Does adenosine play a role in bone formation, resorption and repair?

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Introductory note by the associate editor

There has been increasing interest recently in the role purinergic signalling in the physiology and pathophysiology of musculoskeletal tissues, especially bone. Whereas the role of ATP in bone metabolism [1, 2] has been revealed to some extent and quite a few papers have been published in this respect, functions of its metabolite adenosine are not understood. Bone remodelling is a continuous, life-long process that maintains skeletal integrity. It is orchestrated by the three bone cell types (osteoblasts, osteoclasts and osteocytes), and impairments eventually result in diseases such as osteoporosis. Although there is much insight into the systems that maintain healthy bone, there is still a need to develop new therapies. The hypothesis that the adenosine signalling pathways might provide new targets for bone disease has gained further momentum recently with the publication of the following two papers that are commented on by Bronwen A. J. Evans of Cardiff University. Interestingly enough, the first article deals with findings obtained using CD73 null mice, that have also been the subject of another recently published Highlight [3] that focused on the role of local and systemic adenosine in modulation of anti-tumour responses in vivo. Here, CD73 null mice have been exploited to unveil a potential role of adenosine in osteoblast differentiation. In the second commented article, by using adenosine A2B receptor knockout mice, this receptor is identified as a main target for adenosine in promoting the differentiation of mesenchymal stem cell to osteoblasts.

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Highlight N. 1: CD73-generated Adenosine Promotes Osteoblast Differentiation

Takedachi M, Oohara H, Smith BJ, Iyama M, Kobashi M, Maeda K, Long CL, Humphrey MB, Stoecker BJ, Toyosawa S, Thompson LF, Murakami S. J Cell Physiol 2012; 227: 2622–31.

Article summary

M. Takedachi and colleagues hypothesized that CD73 may be involved in regulating function of the bone forming cell (osteoblast) through modulating nucleotide metabolism and generating extracellular adenosine that can activate adenosine receptors (ARs). To address this hypothesis, they asked whether CD73 functionally regulates bone metabolism in vivo by characterizing the bone phenotype of *cd73*^{−/−} mice. In addition, they investigated the involvement of CD73 and AR signalling in osteoblast differentiation in vitro.

Micro-computed tomography (μCT) showed that 13 week old male mice had osteopenia, and that this was due to reduced trabecular bone volume, decreased trabecular number and thickness, and increased trabecular separation in *cd73*^{−/−} mice when compared to wild-type controls. Subsequent work measuring biochemical markers of bone formation showed that osteocalcin (bone formation marker) was decreased in *cd73*^{−/−} mice whereas bone resorption markers (TRAP5b and fragments of type I collagen) were comparable to wild-type animals. The association of the osteoblast to the impaired bone phenotype seen in *cd73*^{−/−} mice was further substantiated using quantitative RT-PCR. These

results suggested that the involvement of CD73 in bone homeostasis is directed to the osteoblasts.

The second half of the paper describes a series of *ex vivo* experiments using osteoblasts isolated from *cd73*^{−/−} and wild-type animals. They suggest that CD73 plays a role in osteoblast differentiation but not in the development of osteoblast progenitors. Furthermore, the authors describe experiments with a mouse osteoblast cell line (MC-3T3) and conclude that overall their work shows that CD73-generated adenosine positively regulates osteoblast differentiation via A2B AR signalling.

Highlight N. 2: The A2B Adenosine Receptor Promotes Mesenchymal Stem Cell Differentiation to Osteoblasts and Bone Formation *in vivo*

Carroll SM, Wigner NA, Kulkarni N, Johnston-Cox H, Gerstenfeld LC, Ravid K, JBC Papers in Press. Published March 8, 2012 as Manuscript M112.344994. See: <http://www.jbc.org/cgi/doi/10.1074/jbc.M112.344994>

Article Summary

S Carroll and colleagues focus on the differentiation of mesenchymal stem cells to osteoblasts as this pathway is an important component in bone homeostasis, and in bone fracture healing. They build on what others have shown in *in vitro* pharmacological approaches by investigating the role of the A2B AR in osteoblast differentiation and function by studying bone homeostasis *in vivo* using A2B AR knockout (KO) mice.

Ex vivo studies showed that at 9 and 12 days after osteo-induction, bone marrow from A2B AR KO mice showed fewer mineralized nodules, demonstrating a reduction in osteoblast differentiation in the absence of the A2B AR. They then examined the expression of two transcription factors central to osteoblast differentiation, Runx2 and Osterix, at pre- and post-osteogenic induction, and found that with osteogenic induction, the increase in the expression of both Runx2 and Osterix is significantly attenuated in the A2B AR samples. Other studies lead them to conclude that the mechanism of effect involves, at least partially, cAMP. This was indicated by experiments involving activation of the A2B AR or addition of a cAMP analogue during differentiation.

The second part of this paper addresses the role of the A2B AR in an injury response situation by examining callus formation after fracture. Since fracture healing is dependent on the initial mesenchymal stem cell differentiation into osteoblasts and chondrocytes, the authors hypothesized that the reduced potential of A2B AR KO cells to differentiate into osteoblasts would be manifested as reduced bone

formation after fracture. Micro-CT revealed that fracture calluses of A2B AR KO mice showed a smaller overall total volume, as well as a decrease in the ratio of bone volume to total callus volume at 14 and 21 days post-fracture. Analysis of tissue sections supported these findings. In addition, tissue from the fracture callus was analyzed by quantitative RT-PCR. A2B AR KO mice tend to show decreased expression of Runx2 and Osterix at 3 days post-fracture and showed significantly decreased expression at 7 days post-fracture, as compared to wild-type animals. The authors conclude that such findings suggest that the A2B AR is involved in aspects of the skeletal repair process and its absence leads to delayed progression of bone development during fracture repair in the A2B AR KO mice.

The last section of the paper investigates bone structure in A2B AR KO and wild-type mice by μ CT. Interestingly, and unexpectedly, analysis of adult femurs showed lower bone density in A2B AR KO mice as compared to wild-type animals. Furthermore, the A2B AR KO animals have significantly shorter femurs.

Commentary

Many early studies concluded that, in relation to purinergic signalling, ATP and not adenosine was the ‘important’ molecule in bone and joints. Thus, the role of adenosine was largely ignored for decades as it was thought to play very little part in physiology and pathophysiology in these tissues. During the last 6 years, however, a number of papers have gradually changed this view. In 2006, Evans et al. [4] published that osteoprogenitor cells secreted adenosine *in vitro* and that this influenced cell differentiation. The study concluded that the A2B AR was the functionally dominant AR in the human osteoprogenitor cells studied. Furthermore adenosine influenced these cells to secrete factors (e.g., IL-6) that could in turn modulate the differentiation of other bone cells (e.g., osteoclasts). In 2009, Cronstein and colleagues [5] concluded that adenosine A2A receptors play an active role in mouse bone marrow-derived mesenchymal stem cell development. This work included studies from the A2A AR KO mouse. Further insights into the role of adenosine and its receptors in the modulation of mesenchymal stem cell differentiation have come from two *in vitro* cell based studies published last year [6, 7].

Since bone remodelling is orchestrated by osteoblasts, osteoclasts and osteocytes, other studies from Cronstein’s laboratory [8–11] have recently demonstrated a role for the A1 and A2A ARs in osteoclast differentiation and function, again using KO mouse as study models. Furthermore, there

is much recent data on the role of adenosine in joint disease such as arthritis (see e.g., [12, 13]).

The two papers highlighted here thus build on the existing evidence that adenosine signalling pathways are important in musculoskeletal tissues. Both of the papers use an in vivo KO mouse approach—(1) Takedachi et al. [14] use a model where adenosine production is compromised and (2) Carroll et al. [15] use a model where the A2B AR, previously identified to be important for osteoblast differentiation, is missing. It is interesting to note that Takedachi et al. highlight that (1) CD73 expression is regulated by Wnt- β -catenin signalling [16], a known critical pathway in bone metabolism and (2) the hypoxia inducible factor-1 α (HIF-1 α), a transcription factor reported to be important for bone regeneration and skeletal development also regulates CD73 expression. Furthermore, Carroll et al. intriguingly comment that the bone phenotype of the A2B AR KO mouse is similar to observations made in the PTH receptor (PPR) KO mouse, suggesting a possible synergy between the two receptors during bone development. In relation to a link between adenosine signalling with arthritis, this same paper report that A2B AR KO mice tend to display a mild increase in the levels of inflammatory cytokines which could affect bone.

An important aspect to keep in mind is the availability of adenosine for receptor binding/activation in these tissues. Osteoblasts have been reported to release ATP into the extracellular environment. This release has been reported to be increased by mechanical stress and is known to have a paracrine or autocrine effect on osteoblasts and osteoclasts partly via activation of specific P2 receptors. However, this ATP is also known to be rapidly metabolised to adenosine (which can then act via its own receptors) by the sequential actions of a number of enzymes, including ecto-nucleotide pyrophosphatase/phosphodiesterase-1 (ENPP1), CD73 and alkaline phosphatases [4, 17]. Interestingly, a mouse model lacking ENPP1 has recently been shown to have a severe disruption to the architecture and mineralization of long-bones [18]. The pathways involved in the purinergic control of bone metabolism are thus probably complex and the roles played by the three facets involved (metabolising enzymes, P2 receptors, P1 receptors) are probably intertwined. Evidence of this has already been published by Pellegatti and co-workers [19] who show that the P2X7 receptor drives osteoclast fusion by increasing extracellular adenosine concentration. Such complex processes could, of course, be modulated in disease states such as osteoporosis and arthritis.

Taken together, the work published to date on adenosine signalling in bone and joints highlight the intricacies of the pathways involved. Much data have been gathered during the last few years, but there is still much to do before we have a full understanding of the biology involved. We look forward to much more exciting research in the area—watch this space!

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About the author

Bronwen Evans is Senior Lecturer at the School of Medicine, Cardiff University, UK. She has worked in the bone research field for almost 20 years and her current research interests focus on (1) adenosine signalling in bones and joints, (2) osteocyte biology, (3) mechanotransduction and (4) development of 3D model systems for the in vitro study of bone cell biology and interactions. She was the local organiser for the UK Purine Club annual meeting held in Cardiff in November 2011.