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Roles of Kinases in Osteoblast Function

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1. Introduction

Osteoblasts, as well as osteocytes that are a terminally differentiated form of osteoblasts, are responsible for bone formation by producing bone matrix proteins, which subsequently induce tissue mineralization. Osteoblasts differentiate and mature from their progenitors in response to various regulatory factors including bone morphogenetic proteins (BMPs), Insulin-like growth factor 1 (IGF-1), fibroblast growth factor 2 (FGF-2), parathyroid hormone (PTH), tumor necrosis factor-α (TNF-α), Wnts, and extracellular matrix (ECM) signals. Matrix mineral deposition occurs at the terminal stage of osteoblast differentiation and is associated with maximal expression of osteocalcin (Ocn).

Although several transcription factors, such as runt-related transcription factor 2 (Runx2), Osterix (Osx), and activating transcription factor (ATF) 4, have been demonstrated to be crucial to bone development, the molecular details of intracellular signals controlling stage-specific osteoblast differentiation remain enigmatic. It is well recognized that the mitogen activated protein kinase (MAPK) superfamily, including p44/p42 extracellular signal-regulated kinases (ERKs), p38 kinases, and p54/p46 c-Jun N-terminal kinases (JNKs), integrates signals from a diverse range of extracellular stimuli, and plays important roles in cellular functions such as proliferation, differentiation, and cell death in a variety of cell types. Despite intensive investigation, the physiological roles of MAPKs in osteogenic differentiation have not been clearly demonstrated. AMP-activated protein kinase (AMPK) is a serine-threonine kinase widely known as an essential regulator of energy homeostasis of cells. Interestingly, however, non-metabolic functions of AMPK, including its roles in cell differentiation have recently been demonstrated. Several recent studies including ours have indicated that the differentiation of osteoblasts is functionally associated with the AMPK activity.

Being located in the skeletons, osteoblasts are constantly exposed to various forms of mechanical stresses. Many previous studies have indicated that mechanical stresses on osteoblasts affect their cell differentiation process, which may explain why moderate exercises contribute to healthy bone development. However, mechanical stresses often induce tissue inflammation in bones, especially when they are excessive. We and others have recently revealed that osteoblasts respond to mechanical stresses by expressing various inflammatory cytokines and chemokines, which is dependent on the activation of kinases, such as MAPKs.

In this chapter, I will first briefly review recent and basic knowledge about osteoblast functions. I will then try to review the contribution of various kinase signals, including MAPKs and AMPK, to the osteoblast differentiation process. Finally, I will review the known mechanisms of kinase activation by mechanical stresses in osteoblasts, as well as how the mechanical stress-activated kinases affect the physiological functions of osteoblasts, including cell proliferation, differentiation, survival and the expression of chemokines and cytokines.

2. The basic picture of osteoblast functions

In vertebrates, bone tissue constitutes the skeleton and provides mechanical support, motility, and protection of internal organs. Bone tissue also acts as a reservoir of biologically essential minerals including calcium, phosphate, and magnesium. Osteoblasts, as well as osteocytes are responsible for bone formation by producing bone matrix proteins, which subsequently induce tissue mineralization (Raisz and Kream 1983). Osteoblasts are derived from bone marrow mesenchymal stem cells (MSCs), and terminally differentiate into osteocytes in bone tissue. In contrast, osteoclasts, which are derived from bone marrow hematopoietic stem cells, are in charge of bone resorption. The growth and maintenance of bone mass are thus regulated by the coordinated actions of osteoblasts and osteoclasts. Bone remodelling is a dynamic and constant process even in adults, replacing approximately 15% of the trabecular and 3% of the cortical bones every year (Manolagas and Jilka 1995). Disturbance of this delicate balance in bone remodelling leads to various bone disorders. The most well-known is probably osteoporosis which affects over 200 million people world-wide (Cooper, Campion, and Melton 1992) and is considered as a serious public health concern. Osteoporosis is caused by the relative increase of bone resorption resulting in decreased bone density and increased risk of fractures. In contrast, relative increase of bone formation causes disorders such as osteopetrosis and osteosclerosis.

The coordinated balance of bone remodelling is established by multiple coupling mechanisms between osteoblasts and osteoclasts. The most noticeable mechanism is through RANK ligand (RANKL) expressed on the surface of osteoblasts. RANKL signals through RANK, a cell surface receptor expressed on osteoclasts (Yasuda et al. 1998; Lacey et al. 1998; Boyle, Simonet, and Lacey 2003). In a complex interplay with macrophage colony-stimulating factor (M-CSF), which is also produced by osteoblasts, RANKL induces the maturation and activation of osteoclasts. Osteoblasts also secrete osteoprotegerin (Opg) that acts as an inhibitory decoy receptor for RANKL, and the expression ratio of RANKL to Opg by osteoblasts dictates osteoclastogenesis (Manolagas and Jilka 1995; Boyle, Simonet, and Lacey 2003). Thus osteoblasts are the central players of bone metabolism controlling both bone formation and resorption.

2.1 Molecular mechanisms of osteoblast differentiation

Osteoblasts are derived from MSCs, which have the potential to differentiate into myoblasts, fibroblasts, and chondrocytes, in addition to osteoblasts. Osteoblasts differentiate and mature from their progenitors in response to various regulatory factors including BMPs, Insulin-like growth factor 1 (IGF-1), fibroblast growth factor 2 (FGF-2), parathyroid hormone (PTH), tumor necrosis factor-α (TNF-α), Wnts, and ECM signals (Deng et al. 2008). Osteoblasts produce various bone matrix proteins during differentiation (Fig. 1). Matrix mineral deposition occurs at the terminal stage of osteoblast differentiation and is associated with maximal expression of Ocn (Hauschka et al. 1989).

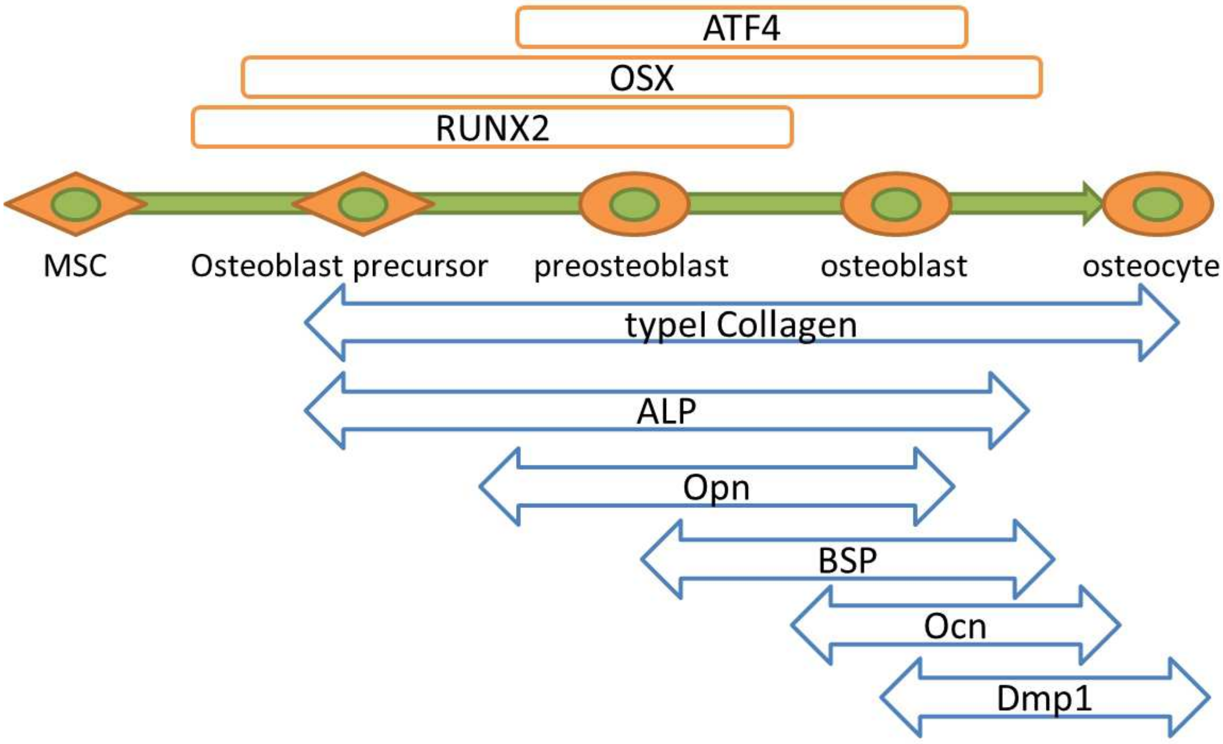

Fig. 1. Schematic presentation of osteoblast differentiation. Osteoblast differentiation is controlled by the expression of several transcription factors including Runx2, Osx, and ATF4. A series of proteins termed “osteogenic markers” contributing to bone matrix formation are produced during osteoblast differentiation in stage-specific manners. These proteins include ColI, Opn, and Ocn.

Differentiation of MSCs into different lineages is governed by the expression of lineage-specific transcription factors. Specifically, osteoblast differentiation is controlled by transcription factors including Runx2 and Osx. Gene deficiency of either Runx2 or Osx causes defective bone formation and lack of mature osteoblasts in mice, suggesting that both of these transcription factors are necessary for osteoblast differentiation in vivo (Ducy et al. 1997; Komori et al. 1997; Nakashima et al. 2002). The Runx regulatory element is found in the gene promoter regions of major bone matrix proteins including collagen type I (ColI), osteopontin (Opn), bone sialoprotein (Bsp), and Ocn (Marie 2008), indicating that the expression of these proteins is directly regulated by Runx2. Furthermore, recent reports have indicated that Runx2 interacts with some other transcription factors, which either activates or repress the transcriptional activity of Runx2 (Karsenty 2008). Thus Runx2 functions as a transducer through which various signals can modulate osteoblast differentiation. A typical example is Runx2-Smad interaction. Two Smad members, Smad1 and Smad5 which are activated by BMPs, interact with Runx2 and enhance its osteoblastogenetic activity.

The essential role of Osx, a zinc-finger protein with homology to the Sp1/Kruppel transcription factor family, in osteogenesis relies on its ability to regulate the expression of a number of osteogenic markers such as Opn, Dickkoph-related protein 1 (Dkk1), and ColI. Furthermore, transcriptional regulators such as Nuclear factor of activated T-cells, cytoplasmic (NFATc) or Nucleolar protein 66 (NO66), a jumonji family histone demethylase, have been shown to interact with Osx and regulate its transcriptional responses (Koga et al. 2005; Sinha et al. 2010). Osx has been generally believed as a downstream transcription factor of Runx2, as Osx-deficient osteoblasts still express Runx2 (Nakashima et al. 2002) and the transcription of Osx is positively regulated by Runx2 (Celil, Hollinger, and Campbell 2005). Interestingly, although expression of Osx in vivo requires Runx2, BMP-2 is still able to stimulate Osx expression in Runx2-deficient cells (Lee et al. 2003). Moreover, recent data have indicated that BMP-2 activates expression of Osx through Runx2-dependent as well as -independent mechanisms involving Dlx5 and Msx2 (Ulsamer et al. 2008; Matsubara et al. 2008).

Other than Runx2 and Osx, various transcription factors have been identified as important regulators of osteoblast differentiation. They include ATF4, activator protein 1 (AP1), homeobox proteins (Msx1, Msx2, Dlx5, Dlx6), and CCAAT/enhancer-binding proteins (C/EBPs) (Marie 2008). ATF4, also known as cAMP-response element-binding protein 2 (CREB2), is a basic leucine-zipper transcriptional factor that belongs to ATF/CREB protein family (Karpinski et al. 1992). Similar to Runx2 and Osx, an essential role of ATF4 in bone development in vivo was revealed by a study using ATF4-deficient mice, which showed a marked reduction in bone mineralization and trabecular development throughout life (Yang et al. 2004). The promoting effects of ATF4 on bone development is not through the modulation of Runx2 or Osx expression level, as ATF4-deficient mice showed normal expression levels of these two transcriptional factors (Yang et al. 2004).

2.2 Osteoblasts as immune-regulatory cells

Bone tissue often becomes the site of inflammation caused by infection, auto-immune responses, and mechanical stresses. Recent lines of evidence have indicated that osteoblasts are important immune-regulatory cells controlling inflammatory responses in bone tissue. We and others have previously shown that osteoblasts express several members of Toll-like receptors (TLRs) which sense microbe infection by binding pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) (Kikuchi et al. 2001; Kikuchi et al. 2003; Amcheslavsky et al. 2005). Stimulation of osteoblasts through TLRs induce the production of RANKL and TNF-α, both of which activate osteoclast-mediated bone resorption (Kikuchi et al. 2001; Kikuchi et al. 2003; Hayashi et al. 2003).

It has been reported that in inflammatory bone tissues, osteoblasts also express chemokines including monocyte chemoattractant protein (MCP)-1, macrophage-inflammatory protein (MIP)-1, RANTES (Lisignoli et al. 2002) and interleukin (IL)-8 (Bendre et al. 2003). These chemokines attract monocytes, macrophages and T cells through CC chemokine receptor 1 (CCR1), CCR2, CCR3, CCR5, and CXC chemokine receptor1 (CXCR1) (Yoshie, Imai, and Nomiyama 2001). Interestingly, IL-8 has recently been shown to increase the motility of osteoclasts and directly induce their differentiation through both RANKL-dependent and independent pathways (Bendre et al. 2003).

2.3 Effects of mechanical stresses on osteoblast functions

Mechanical loading to the skeleton is important for the development and maintenance of strong and healthy bones. Significant bone loss is often encountered due to long-term immobilization, which has become an increasing social problem. Being located in the skeletons, osteoblasts are constantly exposed to various forms of mechanical stresses. Many previous studies have indicated that mechanical stresses on osteoblasts affect their cell proliferation, survival, and differentiation processes, which may explain why moderate exercises contribute to healthy bone development. However, mechanical stresses often induce tissue inflammation in bones, especially when they are excessive. We and others have recently revealed that osteoblasts respond to mechanical stresses by expressing various inflammatory cytokines and chemokines (Bandow et al. 2007; Maeda et al. 2007).

3. Kinases involved in osteoblast differentiation

As briefly discussed in 2.1, several transcription factors including Runx2, Osx, and ATF4 have been demonstrated to be crucial for bone development. However, the molecular details of intracellular kinases controlling stage-specific osteoblast differentiation remain enigmatic. The biggest reason, I presume, is that the expression of most kinases remains relatively constant during osteoblast differentiation, unlike some transcription factors whose expression is clearly induced in differentiation stage-specific manners. Here I list some of the reported kinases whose activities are functionally associated with osteoblast differentiation.

3.1 MAPKs

It is well recognized that the MAPK superfamily, including p44/p42 ERKs, p38 kinases, and p54/p46 JNKs, integrates signals from a diverse range of extracellular stimuli, and plays important roles in cellular functions such as proliferation, differentiation, and cell death in a variety of cell types (Widmann et al. 1999). Activation of MAPKs requires dual phosphorylation of Tyr and Thr residues in the activation loop of the molecules, which is catalized by a family of dual specificity kinases termed MAPK kinases (MKKs). Conversely, their dephosphorylation is catalyzed by dual specificity phosphatases termed MAPK phosphatases (MKPs). Among MKP family members, some show highly selective substrate specificity, whereas others efficiently inactivate all three classes of MAPKs. We previously cloned an MKP termed MKP-M (also referred to as DUSP16 or MKP-7) (Matsuguchi et al. 2001; Masuda et al. 2001; Tanoue et al. 2001), which preferentially inactivates JNKs (Matsuguchi et al. 2001; Masuda et al. 2001).

The role of ERKs in osteoblast differentiation has been studied with inconsistent results. In recent reports, matrix mineralization of MC3T3-E1 cells (a mouse osteoblastic cell line) and preosteocytic MLO-A5 cells was increased by an inhibitor of MEK1/2 that are upstream activators of ERKs (Kono et al. 2007). In contrast, selective expression of a constitutively active form of ERK1 by a transgenic approach accelerated in vitro differentiation of mouse calvaria cells, as well as in vivo bone development in mice (Ge et al. 2007). We additionally observed that matrix mineralization by MC3T3-E1 cells was enhanced by an MEK1/2 inhibitor, whereas the same inhibitor clearly inhibited matrix mineralization of primary osteoblasts from mouse calvaria, indicating the regulatory role of ERKs in osteogenic differentiation may vary depending on the cell types or differentiation stages of osteoblasts (Matsuguchi et al. 2009).

Being similar to ERKs, the role of p38 kinase in osteoblast differentiation is also disputable. BMP2 is known to activate p38 kinase in osteoblasts. Kinase activity of p38 kinase was reported to be stimulatory (Gallea et al. 2001) or inhibitory (Vinals et al. 2002) in BMP-2-induced osteoblast differentiation. Noticeably, it has recently been reported that p38 kinase phosphorylates Osx at Ser-73/77 (Ortuno et al. 2010). Phosphorylatied Osx showed increased ability to recruit coactivators including p300 and SWI/SNF leading to the formation of transcriptionally active complexes, indicating the possibility that p38 exerts promotion of osteoblast differentiation by phosphorylating Osx.

JNK was originally identified by its ability to specifically phosphorylate the transcription factor c-Jun on its N-terminal transactivation domain (Hibi et al. 1993). JNK consists of three isoforms (JNK1, 2 and 3) deriving from distinct genes. Among them, JNK1 and JNK2 are ubiquitously expressed while JNK3 is mainly expressed in brain, testis, and heart (Kyriakis and Avruch 2001). Alternative splicing at the C-terminus yields 46 kDa and 54 kDa polypeptides for each JNK isoform. The 46kDa form is predominant for JNK1, whereas the 54kDa form is predominant for JNK2 and JNK3. Although JNK1 and JNK2 possess structural similarities and many overlapping biological functions, recent evidences have revealed some functional differences between the two kinases (Sabapathy et al. 2004; Liu, Minemoto, and Lin 2004). We have recently found that the terminal osteoblastic differentiation was significantly inhibited by the treatment with a specific JNK inhibitor as well as the over-expression of MKP-M, a JNK-specific MKP (Matsuguchi et al. 2009). Conversely, enhanced matrix mineralization was observed by inducible overexpression of $p54^{JNK2}$ in an isoform-specific manner. More specifically, JNK inhibition significantly suppressed the late stage molecular events of osteoblastic differentiation, such as gene expression of Ocn and Bsp. In contrast, earlier differentiation events including alkaline phosphatase (ALP) activation and Opn expression were not inhibited by JNK inactivation. As for transcriptional factors, induction of ATF4 expression during osteoblastic differentiation was significantly inhibited, whereas the expression levels of Runx2 and Osx, were not significantly affected by JNK inactivation. Thus JNK activity is essential for the late-stage differentiation of osteoblasts.

3.2 Phosphoinositide3-Kinase (PI3K)-akt signaling pathway

Various cytokines and growth factors activate PI3K to produce phosphatidylinositol 3,4,5-phosphate (PIP_3), which then recruits and activates Akt. Akt has been shown to be activated by BMPs in osteoblasts (Ghosh-Choudhury et al. 2002). Deletion of Akt1, the major form of Akt in osteoblasts, in mice results in osteopenia caused by the inhibition of differentiation and the increase of apoptosis of osteoblasts (Kawamura et al. 2007). Conversely, osteoblast-specific deletion of Pten, an inhibitory phosphatase of PI3K, by Ocn-Cre system in mice induced progressive increases in bone volume and density throughout life (Liu et al. 2007). The isolated Pten-defective osteoblasts showed accelerated differentiation capacity and decreased susceptibility to apoptosis. These results indicated that the PI3K-Akt signal is promotive of osteoblasts differentiation and inhibitory to osteoblast apoptosis. Consistently, it has been shown that Akt is essential for Runx2 indcution in osteoblasts by IGF-I, a known osteogenic growth factor.

3.3 Glycogen synthase kinase 3 (GSK3) in Wnt signals

Wnt family proteins play important roles in both embryogenesis and postnatal development. Wnts bind their cell surface receptor, Frizzled (Frz) in combination with the co-receptors, low density lipoprotein receptor-related protein (LRP) 5/6. The activated receptors induce the inactivation of a serine/threonine kinase, GSK3. In the steady-state cells, GSK3 form a complex with β-Catenin and phosphorylates it leading to its ubiquitin-mediated degradation (Cadigan and Liu 2006). When Wnt is signal is on, GSK3-mediated β-Catenin degradation is off, leading to the accumulation of β-Catenin in the nucleus. Nuclear β-Catenin forms a complex with a transcription factor, TCF/LEF, activating the transcription of a series of Wnt-target genes including Runx2 in osteoblasts. This GSK3-mediated cannonical pathway is responsible for the osteogenic activity of Wnts, as inhibitors of GSK3 stimulate osteoblastgenesis in vitro and in vivo (Kulkarni et al. 2006).

Involvemennt of GSK3 in the synergestic effects of Wnt and BMP-4 on osteoblastic differentiation has recently been reported (Fukuda et al. 2010). Wnt3a, a canonical Wnt, synergistically stimulated ColI expression and ALP activity in the presence of BMP-4. Interestingly, overexpression of β-Catenin did not affect BMP-4-induced ALP activity, whereas inhibition or stimulation of GSK3β activity resulted in either stimulation or suppression of ALP activity, respectively, in the presence of BMP-4. This finding indicated that GSK3β mediates synergy of BMP-4 and Wnt3a to promote osteoblastic differentiation through a β-Catenin-independent mechanism.

3.4 AMP-Activated Protein Kinase (AMPK)

AMP-activated protein kinase (AMPK) is a serine-threonine kinase widely known as a regulator of cell metabolism (Hardie 2007). AMPK is a hetero-trimeric protein consisting of the catalytic α subunit and the regulatory β and γ subunits in a 1:1:1 stoichiometric ratio. As several isoforms have been identified for each subunit (α1-2; β1-2; γ1-3), 12 combinations are possible, which show relative tissue specificity (Cheung et al. 2000). AMPK is activated by cellular stresses with increased AMP:ATP ratio as in calorie restriction. Subsequently, ATP-generating pathways are activated, while ATP-consuming mechanisms are inhibited, thereby restoring the cellular AMP:ATP ratio. AMPK is thus considered as an essential regulator of energy homeostasis, and termed as a metabolic “energy sensor” of the biological system. The activation of AMPK is through phosphorylation of the catalytic α subunit by an upstream kinase (Hawley et al. 1996). The most characterized upstream AMPK kinase (AMPKK) is LKB1, which is a reported target of metformin, a type 2 diabetes drug (Shaw et al. 2005).

Intriguingly, recent studies have revealed non-metabolic functions of AMPK, such as the regulation of cell polarity and mitosis (Lee et al. 2007), and mammal longevity (McCarty 2004). Several lines of evidence have also indicated the involvement of AMPK in the regulation of cellular differentiation. Activation of AMPK was suggested to be inhibitory to the differentiation of adipocytes (Dagon, Avraham, and Berry 2006; Giri et al. 2006; Habinowski and Witters 2001; Hwang et al. 2005; Tong et al. 2008), and myoblasts (Fulco et al. 2008), and promotive to the differentiation of endothelial progenitor cells (Li et al. 2008).

We have recently explored the role of AMPK in osteoblast differentiation using in vitro differentiation models (Kasai et al. 2009). We found that the phosphorylation of AMPKα was progressively decreased during osteoblastic differentiation. Conversely, osteoblast differentiation was significantly inhibited by glucose restriction and treatment with metformin, both of which are known activators of AMPK, as well as the forced expression of a constitutively active form of AMPKα. Metformin stimulation of osteoblasts significantly inhibited gene expression of Runx2 along with osteogenic markers including Ocn, Bsp, and Opn. Our result is consistent with a recent report showing the inhibitory effects of AMPK activators on Wnt/β-Catenin signaling in human osteoblastic cells (Takatani et al. 2011). In contrast to our result, treatment by metformin was previously reported to promote osteoblast differentiation (Cortizo et al. 2006; Kanazawa et al. 2008). We presume that the inconsistent results were due to the different dose of metformin used in experiment. The previous reports used metformin at much lower concentrations than we did, which might not be sufficient to induce sustained AMPKα phosphorylation (Kasai et al. 2009). Thus the effects of AMPK activity on osteoblast differentiation may vary in a dose-dependent fashion.

4. Kinases involved in mechanotransduction of osteoblasts

It has been well established that the load-induced mechanical stresses significantly affect cellular functions of osteoblasts. Mechanical stresses have been reported to be associated with increased proliferation, accelerated differentiation, and inhibition of apoptosis of osteoblasts (Aguirre et al. 2006; Robling and Turner 2009; Turner et al. 2009). Recent lines of evidence have also revealed that mechanical stresses induce the expression of RANKL, inflammatory cytokines, and chemokines by osteoblasts (Bandow et al. 2007; Maeda et al. 2007). However, the reception and intracellular signals transducing these stresses (termed as mechanotransduction) of osteoblasts are not well understood. What makes things more complicated is that there are various types of mechanical stresses. They include compression (static or cyclic), tension (static and cyclic), shear stress (including fluid shear stress), ultrasound, and others. It should be considered that the mechanotrasduction mechanisms for different types of stresses may significantly vary. Moreover, intensity and frequency for each type of mechanical stress may also affect the induced molecular events in cells. Here, I will review some of the kinases previously identified as mechanotransducers of osteoblasts.

4.1 MAPKs

4.1.1 ERKs

It has been reported that in osteoblasts ERKs are activated by mechanical stimuli including fluid shear stress (You et al. 2001; Kapur, Baylink, and Lau 2003; Weyts et al. 2002), compression by centrifugation (Hatton et al. 2003), cyclic stretch (Jessop et al. 2002), magnaetic drag force (Pommerenke et al. 2002), and ultrasound (Bandow et al. 2007) (Fig. 2). At least in our hands, it is the MAPK most evidently activated by cyclic stretch (unpublished result) and ultrasound (Bandow et al. 2007). Osteoblast proliferation induced by cyclic stretch requires the activation of ERKs as the treatment with a specific inhibitor of the ERK activating pathway abrogated the proliferation-promoting activity of cyclic stretch (Boutahar et al. 2004). ERK activation has also been reported to be essential for the fluid shear stress to stimulate osteoblast differentiation (Kido et al. 2009). Furthermore, a previous report has indicated that the stretch-induced anti-apoptotic effect on osteoblasts requires the activation of ERKs but not PI3K or p38 kinase (Plotkin et al. 2005). This anti-apoptotic effect involves the nuclear translocation of ERKs and new gene transcription (Plotkin et al. 2005).

The upstream mechanisms mediating mechanical activation of ERKs have been proposed for some experimental systems (Fig. 2). It has previously been shown that integrins are essential

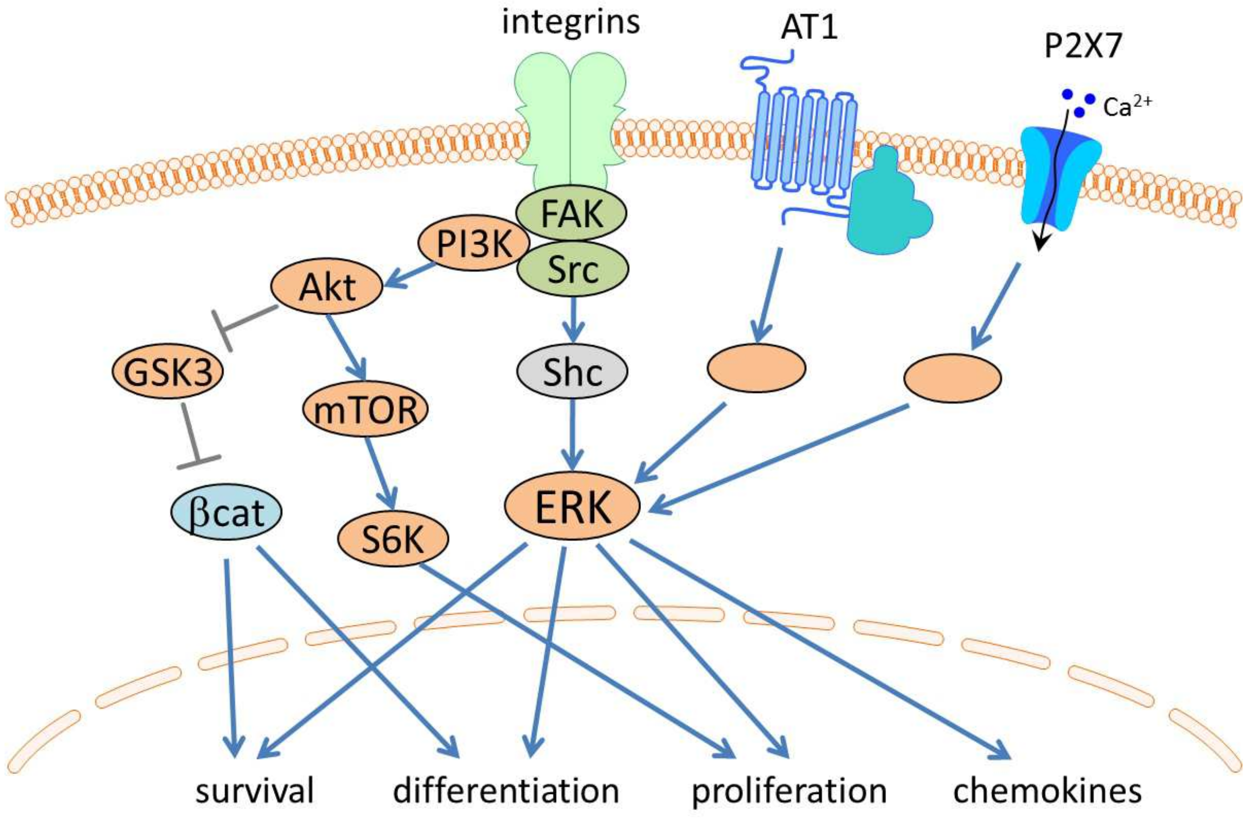

Fig. 2. Working model: Mechanical stress-sensing mechanisms of osteoblasts. ERKs are activated by various types of mechanical stress and initiate multiple cellular functions.

for the activation of ERKs in osteoblasts by cyclic stretch (Schmidt et al. 1998; Zhang et al. 2003). We have recently reported that the treatment of osteoblasts by low-intensity pulsed ultrasound (LIPUS) induced ERK activation and the expression of chemokine and RANKL (Bandow et al. 2007). These LIPUS-induced cellular events were abrogated by candesaltan, a specific inhibitor of angiotensin II type 1 receptor (AT1). AT1 is a known mechanoreceptor in cardiomyocytes (Zou et al. 2004). Osteoblasts express AT1 mRNA and protein and the level of expression increased significantly during osteoblast differentiation (Bandow et al. 2007). These findings indicate that AT1 functions as a mechanoreceptor of in osteoblasts as well as in cardiomyocytes. Another example is P2X7, a two-transmembrane ionotropic receptor. P2X7 has been reported as a mechanotransducer of osteoblasts, which is essential for the ERK activation by fluid shear stress (Liu et al. 2008), indicating that ERK activation by fluid shear stress is dependent on intracellular Ca^{2+}.

In addition, multiple autocrine and paracrine factors have been implicated to transduce ERK activation by mechanical stresses. For example, ERK activation in osteoblasts by fluid shear stress has been reported to be dependent on nitric oxide (NO) synthesis (Rangaswami et al. 2009; Kapur, Baylink, and Lau 2003). According to another report, however, synthesis of NO and prostacyclin is essential for ERK activation in osteoblasts induced by mechanical stretch, but not by fluid shear stress (Jessop et al. 2002).

4.1.2 JNKs and p38 Kinase

Transient activation of JNKs in osteoblasts has been reported to be induced by cyclic stretch (Danciu et al. 2003), compression (Maeda et al. 2007), and fluid shear stress (Lee et al. 2008; Wang et al. 2011). It has been reported that JNK activity is important for compression-induced IL-8 expression (Maeda et al. 2007). On the other hand, transient activation of p38 kinase in osteoblasts has been reported to be induced by cyclic stretch (Kusumi et al. 2005), compression (Maeda et al. 2007), fluid shear stress (Lee et al. 2008; Yeh et al. 2010), and magnetic force (Yuge et al. 2003). Recent reports indicated that p38 activation is essential for Opg expression induced by cyclic stretch (Kusumi et al. 2005), and compression-induce IL-8 expression (Maeda et al. 2007). Intriguingly, acceleration of osteoblast differentiation induced by magnetic force is mainly attributed to p38 kinase activation, while the activity of ERKs or JNKs is dispensable (Yuge et al. 2003). Thus p38 kinase may be specifically involved in the mechanotransduction of magnetic force in osteoblasts.

Activation of JNKs and p38 kinase by cyclic stretch in osteoblasts, however, is not as universally observed as that of ERKs and sometimes controversial. For example, a previous report showed that the application of cyclic stretch to human osteoblasts induced phosphorylation of ERKs, but not JNKs or p38 kinase (Zhu et al. 2009). Additionally, we found that cyclic stretch did not induce JNK or p38 kinase phosphorylation in mouse osteoblast cell lines or mouse primary osteoblasts, whereas it induced significant ERK phosphorylation (unpublished results). Thus, activation of JNKs and p38 kinase by cyclic stretch may be cell line-specific or dependent on stretch conditions.

4.2 Integrin-associated kinases

Cells are surrounded by ECM, which profoundly affects cellular functions. Most effects from ECM are mediated through integrins, cell surface receptors responsible for cell attachment to and reception of signals from ECM. As integrins lack intracellular enzymatic domains, signals from ECM are transduced by various intracellular signaling molecules, the most crucial of which is focal adhesion kinase (FAK) (Fig. 2). Integrin engagement induces activation and the subsequent autophosphorylation of FAK, which then interacts with another tyrosine kinase, Src. These molecular events eventually lead to the activation of ERKs (Bellido 2010). Integrins have been shown as important mechanotransducers in various cell types including osteoblasts.

4.2.1 FAK

As FAK-null mice are embryonic lethal (Furuta et al. 1995), mice with conditional knock-out of FAK in osteoblasts have been generated (Kim et al. 2007). Although these mice were normal in basal osteoblast differentiation, they were defective in bone healing due to decreased matrix deposition, which may be caused by defective mechanotransduction of osteoblasts. The role of FAK in osteoblasts has also been explored in vitro. Fluid shear stress-induced osteoblast differentiation as well as activation of ERK and JNK was blocked by the expression of a dominant negative FAK mutant, indicating the essential role of FAK in mechanotransduction of fluid shear stress (Wang et al. 2011). It has also been reported that FAK$^{-/-}$ osteoblasts are unresponsive to fluid shear stress in ERK activation and Opn expression (Young et al. 2011).

4.2.2 Src

A well-known tyrosine kinase, Src, is involved in integrin signals downstream of FAK. The role of Src in mechanotransduction of osteoblasts has recently been examined. Fluid shear stress induced increased phosphorylation at Tyr-416 of Src in osteoblasts (Morgan et al. 2011). Inhibition of Src, however, had no effects on fluid shear stress-induced Opn expression or ERK activation. These findings indicate that Src is activated by fluid shear stress but not necessary for the proper mechanotransduction of osteoblasts. Another recent report indicated that protein kinase G (PKG), which is activated by NO-mediated production of cGMP, induced the activation of Src in mechanically stimulated osteoblasts (Rangaswami et al. 2010). Activation of Src by PKG is necessary for the interaction of Src with β3 integrin, indicating that Src functions as a converging point of integrin and NO-PKG signals in osteoblasts.

4.3 PI3K-Akt activating pathway

Sustained activation of PI3K in osteoblasts by fluid shear stress has been reported (Lee et al. 2010) (Fig. 2). The resultant activation of Akt, mTOR, and p70S6 kinase leads to cell proliferation. The fluid shear stress-induced activation of PI3K appears to be induced by a complex formation of PI3K p85 subunit with $\alpha_V\beta_3/\beta_1$ integrins, Shc, and FAK. It has also been reported that PI3K-Akt pathway activated by fluid shear stress in osteoblasts and osteocytes, induces increased nuclear translocation of β-Catenin (Kitase et al. 2010; Xia et al. 2010), as Akt phosphorylates and inactivates GSK3 (Wang, Brown, and Martin 2011). The increased β-Catenin signal by fluid shear stress is essential for anti-apoptotic effects against glucocorticoid, indicating a possible mechanism how mechanical loading sustains healthy bone structure.

4.4 LIM Kinase 2 (LIMK2)

When osteoblasts are loaded with mechanical stress, the structure of actin cytoskeleton is reorganized, which results in changes of cell stiffness (Jaasma et al. 2007). LIM kinase (LIMK) is a unique protein kinase that has two LIM domain repeats at the N-terminus, followed by a PDZ domain and a catalytic kinase domain. LIMK protein family includes two members, LIMK1 and LIMK2. LIMK1 is highly expressed in neural tissues (Takahashi, Funakoshi, and Nakamura 2003), whereas LIMK2 is widely expressed in various tissues (Acevedo et al. 2006). LIMK2 phosphorylates and inactivates cofilin, an actin-depolymerizing factor, thereby inducing actin cytoskeleton reorganization (Takahashi, Funakoshi, and Nakamura 2003). A recent report explored the functional role of LIMK2 in osteoblasts treated with fluid shear stress (Fu et al. 2008). Gene silencing of LIMK2 in osteoblasts by siRNA clearly decreased reorganization of actin cytoskeleton induced by fluid shear stress. This finding indicates an important role of LIMK2 in osteoblasts with mechanical loading.

5. Conclusion

Bone is a dynamic tissue where constant remodelling is going on. Osteoblasts are unique cells with multiple aspects. They (1) differentiate into osteocytes, the main cell type in bone tissue, (2) produce proteins for the formation of bone matrix, (3) recruit osteoclasts and control their function, thus playing a regulatory role in bone remodelling, (4) sensing the degree and nature of mechanical loading, which are necessary for the proper maintenance of bone structure suitable for the environment, (5) sense infection and recruit inflammatory cells for host defence. As I have discussed in this review, many of the multiple functions of osteoblasts are controlled by a list of kinases. It should be noted that the same kinases, such as MAPKs and PI3Ks, are involved in many functions of osteoblasts. Thus, in osteoblasts, a lot of crosstalk exists among signal transduction pathways through kinases. If this complicated network is broken due to kinase malfunction, it certainly leads to bone metabolic disorders. In concluding, this review may provide insights into a new list of therapeutic target molecules for diseases that have become threatening social problems, such as osteoporosis.

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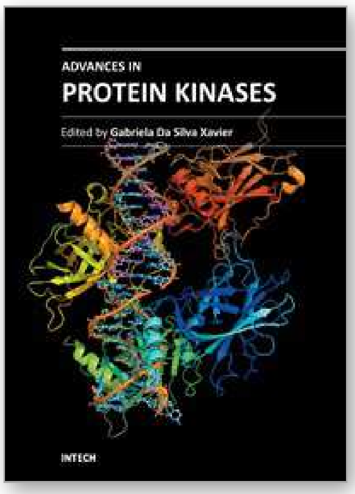

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Proteins are the work horses of the cell. As regulators of protein function, protein kinases are involved in the control of cellular functions via intricate signalling pathways, allowing for fine tuning of physiological functions. This book is a collaborative effort, with contribution from experts in their respective fields, reflecting the spirit of collaboration - across disciplines and borders - that exists in modern science. Here, we review the existing literature and, on occasions, provide novel data on the function of protein kinases in various systems. We also discuss the implications of these findings in the context of disease, treatment, and drug development.

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