

Introduction

Osteocytes: A proposed multifunctional bone cell

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Abstract

Most cell types are ascribed a single function. The osteoclast holds the unique distinction of performing only one function in the body - that of resorbing bone. The osteoblast has been ascribed the major function of bone matrix production. Other less well-defined cell types include progenitor cells and the nebulous cell type that can support osteoclast formation upon stimulation with various bone resorbing cytokines. Obviously, these cells could have other functions. The definition of an osteocyte is descriptive of its location - cells surrounded by mineralized matrix - not its function. For this year's Sun Valley Workshop on osteocytes, several proposed functions will be presented. First, a general consensus exists that osteocytes are most likely sensitive to mechanotransduction and translate mechanical strain into biochemical signals. Consensus does not exist on the nature of the mechanical strain, the form of the biochemical signals, the target cell(s), or the viability status of the osteocyte. Second, it is also proposed that this cell is incredibly adaptable and expresses plasticity in response to mechanical stimuli. In other words, this cell can readjust its responses to strain in the presence of other bone agents such as hormones and bone factors. Third, it will also be presented that osteocytes maintain systemic mineral homeostasis by regulating mineral release and deposition over the enormous surface area over which these cells interface with the surrounding matrix.

Although osteocytes are terminally differentiated osteoblasts, they appear to have separate and distinct properties from their predecessors. Bone cell biologists loaded with an arsenal of bone anabolic and catabolic factors are examining the expression and effects of these factors on osteocytes. Engineers trained in mathematical modeling have generated new models of strain and connectivity to be tested. The unique morphology of osteocytes suggests that the cytoskeleton in these cells may function differently from osteoblasts and other cell types. Osteocytes may consist of different subpopulations; some that possess receptors for parathyroid hormone (PTH) and others that only express receptors for carboxyl terminal PTH suggesting different functions and responses. Osteocytes may respond rapidly to strain through glutamate receptor-like mechanisms, through calcium influxes, through gap junctions, and less rapidly through the production of small molecules and factors. Strain may take the form of substrate stretching and/or fluid flow. Osteocytes may communicate with other osteocytes and/or bone surface cells such as lining cells, stromal cells, osteoblasts, and/or osteoclasts and their precursors. The viability status of the osteocyte may determine the type of signals sent from these cells. If the cells are deprived of oxygen or nutrients, the apoptotic cells may send signals for initiation of resorption. If the cells and/or their dendritic process are ripped or torn by microdamage, they may send signals of both resorption and formation. If the majority of these theories are correct, then the osteocyte is the 'smart' cell that can direct or orchestrate the bone resorbing and bone forming cells even in its death and dying.

Keywords: Osteocytes, Mechanical Strain, Mechanotransduction, PTH, Mineral Homeostasis, Hypoxia

The definition of the osteoclast and osteoblast is their function. The osteoclast holds the unique distinction of performing only one function in the body - that of resorbing

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bone. The osteoblast has been ascribed the major function of bone matrix production. The definition of an osteocyte is descriptive of its location, not its function. For this year's Sun Valley workshop on osteocytes, several proposed functions will be presented. The generally accepted theoretical function of osteocytes is to respond to mechanical stimuli. However, the nature of the response and the function of the osteocyte in response to mechanical stimuli is controversial.

Dennis Cullinane will be presenting his theory for a novel

function of the osteocyte – that of mineral homeostasis. He proposes that osteocytes are responsible for regulating mineral ion homeostasis in response to factors such as PTH through a form of osteolytic osteolysis and reversal of the mineralized surfaces of their lacunae and canaliculi.

Richard Bringham will show evidence that osteocytes have receptors for the carboxyl portion of PTH (CPTHR)-ten fold more than osteoblasts. He will also present his hypothesis that the PTH1R, also found on osteocytes, will upon binding ligand prevent apoptosis, whereas CPTHR upon binding ligand will induce apoptosis. This apoptosis results in proteolysis that may be responsible for increased size of osteocyte lacunae.

Yuko Mikuni-Takagaki proposes that as cells differentiate from progenitors to mature osteoblasts to osteocytes, they respond to various forms of mechanical stimuli differently in terms of calcium influx. For example, calcium channels in early ST2 cells respond to pulsed ultrasound, whereas mature osteoblasts and osteocytes do not. In contrast, osteocytes respond to stretching through calcium channels whereas early cells do not.

Steve Cowin will focus on fluid transport and its role in mechanotransduction. He also proposes that the time course of mechanotransduction is too rapid for secondary signals such as calcium or the passage of molecules through gap junctions, but ideal for electrical signals. He will also address the paradox that strains applied to whole bone are much smaller than the strains necessary to elicit signaling in cell cultures.

Dan Nicollela will also address this paradox by presenting data based on their hypothesis that cell deformation is responsible for the cellular response to either fluid flow or matrix deformation. Local levels of strain in osteocytes in undamaged bone can be over 5 times that in continuum level strains. Microdamage can cause up to 3.0% strain level and osteocyte deformation *in vitro* in response to fluid flow can be as high as 3.0%. Therefore strain applied to the whole bone is amplified at the cellular level.

Clint Rubin will present data that supports his hypothesis that the osteocyte modifies itself and its microenvironment in response to mechanical stimuli. This local adaptive response allows the osteocyte to accommodate mechanical changes without altering bone architecture. Data will be presented showing that in response to an osteogenic loading regimen, collagen type I, osteopontin, integrin $\beta 3$ and connexin 43 are increased but in response to disuse, proteases such as matrix metalloproteinase 1 are increased. Changing the microenvironment allows the cells to ‘normalize’ to the strain in their environment so that bone is not formed or resorbed.

If the majority of these theories are correct, then the osteocyte is the ‘smart’ cell that can direct or orchestrate the bone resorbing and bone forming cells even in its death and dying. However, to prove that these cells are multifunctional *in vivo*, a number of approaches must be utilized. These include various *in vivo* approaches and the use of isolated

primary cells. In addition to these approaches, a number of investigators are utilizing the osteocyte-like cell line MLO-Y4^{1,2} for their studies. First experiments are usually aimed at determining if these cells mimic or reproduce observations made using primary osteocytes. Then using this cell line, studies are performed that are difficult to perform using primary osteocyte cells. Validation studies are required by returning to either *in vivo* models or primary osteocytes. For example, it has been found that MLO-Y4 cells express a K⁺ current not previously described in osteoblast or osteocyte cells³. It will be important to determine if these channels exist in primary osteocytes or if these channels are artifact. These investigators also did not find voltage operated calcium channels in these cells unless treated with hormones such as PTH, estrogen or glucocorticoid⁴. Isolated primary osteocytes do express these channels, perhaps due to exposure to hormones *in vivo*.

Like primary bone cells, but unlike other known osteoblast cell lines, the MLO-Y4 cells will undergo apoptosis in response to glucocorticoids making this cell useful for studying glucocorticoid induced bone loss⁵. This cell line has been used to represent osteocytes for comparison to osteoblast cells to study responses to implant surfaces⁶ and to examine the non-genotropic effects of estrogen⁷, and as representative of osteocytes to study the effects of oxygen deprivation⁸, for the study of gap junctions^{9,10}, the effects of mechanical manipulation using ‘optical tweezers’¹¹ and as representative of ‘bone’ cells in general to study phosphate signaling¹². Hopefully in the future, new cell lines will be generated to represent various stages of osteocyte differentiation to aid in the identification of osteocyte function. These *in vitro* approaches would be strongly complemented by the use of transgenic animals for the targeting of osteocytes.

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