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CONNEXIN 43 AND BONE: NOT JUST A GAP JUNCTION PROTEIN

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Summary

Connexins are essential for the communication of cells among themselves and with their environment. Connexin hexamers assemble at the plasma membrane to form hemichannels that allow the exchange of cellular contents with the extracellular milieu. In addition, hemichannels expressed in neighboring cells align to form gap junction channels that mediate the exchange of contents among cells. Connexin 43 (Cx43) is the most abundant connexin expressed in bone cells and its deletion in all tissues leads to osteoblast dysfunction, as evidenced by reduced expression of osteoblast markers and delayed ossification. Moreover, Cx43 is essential for the survival of osteocytes; and mice lacking Cx43 in these cells exhibit increased prevalence of osteocyte apoptosis and empty lacunae in cortical bone. Work of several groups for the past few years has unveiled the role of Cx43 on the response of bone cells to a variety of stimuli. Thus, the preservation of the viability of osteoblasts and osteocytes by the anti-osteoporotic drugs bisphosphonates depends on Cx43 expression *in vitro* and *in vivo*. This survival effect does not require cell-to-cell communication and is mediated by unopposed hemichannels. Cx43 hemichannels are also required for the release of prostaglandins and ATP by osteocytes induced by mechanical stimulation *in vitro*. More recent evidence showed that the cAMP-mediated survival effect of parathyroid hormone (PTH) also requires Cx43 expression. Moreover, the hormone does not increase bone mineral content in mice haploinsufficient for Cx43 or lacking Cx43 in osteoblastic cells. Since inhibition of osteoblast apoptosis contributes, at least in part, to bone anabolism by PTH, the lack of response to the hormone might be due to the requirement of Cx43 for the effect of PTH on osteoblast survival. In summary, mounting evidence indicate that Cx43 is a key component of the intracellular machinery responsible for the transduction of signals in the skeleton in response to pharmacologic, hormonal and mechanical stimuli.

Key words: connexin43, osteoblast, osteocyte, apoptosis, bone.

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Resumen

CONEXINA 43 Y HUESO: NO SÓLO UNA PROTEÍNA DE LAS UNIONES *GAP*

Las conexinas son esenciales para la comunicación de las células entre ellas mismas y con el medio que las rodea. Hexámeros formados por conexinas se sitúan en la membrana plasmática, constituyendo los llamados hemicanales que permiten el intercambio de componentes celulares con el medio extracelular. Además, hemicanales expresados en células vecinas se alinean para formar uniones *gap* que participan en el intercambio de moléculas entre las células. La conexina 43 (Cx43) es el miembro más abundante de la familia de las conexinas en hueso y su remoción de todos los tejidos lleva a la disfunción de los osteoblastos, como se evidencia por la disminución en la expresión de marcadores osteoblásticos y el retraso en la osificación. Más aún, la Cx43 es esencial para la viabilidad de los osteocitos; y ratones deficientes en Cx43 en estas células exhiben aumento de la prevalencia de osteocitos apoptóticos y lagunas vacías en el hueso cortical. Trabajos realizados por varios grupos durante los últimos años han revelado la participación de la Cx43 en la respuesta de las células óseas ante una variedad de estímulos. En particular, el efecto antiapoptótico sobre los osteoblastos y osteocitos de los bisfosfonatos depende de la expresión de Cx43 *in vitro* e *in vivo*. Este efecto no necesita la comunicación célula-célula y es mediado por hemicanales. Los hemicanales de Cx43 también son necesarios para la liberación de prostaglandinas y ATP en osteocitos sometidos a estimulación mecánica. Estudios más recientes han mostrado que el efecto la hormona paratiroidea (PTH) en la preservación de la viabilidad de los osteoblastos mediado a través de la producción de AMP cíclico también es dependiente de la expresión de Cx43. Más aún, administración de PTH no resulta en un aumento en el contenido mineral óseo en ratones hemicigotas para Cx43 o deficientes de Cx43 en células del linaje osteoblástico. Debido a que la prevención de la apoptosis de los osteoblastos contribuye, por lo menos parcialmente, al efecto anabólico de la PTH, la falta de respuesta a la hormona puede deberse al rol de la Cx43 en el efecto de PTH en la sobrevida de los osteoblastos. En síntesis, existe abundante evidencia que indica que la Cx43 es un componente clave de la maquinaria intracelular responsable de la transducción de señales en el hueso en respuesta a estímulos farmacológicos, hormonales y mecánicos.

Palabras claves: conexina 43, osteoblastos, osteocitos, apoptosis, hueso.

Introduction

Bone cells communicate with each other to properly respond to hormonal and mechanical stimuli. One of the mechanism by which cells this communication is achieved is through gap junctions – clusters of channels that open only transiently and allow the exchange of small (<1 kDalton) molecules between adjacent cells.[1] A gap junction channel is formed by two hemichannels provided by each adjacent cell; and each hemichannel is composed of six molecules of connexin (Figure 1). Hemichannels are also present in unopposed cell membranes and their opening allows exchange of cytoplasmic contents with the extracellular fluid.[2,3] Besides its participation in gap junctions and hemichannels, Cx43 might affect cellular functions by interacting with structural and signaling intracellular molecules (summarized in Table 1). The majority of these interactions are localized in areas of the cytoplasmic C-terminal tail of Cx43, which does not participate in channel formation.[4,5] Moreover, it has been shown that the C-terminus domain of Cx43 (in the absence of channel forming domains of the protein) can inhibit cell proliferation when transfected to HeLa or HEK293 cells,[6,7] or to human glioma cells, in which it also increases cell migration.[8]

Early studies on bone sections and isolated

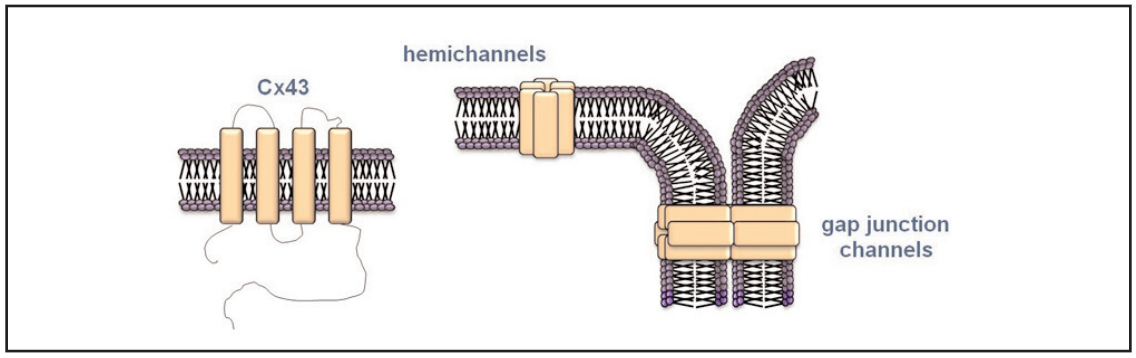

Figure 1. Hemichannels and gap junction channels formed by connexins. Cx43 is a transmembrane protein that forms hexamers on the cell membrane, known as hemichannels. Two hemichannels from opposing cells align to form a gap junction channel.

bone cells showed that gap junctions and connexins are expressed in all the cells types in bone including pre-osteoblasts, osteoblasts, osteocytes and osteoclast.[9-16] This manuscript reviews the evidence collected during the past years pointing towards Cx43 as an essential component of signaling pathways activated in osteoblasts and osteocytes.

Table 1. Molecules that interact with Cx43

Structural	caveolin-1 and 3 [4,82] tight junction protein ZO-1 [4] N-cadherin [5] α-catenin [5] tubulin [4] drebrin [83]
Kinase / phosphatase	Src [4] PKA [4] PKC γ, ε and δ [4,84] Receptor protein tyrosine phosphatase μ [4]
Growth suppression	CCN3/NOV [4]
Cell motility	ephrin [85]
Protein turnover	Nedd4, Eps15 [86] CIP75 [87]
Scaffolding	14-3-3 [88] β-arrestin [40,89]
Other	Cox-2 [5] Hsp90 and TOM [5] β-catenin [5]

Connexin 43 and bone

The connexin family comprises approximately 21 proteins that exhibit high homology.[5] Each connexin molecule is formed by 4 transmembrane domains, 2 extracellular and 1 intracellular loop, and N-terminus and C-terminus tails (Figure 1). Among the members of the connexin family, Cx43, is the most highly expressed in bone cells, although Cx45 and Cx37 have also been detected.[10,17] Inhibition of connexin channel opening or reduced Cx43 function leads to reduced expression of osteoblast specific genes [18,19] and to decreased osteoclast precursor fusion and osteoclastic bone resorption *in vitro*.[20-22] Mice lacking Cx43 ubiquitously die within hours after birth due to cardiac malformations precluding the study of the adult skeleton.[23] Nevertheless, neonatal bones exhibit delayed ossification and osteoblastic cells show low expression of osteoblast markers and deficient mineralization compared to wild type cells.[24] Similarly, immortalized calvaria cells derived from $Cx43^{-/-}$ mice show delay differentiation and mineralization in culture.[25] In addition, mice expressing ubiquitously Cx43 mutants associated with oculodentodigital dysplasia (ODDD), which is unable to form gap junctions and acts as dominant negative for wild type Cx43, exhibit low bone mass and decrease bone strength.[26-28] Moreover, mice in which Cx43 was deleted exclusively from osteochondroprogenitor cells (by using the Dermo1 promoter) have a severe phenotype, with decreased trabecular bone mass and cortical thickness.[29] In contrast, deletion of Cx43 from early osteoblastic cells expressing the 2.3 kb fragment of the collagen1a1 promoter ($Cx43^{fl/-}$;ColCre mice) have a less pronounce phenotype, with mild reduction in bone volume, osteoblast number and bone mass, when compared to control littermates up to 6 months of age.[30] Furthermore, we have found that mice in which Cx43 has been deleted specifically in mature osteoblasts and osteocytes by expression of the Cre recombinase under the control of the human osteocalcin promoter ($Cx43^{fl/-}$;OCNCre mice) exhibit indistinguishable growth, body weight and bone mass (at least between 2 and 4.5 month of age), when compared to littermates expressing Cx43 in osteoblasts and osteocytes.[31] Consistent with the requirement of Cx43 expression in early progenitors, but not mature osteoblast, for full skeletal development, a Cx43 ODDD mutant only prevents osteoblast differentiation when present as a germline mutation and not when it is expressed after osteoblast commitment.[32] Overall, these findings indicate that Cx43 expression in osteoblast precursors (but not mature osteoblasts or osteocytes) is required for normal development of the skeleton.

In addition to Cx43 participation in cell-to-cell communication, we and others have shown that osteoblasts and osteocytes express functional hemichannels that allow the communication of the cells with the extracellular medium.[33-35] This evidence, together with findings in other cell types,[3,36-39] indicate that connexin hemichannels have physiological functions, in addition to be an intermediate in the formation of gap junction channels.[3] Moreover, Cx43 might also function as a scaffold, necessary for the transduction of intracellular signaling. Indeed, the interaction of Src with Cx43 is required for the anti-apoptotic effect of the anti-osteoporotic drugs bisphosphonates on osteoblasts and osteocytes [33] and Cx43 interacts with β-arrestin to modulate parathyroid hormone (PTH) signaling (40). Another protein that interacts with the C-terminal domain of Cx43 is the nephroblastoma overexpressed gene CCN3/NOV, a cell growth suppressor that in turn may be responsible for the tumor suppressor properties of Cx43 over-expression.[4,41-43] Whether Cx43 is involved in the reported effects of CCN3/NOV on osteoblastogenesis and BMP and Wnt signaling remains unknown.[44]

Connexin 43 and osteoblast and osteocyte survival induced by bisphosphonates

In addition to inhibiting osteoclast function, the recognized mechanism by which bisphosphonates stop bone loss, these agents prevent apoptosis of osteoblasts and osteocytes,[45] via a mechanism different from inhibition of the mevalonate pathway or conversion into toxic metabolites, the mechanisms of action of bisphosphonates on osteoclasts (Figure 2).[46] Thus, anti-apoptosis by bisphosphonates is observed at concentrations about 3 orders of magnitude lower than those required to promote osteoclast apoptosis *in vitro*.[45,47-50] Moreover, the anti-apoptotic effect of bisphosphonates on osteoblasts and osteocytes is exerted not only by traditional bisphosphonates but also by compounds such as IG9402 that do not affect osteoclasts and do not inhibit the mevalonate pathway.[45,49-53]

Mechanistic studies demonstrated that bisphosphonates induce the opening of Cx43 hemichannels, but do not affect gap junctions, leading to activation of the survival kinases ERKs in osteoblasts and osteocytes (Figure 3).[33,45] Cx43, but not other connexins, confers the anti-apoptotic response to bisphosphonates. Similar to other ERK activators, bisphosphonates induce activation of the kinase Src. However, unlike most ERKs activating stimuli that induce nuclear accumulation of the kinases, bisphosphonate-activated ERKs are retained outside the nucleus. This leads to the activation of the cytoplasmic ERK substrate p90^{RSK}.[33,54]

We found that bisphosphonates increase the association of Cx43 with β-arrestin, a scaffolding protein that binds to clathrin, and via this association, induce G protein-coupled receptor internalization and cell desensitization to the corresponding agonist. In addition, β-arrestins associate with signaling molecules, and modulate cell motility, chemotaxis and apoptosis.[55] Moreover, recent evidence indicates that β-arrestins are responsible for cytoplasmic retention of ERKs and inhibition of the activation of ERK nuclear targets induced by some ligands of G protein-coupled receptors.[56-58] Consistent with a role of β-arrestins in bisphosphonate-induced extranuclear retention of ERKs, cytoplasmic retention of ERKs and anti-apoptosis induced by the drugs are abolished in cells expressing a dominant

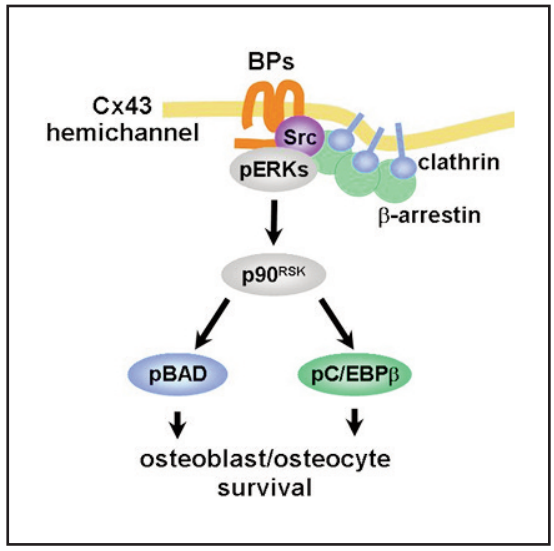

Figure 3. Model for bisphosphonate action in osteoblasts and osteocytes. Bisphosphonates prevent osteoblast and osteocyte apoptosis by a novel mechanism that involves the phosphorylation of cytoplasmic targets of the kinases ERKs and is independent of new gene transcription.

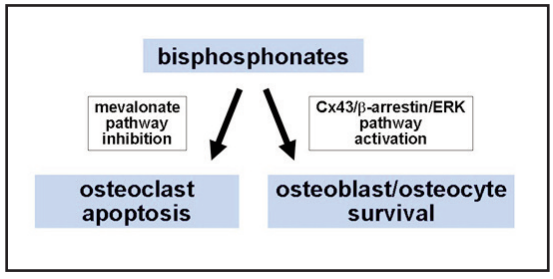

Figure 2. Bisphosphonates actions in osteoclasts and osteoblasts/osteocytes. The anti-osteoporotic drugs bisphosphonates exert opposite actions on osteoclasts and osteoblast/osteocytes, by modulating distinct signaling pathways in these two cell types.

negative form of β-arrestin that prevents the interaction of endogenous β-arrestin with clathrin.[59] Based on this evidence, we propose that opening of Cx43 hemichannels by bisphosphonates results in the formation of a complex containing Cx43, ERKs, β-arrestin and clathrin (Figure 3). This complex is responsible for the retention of ERKs outside the nucleus, leading to activation of the cytoplasmic target of ERKs $p90^{RSK}$ followed by phosphorylation of BAD and C/EBPβ, resulting in osteoblast and osteocyte survival.

Connexin 43 and signaling elicited by mechanical forces

Studies of the last decade have shown that Cx43 is a target of, and mediate the effect of mechanical stimulation in osteoblastic cells. Thus, mechanical loading increases Cx43 expression in osteoblasts and osteocytes *in vitro* and in murine tibiae *in vivo*,[60-62] and increases gap junction communication and leads to opening of Cx43 hemichannels *in vitro*.[35,60,61] It has been also shown that Cx43 expression and opening of hemichannels are required for mechanical stimulation-induced release of 2 in osteocytic cells,[35,63] which may be involved in the anabolic effect of mechanical forces on the skeleton.[64-66] More recent evidence suggests that ATP release through Cx43 hemichannels and the consequent stimulation of purinergic receptor, rather than direct release of 2 through the hemichannels, is required for 2 release induced by mechanical stimulation in osteocytic cells.[67] Taken together, these pieces of evidence suggest that the response to mechanical stimulation depends on Cx43 expression. Consistent with this, $Cx43^{fl/-}$;ColCre mice deficient in Cx43 in osteoblasts and osteocytes, exhibit an attenuated response to the anabolic action of mechanical stimulation.[68] Thus, loading of the tibia resulted in significantly lower increase in endocortical bone formation rate and mineral apposition rate in $Cx43^{fl/-}$;ColCre mice, as compared to wild type littermates. However, the mechanism by which Cx43 mediates the response to mechanical loading remains unknown.

Connexin 43 and PTH signaling

Daily injections of PTH increase osteoblast number and bone mass.[69,70] Studies in mice have shown that the increase in osteoblast number in cancellous bone is due, at least in part, to inhibition of osteoblast apoptosis.[71] Similarly, PTH related protein (PTHrP), the other ligand of the PTH1 receptor, as well as constitutive activation of this receptor in transgenic mice, also increases osteoblast number and decreases the prevalence of osteoblast apoptosis.[72-74] This anti-apoptotic effect of PTH and PTHrP have been reproduced by us and others in murine and human osteoblastic cell lines.[71,75.77] Mechanistic studies showed that the anti-apoptotic effect of PTH on osteoblasts requires the activation of the cAMP/PKA pathway, the phosphorylation of the pro-apoptotic protein BAD and the activity of the transcription factors and Runx2.[71,76] Strikingly, PTH does not prevent apoptosis in osteoblastic cells expressing dominant negative forms of Cx43 or in which Cx43 expression was silenced using small hairpin RNA.[40] Moreover, the response to PTH on cAMP production is blunted in osteoblastic cells in which Cx43 expression has been reduced using anti-sense cDNA.[78] Cx43 expression appears to be required to obtain a full anabolic response to intermittent PTH administration in mice.[30,79] Thus, PTH does not increase bone mass, bone formation and osteoblast number when administered to heterozygous Cx43 deficient mice ($Cx43^{+/-}$).[79] Consistent with this, intermittent PTH administration does not increase bone mineral content in mice lacking Cx43 in osteoblastic cells.[30] Taken together, these pieces of evidence suggests that intermittent PTH administration does not result in a full anabolic response in mice lacking Cx43 due to the inability of the

hormone to prevent apoptosis of osteoblastic cells in the absence of Cx43. Further studies are required to address this possibility.

Conclusion

Mounting evidence suggests the importance of Cx43 for the development and function of several cell types, including bone cells. In addition to its well-recognized function in cell-to-cell communication, Cx43 hemichannels are also active in unopposed plasma membranes, mediating the exchange of molecules between the cells and the extracellular milieu.[3,80] Small molecules released through hemichannels might transmit signals from one cell to others in the vicinity, without requiring direct cell-to-cell contact. Cx43 exhibits also channel independent functions, acting as a scaffolding protein with the ability to foster interactions among molecules of different signaling pathways, thereby regulating intracellular signaling.[81] The importance of Cx43 expression in bone has been demonstrated by the phenotype of embryos of Cx43 null mice.[24] However, the role of Cx43 and, in particular Cx43 hemichannels, in the response of bone to different stimuli, is far from being completely understood. We propose that Cx43 has a central role in the response of the skeleton to pharmacologic, hormonal and mechanical stimuli (Figure 4). Understanding the mechanism of the contribution of Cx43 on the effect of bone-acting stimuli will provide new opportunities to improve the treatment of conditions with increased bone fragility.

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References

1. Doty SB. Morphological evidence of gap junctions between bone cells. *Calcif Tissue Int* 1981; 33:509-12.
2. Evans WH, Martin PE. Gap junctions: structure and function. *Mol Membr Biol* 2002; 19:121-36.
3. Goodenough DA, Paul DL. Beyond the gap: functions of unpaired connexon channels. *Nat Rev Mol Cell Biol* 2003; 4:285-94.

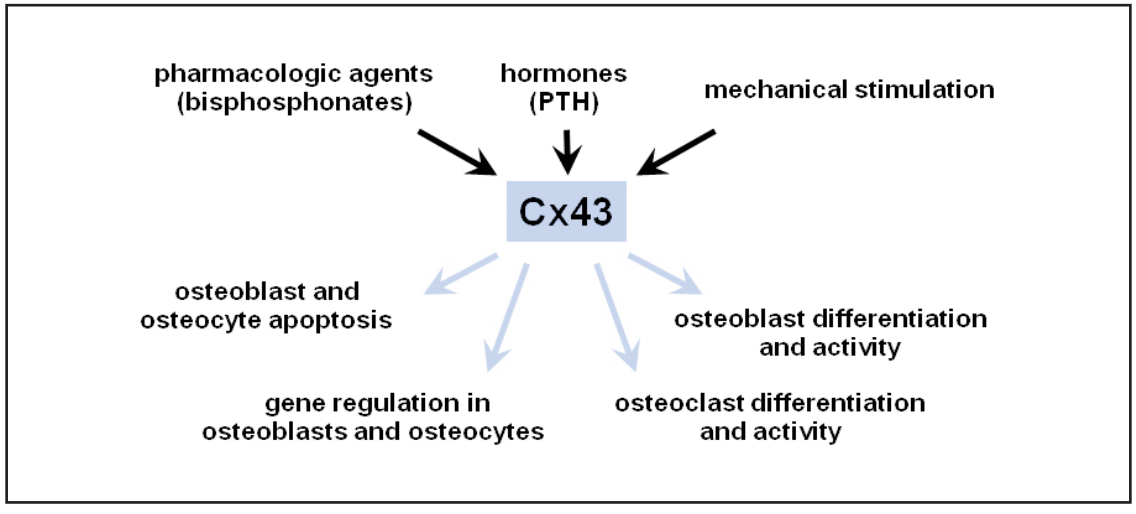

Figure 4. Proposed role of Cx43 in bone. Mounting evidence show that Cx43 is critical for the actions of pharmacologic, hormonal, and mechanical stimuli on bone cells (see text for details).

4. Giepmans BN. Gap junctions and connexin-interacting proteins. *Cardiovasc Res* 2004; 62:233-45.
5. Dbouk HA, Mroue RM, El-Sabban ME, Talhouk RS. Connexins: a myriad of functions extending beyond assembly of gap junction channels. *Cell Commun Signal* 2009; 7:4.
6. Dang X, Doble BW, Kardami E. The carboxy-tail of connexin-43 localizes to the nucleus and inhibits cell growth. *Mol Cell Biochem* 2003; 242:35-38.
7. Dang X, Jeyaraman M, Kardami E. Regulation of connexin-43-mediated growth inhibition by a phosphorylatable amino-acid is independent of gap junction-forming ability. *Mol Cell Biochem* 2006; 289:201-7.
8. Crespin S, Bechberger J, Mesnil M, Naus CC, Sin WC. The carboxy-terminal tail of connexin43 gap junction protein is sufficient to mediate cytoskeleton changes in human glioma cells. *J Cell Biochem* 2010; 110:589-97.
9. Palumbo C, Palazzini S, Marotti G. Morphological study of intercellular junctions during osteocyte differentiation. *Bone* 1990; 11:401-6.
10. Jones SJ, Gray C, Sakamaki H et al. The incidence and size of gap junctions between the bone cells in rat calvaria. *Anat Embryol (Berl)* 1993; 187:343-52.
11. Schirrmacher K, Schmitz I, Winterhager E et al. Characterization of gap junctions between osteoblast-like cells in culture. *Calcif Tissue Int* 1992; 51:285-90.
12. Civitelli R, Beyer EC, Warlow PM, Robertson AJ, Geist ST, Steinberg TH. Connexin43 mediates direct intercellular communication in human osteoblastic cell networks. *J Clin Invest* 1993; 91:1888-96.
13. Kato Y, Windle JJ, Koop BA, Mundy GR, Bonewald LF. Establishment of an osteocyte-like cell line, MLO-Y4. *J Bone Miner Res* 1997; 12:2014-23.
14. Su M, Borke JL, Donahue HJ, et al. Expression of connexin 43 in rat mandibular bone and periodontal ligament (PDL) cells during experimental tooth movement. *J Dent Res* 1997; 76:1357-66.
15. Ilvesaro J, Väänänen K, Tuukkanen J. Bone-resorbing osteoclasts contain gap-junctional connexin-43. *J Bone Min Res* 2000; 15:919-26.
16. Yellowley CE, Li Z, Zhou Z, Jacobs CR, Donahue HJ. Functional gap junctions between osteocytic and osteoblastic cells. *J Bone Miner Res* 2000;15:209-17.
17. Paic F, Igwe JC, Nori R et al. Identification of differentially expressed genes between osteoblasts and osteocytes. *Bone* 2009; 45:682-92.
18. Lecanda F, Towler DA, Ziambaras K et al. Gap junctional communication modulates gene expression in osteoblastic cells. *Mol Biol Cell* 1998; 9:2249-58.
19. Upham BL, Suzuki J, Chen G et al. Reduced gap junctional intercellular communication and altered biological effects in mouse osteoblast and rat liver oval cell lines transfected with dominant-negative connexin 43. *Mol Carcinog* 2003; 37:192-201.
20. Ilvesaro J, Tavi P, Tuukkanen J. Connexin-mimetic peptide Gap 27 decreases osteoclastic activity. *BMC Musculoskelet Disord* 2001; 2:10.
21. Ransjo M, Sahli J, Lie A. Expression of connexin 43 mRNA in microisolated murine osteoclasts and regulation of bone resorption in vitro by gap junction inhibitors. *Biochem Biophys Res Commun* 2003; 303:1179-85.
22. Schilling AF, Filke S, Lange T et al. Gap junctional communication in human osteoclasts in vitro and in vivo. *J Cell Mol Med* 2008; 12:2497-504.
23. Reaume AG, de Sousa PA, Kulkarni S et al. Cardiac malformation in neonatal mice lacking connexin43. *Science* 1995; 267:1831-34.
24. Lecanda F, Warlow PM, Sheikh S, Furlan F, Steinberg TH, Civitelli R. Connexin43

deficiency causes delayed ossification, craniofacial abnormalities, and osteoblast dysfunction. *J Cell Biol* 2000; 151:931-44.
25. Thi MM, Urban-Maldonado M, Spray DC, Suadicani SO. Characterization of human telomerase reverse transcriptase (hTERT) immortalized osteoblast cell lines generated from wildtype and connexin43-null mouse calvaria. *Am J Physiol Cell Physiol* 2010; 299:C994-C1006.
26. Roscoe W, Veitch GI, Gong XQ et al. Oculodentodigital dysplasia-causing connexin43 mutants are non-functional and exhibit dominant effects on wild-type connexin43. *J Biol Chem* 2005; 280: 11458-66.
27. Dobrowolski R, Sasse P, Schrickel JW et al. The conditional connexin43G138R mouse mutant represents a new model of hereditary oculodentodigital dysplasia in humans. *Hum Mol Genet* 2008; 17:539-54.
28. Flenniken AM, Osborne LR, Anderson N et al. A Gja1 missense mutation in a mouse model of oculodentodigital dysplasia. *Development* 2005; 132:4375-86.
29. Watkins M, Ornitz D, Willecke K, Civitelli R. Connexin43 is required for normal skeletal development and bone mass acquisition. *J Bone Min Res* 2006; 21:S56.
30. Chung D, Castro CH, Watkins M et al. Low peak bone mass and attenuated anabolic response to parathyroid hormone in mice with an osteoblast-specific deletion of connexin43. *J Cell Sci* 2006; 119:4187-98.
31. Plotkin LI, Lezcano V, Thostenson J, Weinstein RS, Manolagas SC, Bellido T. Connexin 43 is required for the anti-apoptotic effect of bisphosphonates on osteocytes and osteoblasts in vivo. *J Bone Miner Res* 2008; 23:1712-21.
32. McLachlan E, Plante I, Shao Q et al. ODDD-Linked Cx43 Mutants Reduce Endogenous Cx43 Expression and Function in Osteoblasts and Inhibit Late Stage Differentiation. *J Bone Miner Res* 2008; 23:928-38.
33. Plotkin LI, Manolagas SC, Bellido T. Transduction of cell survival signals by connexin-43 hemichannels. *J Biol Chem* 2002; 277:8648-57.
34. Romanello M, D'Andrea P. Dual mechanism of intercellular communication in HOBIT osteoblastic cells: a role for gap-junctional hemichannels. *J Bone Miner Res* 2001; 16:1465-76.
35. Cherian PP, Siller-Jackson AJ, Gu S et al. Mechanical strain opens connexin 43 hemichannels in osteocytes: a novel mechanism for the release of prostaglandin. *Mol Biol Cell* 2005; 16:3100-3106.
36. Li H, Liu TF, Lazrak A et al. Properties and regulation of gap junctional hemichannels in the plasma membranes of cultured cells. *J Cell Biol* 1996; 134:1019-30.
37. John SA, Kondo R, Wang SY, Goldhaber JI, Weiss JN. Connexin-43 hemichannels opened by metabolic inhibition. *J Biol Chem* 1999; 274:236-40.
38. Quist AP, Rhee SK, Lin H, Lal R. Physiological role of gap-junctional hemichannels. Extracellular calcium-dependent isosmotic volume regulation. *J Cell Biol* 2000; 148:1063-74.
39. Kamermans M, Fahrenfort I, Schultz K, Janssen-Bienhold U, Sjoerdsma T, Weiler R. Hemichannel-mediated inhibition in the outer retina. *Science* 2001;292:1178-80.
40. Bivi N, Lezcano V, Romanello M, Bellido T, Plotkin LI. Connexin43 interacts with β–arrestin: a pre-requisite for osteoblast survival induced by parathyroid hormone. *J Cell Biochem* 2011; DOI 10.1002/jcb.23208.
41. Benini S, Perbal B, Zambelli D, et al. In Ewing's sarcoma CCN3(NOV) inhibits proliferation while promoting migration and invasion of the same cell type. *Oncogene* 2005; 24:4349-61.
42. Brigstock DR. The CCN family: a new stimulus package. *J Endocrinol* 2003; 178:169-75.
43. Gupta N, Wang H, McLeod TL et al. Inhibition of glioma cell growth and tumorigenic potential by CCN3 (NOV). *Mol Pathol* 2001; 54:293-99.

44. Rydziel S, Stadmeyer L, Zanotti S, Durant D, Smerdel-Ramoya A, Canalis E. Nephroblastoma overexpressed (NOV) inhibits osteoblastogenesis and causes osteopenia. *J Biol Chem* 2007; 282:19762-72.
45. Plotkin LI, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T. Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. *J Clin Invest* 1999; 104:1363-74.
46. Rogers MJ. From molds and macrophages to mevalonate: a decade of progress in understanding the molecular mode of action of bisphosphonates. *Calcif Tissue Int* 2004; 75:451-61.
47. Hughes DE, Wright KR, Uy HL et al. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J Bone Min Res* 1995; 10:1478-87.
48. Kogianni G, Mann V, Ebetino F, et al. Fas/CD95 is associated with glucocorticoid-induced osteocyte apoptosis. *Life Sci* 2004; 75:2879-95.
49. Plotkin LI, Manolagas SC, Bellido T. Dissociation of the pro-apoptotic effects of bisphosphonates on osteoclasts from their anti-apoptotic effects on osteoblasts/ osteocytes with novel analogs. *Bone* 2006; 39:443-52.
50. Plotkin LI, Bivi N, Bellido T. A bisphosphonate that does not affect osteoclasts prevents osteoblast and osteocyte apoptosis and the loss of bone strength induced by glucocorticoids in mice. *Bone* 2011; 49:122-27.
51. Dunford JE, Thompson K, Coxon FP et al. Structure-activity relationships for inhibition of farnesyl diphosphate synthase in vitro and inhibition of bone resorption in vivo by nitrogen-containing bisphosphonates. *J Pharmacol Exp Ther* 2001; 296:235-42.
52. Brown RJ, Van Beek E, Watts DJ, Lowik CW, Papapoulos SE. Differential effects of aminosubstituted analogs of hydroxy bisphosphonates on the growth of Dictyostelium discoideum. *J Bone Min Res* 1998; 13:253-58.
53. Van Beek E, Lowik C, Que I, Papapoulos S. Dissociation of binding and antiresorptive properties of hydroxybisphosphonates by substitution of the hydroxyl with an amino group. *J Bone Min Res* 1996; 11:1492-97.
54. Plotkin LI, Aguirre JI, Kousteni S, Manolagas SC, Bellido T. Bisphosphonates and estrogens inhibit osteocyte apoptosis via distinct molecular mechanisms downstream of ERK activation. *J Biol Chem* 2005; 280:7317-25.
55. Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. *Science* 2005; 308:512-17.
56. DeFea KA, Zalevsky J, Thoma MS, Dery O, Mullins RD, Bunnett NW. Beta-arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. *J Cell Biol* 2000; 148:1267-81.
57. Tohgo A, Choy EW, Gesty-Palmer D et al. The stability of the G protein-coupled receptor-β-arrestin interaction determines the mechanism and functional consequence of ERK activation. *J Biol Chem* 2003; 278:6258-67.
58. Ge L, Ly Y, Hollenberg MD, DeFea K. A beta-arrestin-dependent scaffold is associated with prolonged MAPK activation in pseudopodia during protease-activated receptor-2 induced chemotaxis. *J Biol Chem* 2003; 278:34418-26.
59. Krupnick JG, Santini F, Gagnon AW, Keen JH, Benovic JL. Modulation of the arrestin-clathrin interaction in cells. Characterization of β-arrestin dominant-negative mutants. *J Biol Chem* 1997; 272:32507-12.
60. Cheng B, Zhao S, Luo J, Sprague E, Bonewald LF, Jiang JX. Expression of functional gap junctions and regulation by fluid flow in osteocyte-like MLO-Y4 cells. *J Bone Miner Res* 2001; 16:249-59.
61. Ziambaras K, Lecanda F, Steinberg TH, Civitelli R. Cyclic stretch enhances gap

junctional communication between osteoblastic cells. *J Bone Min Res* 1998;13:218-28.
62. Robinson JA, Chatterjee-Kishore M, Yaworsky PJ et al. WNT/beta-catenin signaling is a normal physiological response to mechanical loading in bone. *J Biol Chem* 2006;281:31720-31728.
63. Siller-Jackson AJ, Burra S, Gu S et al. Adaptation of connexin 43-hemichannel prostaglandin release to mechanical loading. *J Biol Chem* 2008;283:26374-82.
64. Li J, Burr DB, Turner CH. Suppression of prostaglandin synthesis with NS-398 has different effects on endocortical and periosteal bone formation induced by mechanical loading. *Calcif Tissue Int* 2002;70:320-329.
65. Chambers TJ, Chow JW, Fox SW, Jagger CJ, Lean JM. The role of prostaglandins and nitric oxide in the response of bone to mechanical stimulation. *Adv Exp Med Biol* 1997;433:295-98.
66. Forwood MR. Inducible cyclo-oxygenase (COX-2) mediates the induction of bone formation by mechanical loading in vivo. *J Bone Miner Res* 1996;11:1688-93.
67. Genetos DC, Kephart CJ, Zhang Y, Yellowley CE, Donahue HJ. Oscillating fluid flow activation of gap junction hemichannels induces ATP release from MLO-Y4 osteocytes. *J Cell Physiol* 2007; 212:207-14.
68. Grimston SK, Brodt MD, Silva MJ, Civitelli R. Attenuated response to in vivo mechanical loading in mice with conditional osteoblast ablation of the Connexin43 gene (Gja1). *J Bone Miner Res* 2008; 23:879-86.
69. Rubin MR, Cosman F, Lindsay R, Bilezikian JP. The anabolic effects of parathyroid hormone. *Osteoporos Int* 2002;13:267-77.
70. Jilka RL. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. *Bone* 2007;40:1434-46.
71. Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM, Manolagas SC. Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. *J Clin Invest* 1999;104:439-46.
72. Miao D, He B, Jiang Y et al. Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH 1-34. *J Clin Invest* 2005;115:2402-11.
73. Martin TJ. Osteoblast-derived PTHrP is a physiological regulator of bone formation. *J Clin Invest* 2005;115:2322-24.
74. Calvi LM, Sims NA, Hunzelman JL et al. Activated parathyroid hormone/ parathyroid hormone-related protein receptor in osteoblastic cells differentially affects cortical and trabecular bone. *J Clin Invest* 2001;107:277-86.
75. Yamamoto T, Kambe F, Cao X, Lu X, Ishiguro N, Seo H. Parathyroid hormone activates phosphoinositide 3-kinase-Akt-Bad cascade in osteoblast-like cells. *Bone* 2007;40:354-59.
76. Bellido T, Ali AA, Plotkin LI et al. Proteasomal degradation of Runx2 shortens parathyroid hormone-induced anti-apoptotic signaling in osteoblasts. A putative explanation for why intermittent administration is needed for bone anabolism. *J Biol Chem* 2003; 278:50259-72.
77. Tobimatsu T, Kaji H, Sowa H et al. Parathyroid hormone increases beta-catenin levels through Smad3 in mouse osteoblastic cells. *Endocrinology* 2006; 147:2583-90.
78. Vander Molen MA, Rubin CT, McLeod KJ, McCauley LK, Donahue HJ. Gap junctional intercellular communication contributes to hormonal responsiveness in osteoblastic networks. *J Biol Chem* 1996;271:12165-71.
79. Castro CHM, Stains JP, Civitelli R. The Anabolic Response to Intermittent PTH (1-34) Requires Connexin43 (Cx43) Mediated Gap Junctional Communication. *J Bone Min Res* 2003;18:S100.

80. Evans WH, De Vuyst E., Leybaert L. The gap junction cellular internet: connexin hemichannels enter the signalling limelight. *Biochem J* 2006;397:1-14.
81. Jiang JX, Gu S. Gap junction- and hemichannel-independent actions of connexins. *Biochim Biophys Acta* 2005;1711:208-14.
82. Liu L, Li Y, Lin J et al. Connexin43 interacts with Caveolin-3 in the heart. *Mol Biol Rep* 2010;37:1685-91.
83. Butkevich E, Hulsmann S, Wenzel D, Shirao T, Duden R, Majoul I. Drebrin is a novel connexin-43 binding partner that links gap junctions to the submembrane cytoskeleton. *Curr Biol* 2004;14:650-658.
84. Niger C, Hebert C, Stains JP. Interaction of connexin43 and protein kinase C-delta during FGF2 signaling. *BMC Biochem* 2010;11:14.
85. Davy A, Bush JO, Soriano P. Inhibition of Gap Junction Communication at Ectopic Eph/ephrin Boundaries Underlies Craniofrontonasal Syndrome. *PLoS Biol* 2006;4:1763-76.
86. Girao H, Catarino S, Pereira P. Eps15 interacts with ubiquitinated Cx43 and mediates its internalization. *Exp Cell Res* 2009;315:3587-97.
87. Li X, Su V, Kurata WE, Jin C, Lau AF. A novel connexin43-interacting protein, CIP75, which belongs to the UbL-UBA protein family, regulates the turnover of connexin43. *J Biol Chem* 2008;283:5748-59.
88. Park DJ, Freitas TA, Wallick CJ, Guyette CV, Warn-Cramer BJ. Molecular dynamics and in vitro analysis of Connexin43: A new 14-3-3 mode-1 interacting protein. *Protein Sci* 2006;15:2344-55.
89. Plotkin LI, Vyas K, Gortazar AR, Manolagas SC, Bellido T. β-arrestin complexes with connexin (Cx) 43 and anchors ERKs outside the nucleus: a requirement for the Cx43/ERK-mediated anti-apoptotic effect of bisphosphonates in osteocytes. *J Bone Miner Res* 2006; 21:S65.