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Dark horse in osteocyte biology

Glycocalyx around the dendrites is critical for osteocyte mechanosensing

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Osteocytes are considered as the major mechanosensory cells of the bone tissue that control the bone remodeling process. Since osteocytes are buried inside mineralized matrix, they maintain a strong communication network with other cells. Long dendritic processes of the osteocytes act as communication cables, conveying mechanical signals to the neighboring osteocytes and the cells on the bone surface; like osteoblasts and osteoclasts. Gap junctions and hemichannels formed by Connexin (Cx) 43 are observed to be involved in responding to the mechanical stimulus and in communicating the mechano-responsive biochemical signals. The contrast in the arrangement of the osteocyte cell body and the dendrites raises an important question of how these parts of the osteocyte respond to mechanical stimulation. We addressed this issue in our recent report through the stimulation of either osteocyte cell body or dendrites and our findings suggest that the osteocyte dendritic processes are sensitive to mechanical stimulation in comparison to the cell body. Most importantly, we observed that the dendritic processes are capable of conveying the mechanical signals to the cell body. Our findings also suggested that the glycocalyx surrounding the dendrites is required for sensing and conveying the mechanical signals. Degradation of the glycocalyx also leads to poor integrin attachment, thereby, affecting dendritic stiffness. These results suggest that the osteocyte dendritic processes are highly responsive towards mechanical stimulation and the glycocalyx surrounding the dendrites is critical in transducing these mechanical signals.

Key words: osteocyte, dendritic processes, hemichannel, glycocalyx, mechanosensing

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Bone is a complex tissue, consisting of different types of cells. Among them, osteocytes with distinct functions typically respond to mechanical stimulation.[1] Bone forming osteoblasts and bone resorbing osteoclasts are located on the bone surface, and majority of the bone cells are osteocytes that are formed due to osteoblast differentiation and their embedding into the mineralized matrix.[2] Local communication between osteocytes, bone forming osteoblasts and bone resorbing osteoclasts are critical for bone remodeling.[3] Due to the position and morphology, osteocytes are considered as the major mechanosensory cells of the bone tissue.[4] Osteocytes have stellate morphology with small cell body and long dendritic processes. The osteocyte dendritic processes and the cell body are surrounded by fluid filled spaces termed as canaliculi and lacuna, respectively. The canaliculi around the dendrites are narrow when compared to that of the lacunar space surrounding osteocyte cell body.[5] Fluid flow through the lacuna-canalicular network due to mechanical stimulation causes shear stress and is considered as the major form of the mechanical stimulation experienced by osteocytes.[4]

Although the exact composition and structure of the fluid around the osteocyte is not known, it is considered to be comprised of extracellular matrix proteins, such as glycocalyx, collagen, fibronectin, vitronectin. These proteins act as ligands for cell membrane receptors, such as integrins, CD44, etc.[6,7] In many cells integrins function as mechanoreceptors. The

integrin attachments on the osteocyte dendrites are suggested to increase sensitivity of dendrites to mechanical stimulation.[8] Recently, it was observed that the sensitivity of dendrites to mechanical stimulation is higher and generates stronger calcium waves due to mechanical stimulation in comparison to the stimulation of osteocyte cell body.[9] Our study addresses the mechanism behind this higher order of sensitivity towards mechanical stimulation displayed by osteocytic dendrites.[10]

Osteocytes also express hemichannels formed by Cx43 that are considered to be mechanosensitive in nature.[11] The other connexin protein, Cx45, is also expressed in osteocytes, but hemichannels formed by Cx43 respond predominantly towards mechanical stimulation.[12,13] Mechanical stimulation of osteocytes results in opening of hemichannels to release molecules, such as PGE_2 and ATP.[14,15] These biochemical signaling molecules play a very important role in maintaining the balance between bone formation and resorption. Disruption of glycocalyx was observed to decrease PGE_2 release by mechanically stimulated osteocytes.[16] These studies suggest that the extracellular matrix proteins along with Cx43 hemichannels play an important role in mechanosensing by osteocytes.

Since osteocyte cell body and dendritic processes are located in different microenvironments, it is important to understand how these parts of the cell respond to mechanical stimulation. This knowledge would aid us in understanding the details of the complex process of mechanosensing by osteocytes. To address this issue, the challenge is to be able to differentially stimulate the dendrites and cell body of the osteocytes. In our report, we used a transwell filter system with a pore size of 1.0 μm to separate the osteocyte dendritic processes and cell body.[10] The smaller pore size of the transwell filter system allows the slender dendrites to transgress but retains the cell body. This system is advantageous to target the mechanical stimulation to cell body and dendritic processes separately.

In order to understand the mechanical forces acting on the cell bodies and dendrites during this experiment, we performed a computational fluid dynamics simulation of the dynamic impact of the fluid drop impacting the transwell filter. Our analysis showed that the loading caused by the drop impacting the filter is a highly dynamic and transient event. As the drop impacts and spreads across the surface of the filter paper, shear stresses are generated at the transwell filter surface that were estimated to be as high as 160 dynes/cm^2 shortly after the impact event reducing to as low as 1 dyne/cm^2 as the droplet spreads to a radial distance of about 0.9 cm from the point of impact. These shear stress values allow us to relate the mechanical environment experienced by osteocytes during this experiment to the mechanical environment thought be acting on osteocytes in bone in vivo.[8,17]

The dendritic growth through the filter pores was confirmed by immunostaining technique using α5 integrin antibody. We observed a ubiquitous expression of α5 integrin and the staining in the dendritic processes overlapped with filter pores. After establishing the penetration of dendritic processes through the filter pores, mechanical stimulation was applied by dropping fluid onto the either side of the transwell filter. Cx43 hemichannel opening as observed by dye uptake analysis was used as a read-out for mechanosensing by osteocytes. Cx43 hemichannel opening on the cell body was observed when either the cell body or the dendritic processes are stimulated. On the contrary, the hemichannel opening was significantly lower on the dendritic side, after the stimulation of either the dendritic or the cell body side. This difference in hemichannel opening could be due to expression of fewer hemichannels on the dendritic processes. Alternatively, the force experienced by the dendrites and the cell body could be different due to their inherent structural differences. These results suggest that both the cell body and the dendritic processes are mechanosensitive, but the hemichannel opening is relatively higher in the osteocyte cell body. This technique of targeted stimulation opens a new concept in cell biology that different parts of the cell function differently and that they can be differentially regulated.

Glycocalyx in cardiac tissue is considered important for mechanosensing by endothelial cells.[18] In general, glycocalyx is mainly formed by a mesh of proteoglycans, glycoproteins and hyaluronic acid. Degradation of endothelial glycocalyx by heparitinase, which digests heparin sulfate glycosaminoglycans or by hyaluronidase that hydrolyze hyaluronic acid results in decreased NO release by endothelial cells.[18,19] These studies suggest that glycocalyx is an important component of the mechanosensory complex in the endothelial cells. Also, endothelial glycocalyx is considered to control the cytoskeletal reorganization during mechanical stimulation and regulates the cellular signaling pathways that are involved in the release of molecules, such as NO.[20]

In osteocyte-like MLO-Y4 cells, glycocalyx degradation by hyaluronidase resulted in decreased shear stress driven PGE_2 release.[16] Based on these studies that highlight the importance of glycocalyx in the extracellular matrix, in our study, we used hyaluronidase to degrade the glycocalyx and studied its role in osteocyte mechanosensing.[10] Hyaluronidase treatment on the dendritic side of the filter resulted in decreased opening of hemichannels on the osteocyte cell body. Interestingly, hyaluronic acid degradation on the cell body side of the filter did not affect hemichannel opening. We also observed that the α5 integrin staining on the dendritic side disappeared with hyaluronidase treatment. These results suggest that the glycocalyx around the dendritic processes of the cell is critical for mechanosensing. Also, intact glycocalyx could be important in maintaining integrin contacts. Attachment of osteocyte dendritic processes to the canalicular wall via integrins is considered to enhance the mechanosensing ability of the dendrites.[8] The reason behind hemichannel opening on the cell body even after degradation of the glycocalyx around the osteocyte cell body is still not clear. One explanation could be that the organization of glycocalyx around the cell body could be different. Also, it is known that the cytoskeletal framework in the osteocyte cell body is different from that of the dendrites, as two actin-bundle forming proteins, alpha-actinin and fimbrin are uniquely expressed in osteocyte dendritic processes.[21] Due to this difference in cytoskeletal organization, components of glycocalyx could be having a differential

effect on the osteocyte cell body during mechanical stimulation.

Another question that remained unanswered is how the signals sensed by dendritic processes reach the cell body and result in hemichannel opening on the cell body? Since glycocalyx is known to change the cytoskeletal organization in response to mechanical stimulation, it could be possible that this event somehow affects hemichannel opening. Integrins expressed on the cell membrane are known to interact with the cytoskeletal proteins such as actins. Changes in the extracellular matrix could affect membrane receptors such as integrins, thereby, affecting cytoskeletal organization. In our study, we observed that the degradation of glycocalyx leads to poor integrin attachment,[10] which could decrease the effect of mechanical stimulation on cytoskeletal reorganization and thereby, result in decreased hemichannel opening. However, a link between cytoskeletal reorganization due to alteration of integrin function and hemichannel opening has to be established.

In order to understand how osteocytes sense the mechanical signals, it is important to understand how glycocalyx acts as a mechanotansducer. It is also important to understand the cellular signaling pathways activated by glycocalyx during mechanical stimulation and the mechanism behind the glycocalyx control of integrin attachment, cytoskeletal reorganization and hemichannel opening.

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