Bone 48 (2011) 514–523

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Distinct mechanisms are responsible for osteopenia and growth retardation in OASIS-deficient mice

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ARTICLE INFO

Article history:
Received 2 July 2010
Revised 14 October 2010
Accepted 27 October 2010
Available online 1 November 2010

Edited by: Bjorn Olsen

Keywords:
Osteopenia
Bone formation
Osteoblast
Endoplasmic reticulum
Growth retardation

ABSTRACT

Old astrocyte specifically induced substance (OASIS), which is a new type of endoplasmic reticulum (ER) stress transducer, is a basic leucine zipper transcription factor of the CREB/ATF family that contains a transmembrane domain and is processed by regulated intramembrane proteolysis in response to ER stress. OASIS is selectively expressed in certain types of cells such as astrocytes and osteoblasts. We have previously demonstrated that OASIS activates transcription of the type I collagen gene *Col1a1* and contributes to the secretion of bone matrix proteins in osteoblasts, and that *OASIS*−/− mice exhibit osteopenia and growth retardation. In the present study, we examined whether osteopenia in *OASIS*−/− mice is rescued by OASIS introduction into osteoblasts. We generated *OASIS*−/− mice that specifically expressed OASIS in osteoblasts using a 2.3-kb osteoblast-specific type I collagen promoter (*OASIS*−/−;Tg mice). Histological analysis of *OASIS*−/−;Tg mice revealed that osteopenia in *OASIS*−/− mice was rescued by osteoblast-specific expression of the *OASIS* transgene. The decreased expression levels of type I collagen mRNAs in the bone tissues of *OASIS*−/− mice were recovered by the *OASIS* transgene accompanied by the rescue of an abnormal expansion of the rough ER in *OASIS*−/− osteoblasts. In contrast, growth retardation in *OASIS*−/− mice did not improve in *OASIS*−/−;Tg mice. Interestingly, the serum levels of growth hormone (GH) and insulin-like growth factor (IGF)-1 were downregulated in *OASIS*−/− mice compared with those in wild-type mice. These decreased GH and IGF-1 levels in *OASIS*−/− mice did not change when OASIS was introduced into osteoblasts. Taken together, these results indicate that OASIS regulates skeletal development by osteoblast-dependent and -independent mechanisms.

Introduction

The endoplasmic reticulum (ER) is a critical cellular compartment in which secretory and transmembrane proteins are folded or processed [1,2]. Various cellular stress conditions lead to the accumulation of unfolded or misfolded proteins in the ER lumen. When unfolded proteins accumulate in the ER lumen, eukaryotic cells activate a system termed the unfolded protein response (UPR) to deal with the unfolded proteins and avoid cellular damage [3–5]. In mammalian cells, monitoring of the ER lumen and signaling for the UPR are mediated by three major transducers, inositol-requiring 1, PKR-like endoplasmic reticulum kinase and activating transcription factor 6 (ATF6). These transducers sense unfolded proteins in the ER lumen and transduce signals to the cytoplasm and nucleus for the transcription of UPR target genes, translational attenuation of global protein synthesis and ER-associated degradation (ERAD). ER stresses caused by genetic mutations and environmental factors are associated with various diseases, such as diabetes, neurodegeneration and osteogenesis imperfecta (OI) [6–8].

Old astrocyte specifically induced substance (OASIS) is a basic leucine zipper (bZIP) transcription factor of the CREB/ATF family that contains a transmembrane domain [9–11]. Its structure is very similar to that of ATF6. OASIS is cleaved at the membrane by regulated intramembrane proteolysis in response to ER stress. Its cleaved cytoplasmic domain, which contains the bZIP domain, translocates into the nucleus where it activates the transcription of target genes. Unlike classical ER stress transducers that are expressed ubiquitously, OASIS has a unique expression pattern that is limited to certain tissues and cells, including astrocytes and osteoblasts [9–14]. CREBH, BBF2H7 and AIbZIP have similar structures to OASIS, and their expression

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doi:10.1016/j.bone.2010.10.176

levels show tissue- or cell type-specific patterns [15–18]. These proteins, including OASIS, have been reported to function as tissue-specific ER stress transducers that convert ER stress to the transcription of target genes for development, differentiation, maturation or other cell type-specific events in various tissues including bone tissues [13–18].

Osteoblasts and osteoclasts are specialized cells responsible for bone formation and resorption, respectively [19,20]. Osteoblasts synthesize type I collagen, which is the major bone matrix protein and it is essential for proper bone formation and strength [21]. Mutations in the genes that encode type I collagen or defects in quality control or posttranslational modification of type I collagen are linked to misfolding or decreased synthesis of type I collagen, and consequently cause OI [22–24]. Therefore, protein quality control in the ER for secreted bone matrix proteins is important for the formation of integrated bone.

We have previously demonstrated that OASIS activates the transcription of the type I collagen gene *Col1a1* and contributes to the secretion of bone matrix proteins in osteoblasts [13]. Expression and activation of OASIS in osteoblasts are promoted by bone morphogenetic protein 2, the signaling of which promotes synthesis of large amounts of bone matrix proteins in osteoblasts and then causes mild ER stress. *OASIS*−/− mice exhibit severe osteopenia involving a decrease in type I collagen in the bone matrix, similar to OI type I, and they also show growth retardation [13,25]. The osteopenia in *OASIS*−/− mice results from a dysfunction of osteoblasts, which show decreased transcription of type I collagen and abnormal expansion of the rough ER with the accumulation of bone matrix proteins. While secretion of bone matrix proteins is impaired in *OASIS*−/− mice, expression levels of bone matrix proteins other than type I collagen are increased and expression level of type I collagen is decreased [13]. Therefore, type I collagen is decreased in the bone matrix of *OASIS*−/− mice. In contrast, it remains unclear whether growth retardation in *OASIS*−/− mice is caused by osteoblast dysfunction.

In the present study, we generated *OASIS*−/− mice that specifically expressed OASIS in osteoblasts (*OASIS*−/−;Tg mice) and investigated whether expression of OASIS in osteoblasts rescues osteopenia, osteoblast dysfunction, and growth retardation in *OASIS*−/− mice.

Material and methods

Generation of OASIS transgenic mice

The generation of *OASIS*−/− mice has been previously described [13]. *OASIS* transgenic (Tg) mice were generated using a pJ251 vector containing an osteoblast-specific 2.3-kb *Col1a1* promoter, FLAG-tagged full-length mouse *OASIS* cDNA and an SV40 polyadenylation signal. The linearized construct was injected into fertilized eggs derived from C57BL/6CrSlc mice by SLC, Inc., Shizuoka, Japan. Transgene integration was identified by genomic polymerase chain reaction (PCR). The primers used for the genomic PCR were 5′-GCCCAGGCCAGTCGTCGGAGCAG-3′ and 5′-CACGCTCCGTGTTCCA-CATC-3′. The tissue specificity of the transgene expression was examined by reverse transcription (RT)-PCR. Although several lines of transgenic mice were obtained, we demonstrated the results from a strain that specifically expressed the *OASIS* transgene in bone tissues (Fig. 1B). The transgenic line was maintained in OASIS+/− [13] background. *OASIS*−/−;Tg, *OASIS*−/− and wild-type (WT) mice were generated by mating *OASIS*+/− and *OASIS*+/−;Tg mice. All experiments were performed with the consent of the Animal Care and Use Committees of Miyazaki University.

Growth hormone treatment

Three-week-old mice were subcutaneously injected with 3 μg/g per day of recombinant growth hormone (GH) (Wako Pure Chemical Industries, Osaka, Japan) for 3 weeks. This dosage of GH treatment was determined according to a previously published protocol [26,27]. Control mice were injected with saline.

Cell cultures

Primary cultures of osteoblasts were prepared from the calvariae of postnatal 3–4-day-old *OASIS*−/− and *OASIS*−/−;Tg mice. The calvariae were digested with 0.1% collagenase (Wako Pure Chemical Industries, Osaka, Japan) and 0.2% dispase (Gibco BRL, Rockville, MD). The isolated cells were grown in alpha-modified Eagle's medium

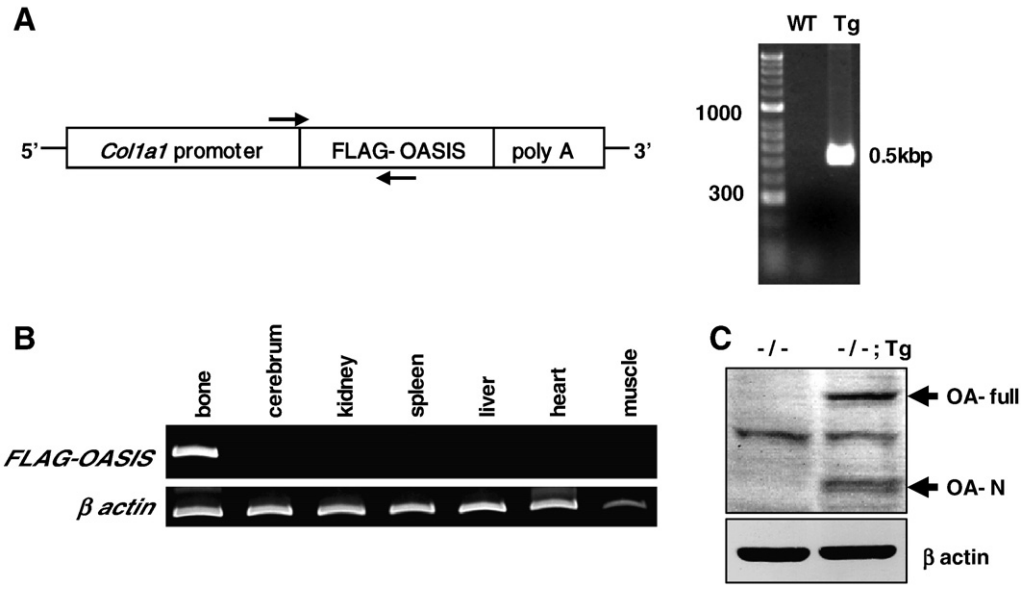

Fig. 1. Generation of *OASIS*−/− mice that specifically express OASIS in osteoblasts. (A) Construction of an *OASIS* transgene containing an osteoblast-specific *Col1a1* promoter and an SV40 polyadenylation signal (Poly A). Arrows indicate the annealing sites of the genomic PCR primer. Tg: *OASIS* transgenic mice. The right panel shows the results of genomic PCR for genotyping of the *OASIS* transgene. The expected band (0.5 kbp) is detected. (B) RT-PCR analysis of RNA from the indicated tissues of 4-week-old *OASIS* Tg mice. *OASIS* is specifically expressed in the bone tissue. (C) Western blot analysis of lysates from primary cultures of osteoblasts using an anti-OASIS antibody. Full-length OASIS (OA-full) and the cleaved N-terminal fragment of OASIS (OA-N) are expressed in *OASIS*−/− osteoblasts by the OASIS transgene.