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Perspective Article

Generation and function of osteocyte dendritic processes

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Osteocytes *in vivo* possess a distinctive morphology – that of dendricity – connecting osteocyte to osteocyte creating the osteocyte syncytium and also connecting osteocytes with cells on the bone surface (see Figure 1). It is thought that bone fluid surrounding the dendrite within the canaliculi is responsible for the transmission of mechanical strain through fluid flow shear stress. Dendrites may be essential for osteocyte function, viability, and response to load.

The cell processes of osteocytes are connected with each other via gap junctions[1], thereby allowing direct cell-to-cell coupling. Osteocytes and MLO-Y4 osteocyte-like cells[2] express large amounts of Connexin 43, the component of gap junctions. But as these cells are only in contact through the tips of their dendritic processes, this raises the question, what is the function of Cx 43 located on the rest of the cell membrane? Recently it has been shown that connexins can complex and function in the form of un-apposed halves of gap junction channels called hemichannels. These channels are localized at the cell surface, independent of physical contact with adjacent cells[3]. Recently evidence of functional hemichannels formed by Cx43 has been reported in neural progenitors and neurons, astrocytes, heart, and especially, osteoblasts and osteocytes. The opening of hemichannels appears to provide a mechanism for ATP and NAD^+ release, which raises intracellular Ca^{2+} levels and promotes Ca^{2+} wave propagation in astrocytes, bone cells, epithelial cells, and outer retina. Hemichannels expressed in bone cells such as MLO-Y4 cells appear to function as essential transducers of the anti-apoptotic effects of bisphosphonates[4] and act as a portal for the extracellular release of PGE2 in osteocytes upon exposure to fluid flow shear stress[5]. Therefore, in osteocytes, gap junctions at the tip of dendrites appear to mediate a form of intracellular communication and

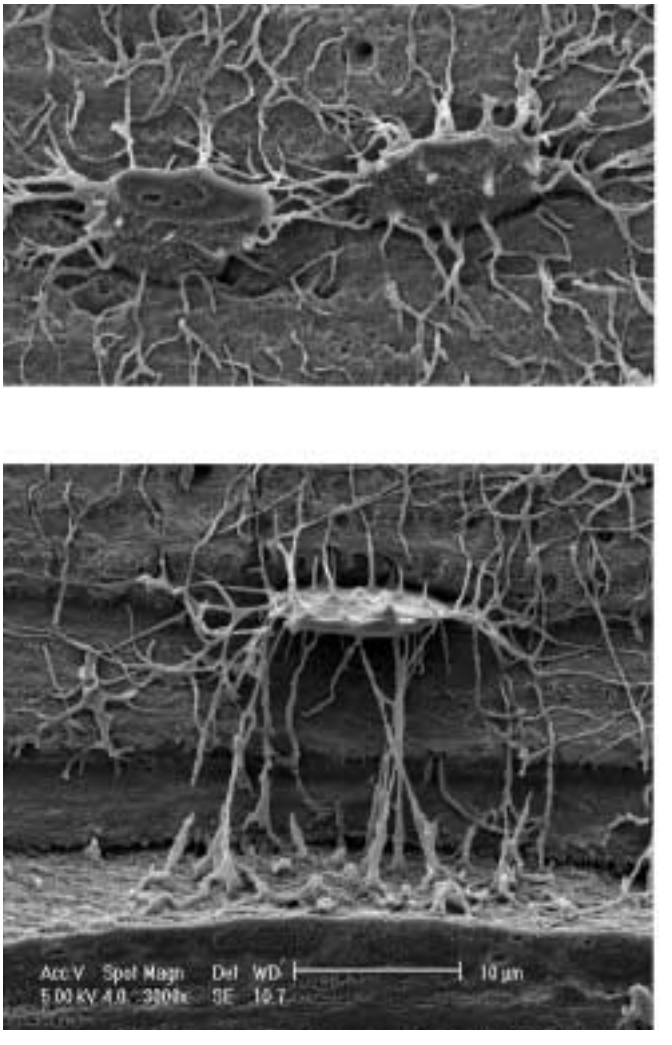

Figure 1. The osteocyte lacuno-canalicular network. Osteocyte cell processes pass through the bone in thin canals called canaliculi connecting osteocytes with each other (top image) and with cells on the bone surface (lower image). Osteocytes are thought to function as a network of sensor cells in bone that can mediate the effects of mechanical loading through their extensive communication network. The images are scanning electron micrographs of resin embedded acid etched mouse bone samples.

The author receives royalties from licensing MLO cell lines.

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hemichannels along the dendrite appear to mediate a form of extracellular communication.

Osteocytogenesis has been thought to be a passive process whereby some osteoblasts become passively encased in osteoid that passively mineralizes. However, Holmbeck and colleagues[6] have shown osteocytogenesis to be an active invasive process requiring cleavage of collagen and potentially other matrix molecules. Osteocytes in null mice for the metalloproteinase MT1-MMP have significantly reduced number and length of dendritic processes. MT1-MMP is a membrane-anchored proteinase that can cleave collagens type I, II, and III, fibrin, fibronectin, and other matrix molecules. In this mouse model, the almost complete lack of dendritic processes did not appear to affect viability or density of osteocytes in contrast to studies by Zhao and co-workers[7] where osteocytes in a mouse model of collagenase resistant type I collagen did show increased apoptosis. However, it was impossible to determine the effect of a lack of dendritic processes on either osteocyte function or effects on the skeleton as the MT1-MMP null mouse exhibits multiple defects such as dwarfism due to a lack of MT1-MMP in other skeletal tissues[8]. These investigators also showed an increase in dendricity of osteocytes in mice between 3 and 4 months of age suggesting that the embedded osteocyte can generate new canaliculi containing new dendrites. Our preliminary studies also suggest increased dendricity with age.

The early formation of dendrites by embedding osteoid-osteocytes is polarized towards the mineralization front to which cellular processes are oriented. Cellular processes towards blood vessels only began to appear when the mineralization begins to spread around the cell[9]. Osteocyte dendricity changes depending on orientation and with static and dynamic bone formation[10]. In undiseased bone, osteocyte connectivity is high and the processes are oriented in the direction of the blood supply[11]. In osteoporotic bone there is a marked decrease in connectivity as well as disorientation of the dendrites which increases in severity. In contrast, in osteoarthritic bone, a decrease in connectivity is observed, but the orientation is intact. In osteomalacic bone, the osteocytes appear viable with high connectivity, but the processes are distorted and the network chaotic[11]. Changes in osteocyte dendricity could not only have a dramatic effect on osteocyte function and viability, but also on the mechanical properties of bone. An equilibrium must be met between number and branching of dendrites to preserve function and viability versus the number that would decrease bone strength.

We propose that a molecule known as E11/gp38 plays a role in the formation of dendritic processes (Figure 2). The earliest description of the gene for E11 was in 1990 as an unknown phorbal ester inducible gene in MC3T3 osteoblast-like cells, called OTS-8[12]. Since that time this gene/protein has become known by several names depending on tissue expression. It is expressed in the choroid plexus, ciliary epithelium of the eye, intestine, kidney podocytes, thyroid, esophagus, type I alveolar lung cells, lymphatic endothelium and osteocytes in bone. The gene cloned from murine

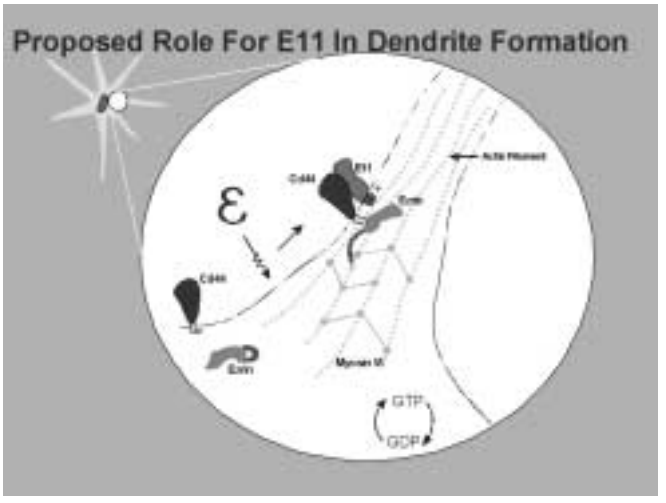

Figure 2. Proposed role for E11 in the formation of dendrites in response to fluid flow shear stress. We propose that E11 associates with membrane proteins such as CD44 and with cytoplasmic molecules such as the ERM complex to generate cellular projections. CD44 is an integral membrane glycoprotein that has the capacity to bind multiple extracellular matrix molecules including hyaluronate, collagen, and fibronectin and has been shown to be highly expressed in osteocytes. ERMS, ezrin, radixin, and moesin are part of a protein complex that acts as a universal linker between the actin cytoskeleton and integral proteins of the plasma membrane. Together these molecules may work together to extend dendritic processes of osteocytes in response to strain.

peripheral lymphoid tissue was called Gp38[13], from rat type I epithelial alveolar lung cells, 'T1alpha'[14], the protein as RTI40[15], and in mouse kidney, as 'podoplanin' as it localizes to the foot processes of podocytes[16]. Expression occurs on the apical surface of lung epithelial cells which are the thin, flat, polarized cells that form the air-blood barrier[14,17]. Deletion of this gene results in mice that die at birth due to respiratory failure as their lungs cannot be inflated to normal volumes[18], due to a failure of type II alveolar lung cells to differentiate into type I alveolar lung cells.

There has been considerable speculation regarding the function of E11. As the molecule is highly negatively charged and resistant to proteases, it may provide a physical barrier protecting cells from environmental agents[19]. The fact that E11 is found in cells that are exposed to an external or internal fluid compartment further supports this hypothesis. Accumulating evidence suggests that the major function of E11/gp38 may be in the formation of dendritic processes. Antibody to podoplanin (E11) causes rapid flattening of podocytes and proteinuria suggesting that the molecule maintains the shape of the podocyte foot processes[20]. Ectopic overexpression of the gene in keratinocytes induces plasma membrane extensions[21] and overexpression in endothelial cells promotes formation of long and thin tube-like structures on Matrigel[22], major reorganization of the

actin cytoskeleton and relocalization of ezrin to cell projections[21]. The molecule co-localizes with ezrin, radixin, and moesin, ERMs[21], molecules that are concentrated in cell-surface projections where they link the actin cytoskeleton to plasma membrane proteins and are involved in cell motility[23]. E11/gp38 was also found to be physically associated with CD44 in tumor vascular endothelial cells[24,25]. Together, this data suggest that E11/gp38 associates with CD44 and the ERMs to induce and regulate the formation of dendritic processes. In osteocytes, the 'gp38/podoplanin/T1alpha' molecule is known as E11, the name given by Wetterwald and co-workers[26]. The E11 antigen was only found on the dendritic processes of osteocytes, not on osteoblasts *in vivo*[27]. Overexpression in an osteoblast-like cell line led to the generation of extended cytoplasmic processes[28]. Other than this information, very little is known about the function of E11 in mineralized tissues.

We have performed studies reproducing previous observations that E11 is expressed in osteocytes and not osteoblasts and have generated E11 null mice that die at birth. We have also shown that E11 is regulated by mechanical strain both *in vivo* and *in vitro*[29]. MLO-Y4 cells express large amounts of E11 protein and gene expression is increased in response to fluid flow shear stress. We had shown previously that fluid flow shear stress increased dendrite lengthening[30] and now have data showing that this increase in dendrite formation can be blocked using siRNA to E11. In the mouse ulna loading model, E11 expression is elevated not only in osteocytes near the cell surface, but also in cells embedded deep in the bone matrix[29]. The fact that dendrite formation is an active, not passive process, suggests that E11 may be critical for the generation of dendritic processes not only upon embedding in osteoid but also while the cell is embedded in a mineralized matrix. Therefore, dendrite formation may be essential for normal bone function.

References

1. Doty SB. Morphological evidence of gap junctions between bone cells. Calcif Tissue Int 1981; 33:509-512.
2. Kato Y, Windle JJ, Koop BA, Mundy GR, Bonewald LF. Establishment of an osteocyte-like cell line, MLO-Y4. J Bone Miner Res 1997; 12:2014-2023.
3. Goodenough DA, Paul DL. Beyond the gap: functions of unpaired connexon channels. Nat Rev Mol Cell Biol 2003; 4:285-294.
4. Plotkin LI, Manolagas SC, Bellido T. Transduction of cell survival signals by connexin-43 hemichannels. J Biol Chem 2002; 277:8648-8657.
5. Jiang JX, Cherian PP. Hemichannels formed by connexin-43 play an important role in the release of prostaglandin E(2) by osteocytes in response to mechanical strain. Cell Commun Adhes 2003; 10:259-264.
6. Holmbeck K, Bianco P, Pidoux I, Inoue S, Billinghurst RC, Wu W, Chrysovergis K, Yamada S, Birkedal-Hansen H, Poole AR. The metalloproteinase MT1-MMP is required for normal development and maintenance of osteocyte processes in bone. J Cell Sci 2005; 118(Pt 1):147-156.
7. Zhao W, Byrne MH, Wang Y, Krane SM. Osteocyte and osteoblast apoptosis and excessive bone deposition accompany failure of collagenase cleavage of collagen. J Clin Invest 2000; 106:941-949.
8. Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, Ward JM, Birkedal-Hansen H. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell 1999; 99:81-92.
9. Palumbo C. A three-dimensional ultrastructural study of osteoid-osteocytes in the tibia of chick embryos. Cell Tissue Res 1986; 246:125-131.
10. Palumbo C, Ferretti M, Marotti G. Osteocyte dendrogenesis in static and dynamic bone formation: an ultrastructural study. Anat Rec A Discov Mol Cell Evol Biol 2004; 278:474-480.
11. Knothe Tate ML, Adamson JR, Tami AE, Bauer TW. The osteocyte. Int J Biochem Cell Biol 2004; 36:1-8.
12. Nose K, Saito H, Kuroki T. Isolation of a gene sequence induced later by tumor-promoting 12-O-tetradecanoylphorbol-13-acetate in mouse osteoblastic cells (MC3T3-E1) and expressed constitutively in ras-transformed cells. Cell Growth Differ 1990; 1:511-518.
13. Farr A, Nelson A, Hosier S. Characterization of an antigenic determinant preferentially expressed by type I epithelial cells in the murine thymus. J Histochem Cytochem 1992; 40:651-664.
14. Rishi AK, Joyce-Brady M, Fisher J, Dobbs LG, Floros J, VanderSpek J, Brody JS, Williams MC. Cloning, characterization, and development expression of a rat lung alveolar type I cell gene in embryonic endodermal and neural derivatives. Dev Biol 1995; 167:294-306.
15. Gonzalez RF, Dobbs LG. Purification and analysis of RTI40, a type I alveolar epithelial cell apical membrane protein. Biochem Biophys Acta 1998; 1429:208-216.
16. Boucherot A, Schreiber R, Pavenstadt H, Kunzelmann K. Cloning and expression of the mouse glomerular podoplanin homologue gp38P. Nephrol Dial Transplant 2002; 17:978-984.
17. Dobbs LG, Williams MC, Gonzalez R. Monoclonal antibodies specific to apical surfaces of rat alveolar type I cells bind to surfaces of cultured, but not freshly isolated, type II cells. Biochim Biophys Acta 1988; 970:146-156.
18. Ramirez MI, Millien G, Hinds A, Cao Y, Seldin DC, Williams MC. T1alpha, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolus formation at birth. Dev Biol 2003; 256:61-72.
19. Zimmer G, Oeffner F, Von Messling V, Tschernig T, Groness HJ, Klenk HD, Herrler G. Cloning and characterization of gp36, a human mucin-type glycoprotein preferentially expressed in vascular endothelium.

Biochem J 1999; 341(Pt 2):277-284.
20. Matsui K, Breiteneder-Geleff S, Kerjaschki D. Epitope-specific antibodies to the 43-kD glomerular membrane protein podoplanin cause proteinuria and rapid flattening of podocytes. J Am Soc Nephrol 1998; 9:2013-2026.
21. Scholl FG, Gamallo C, Vilar S, Quintanilla M. Identification of PA2.26 antigen as a novel cell-surface mucin-type glycoprotein that induces plasma membrane extensions and increased motility in keratinocytes. J Cell Sci 1999; 112(Pt 24):4601-4613.
22. Schacht V, Ramirez MI, Hong YK, Hirakawa S, Feng D, Harvey N, Williams M, Dvorak AM, Dvorak HF, Oliver G, Detmar M. T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. Embo J 2003; 22:3546-3556.
23. Mangeat P, Roy C, Martin M. ERM proteins in cell adhesion and membrane dynamics. Trends Cell Biol 1999; 9:187-192.
24. Ohizumi I, Harada N, Taniguchi K, Tsutsumi Y, Nakagawa S, Kaiho S, Mayumi T. Association of CD44 with OTS-8 in tumor vascular endothelial cells. Biochim Biophys Acta 2000; 1497:197-203.
25. Taniguchi K, Harada N, Ohizumi I, Kinoshita M, Tsutsumi Y, Nakagawa S, Kaiho S, Mayumi T. Molecular cloning and characterization of antigens expressed on rat tumor vascular endothelial cells. Int J Cancer 2000; 86:799-805.
26. Wetterwald A, Hoffstetter W, Cecchini MG, Lanske B, Wagner C, Fleisch H, Atkinson M. Characterization and cloning of the E11 antigen, a marker expressed by rat osteoblasts and osteocytes. Bone 1996; 18:125-132.
27. Schulze E, Witt M, Kasper M, Lowik CW, Funk RH. Immunohistochemical investigations on the differentiation marker protein E11 in rat calvaria, calvaria cell culture and the osteoblastic cell line ROS 17/2.8. Histochem Cell Biol 1999; 111:61-69.
28. Sprague L, Wetterwald A., Heinzman U, Atkinson MJ. Phenotypic changes following over-expression of sense or antisense E11 cDNA in ROS 17/2.8 cells. J Bone Miner Res 1996; 11(Suppl.1):S132.
29. Zhang K, Kotha S, Ye L, Dallas M, Feng J, Bonewald LF. The osteocyte-specific antigen, E11, is responsive to mechanical loading *in vitro* and *in vivo* in bone distant from maximal strain. J Bone Miner Res 2004; 19:S72.
30. Cheng B, Kato Y, Zhao S, Luo J, Sprague E, Bonewald LF, Jiang JX. PGE(2) is essential for gap junction-mediated intercellular communication between osteocyte-like MLO-Y4 cells in response to mechanical strain. Endocrinology 2001; 142:3464-3473.