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Mini review

Signal transduction pathways involved in mechanotransduction in bone cells

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Abstract

Several in vivo and in vitro studies with different loading regimens showed that mechanical stimuli have an influence on proliferation and differentiation of bone cells. Prerequisite for this influence is the transduction of mechanical signals into the cell, a phenomenon that is termed mechanotransduction, which is essential for the maintenance of skeletal homeostasis in adults. Mechanoreceptors, such as the integrins, cadherins, and stretch-activated Ca^{2+} channels, together with various signal transduction pathways, are involved in the mechanotransduction process that ultimately regulates gene expression in the nucleus. Mechanotransduction itself is considered to be regulated by hormones, the extracellular matrix of the osteoblastic cells and the mode of the mechanical stimulus.

Keywords: Mechanotransduction; Signal transduction; Mechanical loading; Osteoblast; Osteocyte

Immobilization and gravity loss lead to atrophy of the bone, whereas mechanical loading is essential for skeletal homeostasis and bone formation [1,2]. Skeletal homeostasis in adults is maintained by a balance between bone forming osteoblasts and bone resorbing osteoclasts. This balance is regulated locally by cytokines and growth factors, and systemically by hormones such as parathyroid hormone and oestrogen. Mechanical loading stimulates bone formation by acting either alone or together with these hormones on bone cells, resulting in changed gene expression. Osteoblasts are directly activated by loading, which leads to an increase in proliferation and matrix synthesis, as well as being indirectly activated by growth factors, and by the release of prostaglandins and NO by the osteocytes [3]. The transformation mechanisms, converting the physical stimulus into the cellular response, called mechanotransduction, are not well known. Integrins, cadherins, and Ca^{2+} channels are proposed to be involved in the transfer mechanism of the mechanical signal, which is subsequently propagated via cellular signal transduction pathways to the nucleus, where gene transcription is modified.

In this review, we focus on signal transduction pathways, which have been considered as being responsible for the cellular response on mechanical loading. We also discuss nitric oxide (NO), and prostaglandin signaling in mechanotransduction, and present aspects of mechanotransduction in bone tissue engineering.

Mechanoreceptors

In the initial phase of mechanotransduction, deformation of the cell membrane by stretch as well as shear stress mediated by the fluid flow in the canaliculi is detected by the osteoblasts and the osteocytes, respectively [2,4]. The surface proteoglycan layer (glycocalix) is a primary sensor of mechanical signals that can transmit force to apical structures such as the plasma membrane or the submembrane cortex (actin cortical skeleton) [5]. Lipid rafts and calveolae may serve as cell surface mechanotransduction sites within the plasma membrane. Transduction can occur here, or at more remote regions of the membrane such as intercellular junctions (adherens junctions), and cell matrix

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contacts (focal adhesions) are activated [4]. In the adhesion complexes recruitment and reorganization of both the integrin and cadherin proteins is induced. Expression of cadherins, proteins of the adherens junctions which interlink the cytoskeleton between neighbouring cells, is increased by mechanical strain. Focal adhesion kinase (pp125FAK) interacts with the large conductance calcium-activated hSlo K^+ channel (BK) in the focal adhesion complexes of human osteoblasts [6]. GTPases, such as RAS, have been shown to be activated by mechanical strain in osteoblast-like cells [7] (Fig. 1). The prevention of osteocyte apoptosis by mechanical stimulation requires integrins and cytoskeletal molecules, together with catalytic molecules such as Src kinases [4,8]. Fluid shear stress has been shown to increase β1-subunit expression and to activate αvβ3 integrins in osteoblasts. Mechanical strain activates L-type voltage-sensitive Ca^{2+} channels to promote Ca^{2+} entry that, in turn, stimulates vesicular ATP release [9]. Stretch-activated Cation channels (SA-CAT) are also thought to be responsible for mechanotransduction in osteoblasts. The expression of the alpha subunit of the epithelial sodium channel (αENaC), known as touch-sensitive protein in *Caenorhabditis elegans*, was demonstrated in human osteoblasts [4]. The expression of connexin (Cx) 43, a major component of gap junctions, is increased by mechanical strain and regulated by prostaglandin E2

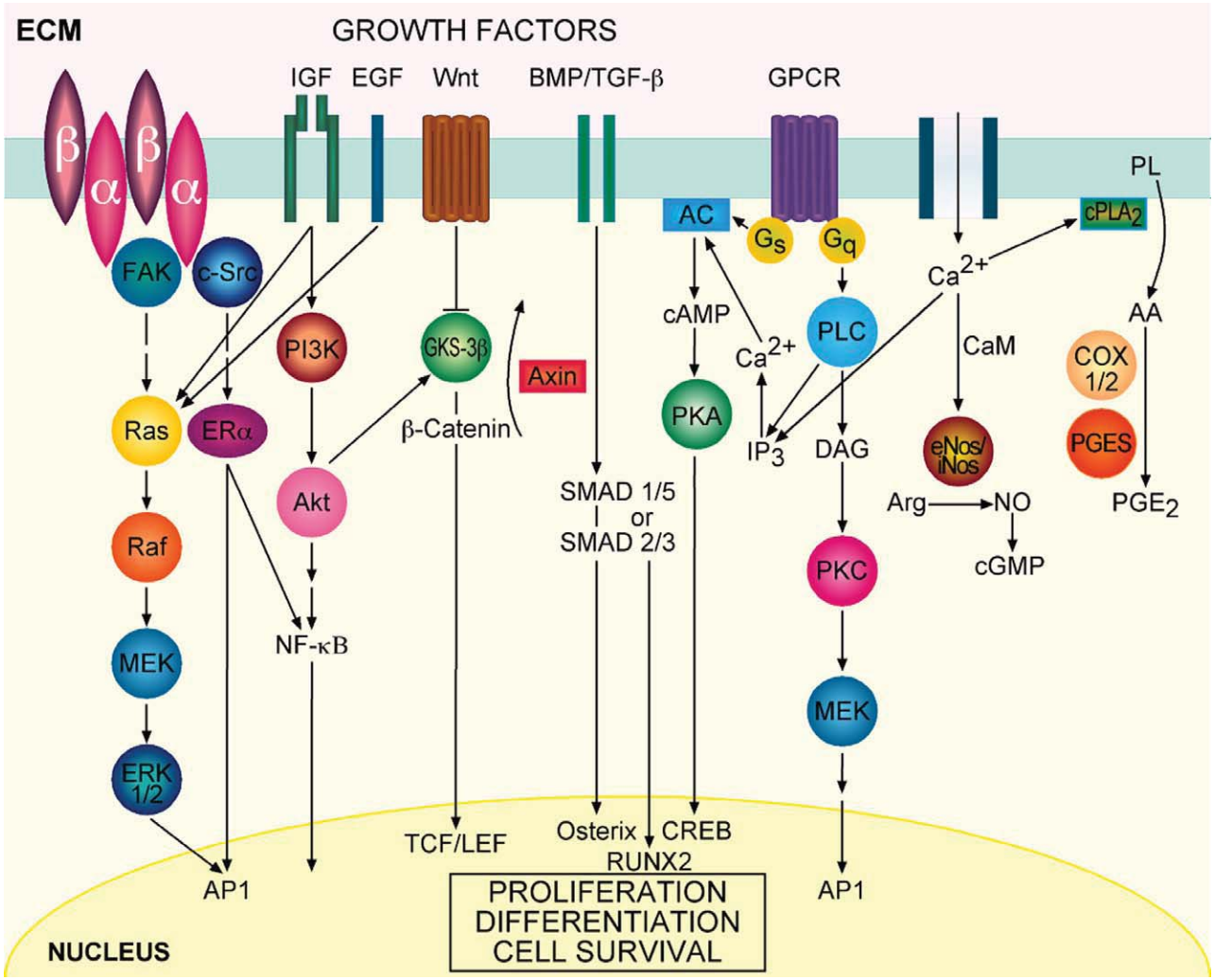

Fig. 1. Mechanotransduction pathways shown to be involved in the mechanical response of osteoblastic cells. *Abbreviations:* AA, arachidonic acid; AC, adenylate cyclase; Akt, akutely transforming (protein kinase B, serin/threonine kinase); AP1, activator protein 1; BMP, bone morphogenetic protein; CAM, calmodulin; COX1/2, cyclooxygenase 1/2; CREB, c-AMP response element-binding protein; c-Src, tyrosine protein kinase; DAG, diacylglycerol; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; ERα, estrogen receptor α; ERK1/2, extracellular signal regulated protein kinase 1/2; FAK, focal adhesion kinase; GSK-3β glycogen synthase kinase-3β; G_s, stimulatory G-protein; GPCR, seven-transmembrane-domain G-protein-coupled receptor; G_q, protein with α_q subunit activates PLC, phospholipase C-β; IGF, insulin-like growth factor; iNOS, inducible nitric oxide synthase; IP3, inositol trisphosphate; LEF, lymphoid enhancer-binding factor; MEK, mitogen-activated protein kinase extracellular signal regulated protein kinase (mitogen-activated kinase kinase); NF-κB, nuclear factor-κB; PI3K, phosphoinositide 3-kinase; PGE_2, prostaglandin E2; PGES, prostaglandin synthase; PKA, protein kinase A; PKC, protein kinase C; PL, phospholipid; Raf, rat fibrosarcoma serin/threonine protein kinase; SMAD, from sma (small) in *Caenorhabditis* and mad (mother against decapentaplegic) in *Drosophila*; Ras, rat sarcoma monomeric GTP-binding protein; TCF, T-cell factor; TGF-β transforming growth factor-β; wnt, from wingless in *Drosophila*; int, (integration)-1 in mouse.

(PGE_2) in an autocrine manner [10]. PGE_2 may be released through unapposed hemichannels formed by Cx43.

Intracellular signal transduction pathways

Numerous signal transduction molecules can localize at, or in the vicinity of adhesion complexes [4]. Signal transduction of mechanical stimuli is dependent on the structural integrity of the microfilament component of the cytoskeleton. Cyclic forces induce an enhanced cytoskeletal anchorage of tyrosine-phosphorylated proteins and an increased activation of FAK and mitogen-activated protein (MAP) kinase. Nuclear matrix proteins including nuclear matrix protein (NMP) 4/cas interacting zinc finger protein (CIZ) are attractive candidates for integrating mechanical signals. These transcription factors localize at adhesion plaques, transfer into the nucleus, bind to consensus DNA sequences, and can activate promoters of mechanosensitive genes. In the model of Pavalko et al. [4] multiproteincomplexes, called mechanosomes, comprised of adhesion proteins acquired from the focal adhesions or adherens junctions, and nucleocytoplasmic shuttling DNA-binding proteins can move between the adhesion complexes and the nucleus, regulating gene expression. Rapid increases of cytoplasmic Ca^{2+} resulted from the influx of Ca^{2+} through activated Ca^{2+} channels in the plasma membrane and from phospholipase C (PLC) activity and inositol-1,4,5-trisphosphate (IP3) signaling, which lead to release of Ca^{2+} from intracellular stores [11]. Ca^{2+} dependent kinases, such as protein kinase C (PKC), and Ras activate the mitogen-activated protein kinase (MAPK) pathway. The activation of extracellular regulated kinases ERK1/2, c-jun kinase (JNK) and p38 mitogen-activated protein kinase results in upregulation of c-fos and c-jun expression [12]. The c-fos gene product together with c-Jun forms the activator protein-1 (AP-1) transcription factor, which binds to the promoter of mechanosensitive genes. An important transcription factor-binding site involved in activating the mechanical response is the shear stress response element (SSRE), which was identified in various genes including c-fos and PDGF-β [13]. The fluid shear stress-induced cyclooxygenase-2 (COX-2) expression in osteoblastic MC3T3-E1 cells is mediated by CCAAT/enhancer-binding protein (C/EBP), AP-1, and c-AMP-response element-binding protein (CREB) [14]. Anti-inflammatory and pro-inflammatory effects of mechanical strain are both regulated by nuclear factor (NF)-κB in osteoblast-like periodontal ligament (PDL) cells [15]. Mechanical stimulation leads to upregulation of growth factors, such as insulin-like growth factor (IGF) I and II, vascular endothelial growth factor (VEGF), transforming growth factor (TGF) β1, and bone morphogenetic protein (BMP) 2 and 4, which act via autocrine and paracrine mechanisms, through their tyrosine and serine/threonine kinase receptors [3,16]. These growth factors activate phosphatidylinositol 3-kinase (PI3K)-Akt (protein kinase B), MAPK, and SMAD signal transduction pathways [16,17]. The BMP-2-induced signaling pathway, for example, leads to the expression of the three osteogenic master transcription factors Osterix, Runx2, and Dlx5 [18]. Released PGE_2, PGI_2 or hormones, such as the parathyroid hormone, bind to seven transmembrane-domain G-protein-coupled receptors (GPCR), and activate adenylate cyclase [16]. Subsequently, protein kinase A (PKA) phosphorylates the CREB transcription factor, which can bind to the promoter of the COX-2 or the FosB/DeltaFosB gene [14]. Phospholipase C is also activated by GPCRs, resulting in synthesis of IP3 and diacylglycerol (DAG), the latter may stimulate PKC [16]. Previously, it was shown that fluid shear stress induces translocation of β-catenin, a component of the canonical wnt signal transduction pathway, to the nucleus [19]. Moreover, the lipoprotein receptor-related protein (LRP) 5 G171V mutation results in an increased responsiveness of bone to mechanical load, and reduces the threshold of load required to elicit a response [20]. Additionally, this mutation leads to an increased transcription of osteoprotegerin (OPG), which may result in a reduction of osteoclastogenesis. There is evidence of signal transduction pathways interacting, such as for IGF-I with the estrogen receptor (ER) signaling pathway in the proliferative response to mechanical strain [21]. A crosstalk among anabolic, intracellular pathways may enhance the upregulation of these signaling pathways, ultimately leading to the cellular response on mechanical loading.

Nitric oxide and prostaglandin signaling

An enhancement of cellular reaction to mechanical loading is achieved by intercellular signaling through mechanically induced release of mediating signaling molecules from bone cells. Prostaglandins (PGs) and nitric oxide (NO), which are involved in the response of bone tissue and cells to stress [22–24], seem to be interesting candidates for intercellular communication within the three dimensional network of bone cells as they are rapidly released by mechanically stressed bone cells.

In osteocytes the mechanically induced synthesis and release of PGE_2 are transmitted via the cytoskeleton, which is physically linked to ion channels, as well as protein kinase C, and phospholipase A_2 [22]. PGE_2 mediates the gap junctional intercellular communication between osteocyte-like cells in response to mechanical strain by increasing the number of functional gap junctions and the amount of connexin 43 protein [10]. This effect has been shown to be mediated through prostaglandin EP_2 receptor activation of cAMP-dependent protein kinase A. The expression of COX-2, the inducible isozyme for PGE_2 synthesis, is also directly upregulated by mechanical loading through the formation of focal adhesions and subsequent ERK and PKA signaling pathways in osteoblasts [16]. However, mechanotransduction does not necessarily require a functional COX-2 gene [25]. Beside mediating intercellular signaling, PGE_2 has been shown to have a regulatory effect on

RANKL expression and therefore on osteoclastogenesis, as has been shown in periodontal ligament cells [26]. Physiological levels of mechanical stress decrease RANKL expression and increase endothelial NO synthase (eNOS) expression, which is mediated through ERK1/2, in primary bone stromal cells and conditionally immortalized murine calvarial cells (CIMC-4), respectively [7,27].

Burger et al. [23] assumed that low canalicular flow reduces NO production by local osteocytes, leading to apoptosis and the attraction of osteoclasts that resorb both bone matrix and dying osteocytes. In contrast, high amounts of NO produced by osteocytes at high canalicular flow may prevent osteocyte apoptosis, as has been shown for endothelial cells [28], and promote retraction and detachment of osteoclasts [29]. Bakker et al. [30] showed that the NO response to mechanical strain is higher in bone cells from osteoporotic than from osteoarthritic donors, while the PGE2 response was higher in the osteoarthritic cells. They also demonstrated interactive effects of parathyroid hormone (PTH) and estrogen with mechanical stress on NO and PGE2 production, respectively [31,32].

Perspectives of mechanotransduction in bone tissue engineering

The identification of signal transduction pathways and mechanosensitive genes may contribute to bone tissue engineering in vitro. It has been shown that mechanical stimulation of specific pathways and genes enhanced proliferation and differentiation of osteoblasts [21]. Therefore, a defined mechanical environment may be an adequate mitogenic and osteogenic stimulus for bone tissue engineering. An expansion of osteoblastic precursor cells to obtain adequate cell numbers together with culture conditions which promote osteogenic differentiation of these cells, could be an applicable pretreatment of cells before reimplantation in bone defects. Various three dimensional (3-D) constructs have already been used to study mechanical effects on osteoblastic cells and their adequacy in improving the above mentioned properties [33]. It was shown that murine osteoblastic cells in polydimethylsiloxane (PDMS) microdevices and in human trabecular 3-D bone scaffolds cultured under dynamic conditions enhanced their alkaline phosphatase (ALP) activity, and their proliferation, as well as Runx2, osteocalcin, and ALP expression [34,35]. Moreover, mechanical stimulation resulted in increased mineralized matrix production by human bone marrow stromal cells cultured in 3-D, partially demineralized bone scaffolds [36]. Mechanical stimulation of osteoblastic cells seeded in a collagen type I matrix influenced the proliferation and differentiation of these cells, too [37]. However, a deeper understanding of the mechanotransduction mechanisms, which is dependent on nutrient supply, cell-matrix interactions (scaffold material) and the mode of the mechanical stimulus, is essential to generate bone tissue for tissue replacement purposes.

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