Current Osteoporosis Reports (2019) 17:195–206
https://doi.org/10.1007/s11914-019-00520-2

OSTEOCYTES (J KLEIN-NULEND, SECTION EDITOR)

Integrins in Osteocyte Biology and Mechanotransduction

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Published online: 27 June 2019

Abstract

Purpose of Review Osteocytes are the main mechanosensitive cells in bone. Integrin-based adhesions have been shown to facilitate mechanotransduction, and therefore play an important role in load-induced bone formation. This review outlines the role of integrins in osteocyte function (cell adhesion, signalling, and mechanotransduction) and possible role in disease.

Recent Findings Both β_1 and β_3 integrins subunits have been shown to be required for osteocyte mechanotransduction. Antagonism of these integrin subunits in osteocytes resulted in impaired responses to fluid shear stress. Various disease states (osteoporosis, osteoarthritis, bone metastases) have been shown to result in altered integrin expression and function.

Summary Osteocyte integrins are required for normal cell function, with dysregulation of integrins seen in disease. Understanding the mechanism of faulty integrins in disease may aid in the creation of novel therapeutic approaches.

Keywords Osteocyte · Integrin · Mechanotransduction · Osteoporosis · Osteoarthritis · Bone metastasis

Introduction

Bone is a metabolically active organ that undergoes continuous remodelling due to the synchronised action of osteoblasts, osteoclasts and osteocytes. Osteoblasts and osteoclasts deposit and resorb bone mineral respectively. Osteocytes produce specific proteins that exert many systemic and local effects on bone physiology, most notably receptor activator of nuclear factor-κB ligand (RANKL), osteoprotegerin (OPG) and sclerostin have a direct effect on bone remodelling. RANKL is a factor that binds to the RANK receptor on osteoclasts thereby promoting osteoclastogenesis and bone resorption [1•, 2]. OPG is a decoy receptor for RANK that can bind to RANKL and antagonise its effects on osteoclasts [1•]. Sclerostin is an antagonist of the canonical WNT signalling pathway, a major driver of bone formation by osteoblasts [3–5]. This paracrine regulation by osteocytes of other cell types within the bone niche allows for the maintenance of a fine balance in bone remodelling. Excessive bone resorption and deposition are hallmarks of conditions such as osteoporosis or osteosclerosis respectively [6, 7].

Mechanical loading is a potent regulator of bone remodelling, whereby reduced physical activity and ensuing cellular-level mechanical stimuli activate bone resorption, whereas they conversely increases in mechanical stimulation result in net bone deposition [6, 8]. The mechanical stimuli are transmitted from the organ level and result in matrix and cellular strains or fluid shear stress that directly stimulates the osteocyte cell membrane [9–11]. These mechanical stimuli are transduced into biochemical signals with the aid of specialised structures known as mechanosensors [12, 13]. One such mechanosensor, integrins, allow for the transduction of force from the ECM to the cell [13, 14]. The role of integrins in mechanotransduction has been studied in many cell types but only recently has the focus turned to the role of integrins in osteocyte mechanobiology [15••, 16••, 17, 18••]. In this review, we will outline the role of osteocytes in bone

This article is part of the Topical Collection on *Osteocytes*

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physiology, discuss the importance of integrins in cell function, describe the mechanosensory role of integrins in osteocytes and highlight recent studies that have shown that integrin spatial distribution and function are altered in bone tissue under certain pathological conditions.

The Role of Osteocytes in Bone

Osteocytes are the most numerous bone cells in adult bone (90–95%) and are the most long-lived of all the bone cells, some of which survive for decades [19•, 20]. A recent study of osteocyte lacunar density from ribs, femurs and radii of human cadaveric origin measured a range of 500–1300 osteocyte lacunae per mm^2 of bone in the cortex [21].

Osteocytes develop during the formation and maturation of new bone, through differentiation of osteoblasts, by means of a process that involves rearrangement of the cytoskeleton, intracellular machinery and a loss of the apical and basolateral plasma membrane polarisation found in osteoblasts, before becoming embedded within mineralised tissue of bone [22, 23]. Osteocytes are biologically distinct from osteoblasts and can be distinguished through phenotypic markers (dentin matrix protein 1, E11, phosphate-regulating neutral endopeptidase on chromosome X, matrix extracellular phosphoglycoprotein, Dickkopf-related protein 1, sclerostin, fibroblast growth factor 23), but also produce OPG, and RANKL in common with osteoblasts [22, 24, 25]. Osteocytes regulate mineral in local bone matrix by perilacunar remodelling or micropetrosis activated during apoptosis.

Osteocytes are complex cells with chemosensory [5, 19•, 26] and endocrine functions [1•, 27, 28] but are primarily regarded to be the master coordinator of loading-induced bone formation [1•, 5, 22, 26, 29, 30•]. Bone cell mechanobiology has been widely studied in vitro, using various experimental approaches, but fluid shear stress is the most well studied and elicits the most robust response in vitro [15••, 16••, 18••, 31, 32•, 33, 34•, 35–40]. Osteocytes have also been shown to respond to the mechanical properties of their substrate (ECM) [41, 42]. It has been shown that osteoblasts cultured on softer substrates differentiated into osteocyte-like cells, as seen by an osteocytic dendritic phenotype and a downregulation in osteoblastic gene markers [42]. Osteocytes respond to such mechanical stimuli by initiating intracellular signalling and producing biochemicals and proteins that activate osteoblasts and osteoclasts to remodel bone [43–46]. In particular, WNT/β-catenin signalling regulates bone formation by osteoblasts and stimulates production of biochemicals (RANKL, OPG) to inhibit resorption. Sclerostin is a protein produced by osteocytes [43, 44, 47–50], which inhibits WNT/β-catenin signalling and bone formation but promotes resorption. Mechanical loading decreases sclerostin production by osteocytes [4, 44, 46, 51], enabling bone formation, but this response is limited to certain areas of the bone [52]. Oestrogen enhances the osteogenic response of osteoblasts and osteocytes to mechanical stress [33, 34•, 53–60] and protects against cell death by apoptosis [56, 58].

Osteocytes have a large cell body with multiple cell processes and are found embedded in mineralised bone [61]. These cell processes join neighbouring osteocytes together at gap junctions and form a function syncytium as well as interacting with surface osteoblasts, vasculature and possibly even bone marrow [20, 27, 62]. Moreover, osteocyte cell processes are surrounded by a pericellular matrix (or glycocalyx) which tethers the cell processes to ECM projections (collagen hillocks) found along the bone mineral and allows for amplification of strain applied on a whole bone level to strains required to produce a mechanobiological response, as seen in cell culture experiments [63••, 64••, 65, 66••]. Several mechanosensors have been shown to be important in osteocyte mechanotransduction including the primary cilium [35, 36, 67], gap junctions [37], stretch-activated ion channel, TRPV4 [38], plasma membrane disruptions [39], PTH1R [40], the glycocalyx [31, 68, 69] and in particular, integrins [15••, 16••, 18••]. Computational investigations of the osteocyte mechanical environment confirmed that the cell processes and in particular, the ECM projections where integrin binding sites are found displayed the highest degree of stimuli, and were within the range required to produce a mechanobiological response in vitro [10••].

Integrins

Integrins are a family of heterodimeric transmembrane proteins comprised of α and β subunits [70•], which bind intracellular proteins of the internal cytoskeleton to extracellular matrix proteins (ligands) [71] but also facilitate interaction with other cells and act as signalling receptors [71–74]. Integrin-based focal adhesions occur between the cell body and the underlying matrix, and lamellipodia and filopodia protrusions of the cell membrane and cytoskeleton also attach to the extracellular matrix by means of such adhesions. Integrins form focal adhesions, in combination with intracellular proteins such as vinculin, α-actinin, talin and paxillin, and thereby connect the internal cytoskeleton to the extracellular matrix [70•, 75]. The formation of focal adhesions begins with the activation of integrins from a low-affinity state to a high-affinity state [76•]. Next, two adapter proteins, talin and kindlin bind to the β integrin subunit [76•, 77]. This activation leads to an aggregation of integrins and associated proteins leading to the formation of a nascent adhesion, visible under the microscope [76•]. A number of stable nascent adhesions aggregate to form a focal complex, which over time aggregates further and mature to form focal adhesions [77]. Other focal adhesion proteins such as vinculin and paxillin are recruited during this maturation process and aid in the

stabilisation of new focal adhesion [76•, 78•]. Assembly and disassembly of focal adhesions occur frequently and is a vital mechanism for cell migration [79].

Integrins work in concert with the cytoskeleton to [1•] perceive external mechanical stimuli, [2] facilitate movement by cells, [3] generate tension on their extracellular environment and [4] activate intracellular signalling pathways and elicit biochemical responses [14, 72, 73, 80, 81]. Integrins and their ligands (major constituents of the ECM including collagen, vitronectin, fibronectin, fibrinogen, laminin, etc.) act together to mediate cell-matrix interactions and sense mechanical stimuli for many cells of the body by activating intracellular signalling pathways and eliciting a biochemical response [13, 14, 82]. Together with intracellular myosin motors, cells exert contractile forces on the matrix through these adhesions to facilitate cell movement [83, 84] and to explore their environment [85, 86]. The stiffness of the underlying substrate dictates how much force a cell exerts and regulates formation of integrin adhesions [87, 88] and cytoskeletal tension [89], which in turn initiate cytoskeleton adaptation and regulate cell behaviour. "Durotaxis" refers to cell behaviour whereby cells migrate toward areas of higher stiffness, which is enabled by focal adhesion traction [88]. Mechanoresponsiveness and matrix attachment in many cell types is mediated by integrins [72–74], and these attachments are crucial for cell survival and proliferation.

Integrins in Bone

Integrins are ubiquitous in bone. In particular, osteoclasts attach to the bone matrix by means of integrins $\alpha_v\beta_1$, $\alpha_2\beta_1$ and $\alpha_v\beta_3$ during bone resorption [90, 91]. $\alpha_\upsilon\beta_3$ integrin is strongly linked to osteoclastogenesis and osteoclastic resorptive activity, as $\alpha_\upsilon\beta_3$ forms the sealing zone for resorption pit formation [92]. Osteoblasts and osteocytes express β_1 integrin subunits in conjunction with α_1, α_2, α_3, α_4 and α_5 integrin subunits [93–95]. β_3 integrins are associated with α_v in osteoblasts [96] and osteocytes [15••]. By immunohistochemistry, it was shown that osteocyte cell processes have distinct clusters of integrin $\alpha_v\beta_3$ in vivo and it has been proposed that these attach to the extracellular matrix (ECM) to facilitate mechanosensation [63••, 64••].

$\alpha_v\beta_3$ integrins are more numerous in osteocyte cell processes in comparison to the cell body, whereas β_1 integrins have been found along osteocyte cell bodies where they interact with a loose pericellular matrix [63••]. This difference in integrin location indicate that they are highly specialised structures and therefore may play different roles in bone biology [63••]. Interestingly, along cell processes, β_3 integrins have been found to co-localise with specialised structures containing pannexin1, P2X7R, and CaV3.2–1 [97••]. Larger focal adhesion proteins such as vinculin and paxillin have been found around the cell body and not the tightly packed cell processes. *Cabahug-Zuckerman* et al. proposed that the lack of focal adhesion proteins in osteocyte cell processes was due to space constraints [97••], as the cytoplasmic space between the osteocyte process membrane and the actin filaments found within was much smaller (< 20 nm) than the cytoplasmic depth (> 40 nm) taken up by the array of adaptor proteins seen at typical focal adhesion sites [98]. The β_3 containing foci seen in vivo are distinctly different from typical focal adhesions seen in vitro, and as such, *Cabahug-Zuckerman* et al. propose that they may result in altered downstream signalling [97••]. However, this has not been investigated in vitro.

Integrin-Based Signalling

In addition to playing a structural role in the cell, focal adhesions are responsible for transducing signalling pathways including focal adhesion kinase (FAK)) and Shc signalling [99•, 100]. FAK is a protein tyrosine kinase ubiquitously expressed in focal adhesions and composed of a central kinase domain, an N-terminal FERM domain and a C-terminal domain that includes the focal adhesion-targeting (FAT) sequence [100, 101]. Prior to integrin-mediated cell adhesion, FAK is in its inactive, closed conformation [101]. However, upon integrin-mediated cell adhesion, the FERM domain of FAK is displaced by an activation protein, such as the β cytoplasmic tail of an integrin, and allows for FAK autophosphorylation at the tyrosine phosphorylation site, Y397, and phosphorylation at other sites, which results in complete FAK activation [101]. Activation of FAK is necessary for a multitude of cell functions, including cell migration, spreading and adhesion [100].

FAK signalling has been shown to play a role in osteogenic differentiation of mesenchymal stem cells (MSCs) [102] and in osteocytes preventing dexamethasone-induced apoptosis [103]. Interestingly, in osteoblasts, FAK signalling (via β_1 integrins) has been shown to be necessary for RANKL expression [104]. In ovarian cancer cells, FAK has been shown to be required for OPG expression [105], a well-known paracrine factor involved in bone remodelling, but the role of FAK in OPG expression in bone cells remains to be studied. Activation of FAK signalling in response to mechanical stimulation occurs in many cell types including osteoblasts [99•, 106•]. Shc is an adaptor protein found to be important in many signalling events, including integrin-based signalling [107]. Shc isoforms contain two distinct domains, which allow for the binding of phosphotyrosine containing sequences and a central region which contains tyrosine phosphorylation sites, with this phosphorylation site known to be activated at many cell surface receptor sites [107]. The anti-apoptotic activity of the oestrogen receptor and androgen receptor was shown to be mediated by the Src/Shc/ERK pathway in osteocytes [108]. In osteoblasts, β_1 and $\alpha_v\beta_3$ integrins co-localise with Shc, in response to fluid shear stress [109]. Given the fact that Shc

is involved in signalling events from so many receptors, it might be difficult to determine an exact role of integrin-based focal adhesions in these processes.

While the FAK pathway and the Shc pathway are independent pathways activated by integrin-based focal adhesion sites, they have been known to be synergistically activated and ultimately lead to the activation of other pathways, such as the PI3K/Akt/mTOR pathway and MAPK pathways [99•, 107, 110, 111]. The interconnectedness of these pathways is most apparent in a study where FAK was shown to be necessary for the flow-induced PGE_2 response in osteoblasts in vitro, but deletion of FAK in a mouse model resulted in no change in mechanically induced bone loading, possibly due to a compensatory mechanism involving another of the aforementioned pathways [106•]. Delineating the roles of this intricate network of pathways may aid in a greater understanding of osteocyte biology, especially in disease states.

Integrin Mechanotransduction

While many focal adhesion proteins are known to play mechanosensory roles [112, 113], integrins are thought to be of particular importance in osteocyte mechanotransduction [15••, 16••, 18••]. The role of β_1 and β_3 integrins has been a recent focus in osteocyte mechanotransduction. Such studies have utilised the MLO-Y4 cell line, which is isolated from transgenic murine long bones and exhibits certain behaviours of osteocytes (low alkaline phosphatase expression, high expression of connexin 43 and the antigen, E11, low levels of DMP1 and a dendritic phenotype [19•, 114•].

MLO-Y4 cells were transfected with a dominant negative form of β_1 integrin and subjected to oscillatory fluid flow using a parallel plate flow chamber [18••]. There was no difference in Ca^{2+} signalling between the control and β_1 negative cells, but an abrogated response in *Cox-2* and PGE_2 expression was seen following fluid flow, compared to controls. Actin organisation was unaffected in β_1-negative cells, but a reduction in vinculin co-localisation to focal adhesions was seen. Interestingly, in a separate study, it was reported that cyclic stretching of MLO-Y4 cells led to ERK activation, via β_1 integrins, actin and tubulin cytoskeletal proteins, and Src kinases, which were likely assembled in small invaginations in the plasma membrane known as caveolae [115]. An in vivo study of mice with a conditional knockout of cortical osteocyte β_1 integrins showed an altered cell morphology with fewer β_1 integrins present along the cell body and a reduction in visible cell processes [17]. The mice were subjected to 3 days of cyclic axial ulnar loading, and this resulted in a reduced mineralised bone formation rate in the ulna in the mice with β_1 integrin conditional knockout, compared to controls. These results indicate the importance of osteocyte β_1 integrins in mechanically induced bone formation in vivo. β_1 integrins, in the form of $\alpha_5\beta_1$, have been implicated in gap junction function in osteocytes, whereby fluid shear stress led to $\alpha_5\beta_1$ activation and the opening of connexin 43 hemichannels [116].

β_3 integrins have also been shown to play an important role in osteocyte mechanotransduction. The application of different fluid flow stimuli (e.g., oscillatory) on MLO-Y4 cells was shown to produce a Ca^{2+} response or *Cox-2* and PGE_2 responses respectively (Fig. 1a, c) [15••, 16••]. When the integrin $\alpha_v\beta_3$ was antagonised with a small molecule inhibitor, it led to perturbed flow responses (Fig. 1b, c). Given the localisation of integrin $\alpha_v\beta_3$ along osteocyte cell processes in vivo [63••], particular focus was given to osteocyte cell processes. *Thi* et al. showed that the Ca^{2+} response was highly polarised along the cell processes, with stimulation of the cell processes leading to a higher Ca^{2+} response than stimulation of the cell body (Fig. 1a) [15••]. *Haugh* et al. showed that $\alpha_v\beta_3$ antagonism led to an altered cell morphology, with a smaller cell area and fewer cell processes (Fig. 1d) [16••]. In primary osteocytes, $\alpha_v\beta_3$ integrin activation by mechanical stimulation was shown to lead to an upregulation in c-fos, IGF-1 and Cox-2 [117••], demonstrating a potent role for this integrin in osteocyte mechanobiology.

As mentioned previously, osteocyte cell processes are surrounded by a pericellular matrix (or glycocalyx) in vivo which has been proposed to amplify the whole bone level strains [63••, 64••, 65, 66••]. Immunocytochemistry revealed that hyaluronic acid was a major component of the MLO-Y4 glycocalyx [31]. Degradation of the glycocalyx with hyaluronidase resulted in an abrogated PGE_2 release in response to fluid shear stress, indicating the importance of the glycocalyx for normal MLO-Y4 mechanoresponses [31]. Of relevance here, glycocalyx degradation has also been shown to lead to poor integrin attachment and a diminished ability to open cell body hemichannels [68]. Perlecan was also found to be a key constituent of the pericellular matrix [69]. In perlecan-deficient mice, the density of the pericellular matrix fibres was lower and this, in turn, resulted in a lack of anabolic bone formation in response to uniaxial compressive tibial loading in vivo, compared to control mice [69]. Interestingly, a decrease in pericellular matrix fibre density was seen in aged mice (12–13 months), in comparison to young mice (4–5 months), indicating a possible source of decreased mechanosensitivity in ageing [69].

β_1 and β_3 activation in osteoblasts has been more extensively studied compared to osteocytes, and it has been shown that the application of fluid shear stress activates FAK and Shc, resulting in the activation of PI3-K and Akt/mTOR/p70S6K pathways [99•]. While FAK, PI3-K, and Akt have been shown to be activated in response to fluid shear stress in osteocytes, it is unknown whether this is mediated by β_1 or β_3 integrins [118]. Deletion of specific integrin subunits (β_1 or β_3) has led to an attenuated mechanosensitivity in osteocytes in vitro [15••, 16••, 18••] but does not lead to a complete loss of mechanosensation in vivo [17]. One hypothesis for this

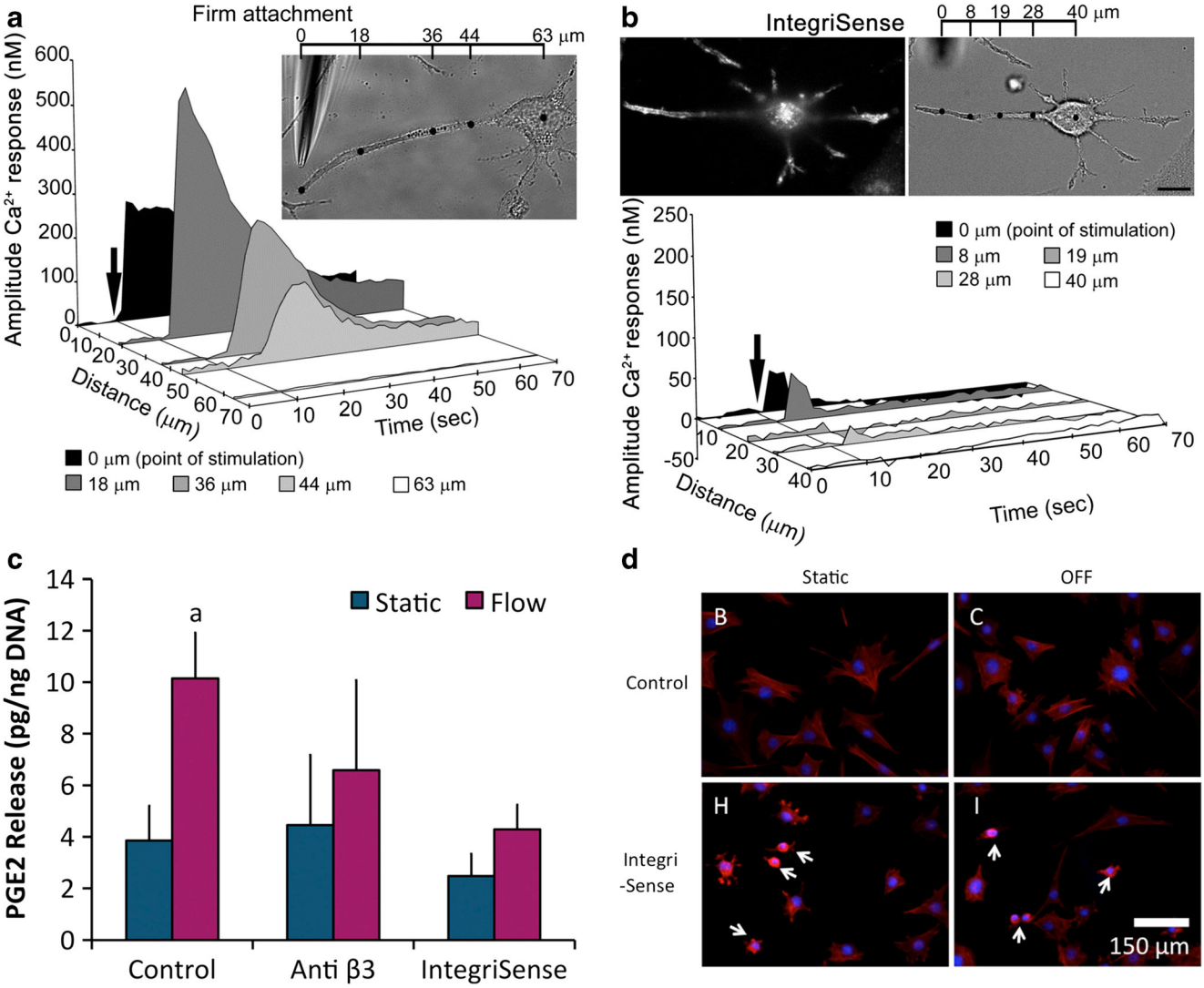

Fig. 1 The role of β_3 integrin in osteocyte mechanotransduction. **a** Ca^{2+} responses in MLO-Y4 cells to a Stokesian flow stimulus is highly polarised along the cell processes (adapted from Thi et al. [15]), with permission from PNAS. **b** Antagonism of the integrin $\alpha_v\beta_3$ with a small-molecule inhibitor, IntegriSense, leads to a disruption in this Ca^{2+} response (adapted from Thi et al. [15]) with permission from PNAS. **c** Oscillatory fluid flow leads to an increase PGE_2 response, with antagonism of the integrin $\alpha_v\beta_3$ resulting in a perturbed PGE_2 response to flow (adapted from Haugh et al. [16]). **d** Antagonism of the integrin $\alpha_v\beta_3$ led to an altered osteocyte cell morphology (adapted from Haugh et al. [16]) with permission from Elsevier

is that in response to the loss of a specific integrin subunit in vivo, alternative integrin subunits compensate for the loss of function [119].

Integrins in Disease

Given the importance of integrins in osteocyte mechanotransduction, recent studies have focused on the effect of various disease states on integrin function, most notably with respect to post-menopausal osteoporosis, osteoarthritis and bone metastases.

Post-menopausal osteoporosis is a disease characterised by lower circulating oestrogen levels and an imbalance in bone remodelling, which leads to bone loss and ultimately an increased likelihood of fracture [120]. Given the potent effects of RANKL and sclerostin on bone remodelling, anti-RANKL [121] and anti-sclerostin [122]-based immunotherapies have been investigated for their potential therapeutic roles in post-menopausal osteoporosis. It has been shown that the α_2 integrin, as part of a $\alpha_2\beta_1$ heterodimer, was downregulated, unlike other β_1 heterodimers ($\alpha_1\beta_1$ and $\alpha_{11}\beta_1$), in osteoporotic versus aged human patients [123]. In an oestrogen-deficient ovariectomised rat model of post-menopausal osteoporosis, it was reported that

there was a reduction in β_3 integrin-positive cells in cortical bone compared to controls, but there was no change in the amount of β_1 integrin-positive cells [124••]. Interestingly, in vitro investigations of MLO-Y4 cells following oestrogen withdrawal had an attenuated mechanosensitivity [34•] and in a separate study, oestrogen deficiency was associated with a higher incidence of osteocyte apoptosis, when compared to oestrogen-treated cells [125]. Immunocytochemistry staining of MLO-Y4 cells that had undergone an oestrogen withdrawal culture regime, revealed a lower $\alpha_v\beta_3$ intensity at the cell level and at focal adhesion sites, along with a disrupted focal adhesion assembly [33]. MLO-Y4 cells that underwent oestrogen withdrawal also had a higher *Rankl*/*Opg* gene ratio and an abrogated *Cox-2* response to flow, when compared to oestrogen-treated cells. To understand whether the integrin $\alpha_v\beta_3$ was responsible for these changes in gene expression, the integrin $\alpha_v\beta_3$ was blocked using an antagonist against $\alpha_v\beta_3$, IntegriSense 750. Interestingly, the $\alpha_v\beta_3$ blocked MLO-Y4 cells also exhibited disrupted focal adhesion assembly, an abrogated *Cox-2* response to flow and there was no change in *Rankl*/*Opg* ratio between oestrogen and oestrogen withdrawal cells, under static conditions, indicating the importance of $\alpha_v\beta_3$ for *Rankl*/*Opg* ratio. Whether other integrin subunits or other mechanosensors in osteocytes are also affected by oestrogen withdrawal remains to be elucidated.

Osteoarthritis (OA) is a common degenerative joint disease which affects both cartilage and bone, as seen by progressive loss of the articular cartilage, thickening of the subchondral bone, formation of osteophytes and inflammation of the synovium [126]. In a rat model of OA, induced by partial menisectomy of the right back leg, increases in α_4, α_5 and α_2 integrin expression was measured in cartilage over time, along with changes in proteoglycan and fibronectin content, possibly caused by increased matrix metalloproteinases (MMP) activity [127]. Crosstalk has been shown to occur between the subchondral bone and cartilage, whereby conditioned media collected from cyclically compressed primary mouse osteoblasts/osteocytes induced the release of MMPs in primary mouse chondrocytes [128]. In the subchondral bone of OA patients, osteocyte morphology was altered, with a rounded and rough cell body and fewer and disorganised dendrites, compared to controls [129]. MLO-Y4 cells cultured on ECM derived from OA patients showed a lower β_1 expression and deactivated FAK signalling, compared to cells cultured on healthy ECM [130••]. Different ECM mechanical properties, such as stiffness, have been shown previously to alter osteocyte behaviour [41, 42]. Elucidating the exact mechanism and timeline by which changes in osteocytes in subchondral bone lead to OA phenotype in cartilage, and the possible role of integrins in this process, may prove useful for the future of OA research.

Bone is a common site of metastases, with the prostate and breast cancer responsible for up to 70% of bone metastases [131]. The relationship between bone cells and invading tumour cells has been dubbed a "vicious cycle" [132, 133]. The first step in this vicious cycle of bone metastases is when invading tumour cells begin expressing integrins, which facilitate tumour invasion, metastasis and angiogenesis. In breast cancer, tumour cells are directed towards bone by chemokines expressed by osteoblasts (CXCL12/SDF-1) [134]. Osteoblasts are also stimulated by tumour cells to produce RANKL, to allow for osteoclast-mediated bone resorption to facilitate for a site to tumour migration and growth factors to stimulate tumour growth and activate osteoclasts, further contributing to the development of a metastatic lesion [135]. Bone matrix has been proposed to provide a favourable environment for bone metastases [132, 133]. A human 3D ex vivo model of prostate cancer co-cultured with primary osteocytes demonstrated a compromised tissue morphology, and rounded osteocyte cells with fewer dendrites, compared to osteocytes cultured alone in the 3D model [136•]. The 3D prostate cancer cell-osteocyte co-culture also showed altered WNT signalling (sclerostin and DKK1) gene and protein expression and higher ALP gene and protein expression in the osteocytes, compared to the 3D osteocyte monoculture. Interestingly, no difference was seen in these responses between the monoculture and co-culture experiments in a 2D environment. Integrins have been implicated in tumour invasion, metastasis and angiogenesis in many cell types [137, 138]. Osteoclast-mediated invasion into bone tissue is an important step in bone metastasis, and the integrin $\alpha_v\beta_3$ plays a key role in this process [139]. Recent investigations into the role of osteocytes in breast cancer metastasis have proven enlightening. Firstly, conditioned media collected from MLO-Y4 cells were shown to promote MDA-MB231 cell migration and proliferation [140]. Next, it was shown that MLO-Y4 cells cultured with conditioned media from mechanically loaded MDA-MB231 breast cancer cells exhibited a greater number of osteocyte cell processes, when compared to osteocytic cells cultured with conditioned media from unloaded MDA-MB231 breast cancer cells [141•]. These changes were comparable to those seen when MLO-Y4 cells were directly mechanically stimulated. Interestingly, the MLO-Y4 cells cultured with conditioned media from mechanically loaded MDA-MB231 breast cancer cells showed a higher *Rankl*/*Opg* gene ratio, compared to the conditioned media from unloaded MDA-MB231 breast cancer cells. This shows that factors released from breast cancer cells are affecting the number of cell processes, where the mechanosensitive integrin $\alpha_v\beta_3$ is found, and resulting in increased osteoclastogenic paracrine signalling. However, it is not yet known how this affects the expression of $\alpha_v\beta_3$ integrins and the role they play in cancer metastases.

Future Directions

Integrins play an important role in osteocyte biology, as is evident from recent in vitro studies of osteocyte-like cells in 2D culture and animal studies. But the reliance on 2D

in vitro models has failed to account for essential mechanical cues from the matrix and daily loading, due to the loss of native morphology and interactions with the surrounding matrix [142], which is particularly relevant in the case of osteocytes whose biological activity are governed by 3D interactions with the surrounding bone matrix and the mechanical environment. Animal models do not fully embody human biology. In the future, 3D cell culture techniques may enable studies that will improve our understanding of osteocyte biology. While the role of integrins in MLO-Y4 cells has been well studied, integrin function in other osteocyte cell lines, such as MLO-A5 cells [143], IDG-SW3 cells [144], C59 cells [145] and Ocy454 cells [49], has not been studied. These osteocyte cell lines capture osteocyte function at different stages of osteocyte maturity, and as such, understanding integrin function in these cell lines will help illuminate osteocyte integrin function in a more complete manner. Moreover, further investigation of the integrin-mediated pathways activated following mechanical stimulation of osteocytes, such as FAK, Shc and other downstream pathways, is necessary and in particular to investigate whether these pathways are implicated in disease states. Given the effect of integrin-based therapeutics in inflammatory bowel disease [146], multiple sclerosis [146] and other diseases, such investigations may form the basis for the creation of the next generation of integrin-based therapeutics for bone.

Conclusion

Recent studies have identified the importance of integrins, in particular, β_1 and β_3 integrins, in osteocyte mechanotransduction. Antagonism of these integrin subunits have been shown to alter osteocyte morphology and result in impaired osteocyte paracrine signalling, particularly *Rankl* and *Opg* expression. The oestrogen deprived conditions of osteoporosis have been shown to alter integrin distribution and function in osteocytes. The altered mechanical environment of osteoarthritis results in lowered integrin expression and impaired downstream signalling. Invasion of metastatic cancer cells in bone alters osteocyte phenotype and may affect integrin-signalling. Taken together, the effect of these disease states on integrin function highlights the importance of integrins in normal osteocyte function and suggests that integrin-based therapeutics could be an exciting area of research.

Funding information This publication has emanated from research conducted with the financial support of Science Foundation Ireland (SFI) and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073 and Grant Number 14/IA/2884. The authors received funding from European Research Council (ERC) Starting Grants (336882 and 258992) and Science Foundation Ireland (SFI) Grants 13/ERC/L2864 and 12/RC/2278.

Compliance with Ethical Standards

Conflict of Interest Ivor P. Geoghegan, David A. Hoey and Laoise M. McNamara declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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