Communicative & Integrative Biology 5:5, 516-518; September/October 2012; ©2012 Landes Bioscience

"INTEGRINating" the connexin hemichannel function in bone osteocytes through the action of integrin α5

Nidhi Batra and Jean X. Jiang*
Department of Biochemistry; University of Texas Health Science Center; San Antonio, TX USA

Mechanical loading influences skeletal structural integrity and bone remodeling. Application of a mechanical stimulus such as fluid flow shear stress to the bone osteocytes activates the cascade of mechanotransduction mediated by multiple signaling molecules. Hemichannels formed by connexin molecules are emerging as a candidate mechanosensor. Connexin 43 (Cx43) hemichannels open in response to mechanical stimulation to release bone modulators which influence bone remodeling. Our study identified a direct interaction between integrin α5 and Cx43 which was essential for hemichannels to open. Uncoupling the interaction blocked the hemichannels and shear stress enhanced the interaction between the two proteins to promote channel opening. More importantly, integrin α5, independent of its association with fibronectin, was activated upon shear stress through a PI3K signaling pathway. These results suggest a critical regulatory mechanism for Cx43 hemichannel opening through the association of integrin α5, resulting in release of bone anabolic factors required for bone development.

Keywords: connexin, integrin, hemichannel, mechanotransduction, bone osteocytes

Submitted: 06/29/12

Accepted: 07/02/12

http://dx.doi.org/cib.21322

*Correspondence to: Jean X. Jiang; Email: jiangj@uthscsa.edu

Addendum to: Batra N, Burra S, Siller-Jackson AJ, Gu S, Xia X, Weber GF, et al.; Addenda to. Mechanical stress-activated integrin α5β1 induces opening of connexin 43 hemichannels. Proc Natl Acad Sci U S A 2012; 109:3359-64; PMID:22331870; http://dx.doi.org/10.1073/pnas.1115967109.

Improvement in the bone strength and overall health of the body is highly responsive to exercise and/or physical activity. Regular or strenuous exercise is a form of mechanical loading on the bone that generates mechanical signals. These signals are sensed by the bone cells and converted to biochemical events which regulate bone modeling and remodeling. The cells of the bone which are thought to sense the signals are osteocytes. Occupying nearly 95% of the space, these cells are the major mechanosensors of the bone which sense the mechanical signals and pass it onto the other types of bone cells that either form the bone (osteoblasts) or resorb the bone (osteoclasts).[1,2] The osteocytes are susceptible not only to biochemical stimuli, but also to physical changes imposed by mechanical forces. Identifying the detailed molecular mechanisms for conversion of the mechanical signals into biochemical events is thus of great interest. Evidence from various studies have indicated that cells sense mechanical signals and turn physical alterations into biochemical events that differentially regulate cell signaling pathways.[3,4]

The mechanoresponsiveness in many cell types is mediated by the heterodimeric signal transducing transmembrane glycoproteins called integrins that link extracellular matrix constituents to intracellular elements of the actin cytoskeleton.[5] All bone cell types express integrins,[6,7] and osteocytes in particular express integrins of both β1 and β3 families.[7] Yet, how osteocytic integrins participate in the response to mechanical signaling remained an open question.

Several views have been put forth to determine how the osteocytes function as mechanosensors. Osteocytes experience force from two different sources; fluid flow shear stress (FFSS) and cellular deformations.[8] When shear stress is applied to the osteocytes, hemichannels formed by connexin 43 (Cx43) open to serve as a portal for the release of prostaglandins.[9] Various studies have shown the increase in expression of connexins in vitro and in vivo upon mechanical stimulation, suggesting

a possible role of this protein in mechanotransduction of bone.[10,11] Connexins are gap junction and hemichannel forming proteins and Cx43 is abundantly expressed in osteocytes.[10,12,13] The surface expression of Cx43 and formation of Cx43 gap junctions increases upon mechanical loading in areas of bone exposed to tension.[14] Although, we and others have shown that Cx43 hemichannels open in response to FFSS, the mechanisms and molecules that are involved in the shear stress-mediated opening of the hemichannels in these cells is poorly understood. Understanding the role of Cx43 in bone modeling and remodeling, and identifying the molecular mechanism for regulation of Cx43 hemichannel opening in response to mechanical loading for appropriate release of bone modulators is an endeavor of several laboratories including ours.

Sometime ago, it was speculated that strain amplification mediated by integrins would be most effective if mechanosensitive membrane channels were localized at adhesion sites. Also, several lines of evidence indicate that integrins can regulate ion channel activity.[15] Stress-activated ion channels on the cell surface can be activated through the forces transmitted by the integrins to the channel elements, either directly or through the cytoskeleton.[16] In our search of potential mechanosensors for the opening of hemichannels, we identified integrin α5 as a candidate mechanosensor. Among the different integrins, integrin α5β1 and αvβ3 are expressed in the bone[7,17] and β1 integrin has been shown to mediate the cellular response of bone osteocytic cells called MLO-Y4 (murine long bone osteocytic cells) to FFSS.[18] In the present study, we noticed that integrin α5 and Cx43 are colocalized in MLO-Y4 cells and primary osteocytes isolated from the chicken calvarial bone.[19] Since, integrin α5 is known to be consistently associated in a heterodimeric composition with β1 subunit, we identified the β1 binding partner for integrin α5 in these cells. The known repertoire of the proteins associated with Cx43 demonstrates that majority of the interactions occur at the C-terminal domain due to its variability and participation in protein-protein interaction, post-translational modifications and other functions.[20] Indeed, we noticed a direct interaction between Cx43 and integrin α5, as demonstrated through GST pull down assay using a peptide to the C-terminus of integrin α5 to pull down GST-tagged C terminus of Cx43. This interaction was further confirmed by estimating the binding constant through surface plasmon resonance (SPR). While the binding constant (Kd) obtained through SPR was relatively weak; however, the association between the two proteins was direct and specific. Our analysis showed that the weak Kd was a result of a fast association followed by an equally rapid dissociation of the two proteins. Interestingly, mechanical stimulation strengthened the interaction between these proteins. Furthermore, the requirement of integrin α5 for the hemichannel opening was confirmed when expression of integrin α5 was inhibited using siRNA, and hemichannel opening was blocked. The importance of the interaction between the two proteins for the mechanical stress induced hemichannel opening was determined by uncoupling the interaction. Overexpression of the C-terminal domain of Cx43 in these cells successfully uncoupled the interaction, which clearly blocked the hemichannels from opening.

The bone matrix is comprised of various matrix proteins like collagen, fibronectin (FN), osteopontin, and FN is a known receptor for integrin α5β1 that helps in cell attachment.[21] However, we found that although these osteocytic cells need to attach to the surface, the opening of hemichannels is independent of a specific binding of integrin α5β1 to FN. The cell has similar dye uptake regardless of their attachment to collagen or FN matrix. These experiments indicated the importance of integrin α5 for the channel opening; however, the shear stress-induced mechanistic effect on integrin α5 that facilitated the opening of the hemichannels was not yet clear.

Many studies have evidence to show that integrins undergo conformational rearrangements which govern the affinity to the extracellular ligand and binding with cytoskeletal proteins.[22-24] We performed magnetic perturbation of integrin α5β1 in the cell using magnetic beads coated with either integrin α5 antibody or the primary substrate of integrin α5, FN. The opening of hemichannels was induced under the influence of magnetic field in these cells, suggesting that physical perturbation of integrin α5 led to the opening of the hemichannels. These observations implied that mechanical force may trigger the conversion of integrin α5β1 to an activated state that is competent to signal, which is in line with previous reports.[25] Indeed, the conformational activation of integrin α5β1 is sufficient for hemichannel opening was further supported by our finding where hemichannels were induced open without any FFSS but merely in the presence of a β1 integrin activating antibody (TS2/16), known to bind and activate β1 integrin molecules.[26] In addition, a reporter molecule which selectively binds active integrin α5β1 called GST-$FNIII_{9\text{-}11}$, bound only to the cells treated for FFSS and not the control, further confirming that FFSS induced a conformational change that activated integrin α5β1. However, some studies indicate that shear stress does not directly activate the integrins, rather PI3K has been implicated as the primary transducer of the force. PI3K inhibitors can completely block the force induced activation of an integrin αvβ3 suggesting that shear stress-stimulated PI3K mediates integrin activation.[27,28] On the similar lines, we recently showed that FFSS activated PI3K signaling in osteocytic cells had a stimulatory effect on the mRNA expression of Cx43.[29] Interestingly, inhibition of active PI3K disrupted the interaction between Cx43 and integrin α5β1 in our study, and dramatically reduced the hemichannel opening. Inhibition of PI3K signaling using PI3K inhibitors in the osteocytic cells prior to FFSS significantly decreased the binding of GST-$FNIII_{9\text{-}11}$ indicating the reduced activation of integrin α5β1. This data suggests that PI3K is an important player that transmits the effect of mechanical stimulation on the integrin through its activation, which is sensed by the Cx43 hemichannel to result in their opening and release of anabolic bone modulators. Abolition of the interaction between the two proteins due to absence of active PI3K indicated that the interacting C terminal domains of integrin α5β1 and Cx43 may undergo changes in response to force and

mediate mechanotransduction. Activation of integrin involves the separation of the cytoplasmic tails of α and β subunits.[30] Parting between the tails of the integrin molecule may allow the C-terminus of integrin α5 to be available for interaction with other proteins, such as Cx43 and facilitate hemichannel opening.

Although our study has brought to focus a new player, integrin α5 in the hemichannel induced mechanotransduction of bone, it is rather difficult to ignore the fact that mechanical load applied to the bone cells in vitro are typically much higher strains compared to those required in vivo. As a result, bone cells are subjected to much more strain in vitro to induce cellular deformations for activating measurable cellular responses. Part of this inconsistency between in vitro and in vivo mechanical load magnitude could occur due to the non-physiological conditions during cell culture, such as two dimensional culture conditions which lack the connections and constraints exerted on the bone architecture due to the presence of mineralized matrix. Hence, switching to a closer mimic of the bone structure to conduct these studies is a future call. Some of the other unanswered questions that need to be addressed include determining how FFSS activates PI3K and how PI3K activates integrin α5 which facilitates Cx43 hemichannel opening, a critical step for the release of anabolic factors responsible for bone modeling and remodeling.

Acknowledgments

This work was supported by National Institutes of Health Grant (AR46798) and Welch Foundation grant (AQ-1507).

References

1. Bonewald LF. Osteocytes as dynamic multifucntional cells. Ann N Y Acad Sci 2007; 1116:281-90; http://dx.doi.org/10.1196/annals.1402.018.
2. Klein-Nulend J, Bacabac RG, Mullender MG. Mechanobiology of bone tissue. Pathol Biol (Paris) 2005; 53:576-80; PMID:16364809; http://dx.doi.org/10.1016/j.patbio.2004.12.005.
3. Tamada M, Sheetz MP, Sawada Y. Activation of a signaling cascade by cytoskeleton stretch. Dev Cell 2004; 7:709-18; PMID:15525532; http://dx.doi.org/10.1016/j.devcel.2004.08.021.
4. Sukharev S, Corey DP. Mechanosensitive channels: multiplicity of families and gating paradigms. Sci STKE 2004; 2004:re4; PMID:14872099; http://dx.doi.org/10.1126/stke.2192004re4.
5. Yamada KM. Integrin signaling. Matrix Biol 1997; 16:137-41; PMID:9402002; http://dx.doi.org/10.1016/S0945-053X(97)90001-9.
6. Horton MA, Davies J. Perspectives: adhesion receptors in bone. J Bone Miner Res 1989; 4:803-8; PMID:2481941; http://dx.doi.org/10.1002/jbmr.5650040603.
7. Hughes DE, Salter DM, Dedhar S, Simpson R. Integrin expression in human bone. J Bone Miner Res 1993; 8:527-33; PMID:8511980; http://dx.doi.org/10.1002/jbmr.5650080503.
8. McGarry JG, Klein-Nulend J, Mullender MG, Prendergast PJ. A comparison of strain and fluid shear stress in stimulating bone cell responses--a computational and experimental study. FASEB J 2005; 19:482-4; PMID:15625080.
9. Cherian PP, Siller-Jackson AJ, Gu S, Wang X, Bonewald LF, Sprague E, et al. Mechanical strain opens connexin 43 hemichannels in osteocytes: a novel mechanism for the release of prostaglandin. Mol Biol Cell 2005; 16:3100-6; PMID:15843434; http://dx.doi.org/10.1091/mbc.E04-10-0912.
10. 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; PMID:11204425; http://dx.doi.org/10.1359/jbmr.2001.16.2.249.
11. Gluhak-Heinrich J, Gu S, Pavlin D, Jiang JX. Mechanical loading stimulates expression of connexin 43 in alveolar bone cells in the tooth movement model. Cell Commun Adhes 2006; 13:115-25; PMID:16613785; http://dx.doi.org/10.1080/15419060600634619.
12. Goodenough DA, Paul DL. Beyond the gap: functions of unpaired connexon channels. Nat Rev Mol Cell Biol 2003; 4:285-94; PMID:12671651; http://dx.doi.org/10.1038/nrm1072.
13. Kamioka H, Ishihara Y, Ris H, Murshid SA, Sugawara Y, Takano-Yamamoto T, et al. Primary cultures of chick osteocytes retain functional gap junctions between osteocytes and between osteocytes and osteoblasts. Microsc Microanal 2007; 13:108-17; PMID:17367550; http://dx.doi.org/10.1017/S143192760707016X.
14. Taylor AF, Saunders MM, Shingle DL, Cimbala JM, Zhou Z, Donahue HJ. Mechanically stimulated osteocytes regulate osteoblastic activity via gap junctions. Am J Physiol Cell Physiol 2007; 292:C545-52; PMID:16885390; http://dx.doi.org/10.1152/ajpcell.00611.2005.
15. McNamara LM, Majeska RJ, Weinbaum S, Friedrich V, Schaffler MB. Attachment of osteocyte cell processes to the bone matrix. Anat Rec (Hoboken) 2009; 292:355-63; PMID:19248169; http://dx.doi.org/10.1002/ar.20869.
16. Ingber DE. Cellular mechanotransduction: putting all the pieces together again. FASEB J 2006; 20:811-27; PMID:16675838; http://dx.doi.org/10.1096/fj.05-5424rev.
17. Bennett JH, Carter DH, Alavi AL, Beresford JN, Walsh S. Patterns of integrin expression in a human mandibular explant model of osteoblast differentiation. Arch Oral Biol 2001; 46:229-38; PMID:11165569; http://dx.doi.org/10.1016/S0003-9969(00)00114-X.
18. Litzenberger JB, Kim JB, Tummala P, Jacobs CR. β1 integrin mediate mechanosensitive signaling pathways in osteocytes. Calcif.Tissue Int. 2010. PMID:20213106
19. Batra N, Burra S, Siller-Jackson AJ, Gu S, Xia X, Weber GF, et al. Mechanical stress-activated integrin α5β1 induces opening of connexin 43 hemichannels. Proc Natl Acad Sci U S A 2012; 109:3359-64; PMID:22331870; http://dx.doi.org/10.1073/pnas.1115967109.
20. Hervé JC, Bourmeyster N, Sarrouilhe D, Duffy HS. Gap junctional complexes: from partners to functions. Prog Biophys Mol Biol 2007; 94:29-65; PMID:17507078; http://dx.doi.org/10.1016/j.pbiomolbio.2007.03.010.
21. Takagi J. Structural basis for ligand recognition by RGD (Arg-Gly-Asp)-dependent integrins. Biochem Soc Trans 2004; 32:403-6; PMID:15157147; http://dx.doi.org/10.1042/BST0320403.
22. Calvete JJ. Structures of integrin domains and concerted conformational changes in the bidirectional signaling mechanism of alphaIIbbeta3. Exp Biol Med (Maywood) 2004; 229:732-44; PMID:15337827.
23. Wegener KL, Campbell ID. Transmembrane and cytoplasmic domains in integrin activation and protein-protein interactions (review). [review]. Mol Membr Biol 2008; 25:376-87; PMID:18654929; http://dx.doi.org/10.1080/09687680802269886.
24. Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. Cold Spring Harb Perspect Biol 2011; 3:a004994; PMID:21421922; http://dx.doi.org/10.1101/cshperspect.a004994.
25. Friedland JC, Lee MH, Boettiger D. Mechanically activated integrin switch controls α5β1 function. Science 2009; 323:642-4; PMID:19179533; http://dx.doi.org/10.1126/science.1168441.
26. Tsuchida J, Ueki S, Saito Y, Takagi J. Classification of 'activation' antibodies against integrin beta1 chain. FEBS Lett 1997; 416:212-6; PMID:9369217; http://dx.doi.org/10.1016/S0014-5793(97)01206-4.
27. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature 2005; 437:426-31; PMID:16163360; http://dx.doi.org/10.1038/nature03952.
28. Katsumi A, Naoe T, Matsushita T, Kaibuchi K, Schwartz MA. Integrin activation and matrix binding mediate cellular responses to mechanical stretch. J Biol Chem 2005; 280:16546-9; PMID:15760908; http://dx.doi.org/10.1074/jbc.C400455200.
29. Xia X, Batra N, Shi Q, Bonewald LF, Sprague E, Jiang JX. Prostaglandin promotion of osteocyte gap junction function through transcriptional regulation of connexin 43 by glycogen synthase kinase 3/β-catenin signaling. Mol Cell Biol 2010; 30:206-19; PMID:19841066; http://dx.doi.org/10.1128/MCB.01844-08.
30. Kim M, Carman CV, Springer TA. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. Science 2003; 301:1720-5; PMID:14500982; http://dx.doi.org/10.1126/science.1084174.