Osterix finds its master

Stavroula Kousteni

Bone formation requires generation of osteoblasts from progenitor cells. It also depends on the ability of mature osteoblasts to synthesize bone extracellular matrix (ECM). In osteoblasts, procollagen type I $\alpha 1$ and $\alpha 2$ chains are imported into the endoplasmic reticulum (ER), where they are post-translationally modified to form procollagen triple helices. These are then secreted to form mature type I collagen, the main protein component of bone ECM. Evidence for the regulation of protein synthesis in secretory cells has come from investigation of the unfolded protein response (UPR; Ron & Walter, 2007), a mechanism that counteracts the deleterious effects of accumulating unfolded proteins in the ER. The UPR in higher eukaryotes involves activation of chaperone proteins along with repression of global protein synthesis and ER-associated protein degradation. The three main transducers of the UPR—eIF-2 α kinase PERK (PKR-like ER kinase), IRE1 (inositol-requiring endonuclease 1) and ATF6 (activating transcription factor 6)—sense unfolded proteins in the ER lumen and transduce signals to the nucleus.

...IRE1–XBP1 signalling is activated during BMP2-induced osteoblast differentiation

The role of PERK and ATF6 in relation to osteoblast function has been investigated previously. PERK is required for prenatal and postnatal bone development (Zhang *et al*, 2002). In its absence, the secretory pathway in osteoblasts cannot efficiently process and secrete procollagen. OASIS—an ATF6 structural homologue processed in response to ER stress—induces transcription of *Col1 $\alpha 1$* through an UPR element (UPRE)-like sequence in the *Col1 $\alpha 1$* promoter (Murakami *et al*, 2009). *OASIS*^{−/−} mice are severely osteopenic, due to a decrease in

Col1 $\alpha 1$ levels and a decline in the activity of osteoblasts due to the accumulation of ECM proteins in the ER.

In this issue of *EMBO reports*, the Horiuchi group reveals a new mechanism by which the UPR response regulates differentiation and the secretory function of osteoblasts (Tohmonda *et al*, 2011). This new pathway involves activation of the third arm of the UPR response, IRE1 α signalling. IRE1 α is a transmembrane kinase/endonuclease in the ER membrane that cleaves X-box binding protein 1 (XBP1) prior to its translocation to the nucleus, where it binds to and activates the expression of the UPRE-containing genes needed to alleviate ER stress (Costa-Alvear *et al*, 2007).

Tohmonda and colleagues induced osteoblastic lineage with bone morphogenetic protein 2 (BMP2) in mouse embryonic fibroblasts (MEFs). Osteoblastogenesis was verified by increased expression of the transcription factor *Runx2*, which triggers osteoblast differentiation (Ducy *et al*, 1997) as well as the bone ECM proteins COL1A1 and osteocalcin. Differentiation occurred in parallel with induction of the UPR response. Protein levels of both spliced and unspliced *Xbp1* increased in the nucleus of BMP2-treated MEFs. XBP1 processing was confirmed in *ex vivo* cultures of BMP2-treated calvaria explants. Thus, IRE1–XBP1 signalling is activated during BMP2-induced osteoblast differentiation.

As distention of the ER due to accumulation of proteins can compromise many cellular functions, a key question is whether IRE1 α is directly involved in these events. Two processes are involved in formation of mature osteoblasts from MEFs: commitment to the osteoblastic lineage and differentiation of ECM-producing osteoblasts. Tohmonda and colleagues report that inactivation of IRE1 α in BMP2-treated MEFs leads to increased expression of the early osteoblast differentiation markers *Runx2* and *Col1a*. By contrast, expression of later

differentiation markers—some of which facilitate the synthesis of ECM components such as bone sialoprotein or alkaline phosphatase—was not induced. This indicates that osteoblast maturation is defective in the absence of IRE1 α .

The authors identify the osteoblast-specific transcription factor Osterix (Nakashima *et al*, 2002), but not *Runx2*, as a direct target of XBP1. XBP1 regulates Osterix expression by binding to an XBP1-binding site in the Osterix promoter. In addition, a UPRE-like sequence is required for maximal transcriptional activity in response to BMP2. Osterix expression in *Ire1 α* -deficient MEFs partly rescues the decrease in alkaline phosphatase activity.

XBP1 regulates Osterix expression by binding to an XBP1-binding site in the Osterix promoter

Osterix is expressed at the osteochondroprogenitor stage, in a population of cells that express *Runx2* (Ducy *et al*, 1997) and that are able to differentiate into osteoblasts or chondrocytes. Osterix acts downstream from *Runx2* to potentiate the transition to the osteoblast lineage (Nakashima *et al*, 2002). Thus, it was interesting to test whether IRE1 α –XBP1 signalling regulates Osterix transcription before or after the transition of the osteochondroprogenitor cell to the committed pre-osteoblast. To address this question *in vitro*, two cell populations were used: multipotent, uncommitted MEFs and committed pre-osteoblasts. Induction of ER stress promoted Osterix reporter activity in both MEFs and pre-osteoblasts, but only increased Osterix expression in pre-osteoblasts, an effect that was abolished by knocking down *Xbp1*. Thus, XBP1-mediated Osterix regulation seems to be functional in committed osteoblast progenitors.

Fig 1 | During osteoblast differentiation, ER stress triggers splicing of *Xbp1* messenger RNA by IRE1 α , resulting in activation of the unfolded protein response, which maintains ER homeostasis. Spliced XBP1 translocates to the nucleus where it binds to the *Osterix* promoter and increases its expression. Osterix in turn promotes differentiation of committed pre-osteoblasts and ECM deposition. ECM, extracellular matrix; ER, endoplasmic reticulum; XBP1, X-box binding protein 1; *XBP1u*, unspliced XBP1.

In hindsight, the fact that Osterix is induced by ER stress responses triggered by osteoblast differentiation is not surprising, as it is also required for deposition of bone matrix. In relation to this, it would have been interesting to test the possibility of a direct XBP1–ATF4 interaction. ATF4 is one of three transcription factors required for osteoblast formation and mineralization (Yang *et al*, 2004). Tohmonda and colleagues (2011) show that expression of both *Perk* and *Atf4* is upregulated in BMP2-treated MEFs; yet, although the increase in *Atf4* expression is IRE1 α -dependent, that of *Perk* is not. PERK function is often assessed by its activation status rather than expression levels; however, the possibility that XBP1 might also target ATF4 remains important to examine.

One limitation of the present study is that all experiments were performed *in vitro*, using an artificial system in which osteoblast differentiation was induced by BMP2. Additional studies involving cell-specific deletion of the molecules involved are required to further confirm the role of the proteins, and the interactions between them, in skeletal physiology. This, however, does not undermine the importance of the findings presented here. First, the current study implicates the IRE1 α –XBP1 pathway in osteoblast UPR and osteoblast function. Second, it links ER-stress-induced signalling to transcriptional regulation of Osterix. Finally, it identifies for the first time a regulator of Osterix expression, by showing that XBP1 binds directly to its promoter (Fig 1).

The finding that, in addition to IRE1 α –XBP1 signalling, the other two branches of the UPR response—PERK–ATF4 and ATF6—might also be activated in response to BMP2, indicates that the UPR response is universally upregulated during osteoblast differentiation. At a time when the need to translate basic science to clinical applications is increasingly being realized, observations linking UPR response mechanisms to bone homeostasis are even more important, because they might be relevant to human disease. Indeed, mineralization defects observed in *Perk*^{−/−} mice are similar to those seen in patients with osteogenesis imperfecta, a disease associated with defective collagen synthesis. In that respect, it is interesting to note that a form of this disease has recently been associated with a frameshift mutation in Osterix (Lapunzina *et al*, 2010). Similarly, Wolcott–Rallison syndrome, a rare disorder that is characterized by permanent neonatal insulin-dependent diabetes, severe epiphyseal dysplasia and osteoporosis, results from mutations in *Perk* (Delepine *et al*, 2000). Furthermore, ATF4 is implicated in Coffin–Lowry syndrome, an X-linked condition associated with mental retardation and skeletal abnormalities (Yang *et al*, 2004).

REFERENCES

- Costa-Alvarez D *et al* (2007) *Mol Cell* **27**: 53–66
- Delepine M *et al* (2000) *Nat Genet* **25**: 406–409
- Ducy P *et al* (1997) *Cell* **89**: 747–754
- Lapunzina P *et al* (2010) *Am J Hum Genet* **87**: 110–114
- Murakami T *et al* (2009) *Nat Cell Biol* **11**: 1205–1211
- Nakashima K *et al* (2002) *Cell* **108**: 17–29
- Tohmonda T *et al* (2011) *EMBO Rep* **12**: 451–457
- Ron D, Walter P (2007) *Nat Rev Mol Cell Biol* **8**: 519–529
- Yang X *et al* (2004) *Cell* **117**: 387–398
- Zhang P *et al* (2002) *Mol Cell Biol* **22**: 3864–3874

Stavroula Kousteni is at the Department of Medicine, Division of Endocrinology, College of Physicians & Surgeons, Columbia University, New York, New York, USA
E-mail: sk2836@columbia.edu

Published online 8 April 2011

EMBO reports (2011) **12**, 382–383.
 doi:10.1038/embo.2011.61