Life Sciences 145 (2016) 152–160

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Melatonin, bone regulation and the ubiquitin-proteasome connection: A review

Jerry Vriend [a,*], Russel J. Reiter [b]

[a] Department of Human Anatomy and Cell Science, University of Manitoba, 745 Bannatyne Avenue, Winnipeg, Manitoba R3E 0W3, Canada
[b] Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 2 December 2015
Accepted 14 December 2015
Available online 17 December 2015

Chemical compounds studied in this article:
Melatonin (PubChem CID: 896)
Bortezomib (PubChem CID: 387447)
Lactacystin (PubChem CID: 6610292)

Keywords:
Osteoblasts
Osteoclasts
Parathyroid hormone
Rankl
$SCF^{B\text{-}TrCP}$
Bortezomib

ABSTRACT

Recently, investigators have shown that ubiquitin-proteasome-mediated protein degradation is critical in regulating the balance between bone formation and bone resorption. The major signal transduction pathways regulating bone formation are the RANK/NF-κB pathway and the Wnt/β-catenin pathway. These signal transduction pathways regulate the activity of mature osteoblasts and osteoclasts. In addition, the Wnt/β-catenin pathway is one of the major signaling pathways in the differentiation of osteoblasts. The ubiquitin ligases that are reported to be of major significance in regulating these pathways are the ubiquitin $SCF^{B\text{-}TrCP}$ ligase (which regulates activation of NF-κB via degradation of IkBα in osteoclasts, and regulates bone transcription factors via degradation of β-catenin), the Keap–Cul3–Rbx1 ligase (which regulates degradation of IkB kinase, Nrf2, and the antiapoptotic factor Bcl-2), and Smurf1. Also of significance in regulating osteoclastogenesis is the deubiquitinase, CYLD (cylindramatosis protein), which facilitates the separation of NF-κB from IkBα. The degradation of CYLD is also under the regulation of $SCF^{B\text{-}TrCP}$. Proteasome inhibitors influence the activity of mature osteoblasts and osteoclasts, but also modulate the differentiation of precursor cells into osteoblasts. Preclinical studies show that melatonin also influences bone metabolism by stimulating bone growth and inhibiting osteoclast activity. These actions of melatonin could be interpreted as being mediated by the ubiquitin ligases $SCF^{B\text{-}TrCP}$ and Keap–Cul3–Rbx, or as an inhibitory effect on proteasomes. Clinical trials of the use of melatonin in the treatment of bone disease, including multiple myeloma, using both continuous and intermittent modes of administration, are warranted.

Contents

* Corresponding author.
E-mail address: Vriend@ms.umanitoba.ca (J. Vriend).

http://dx.doi.org/10.1016/j.lfs.2015.12.031
0024-3205/

1. Introduction

The ubiquitin-proteasome system provides a mechanism for regulating proteins that control osteoblasts and osteoclasts [103]. The ability of the ubiquitin-proteasome system to degrade signaling proteins in a substrate specific manner is essential in maintaining a balance between bone formation and bone resorption, allowing for bone remodeling, and preventing bone disease. The ubiquitin-proteasome system also regulates cellular concentrations of signal transduction factors that are required for differentiation of osteoprogenitor cells [79].

A number of drugs are available to stimulate bone formation and reduce the risk of fractures. These include parathyroid hormone, estrogen, calcium, vitamin D, bisphosphonates, and denosumab (Prolia) [76,101]. These agents act by promoting bone formation more than bone resorption, or by inhibiting bone resorption alone [101]. In some clinical conditions, proteasome inhibitors such as bortezomib can be used to stimulate bone formation or to prevent excessive bone resorption [103]. The proteasome inhibitor, lactacystin, is reported to enhance osteoblast differentiation [48].

The indole melatonin, a well known secretory product of the mammalian pineal gland [114] influences a vast number of cellular proteins involved in a variety of physiological processes [38,98,99] including bone formation [58,69,74,108]. Considering the fact that the degradation of most cellular proteins are regulated by the ubiquitin-proteasome system [34,135], it can be fairly assumed (but not generally recognized) that many of the proteins influenced by melatonin, are likely regulated through their degradation by the ubiquitin-proteasome system. This assumption is supported by the number of signal transduction factors that are similarly influenced by proteasome inhibitors and by melatonin [129].

In the current manuscript, we discuss the major proteins that modulate osteoblast and osteoclast activities in terms of their regulation by the ubiquitin-proteasome system and we review the literature related to the actions of melatonin on bone. We review the similarities between the function of melatonin and the action of proteasome inhibitors on bone formation and suggest a model in which melatonin interacts with the ubiquitin-proteasome system to contribute to bone regulation. In this model, we examine the specific ubiquitin E3 ligases controlling transcription and those controlling degradation of proteins that regulate differentiation of osteoblasts and osteoclasts and those controlling bone turnover. This model should be useful in testing whether targeting ubiquitin ligases is clinically useful and whether melatonin administration would be clinically effective in treating bone diseases resulting from an imbalance of osteoblast and osteoclast activity. Cardinali et al. [16] have suggested a relationship between age-related bone loss and age-related decline in circulating levels of melatonin.

Bortezomib, the first proteasome inhibitor to be approved for clinical use by the US Food and Drug Administration in 2004, is used in the treatment of multiple myeloma (MM). MM is often associated with destructive bone disease due to increased bone resorption [24]. Bortezomib treatment reduces tumor mass, but it also lowers bone resorption and increases bone mineral density in MM patients [78]. The proteasome inhibitor lactacystin also has anti-myeloma activity [138]. Herein, we also provide a rationale (based on preclinical data) for testing the effects of melatonin on bone resorption and bone mineral density in MM patients.

2. Differentiation of mesenchymal stem cells into osteoblasts: role of ubiquitin ligases

Uyama et al. [124] concluded that the proteasome plays a key role in osteoblast differentiation by controlling the degradation of transcription factors. Table 1 lists the ubiquitin ligases which influence proteins involved in differentiation and turnover of bone. The main factors controlling osteoblast differentiation from mesenchymal stem cells are the Wnt/β-catenin pathway [33], the transcription factor Runx2 [86], the transcription factor ATF4 (activating transcription factor 4) [26], BMP-2 (bone morphogenetic protein-2) and the transcription factor JunB [149]. While several signal transduction proteins are required for osteoblast differentiation, the Wnt/β-catenin pathway is of particular significance in osteoblast differentiation and bone formation [6,45,102] (Fig. 1). Indeed, this pathway has been referred to as the master regulator of osteogenesis [33]. Interference with the co-receptor for Wnt, LRP5, results in very low bone mass, whereas an increase of LRP5 culminates in a high bone mass [7]. Paradoxically, one of the targets of β-catenin as a transcriptional factor is osteoprotegerin (OPG), a natural decoy receptor for RANKL [12,32]. Thus, by stimulating production of OPG, β-catenin signaling can also inhibit osteoclast activity indirectly. Members of the WNT/β-catenin pathway have been reported to be regulated by melatonin and by the photoperiod in rats [40].

Table 1
Substrates of ubiquitin ligases that influence bone metabolism.

Ubiquitin ligase	Substrates	Function	References
B-TrCP	ATF4	Differentiation factor	[142]
	B-catenin	Differentiation factor osteoblast differentiation; stimulates OPG	[12]; [88]; [96]
	IkBα	Inhibitor of NF-κB	[19]; [52]
	PER	Circadian clock protein	[115]
	Gli2	Transcription factor; mediates BMP2 expression	[89]; [9]
	Smad4	Transcription factor regulated by BMP	[132]
	CYLD	TRAF6 inhibitor	[137]
	NRF2	Transcription factor for antioxidant enzymes	[21]
	GHR	Growth hormone receptor	[125]
Keap1–Cul3–Rbx1	IKK	IkB kinase	[64]; [120]
	Nrf2	Transcription factor for antioxidants	[120]
	Bcl-2	Antiapoptotic factor	[120]
TRAF6	IKK kinase	Activate kinase to phosphorylate IkBα	[137]
Fbxl12	P57	Cell cycle cdk inhibitor	[56]
Smurf1	Smad1	Transcription factor	[112]
	Runx2	Early differentiation of osteoblasts	[151]; [113];[139]
	BMP-2	Bone morphogenetic factor	[15]
	Jun B	Transcription factor	[149]
	Traf6	Ubiquitin ligase	[67]
	MEKK2	Kinase	[141]
c-Cbl	α5 Integrin	Transmembrane receptor	[112]
	EGFR/FGFR	Membrane receptors	[112]
	PI3K	Akt signaling	[122]
	Lyn/Fyn	Osteoblast differentiation	[112]
	Stat-5	Osteoblast differentiation from mesenchymal cells	[23]
Chip	Smad1	Transcription factor	[68]

Since degradation of β-catenin is influenced by the ubiquitin ligase $SCF^{B\text{-}TrCP}$ [88], this ligase is also a significant factor in regulation of osteoblast differentiation. Table 1 illustrates that several signaling proteins, in addition to β-catenin, are substrates for the ubiquitin ligase $SCF^{B\text{-}TrCP}$ and contribute to regulation of bone formation and bone remodeling.

Runx2 is a transcription factor that is essential for differentiation of osteoblasts [4,54]. Its expression is stimulated by β-catenin (see Fig. 1). Runx2 interacts with a vitamin D3 responsive element to facilitate transcription of the osteocalcin (OC) gene (and the genes for other proteins) in osteoblasts [90]. Expression of the osterix (Osx) gene also depends on Runx2 [4]. Osterix, reportedly contributes to the differentiation of preosteoblasts into osteoblasts, and is of functional significance in the osteocyte [146]. Runx2 is a substrate for the ubiquitin ligase Smurf1 (Table 1) [112].

Melatonin enhances osteoblast differentiation both in vitro and in vivo [105,109,147,148]. Sethi et al. [111] determined that 50 nM for 21 days induced osteoblast differentiation from human mesenchymal stem cells. This protocol of melatonin administration stimulated the