with ATF4 and proteasomal degradation of ATF4 protein while inactivation of β TrCP1 [29]. (2) Acetylation has also been reported to alter protein stability. HAT p300 is a member of nuclear histone acetyltransferases (HATs) that is able to stabilize ATF4 independently of its enzymatic activity while overexpression of p300 leads to ATF4 stabilization. The

mechanism under this is that ATF4 protein can interact with p300 through its N-terminal domain and this interaction leads to recruitment of ATF4 into nuclear speckles where it is unavailable for β TrCP1 binding to ATF4 and thus protects ATF4 from proteasomal degradation [43]. (3) TFA γ can also increase ATF4 protein stability and ATF4 protein was rapidly degraded and almost undetectable on Western blot in the absence of TFA γ , while overexpression of which greatly delayed the degradation process with the levels of ATF4 protein only slightly reduced [44]. (4) M-CSF is derived from osteoblasts, stromal cells and hypertrophic chondrocytes that can regulate osteoclast formation and maturation. BMMs were cultured in the presence and absence of M-CSF and the level of ATF4 protein was dramatically reduced in the absence of M-CSF, while the level of ATF4mRNA was not. Subsequently using LY294002, a specific inhibitor of the PI3K/AKT pathway, dramatically reduced total and phosphorylated ATF4 protein. Thus, the M-CSF/PI3K/AKT pathway is involved in the de novo protein biosynthesis as well as modulation of ATF4 protein stability [34].

Factors that interact with ATF4 protein

A lot of factors have been reported to interact with ATF4 protein to form a complex, and this interaction could change ATF4 transcriptional activity. The interaction between distinct families of DNA-binding transcription factors was observed in the context of transcriptional activation or repression, and this notion remains valid nowadays. Here, we list some well-known interactions, either activating or inhibiting ATF4, to exhibit a more complicated regulation network in the ATF4-dependent transcriptional modification of osteoblast specific genes.

Combinatorial interactions activating ATF4

RUNX2 is also an osteogenic transcription factor that can bind to the OSE2 sequence on osteocalcin promoter. It was observed that ATF4 activates the osteocalcin gene promoter via cooperative interaction with RUNX2, and this costimulation required an intact OSE2. While the activation was maximal when both the OSE1 and OSE2 were present, it was still observed in the absence of the ATF4 binding site OSE1 [28].

C/EBP β is a member of CCAAT/enhancer-binding protein family and can form heterodimerization with ATF4, which could allow ATF4 to synergize with RUNX2 to increase osteocalcin expression [45]. Although Runx2 and ATF4 interact in osteoblasts, this interaction is not directed, provide by the Co-Immunoprecipitation assay. Since TF II A γ can bind to ATF4 and RUNX2 directly, it tend to be the bridge between the two factors and maximizes the osteocalcin gene transcription by promoting the formation of the complex containing TF II A γ , ATF4 and RUNX2 [44].

SATB2 is a member of the special AT-rich binding protein family. SATB2-deficient mice have compromised osteoblast differentiation and function, with decreased expression of osteoblast-specific genes such as OCN and BSP. Except for the ability of SATB2 binding to BSP promoter to activate its expression, direct protein-protein interactions between SATB2 and RUNX2, as well as SATB2 and ATF4 were

provided by GST-pulldown experiments and coimmunoprecipitation. So SATB2 appear to stabilize the interaction between ATF4 and RUNX2, resulting in enhanced DNA binding and transactivation by the two factors [46].

FoxO1, a member of the ubiquitously expressed FoxO family of transcription factors, could affect osteoblast proliferation and function by regulating amino acid import and thus protein synthesis. This function results from the physical interaction of FoxO1 with ATF4. Both the factors predominantly located in the cytoplasm initially in the absence of any stimuli, and stress signals stimulate their translocation into the nucleus, where they actively associate with each other to transactivate some target genes such as OCN [47].

Combinatorial interactions inhibiting ATF4

Factor Inhibiting ATF4-mediated Transcription (FIAT) is a novel leucine zipper factor that can interact with ATF4, and then subsequently block ATF4 triggered osteocalcin gene transcription. FIAT contains three leucine zipper domains but no identifiable basic DNA recognition sequences, and it can heterodimerize with ATF4 through the second leucine zipper domain and thereby stop ATF4 from binding to its cognate DNA sequences such as OSE1 exists on the OCN promoter [48]. Stable overexpression of FIAT transgene was shown to prohibit transcription of OCN and BSP, and to reduce mineralization, both in cultures of primary osteoblasts or in MC3T3-E1 cells [48,49]. FIAT transgenic mice were osteopenic with decreased bone mineral density, bone volume, mineralized volume, mineral apposition rates, and reduced rigidity of long bones, and this is due to a perturbation of osteoblastic activity, rather than the reduced proliferation and increased apoptosis of osteoblast [48]. Conversely, using RNA interference that effectively down-regulate FIAT expression at the mRNA and protein level leads to a relief of its inhibition on ATF4 activity, thereby increase ATF4 binding to its target gene and augmentation of expression and promoter occupancy of OCN and BSP, as well as type I collagen synthesis [50]. FIAT is expressed in early differentiated osteoblasts as well as osteocytes, but not in mature osteoblasts. This is in adverse with the expression of ATF4, which is expressed highest in mature osteoblasts during the osteoblast differentiation. Thereby, an excess of ATF4 over FIAT in mature osteoblasts releases the inhibitory effect of FIAT on ATF4, allowing ATF4 to subsequently transactivate the osteocalcin gene and possibly other downstream signalling targets important for bone formation [51].

Twist1, one of the basic helix-loop-helix (bHLH)-containing transcription factors, could prohibit ATF4 function of transactivating osteocalcin promoter through interaction between those two proteins. Overexpression of Twist1 in ROS cells attenuated PTH-induced ATF4 binding to the osteocalcin promoter, while had no effect on ATF4 protein level. The Twist domain is necessary for Twist1 to interact with ATF4, while the bHLH domain is sufficient but unnecessary for the interaction. In ATF4 protein, domain necessary for interacting with Twist1 is located between aa 1 and 276 and that the basic, ZIP, and the carboxy-terminal domains are dispensable for this interaction [52].

Vimentin is a type III intermediate filament (IF) protein, which belongs to cytoskeletal proteins that have been well recognized to be localized in the cytoplasm and perinucleus, but can also transport into the nucleus by DNA oligonucleotide mediation. The interaction between ATF4 and Vimentin is mediated by the LZ1 (aa124-138) domain existing on Vimentin protein, and the C-LZ(aa110-221) domain existing on ATF4 protein. Vimentin inhibit ATF4 transcriptional activity by affecting its ability of binding to osteoblast genes, such as BSP and OCN, and inhibition of Vimentin expression using siRNA-mediated knockdown induces endogenous OCN expression [53].

The inducible cAMP early repressor (ICER) is transcribed from the P2 promoter of the *Crem* gene, which encodes the cAMP responsive element modulator (CREM). When ATF4 and ICER were co-transfected, ICER can profoundly repress the ATF4 activity at the OSE1 site without affecting the RUNX2 activity at the OSE2 site. The mechanism under this may rely on the fact that ICER only consists the bZIP domain and is devoid of transactivation domain. The high affinity of ICER to the cAMP responsive element (CRE) sites precludes the recruitment of other transcription factors necessary for transcriptional initiation of their target genes. This may explain the reduced bone mass and impaired osteoblast differentiation observed in ICER transgenic mice.

Summary

In vitro and in vivo studies all showed that ATF4 is a crucial transcription factor for osteoblast and osteoclast differentiation. As a hinge in the exquisitely regulated network controlling osteogenic genes expression, ATF4 not only assembles multiple signal pathways in promoting skeletal remodeling and development, but also interacts with lots of well-known factors to form steric regulation of osteoblast differentiation. Although ATF4 and its regulation on skeletal development have been widely accepted, the mechanism on how exactly it works and how the factors influence ATF4 expression or activity still need further studies. Thus, more genes regulated by ATF4 and more signal pathways that affect ATF4 will need to be discovered, which may allow us to fully evaluate the genetic cascade controlling the skeletal homeostasis. This could shed some light on the therapeutic treatment for osteoporosis and other diseases of altered bone mass by stimulating or suppressing ATF4 expression and then affect its function on skeletal biology. We envision that it will be possible to take the orthodontists a step further in the process of understanding the intriguing orthodontics treatment controlling the skeletal remodeling process. More generally, this will have profound potential in skeletal biology.

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