HtrA1: a novel regulator of physiological and pathological matrix mineralization?

A.E. Canfield1, K.D. Hadfield, C.F. Rock, E.C. Wylie and F.L. Wilkinson
Wellcome Trust Centre for Cell-Matrix Research, Faculty of Medical and Human Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, U.K.

Abstract

HtrA1 (high-temperature requirement protein A1) is a secreted multidomain protein with proven serine protease activity and the ability to regulate TGF-β (transforming growth factor-β)/BMP (bone morphogenetic protein) signalling. There is increasing evidence that HtrA1 regulates several pathological processes, including tumour development, Alzheimer’s disease, age-related macular degeneration and osteoarthritis, although the mechanism(s) by which it regulates these processes have not been fully elucidated. Using overexpression and knock-down strategies, we have evidence demonstrating that HtrA1 is also a key regulator of physiological and pathological matrix mineralization in vitro. We propose that HtrA1 regulates mineralization by inhibiting TGF-β/BMP signalling and/or by cleaving specific matrix proteins, including decorin and MGP (matrix Gla protein). Taken together, these studies suggest that HtrA1 may be a novel therapeutic target for several diseases.

Introduction

Mammalian HtrA1 (high-temperature requirement protein A1) is a member of the family of HtrA (high-temperature requirement A) proteins originally identified in bacteria (reviewed in [1]). These proteins contain a trypsin-like serine protease domain and one or two PDZ domains. In contrast with bacterial HtrA, mammalian HtrA1 is secreted and it also contains an IGFBP (insulin-like growth factor-binding protein)/follistatin/Mac25-like domain and a Kazal-type inhibitor domain at the N-terminus [1] (Figure 1). The functions of these domains are not known. Four forms of mammalian HtrA have been described in the literature, HtrA1–4 [1]. HtrA3 structurally resembles HtrA1 [2] and it has been proposed that they may serve similar functions in vivo [3]. In contrast, HtrA2 does not contain a signal peptide or the IGFBP/follistatin/Mac25 and Kazal-type inhibitor domains [1]. Instead, this protease contains a mitochondrial localization signal and it has been proposed that HtrA2 regulates protein quality control in the mitochondria [4,5]. In addition, HtrA2 has been shown to induce apoptosis in mammalian cells [5]. Very little is currently known about HtrA4.

HtrA1

HtrA1 (formerly termed L56) was originally identified as a gene that was down-regulated in transformed fibroblasts [6]. Subsequently, we identified HtrA1 as a gene that was up-regulated when pericytes underwent osteogenic differentiation [7], and Hu et al. [8] demonstrated that this gene was also up-regulated in osteoarthritic cartilage. Interestingly, evidence is now accumulating to suggest that HtrA1 is involved in the development and progression of several pathologies as its expression is markedly up-regulated in osteoarthritis, rheumatoid arthritis and Alzheimer’s disease [8–11]. A polymorphism in the HtrA1 promoter has recently been shown to be strongly associated with age-related macular degeneration, and elevated HtrA1 expression has been demonstrated in patients with this disease [12,13]. Conversely, HtrA1 expression is decreased in several cancers and overexpression of HtrA1 inhibits tumour growth in vitro and in vivo, suggesting that this protease may also function as a tumour suppressor [14].

The mechanisms by which HtrA1 may exert its effects have recently started to become elucidated. Recombinant HtrA1 has been shown to degrade several matrix proteins, including decorin, fibromodulin, fibronectin, aggrecan and type II collagen, by using in vitro assays [3,9,10]. It has also been demonstrated that HtrA1 binds to and inhibits signalling mediated by several members of the TGF-β (transforming growth factor-β) superfamily [15]. Using GST (glutathione S-transferase)-pull-down assays, Oka et al. [15] demonstrated that HtrA1 binds to BMP-4 (bone morphogenetic protein 4), TGF-β2, TGF-β1, activin, BMP-2 and Gdf5 (growth differentiation factor 5); in contrast, this protease does not bind EGF (epidermal growth factor) or FGF-2 (fibroblast growth factor 2). This group also demonstrated that HtrA1 inhibits TGF-β/BMP signalling and that the protease and linker domains are essential for this activity [15]. Interestingly, TGF-β, BMP-2 and BMP-4 are not degraded by HtrA1 in vitro and this protease does not inhibit signalling from the...
Our recent studies have now demonstrated a new role for HtrA1 as an inhibitor of matrix mineralization (A.E. Canfield, K.D. Hadfield, C.F. Rock, E.C. Wylie and F.L. Wilkinson, unpublished work). We have shown that HtrA1 protein is detected at sites of mineralization in human femoral arteries, in a similar location to osteopontin and MGP (matrix Gla protein), two proteins that have been proposed to regulate vascular calcification. We have also shown that HtrA1 expression is markedly up-regulated when VSMCs (vascular smooth muscle cells), pericytes and osteoblasts undergo osteogenic differentiation and is then down-regulated when their matrix is mineralized [7]. Finally, using several complementary approaches, we have demonstrated a functional role for HtrA1 in matrix mineralization. First, we have shown that mineralization is significantly inhibited when osteoblasts are induced to overexpress HtrA1 and is significantly enhanced when the expression of HtrA1 is knocked-down using RNAi (RNA interference). Secondly, using a series of deletion constructs of recombinant HtrA1, we have shown that HtrA1 inhibits mineral deposition by VSMCs and osteoblasts and that the protease domain is required for this effect. Thirdly, overexpression of HtrA1 prevents BMP-2-induced mineralization of osteoblasts. Finally, recombinant HtrA1 cleaves MGP and decorin, two proteins that have previously been shown to modulate mineralization directly and indirectly by regulating BMP-2/TGF-β bioavailability and activity.

Together, these studies suggest that HtrA1 is a novel regulator of physiological and pathological matrix mineralization by osteoblasts and VSMCs. Current evidence suggests that HtrA1 exerts its effects by inhibiting growth factor signalling and/or by cleaving specific matrix proteins and that these effects may occur in a context-dependent manner. We propose that the deregulation of HtrA1 expression in vivo may contribute to pathologies associated with aberrant matrix mineralization.

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References

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