MINI-REVIEW

The Emerging Roles of HTRA1 in Musculoskeletal Disease

André Nicki Tiaden and Peter James Richards

From the Bone and Stem Cell Research Group, Center for Applied Biotechnology and Molecular Medicine, University of Zurich, Zurich, Switzerland

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Address correspondence to Peter J. Richards, Ph.D., CABMM, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. E-mail: peter.richards@cabmm.uzh.ch.

The high-temperature requirement serine protease A1 (HTRA1) is one of four known proteases belonging to the broadly conserved family of HTRA proteins. Although it was originally considered as representing an important modulator of tumorigenesis, an increasing number of reports have suggested that its influence on human disease may extend beyond cancer. HTRA1 has the capacity to degrade numerous extracellular matrix proteins, and as such, its potential involvement in diseases of the musculoskeletal system has been gaining increased attention. Musculoskeletal disease constitutes a wide variety of degenerative conditions that can manifest themselves in different ways such as joint and back pain, as well as deficiencies in skeletal bone quality, and ultimately result in significant suffering and reduced quality of life. Convincing data now exist to support a detrimental role for HTRA1 in the pathogenesis of joint and intervertebral disk degeneration. However, the function of HTRA1 in other closely related musculoskeletal diseases affecting bone and muscle remains unclear and largely unexplored. To help set the stage for future research, we discuss here some of the recent advances in our understanding of the role played by HTRA1 in musculoskeletal pathology.


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This has encouraged further research into the potential role of HTRA1 in human diseases in which breakdown of the ECM is considered to be of significant importance. Subsequently, new evidence is emerging that implicates HTRA1 in several musculoskeletal diseases (MSDs) affecting bone, joint, and muscle.

The aim of this review is therefore to expand on our current knowledge relating to the involvement of HTRA1 in human disease. Our understanding of the role of HTRA1 in disease is continually being challenged and requires regular updating to afford us with the necessary information to allow for the evaluation of HTRA1 as a potential therapeutic target. The structural and functional properties associated with HTRA1, and how these relate to its role in human disease, have already been discussed elsewhere and will not be readdressed here. We will instead focus more on examining the evidence available to implicate HTRA1 in MSD pathology and how we can exploit this knowledge to help combat such debilitating disorders.

Musculoskeletal Disease

Musculoskeletal is defined as relating to muscles and skeleton, and encompasses a wide variety of tissues, including muscles, bones, cartilage, joints, tendons, and ligaments. MSDs are generally considered to be, although perhaps wrongly, an inevitable part of growing old and are seen to be the most widespread conditions affecting the aged population. Due to their high prevalence, MSDs are seen to represent a significant burden, both to the affected individual and to society as a whole. Furthermore, this burden is likely to increase over the coming years due to rising longevity and shifts in the population pyramid favoring a higher proportion of senior citizens. Significant efforts are therefore being made to try and combat the most debilitating of the MSDs, including conditions relating to rheumatism, back pain, and osteoporosis, and involve basic and applied research approaches geared toward improving our understanding of the biological processes that govern these diseases. To this end, an increasing number of researchers are now focusing their attention on HTRA1 as a possible causal agent in the pathology of several closely related MSDs. To date, the most convincing evidence for the involvement of HTRA1 in MSD comes from studies involving patients with rheumatoid arthritis (RA) or osteoarthritis (OA), although there is now speculation that HTRA1 may also play a role in spine disease and possibly even osteoporosis and muscular dystrophy. Each of these conditions and their association with HTRA1 will be discussed in turn during the course of this Mini-Review.

Rheumatic Disorders

The term rheumatic disorder covers a wide variety of conditions, of which OA and RA are considered to have the greatest impact on public health. OA is the most prevalent form of arthritis and is characterized by a breakdown in articular cartilage as well as degeneration of other joint tissues, including synovium and subchondral bone. Originally considered to be a noninflammatory disorder, OA has since been associated with a low-level inflammatory response. It has been speculated that inflammation brought about through joint trauma may in fact be one of the main instigators of OA. The supervening wave of cytokines released during this initiation phase is important, not only in the development of pain-related symptoms, but also in cartilage destruction via the up-regulation of catabolic agents such as proteolytic enzymes, the chief being the matrix metalloproteinases (MMPs).

Hu et al were the first to demonstrate that in addition to the known enzymes up-regulated in OA patients, HTRA1 production is also significantly enhanced, with increases in both HTRA1 mRNA and protein being measured in arthritic cartilage explants. This finding was confirmed almost a decade later in a study examining soluble protein levels in articular cartilage tissue using a comparative proteomics analysis approach. Of the 814 proteins identified within tissue samples taken from normal and OA patient groups, 59 were considered as being differentially expressed. HTRA1 represented one such protein and was shown to be up-regulated by up to eightfold in OA cartilage as compared to normal cartilage. This was in good agreement with the sevenfold increase in the expression of HTRA1 mRNA originally reported by Hu et al. These observations were later corroborated by findings from similar studies using genetic or proteomic approaches to assess mechanisms underlying OA development. Although HTRA1 is firmly established as being up-regulated in joint degeneration, there still remained the question as to the role of HTRA1 in arthritis pathology.

Synovial fibroblasts play a key role in mediating inflammation and joint destruction as observed in RA. In addition to being a potent source of cytokines and chemokines, synovial fibroblasts also have the potential to secrete a barrage of matrix-degrading enzymes of which the MMPs are considered to be of primary importance in the pathogenesis of both OA and RA. It has previously been demonstrated that in addition to HTRA1 being up-regulated in both OA and RA patients, it was also capable of inducing MMP production by synovial fibroblasts isolated from OA and RA patients. Furthermore, the stimulatory effects of HTRA1 were shown to be reliant on its proteolytic activity and were mediated in part through the generation of fibronectin fragments. These findings, not only are suggestive of HTRA1 as having a detrimental role in arthritis pathology, but also offer a novel mechanism through which its actions may be mediated. Certainly, fibronectin fragments are considered to be of central importance in driving the arthritic process through their ability to stimulate MMP secretion. Clearly, based on the diverse proteolytic nature of HTRA1, this may be only part of the story, and its
interaction with other proteoglycans within the joint cannot be ruled out as a possible cause for its effects in arthritic disease. For instance, type-II collagen is a known HTRA1 substrate, and its degradation products are up-regulated in OA and can themselves induce ECM breakdown. More direct evidence comes from studies investigating the ability of HTRA1 to cleave the proteoglycan aggrecan, a major component of the articular cartilage ECM. HTRA1 digested aggrecan fragments were found to be up-regulated in OA cartilage, being localized to chondrocytes and areas of cartilage where proteoglycan loss was greatest. At present, it is unclear as to whether such fragments play any functional role in mediating cellular function, as do fibronectin fragments.

The relevance of HTRA1 to arthritic disease progression has also been investigated in experimental models that closely simulate the human condition. Initial evidence for its involvement came from studies performed in mice with collagen-induced arthritis, where levels of HTRA1 mRNA were shown to be elevated in accordance with the appearance of joint swelling. Furthermore, immunohistochemical analysis revealed that HTRA1 protein localized to hypertrophic chondrocytes in the deep layers of degraded articular cartilage, although it remained largely absent from cells undergoing apoptosis. Interestingly, HTRA3, another secreted HTRA member with high homology to HTRA1, was also found to be overexpressed in the articular cartilage of arthritic mice, although this was confined mainly to the superficial layer. In both cases, the proteolytic action of HTRA1 and HTRA3 on various ECM proteoglycans, including decorin and biglycan, was implicated as being a potential contributory factor to disease progression. These observations have since been extended to studies in mouse OA models, where increased levels of HTRA1 were identified in articular cartilage from diseased mice in association with both MMP13 and discoidin domain-containing receptor 2 (Ddr2), both of which are considered key elements in the cartilage breakdown cascade. It was proposed that HTRA1 might mediate its effects through removal of the protective pericellular matrix surrounding chondrocytes, leading to exposure of cells to the network of surrounding collagen fibers and induction of chondrocyte hypertrophy. HTRA1 may therefore represent an additional component of what may be a central molecular pathway responsible for initiating OA cartilage degeneration.

### Spinal Disk Degeneration

Acquired degenerative stenosis is by far the most common cause of chronic back pain, arising mainly from a breakdown in the structural integrity of lumbar intervertebral disks (IVDs). IVDs are primarily designed to counteract compressive forces on the spine and to maintain vertebral separation, thus allowing for unrestricted movement. IVD degeneration is considered to be predominantly an age-related phenomenon, resulting in loss of disk height as well as increases in disk bulging and subsequent impingement of nerve roots. Changes in matrix turnover and cell activity are believed to be two of the major contributors of structural integrity failure encountered in degenerating IVDs and are closely associated with an overproduction of matrix-degrading enzymes. The localization of both MMPs and their inhibitors to cells of the nucleus pulposus and inner fibrosus compartments of the IVD would tend to suggest that these cells are of central importance in the development and progression of IVD disease.

With regard to the involvement of HTRA1 in IVD degeneration, initial insights came from studies examining genetic changes in the HTRA1 promoter region of Japanese women with IVD degeneration, where a single nucleotide polymorphism, rs11200638, was identified as being a risk factor for spinal disk space narrowing. This interesting observation was impetus for further studies undertaken by our laboratory in which we assessed the role HTRA1 in IVD degeneration in a small European patient population. A detrimental role for HTRA1 in IVD degeneration was alluded to be based on its ability to stimulate MMP and aggrecanase production by cultured IVD cells. Furthermore, despite our being unable to show any association of rs11200638 with disease severity, we were able to demonstrate that HTRA1 production was significantly up-regulated in degenerated IVD tissue explants and was directly correlated with appearance of both C- and N-terminal fibronectin fragments. This finding is considered to be of particular relevance based on the fact that fibronectin fragments have already been closely linked with the detrimental effects of HTRA1 in arthritis. As with arthritis, fibronectin fragments are believed to have a significant influence on disease progression. Therefore, it is possible that HTRA1 mediates its destructive effects in both spine and joint disease through a common mechanism involving the generation of reactive fibronectin fragment species. Although, as already alluded to earlier in this Mini-Review, one cannot exclude the possibility that other proteoglycans within the ECM are also susceptible to the actions of HTRA1. As with OA cartilage, increases in type-II collagen degradation products are common features of IVD degeneration and have the potential to activate resident cells. Evidently, further investigations are needed to better define the role of such breakdown products in the destruction of both cartilage and IVD tissue.

### Age-Related Osteoporosis

Osteoporosis is defined as a systemic disease characterized by reduced bone mass and low bone mineral density, with a subsequent increase in bone fragility and vulnerability to
Bone formation is reliant on the presence of functionally active mature osteoblasts derived from progenitor cells within the bone marrow, termed bone marrow stromal cells (BMSCs), through a process of osteogenic differentiation. The observation that BMSCs isolated from aged osteoporotic patients have a higher propensity toward adipogenesis than osteogenesis implies that the structural abnormalities associated with osteoporotic bone may be a consequence of inadequacies in bone cell differentiation. This is further supported by data from experimental models of osteoporosis. Clearly, therefore, identifying factors involved in controlling BMSC lineage commitment and subsequent bone formation may be a key step in developing successful therapeutic strategies for the treatment of age-related osteoporotic bone loss and prevention of associated fractures.

The suggestion that HTRA1 may play a role in bone formation originally came from detailed in situ hybridization and immunohistochemical studies performed in tissues from mice at various stages of development. HtrA1 gene expression was identified in undifferentiated mesenchymal cells in the vicinity of pre-cartilage condensations, as well as in osteocytes within bone matrix. Positive staining for HTRA1 protein was visualized in areas associated with both endochondral ossification and intramembranous ossification, suggestive of a central role for HTRA1 in bone remodeling. This concept is supported by findings from our own studies examining HTRA1 levels during fracture healing in mice, where we identified a noticeable increase in HTRA1 protein expression at sites of active bone formation within both osteoblasts and hypertrophic chondrocytes. Similarly, enhanced HTRA1 expression has also been observed in dentin during reparative dentin formation induced in rats.

Until recently, the role of HTRA1 in bone formation and disease was generally considered to be a negative one. This was based on the findings of Hadfield et al, where over-expression of HTRA1 in immortalized mouse 2T3 osteoblasts was associated with decreases in matrix mineralization. However, recent findings from our own studies investigating human BMSC (hBMSC) osteogenesis somewhat contradict this model. We could show that hBMSCs induced to undergo osteogenic differentiation were reliant on functional HTRA1 and that when added in excess, could greatly enhance osteoblast formation and mineralized matrix deposition. Although the exact mechanisms by which HTRA1 mediated its osteoanabolic effects were not fully investigated, strong evidence was given implicating HTRA1-dependent HTRA1 is regarded as having a negative role in both IVD and joint degeneration through its direct and indirect effects on ECM components and MMP production, respectively. HTRA1 is therefore strongly implicated in the pathology of spine and joint disease alike. The ability of HTRA1 to dictate BMSC lineage commitment in favor of osteogenesis may impart to it a positive role in the maintenance of bone quality and development of age-related bone loss. The up-regulation of HTRA1 in degenerating muscle, along with its capacity to alter the activation status of specific growth factors (GFs) involved in regulating muscle growth, are suggestive of a role in regulating muscle disease. However, it remains unknown as to whether HTRA1 acts as a negative or positive mediator of muscle quality.

**Figure 1** Proposed scenario for the influence of HTRA1 on the musculoskeletal system and its contribution to MSD. HTRA1 is regarded as having a negative role in both IVD and joint degeneration through its direct and indirect effects on ECM components and MMP production, respectively. HTRA1 is therefore strongly implicated in the pathology of spine and joint disease alike. The ability of HTRA1 to dictate BMSC lineage commitment in favor of osteogenesis may impart to it a positive role in the maintenance of bone quality and development of age-related bone loss. The up-regulation of HTRA1 in degenerating muscle, along with its capacity to alter the activation status of specific growth factors (GFs) involved in regulating muscle growth, are suggestive of a role in regulating muscle disease. However, it remains unknown as to whether HTRA1 acts as a negative or positive mediator of muscle quality.
matrix protein modulation as being a causative factor. In direct contrast to its influence on hBMSC osteogenesis, HTRA1 was also shown to act as a negative regulator of hBMSC adipogenesis and may therefore additionally represent a key mediator of fat marrow adiposity. If true, this may have significant implications in terms of the potential of HTRA1 as a therapeutic target for treating osteoporosis, where such imbalances in bone and fat marrow are wholly evident. One could even contemplate a scenario where deficiencies in HTRA1 activity may in fact be part of the underlying pathology. However, it still remains to be seen whether abnormalities in HTRA1 regulation can be linked to osteoporosis development. Therefore, although further studies are necessary to define its exact role in bone pathology, the majority of evidence to date points toward HTRA1 as having a positive influence on bone formation.

Muscular Dystrophy

The degenerative muscle disease Duchenne muscular dystrophy (DMD) affects 1 in 3500 males and is due to a mutation in the dystrophin gene, leading to a high susceptibility to skeletal muscle injury in patient sufferers. This results in the degeneration of muscle fibers and their subsequent replacement by adipose and fibrous tissue. The pathophysiological pathways governing these downstream events are undoubtedly complex and overlapping, although some insights into these processes have already been gleaned through the use of microarray analysis. In one such study, HTRA1 gene expression was found to be increased by 4.4- to 5.5-fold in DMD muscle as compared to normal control tissue. Although no functional analyses were performed in this study, it was speculated that high levels of HTRA1 within dystrophin-deficient muscle may actually contribute indirectly to muscle degeneration through inactivation of IGFBP5, a well-recognized HTRA1 substrate. IGFBP5 is considered to be a positive regulator of muscle regeneration through its ability to augment insulin-like growth factor-1 (IGF-1) signaling. As such, down-regulation of IGFBP5 activity by HTRA1 may have negative functional consequences on the potential of IGF-1 to stimulate muscle growth. However, it is also conceivable that HTRA1 could act to counter the effects of dystrophin deficiency in muscle tissue. This notion is based on the ability of HTRA1 to inhibit the activities of several members of the transforming growth factor-β superfamily, including bone morphogenetic proteins, activin, and growth differentiation factors, and the suggestion that transforming growth factor-β signaling is causally linked to defects in myogenesis and the subsequent development of DMD. Based on previous observations regarding the influence of HTRA1 over mesenchymal stromal cell differentiation, investigations into HTRA1’s involvement in myogenic differentiation may therefore represent a logical next step when considering how best to elucidate the role of HTRA1 in DMD development and progression.

Concluding Remarks

Despite increases in our understanding of the pathophysiological processes governing the majority of the most debilitating MSDs, new and more efficacious treatment regimens are still needed. The fact that HTRA1 has been implicated as an important contributory factor in the pathogenesis of several MSDs, including RA, OA, and IVD degeneration, suggests that it may well represent a novel therapeutic target for the treatment of such diseases. However, the potential for HTRA1 to deliver beneficial effects in other closely related MSDs, such as osteoporosis and DMD, should give pause for thought in terms of how treatment strategies are to be designed and implemented (Figure 1). As previously mentioned, MSD encompasses a wide variety of pathological conditions affecting numerous different skeletal tissues. It is highly likely therefore that the effects of HTRA1 in MSD extend beyond what has been discussed in this Mini-Review. Certainly, the promiscuous nature of the substrate selectivity of HTRA1 leaves open the possibility of it being a central player in a variety of diseases involving modifications of the ECM. Future consideration should therefore be given to other major MSDs where alterations in matrix turnover and composition are contributing factors to disease development. We propose tendinopathy as being one such MSD, which, as far as we are aware, has not yet been linked with changes in HTRA1 production. However, new studies may be warranted due to the close association between tendinopathy and other MSDs such as OA, in which HTRA1 is considered a key player. On the basis of the information presented in this Mini-Review, we are in no doubt that the number of MSDs linked to HTRA1 production and/or activity will continue to rise, although it remains uncertain as to whether HTRA1 will be deemed beneficial or detrimental to disease pathology.

References


