Bone 51 (2012) 961–968

Contents lists available at SciVerse ScienceDirect

Bone

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

Review

New insights into NPP1 function: Lessons from clinical and animal studies

N.C.W. Mackenzie [a], C. Huesa [a], F. Rutsch [b], V.E. MacRae [a,*]

[a] The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Roslin, Midlothian, EH25 9RG, Scotland, UK
[b] Department of General Pediatrics, University Children's Hospital, Münster, Germany

ARTICLE INFO

Article history:
Received 15 June 2012
Revised 13 July 2012
Accepted 17 July 2012
Available online 26 July 2012

Edited by: T. Jack Martin

Keywords:
ENPP1
GACI
Vascular calcification
Insulin
Hypophosphataemia
FGF-23

ABSTRACT

The recent elucidation of rare human genetic disorders resulting from mutations in ectonucleotide pyrophosphotase/phosphodiesterase (*ENPP1*), also known as plasma cell membrane glycoprotein 1 (PC-1), has highlighted the vital importance of this molecule in human health and disease.

Generalised arterial calcification in infants (GACI), a frequently lethal disease, has been reported in recessive inactivating mutations in *ENPP1*. Recent findings have also linked hypophosphataemia to a lack of NPP1 function. A number of human genetic studies have indicated that NPP1 is a vital regulator that influences a wide range of tissues through various signalling pathways and when disrupted can lead to significant pathology.

The function of *Enpp1* has been widely studied in rodent models, where both the mutant tiptoe walking (*ttw/ttw*) mouse and genetically engineered $Enpp1^{-/-}$ mice show significant alterations in skeletal and soft tissue mineralisation, calcium/phosphate balance and glucose homeostasis. These models therefore provide important tools with which to study the potential mechanisms underpinning the human diseases associated with altered NPP1.

This review will focus on the recent advances in our current knowledge of the actions of NPP1 in relation to bone disease, cardiovascular pathologies and diabetes. A fuller understanding of the mechanisms through which NPP1 exerts its pathological effects may stimulate the development of novel therapeutic strategies for patients at risk from the devastating clinical outcomes associated with disrupted NPP1 function.

Contents

Introduction

Rare human genetic disorders resulting from loss-of-function mutations in the ectonucleotide pyrophosphotase/phosphodiesterase (*ENPP1*) gene, also known as plasma cell membrane glycoprotein 1 (PC-1), have highlighted the importance of this molecule in human health and disease. Generalised arterial calcification in infants (GACI) and severe hypophosphataemia have been reported in recessive inactivating mutations in the *ENPP1* gene [1–4]. Together with the association between polymorphisms in *ENPP1* and *ALPL*, the gene encoding for tissue non-specific alkaline phosphatase (TNAP), and reduced bone size and mineral density in the Caucasian population [5] these findings indicate that the *ENPP1* gene is required for normal inhibition of ectopic

* Corresponding author at: The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Roslin, Midlothian, EH25 9RG, UK. Fax: +44 131 651 9105.
E-mail address: vicky.macrae@roslin.ed.ac.uk (V.E. MacRae).

8756-3282/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bone.2012.07.014

mineralisation while also being essential for mineralisation in the bone. Furthermore, levels of *ENPP1* expression have been reported to be elevated in humans showing high levels of insulin resistance [6–8] suggesting an important role in glucose homeostasis and insulin signalling. These human studies indicate that the NPP1 protein is a vital regulator that influences a wide range of tissues through various signalling pathways and when disrupted can lead to significant pathology.

The function of NPP1 has been widely studied in rodent models, where both the mutant tiptoe walking (*ttw*/*ttw*) mouse [9–14], and the transgenically engineered *Enpp1*$^{-/-}$ mice [15,16], show changes in skeletal and soft tissue mineralisation, calcium/phosphate and glucose homeostasis, mimicking the diseases seen in human subjects. Furthermore, by acting remotely on the balance of circulating minerals and glucose, NPP1 has a wider reaching impact on both skeletal and soft tissue structure and metabolism. This review will focus on the recent advances in current understanding of the role of the NPP1 protein in these pathways and outline the importance of this research in bone diseases, cardiovascular diseases and diabetes.

Genetics and function of NPP1

The nucleoside pyrophosphatase/phosphodiesterases (NPPs) are an important group of enzymes with an extensive functional range that are distributed widely and are highly conserved between species. In humans the NPP family consists of 5 proteins of which NPP1 and NPP3 show similar structure and function and the genes encoding for these two proteins have been mapped to human chromosome 6q22-23 [17,18]. Despite the close sequence homology of the *NPP* genes between species it has been reported that the 5′ flanking region is far less conserved, leading to different regulation and gene expression patterns in different species [19].

The NPP1 protein is a membrane spanning homodimer and, when cleaved, the extracellular domain can function as a secreted circulating protein. In a very revealing review Bollen and colleagues have discussed the biochemistry of the NPP family and have summarised the localisation of *ENPP1* gene expression [19]. *ENPP1* is expressed in a wide range of tissues including cartilage, heart, kidney, parathyroid and skeletal muscle, and it is highly expressed in vascular smooth muscle cells (VSMCs), osteoblasts and chondrocytes [20–22].

NPPs have wide substrate specificity, and the hydrolysis of pyrophosphate bonds (for example, in ATP) and phosphodiester bonds (for example, in oligonucleotides) to produce nucleoside 5′-monophosphates makes NPPs extremely important in extracellular nucleotide metabolism and extracellular signalling. NPP1 (EC3.1.4.1) is a 104 kDa type II transmembrane protein consisting of a small intracellular region (between 10 and 80 residues) and a larger extracellular domain (830 residues) which contains the catalytic site [23]. Phosphodiesterases are classified as enzymes that hydrolyse diesters of phosphoric acid into phosphomonesters, and can be classified into two main groups — those that act on lipids or on nucleotides. Pyrophosphatases are acid anhydride hydrolases that catalyse the breakdown of diphosphate bonds and are biologically important in the cleavage of ATP. NPP1 hydrolyses ATP to generate either inorganic pyrophosphate (PP_i) plus AMP or inorganic phosphate (P_i) plus ADP in a two stage process via either ADP or a phosphate bound intermediate, respectively (Fig. 1) [19,24]. It has also been reported that NPPs can convert AMP into adenosine and P_i [25,26] although conflicting reports suggest that AMP competitively inhibits NPP activity [27]. All of the products of these hydrolysis reactions are essential in cellular signalling and function, the effects of which vary between tissues.

Basic mechanisms of bone formation and the role of NPP1 in skeletal mineralisation

In order to understand the functions of NPP1 it is important to appreciate the physiological process of mineralisation in bone. This relies on the deposition of hydroxyapatite (HA) onto a collagenous matrix, and is a highly regulated process that requires the correct concentration of calcium (Ca^{2+}) and P_i to precipitate as HA crystals. Mineralisation is thought to be a two stage process, the first of which occurs within matrix vesicles (MVs) [28] where the conditions are optimal for the initial precipitation of HA. The second stage consists of the propagation of HA formation onto the extracellular matrix

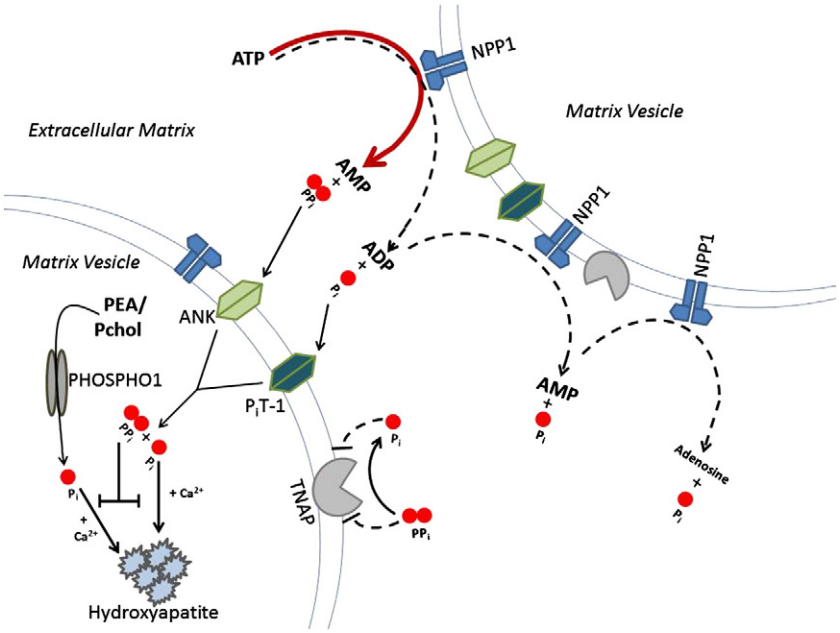

Fig. 1. Schematic showing the role of NPP1 in ATP hydrolysis and the downstream effects on bone mineralisation. The primary function of NPP1 is the hydrolysis of ATP into AMP and PP_i, although it is involved in further degradation of pyrophosphate bonds to generate ADP, adenosine and P_i (secondary reactions denoted by dotted lines). PP_i is converted into P_i by TNAP and the transport of PP_i and P_i through the cell membrane is mediated by ANK and P_iT-1 respectively. Within the matrix vesicle PHOSPHO1 can generate further P_i by the hydrolysis of PEA and Pchol. PP_i acts to inhibit hydroxyapatite formation, while P_i promotes this process, thus the balance of these two mediators is highly important in regulating mineralisation.