Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

New perspectives on rare connective tissue calcifying diseases

Nabil A Rashdan[1], Frank Rutsch[2], Hervé Kempf[3], András Váradi[4], Georges Lefthériotis[5,6] and Vicky E MacRae[1]

Connective tissue calcifying diseases (CTCs) are characterized by abnormal calcium deposition in connective tissues. CTCs are caused by multiple factors including chronic diseases (Type II diabetes mellitus, chronic kidney disease), the use of pharmaceuticals (e.g. warfarin, glucocorticoids) and inherited rare genetic diseases such as pseudoxanthoma elasticum (PXE), generalized arterial calcification in infancy (GACI) and Keutel syndrome (KTLS). This review explores our current knowledge of these rare inherited CTCs, and highlights the most promising avenues for pharmaceutical intervention. Advancing our understanding of rare inherited forms of CTC is not only essential for the development of therapeutic strategies for patients suffering from these diseases, but also fundamental to delineating the mechanisms underpinning acquired chronic forms of CTC.

Addresses
[1] The Roslin Institute, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Midlothian, EH25 9RG Scotland, United Kingdom
[2] Department of General Pediatrics, Muenster University Children's Hospital, Muenster, Germany
[3] French Institute of Health and Medical Research, UMR 7365 CNRS-Université de Lorraine, Paris, France
[4] Institute of Enzymology, RCNS, Hungarian Academy of Sciences, Magyar tudosok krt., Budapest 1117, Hungary
[5] PRES L'UNAM, University Hospital of Angers, PXE Health and Care Centre, 49933 Angers, France
[6] PRES L'UNAM, Medical School, UMR CNRS 6214 – Inserm 1083, 49935 Angers, France

Corresponding author: MacRae, Vicky E (vicky.macrae@roslin.ed.ac.uk)

Current Opinion in Pharmacology 2016, **28**:14–23

This review comes from a themed issue on **Musculoskeletal**

Edited by **Isabel R Orriss** and **Vicky E MacRae**

For a complete overview see the Issue and the Editorial

Available online 27th February 2016

http://dx.doi.org/10.1016/j.coph.2016.02.002

Introduction

A disease or disorder is defined as rare in Europe when it affects less than 1 in 2000 people. In the EU, as many as 30 million people alone may be affected by one of over 6000 existing rare diseases (http://www.eurordis.org/about-rare-diseases). A number of rare inherited forms of Connective Tissue Calcifying diseases (CTCs) have been identified, and are characterized by abnormal calcium mineral deposition in connective tissues. Although all tissue has the potential to undergo calcification, several tissues have a higher propensity to calcify including skin, kidney, blood vessels and cardiac valves [1]. There are multiple mechanisms by which connective tissue calcification can progress, however these mechanisms are not exclusive and multiple pathologies can concurrently promote aberrant calcification. Common causes of connective tissue calcification include aging, as well as diseases such as atherosclerosis, chronic kidney disease (CKD) and Type II diabetes mellitus. Connective tissue calcification is also the result of specific rare congenital diseases such as generalized arterial calcification of infancy (GACI), pseudoxanthoma elasticum (PXE), Hutchinson–Gilford progeria syndrome (HGPS), arterial calcification due to deficiency of CD73 (ACDC) and Keutel syndrome (KTLS) [2]. Despite scarcity of cases, these diseases provide significant insight into the complex biological processes underpinning connective tissue calcification. The single gene deficiencies of rare inherited forms of CTC have allowed the identification of specific targets and the development of novel animal models to further study the process of connective tissue calcification. Furthermore, data from patients and animal models has resulted in the elucidation of pathways involved in both the promotion and inhibition of connective tissue calcification.

Basic mechanisms of bone mineralization

In order to understand more fully the mechanisms underpinning connective tissue calcification, it is important to appreciate the physiological process of bone mineralization, which occurs through the deposition of hydroxyapatite (HA) onto a collagenous extracellular matrix (ECM). HA crystal formation is regulated by matrix vesicles (MVs), which maintain calcium (Ca^{2+}) and inorganic phosphate (P_i) concentrations at levels optimal for HA nucleation. As the HA crystals grow they disrupt the MV and deposit onto the ECM where they continue to grow [3]. The transport of Ca^{2+} into MVs is primarily controlled by annexin channels, whereas P_i is transported into the MV by the type III sodium-dependent P_i co-transporter-1 (PiT-1) [4]. Intracellular to extracellular channelling of pyrophosphate (PP_i) is mediated by ANK [5].

Whilst P_i acts to promote HA crystal formation, PP_i, generated by ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), has a dual role as an inhibitor of HA

generation and as a precursor to P_i [6]. The ratio of P_i to PP_i is controlled by a complex interaction between the regulatory phosphatases tissue non-specific alkaline phosphatase (TNAP) and phosphoethanolamine/phosphocholine phosphatase (PHOSPHO1). TNAP hydrolyses PP_i in the ECM to release P_i and PHOSPHO1 hydrolyses phosphocholine and phosphoethanolamine to produce P_i inside the MVs. Together these phosphatases control the P_i/PP_i balance during the mineralization process [7]. Further feedback signalling allows modulation of mineralization; inorganic pyrophosphatase stimulates mineralization without reducing PP_i levels [8]. Both exogenous P_i and PP_i upregulate the bone sialoprotein osteopontin (OPN), which in turn inhibits mineralization through restricting HA crystal formation and growth [9]. Intriguingly, a clear dissociation in the hierarchical roles of PP_i and OPN has recently been highlighted [10•].

Overview of mechanisms of connective tissue calcification

The control and regulation of connective tissue calcification is a multifactorial process which shares many similarities with that of the physiological matrix mineralization during skeletal development described previously. A wealth of knowledge emanates from research into vascular calcification, a strong and independent predictor of morbidity and mortality in cardiovascular disease [2]. Indeed normal vascular smooth muscle cell (VSMC) populations contain cells that undergo phenotypic transition to osteocytic, osteoblastic and chondrocytic cells in a calcified environment [11]. In VSMCs, MVs have been shown to nucleate hydroxyapatite crystals that contain calcium and inorganic phosphate [12••] forming the first nidus for calcification. This nucleation occurs via a tightly controlled balance of inhibitors and inducers comparable to that seen in bone, with PHOSPHO1, sphingomyelinase 3 (SMPD3), TNAP, annexins, ANK and ENPP1 playing key regulatory roles [2,12••]. Furthermore, MVs derived from VSMCs have been shown to contain negative regulators of hydroxyapatite crystal nucleation and growth, such as fetuin-A and matrix gla protein (MGP) [13•]. In cooperation with local mediators such as PP_i [6], these molecules protect the arteries from mineral deposition and growth. In the absence of these inhibitors, or following the stimulation of apoptotic processes [14•], together with the osteogenic activity of VSMCs, vascular calcification readily proceeds.

A key role for pyrophosphate (PP_i) in rare CTCs

It has recently been established that connective tissue calcification is contingent on circulating PP_i levels rather than local PPi production [15]. As previously highlighted, PP_i not only acts as a potent inhibitor of connective tissue calcification, but also contributes directly to the calcification process. Intriguingly, one of the identified sources of systemic PP_i is through ATP binding cassette sub-family C member 6 (ABCC6)-mediated ATP release from hepatocytes [16]. Still within the vasculature of the liver, released ATP is rapidly converted to PP_i and AMP by ENPP1, which are in turn distributed throughout the body via the circulation. In connective tissues the metabolite AMP is further hydrolysed into P_i and adenosine by ecto-5′-nucleotidase (CD73). Adenosine in turn inhibits TNAP transcription, thus decreasing P_i production and more importantly maintaining PP_i levels [17]. Different perturbations in this mechanism can contribute to several rare CTCs (Table 1) which whilst varying in degree of severity and phenotype, show notable overlap.

Table 1

The cause and phenotype of significant rare inherited CTCs

CTC disease	Cause	Phenotype
Pseudoxanthoma elasticum (PXE)	ABCC6 deficiency	Elastic fibre mineralization in skin, eyes, and arteries.
Generalized arterial calcification in infancy (GACI)	ENPP1 deficiency	Widespread mineralization of arteries, and to a lesser extent joints.
Arterial calcification due to deficiency of CD73 (ACDC)	CD73 deficiency	Mineralization of arteries and joints in the extremities.
Hutchinson–Gilford progeria syndrome (HGPS)	Progerin (lamin A mutant)	Premature aging, atherosclerosis and calcification of blood vessels and the aortic valve.
Keutel syndrome (KTLS)	MGP deficiency	Facial abnormalities, calcification of the larynx trachea and bronchi, along with auricular, nasal and rib cartilage.
Fibrodysplasia ossificans progressiva (FOP)	ACVR1 gain of function	Progressive heterotopic endochondral ossification of skeletal muscle, fascia, tendons, and ligaments.
Coeliac disease with epilepsy and cerebral calcifications (CEC)	Unknown	Occipital epilepsy, with bilateral occipital calcifications and coeliac disease.
Idiopathic basal ganglia calcification (IBGC)	PiT-2 and/or PDGFR-B deficiency?	Calcification of the basal ganglia as well as the thalamus and cerebellum.