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Minireview

The important role of matrix metalloproteinases in nematode parasites

M. AZAMI[1], M. ANVARINEJAD[2*], M. JAMSHIDIAN GHALESEFIDI[3]

[1]Skin Diseases and Leishmaniasis Research Center, Isfahan University of Medical Science, Isfahan, Iran;
[2]Professor Alborzi Clinical Microbiology Research Center, Nemazee Teaching Hospital, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, E-mail: *anvarinejad@yahoo.com*;
[3]Moddares Hospital, Isfahan University of Medical Sciences, Isfahan, Iran

Summary

Matrix metalloproteinases (MMPs) represent a large family of over twenty different secreted or membrane-bound endopeptidases, involved in many physiological (embryogenesis, precursor or stem cell mobilization, tissue remodeling during wound healing, etc.), as well as pathological (inflammation, tumor progression and metastasis in cancer, vascular pathology, etc.) conditions. For a long [illegible] MMPs were considered only for the ability to degrade extracellular matrix (ECM) molecules (e.g., collagen, laminin, fibronectin) and to release hidden epitopes from the ECM. However, expressions of many MMPs have been associated with several pathological conditions. It has been established that the MMPs are conserved through animal kingdom and studies of invertebrates have demonstrated that primarily they are involved in various developing functions in hydra, *Drosophila*, sea urchin and nematodes. The syntheses of these proteolytic enzymes and their release as excretory and secretory products have been reported in various parasitic nematodes. Host invasion and tissue migration of several nematodes have been linked to the expression and release of parasite-derived proteases. Studies on enzyme inhibitors suggest that the enzyme may be a metalloproteinase. Moreover, substrate impregnated zymographic analysis of extracts and excretory and secretory products of different nematode parasites have revealed the multiple enzyme activities of MMPs with various molecular weights. More research on MMP degradome in nematode parasites can provide valuable information for intense evaluation of pathogenesis caused by these parasites.

Keywords: matrix metalloproteinase; endopeptidases; nematode parasites

Introduction

Matrix metalloproteinases (MMPs) are a family of multidomain $Ca2^{+}$-dependent and $Zn2^{+}$-containing endopeptidases, strictly related, which can degrade almost all components of the extracellular matrix (ECM), but also non-matrix proteins (Visse & Nagase, 2003). It has been established that various biological processes including cell migration, wound healing, tissue differentiation, immune system have been accomplished by morphological changes in extracellular matrix (Parks & Shapiro, 2000).

MMPs are the major group of enzymes with ability for degrading extra cellular matrix proteins and this process of ECM remodeling is responsible for all these necessary processes (Shapiro, 1998). These proteins may be found from bacteria to advanced forms of life even in several viruses and have been considered as a group of highly conserved proteins throughout the course of evolution (Clark, 2001).

These enzymes seem to play various functional roles and touch many aspects of physiological and pathological processes not only in vertebrates but also in invertebrates (Mannello *et al.*, 2005). The parasitic nematodes are the major group of invertebrates, which dwell in different organs of vertebrate hosts (Anderson & Anderson, 2000). Many of these parasites to complete their life cycle have to migrate or invade through various organs of their hosts. During this journey, expression and release of MMPs by various parasitic nematodes have been associated with the pathology resulting from histolysis (Williamson *et al.*, 2006). Genetic analysis and cDNA cloning of MMPs have been evaluated in few parasitic nematodes (Salter *et al.*, 2002; Uparanukraw *et al.*, 2001; Wang *et al.*, 2001).

The aim of this review is to focus the attention the role played by MMPs in the pathogenesis process that caused by nematode parasites.

MMPs and nematode parasites

Metalloproteinases comprise a heterogeneous group of proteolytic enzymes whose main characteristic is the utilization of a metal ion to polarize a water molecule and perform hydrolytic reactions. MMPs are a major group of zinc-dependent endopepdidases with a ability to cleave one or more extracellular matrix constituents as well as non-matrix proteins (Williamson *et al.*, 2006). These enzymes include a wide range of proteases in many organisms and play essential roles in multiple biological processes. By virtue of these proteolytic property proteases regulate a variety of cellular processes such as cell proliferation, cell-cycle progression, tissue differentiation and migration, apoptosis, senescence, DNA replication and autophagy (Yoshizaki *et al.*, 2002). In metazoans, proteolytic activities are also involved in the maintenance of tissue homeostasis and in the regulation of different physiological processes such as fertilization and fecundation, embryonic development, wound healing, tissue remodeling, immune response and angiogenesis (Ugalde *et al.*, 2010). However, expressions of some of the MMPs have been associated with different pathological manifestations, which are predominantly associated with inflammation, arthritis, cancer metastasis and parasitic invasion through host's tissues (Aggarwal *et al.*, 2006; McDonnell *et al.*, 1992; Ugalde *et al.*, 2010). MMPs can be categorized as collagenases, gelatinases, elastases, stromelysins, matrilysins, and membrane-type MMPs based on their substrate specificity and their amino acid sequence similarity (Hijova, 2005; Nagase *et al.*, 2006).

Life cycles of many nematode parasites consist of different migratory as well as invasive larval stages in host environment, even sometimes young and adult may be invasive in their nature (Lai *et al.*, 2005b). Host invasion and tissue migration of several nematodes have been linked to the expression and release of parasite derived proteases. In nematodes, MMPs are the proteases which are thought to play an important and essential role in tissue migratory and invasive phenomena (Babu, 2012). Nematode MMPs generally include collagenases, gelatinases and elastases (Healer *et al.*, 1991; Matrova *et al.*, 1998).

Most of researches evaluated MMPs from excretory and secretory products as well as the extracts of parasites (Lai *et al.*, 2005b). Initial period of MMPs research in nematodes include detection and measurement of activity in different life stages of nematode parasites. Researchers determined collagenilytic activity in extracts of adult worms, in live microfilariae of *Onchocerca volvulus* and in live infective larvae and adult female worms of *Brugi malayi* (Petralanda *et al.*, 1986). In culture, infective larvae of *B. malayi* also secreted large amounts of collagenase. Studies with enzyme inhibitors, antigen-antibody reactions and immunoprecipitation indicated that this protease might be a metalloproteinase. In another study scientists did characterize this metalloproteinase in *Toxocara canis* by using different techniques (Lai *et al.*, 2005a).

Various forms of enzyme have been determined by zymography where they found two bands with molecular weights of 120 and 32 kDs. This group has optimum activity at pH 9 with less activity at pH 5 and 7. As a potent inhibitor Phenylmethylsulfonyl fluoride was used to characterize the metalloproteinase nature of these proteins (Takino *et al.*, 1995).

Third stage larva of *Strongyloides stercoralis* have an ability to migrate through tissue at a speed of 5 – 15 cm per hour (Greaves *et al.*, 2013) that this process of migration was facilitated by a metalloproteinase with elastase activity. This invasive property of elastase has also been determined by inhibition study and authors indicated that the parasitic virulence factor may be regulated by metalloproteinase and could be a molecule for therapeutic purpose (Gomez Gallego *et al.*, 2005). In another study inhibitor as zinc-chelator like 1, 10-phenanthroline has been used to prove that proteolytic activity of MMP (Hawdon *et al.*, 1995). However the study concentrated on the role of MMPs in transition from free living to parasitic mode of life. In 1990 Hotez and colleagues published a work including the study of metalloproteinase from a human hookworm species *Ancylostoma duodenale and* and zoonotic species *A. caninum* and they found that these two species could synthesis MMPs that have similar molecular weights (68 and 25 kDs) and might cause tissue degradation and ecdysis (Hotez *et al.*, 1990). In their study, radiolabelled fibronectin has been degraded by both species of *Ancylostoma* larvae, which could be corroborated with the activity of MMPs found in *S. stercoralis* larvae and *Serratia marcescen* (Hotez *et al.*, 1990). Healer and colleague also (Healer *et al.*, 1991) characterized the zymogen form of MMP in larval somatic extract. However, it has been found that uninfected rat intestinal tissue has similar kind of protein with identical molecular weight, suggesting the uptake of this metalloproteinase by the parasite.

A high pressure liquid chromatography technique has been used to purify a zinc metalloproteinase, isolated from in vitro culture fluids of *Trichuris suis* adults (Hill *et al.*, 1993). Determination of isoelectric point and immunohistochemistry of MMPs has also been done in this experiment.

Analysis of tissue extracts of excretory and secretory products of *Angiostrongylus cantonensis* showed the activity of different form of MMPs (Lai *et al.*, 2005b). They examined gelatinase activities from extracts of L1, L3, young and adult stages of this parasite. Five gelatinase bands (94, 86, 66, 42 and 30 kDs) were observed in L3 larval stages, and minimum number of bands (94 and 72 kDs) was seen in young and adults. All these bands of various stages showed common characteristics with MMP, cysteine and serine proteinase. Zymographic bands of L1 (105, 94 and 42 kDs) and L3 (105, 66, 50 and 30 kDs) excretory and secretory products were proved to have proteolytic activity whereas adult and young excretory and secretory products had no gelatinase activity. Of these bands obtained from larval stages, 94 and 105 kDs were gelatinase and others were metalloproteinases. In this experiment about 2000 larvae were used to detect enzyme activity while to another

experiments 60 larvae were used to infected mice and MMPs activity was determined (Lee *et al.*, 2004). This differentiation led to suggest that MMPs were secreted by the host not by parasite. Simultaneously, several authors provided information related with the activities of MMPs in different helminthes and MMP mediated histolysis of skin and intestinal walls (Tort *et al.*, 1999) and degradation of ECM proteins (Petralanda *et al.*, 1986). It has been evident that after ingestion, *A. cantonensis* larvae could invade and penetrate host stomach or intestinal wall (Lai *et al.*, 2004). Lai and group ultimately suggested that MMPs secreted by larvae could be associated with parasite spreading and pathogenesis in host (Lai *et al.*, 2005b).

In developing countries 740 million people are infected with hookworms (Hotez *et al.*, 2004). In most of the cases hookworm infect took place by penetrating host's skin and after getting inside of host, larvae migrate by invading tissues of various organs (Hotez *et al.*, 2004). Proteases have been considered as invasive arm for tissue penetration by parasitic helminthes and many experiments revealed that characterization of protease enzyme activity has been evaluated from larval, young and adult crude extract as well as excretory and secretory products of many parasites (Lai *et al.*, 2005b).

Williamson et al. (Williamson *et al.*, 2006) did an experiment where they found an astacin-like metalloproteinase (Ac-MTP-1) activity in excretory and secretory product of *A. caninum* L3 larvae. This Ac-MTP-1 has a sequence similarity with zinc-metalloproteinase. L3 larval stage of *A. caninum* can exclusively express Ac-MTP-1and its activity in culture medium indicates its role in host tissue invasion.

In this study, activity of metalloproteinase has been proved by native and recombinant enzymes and using immunoelectron microscopy, site of synthesis in secretory granules of the glandular oesophagus of L3 and its course of transportation to cuticle and followed by secretion from cuticle have been evaluated. More recently an orthologous of Ac-MTP-1 known as Ay-MTP-1 has also been detected in *A. ceylanicum*. This group of scientists suggested a probability of using MTP as the target molecule for developing vaccine to prevent larval migration through tissues.

Similar kind of metalloproteinase has been characterized from sheep barber's pole worm, *Hemonchus contortus* (Yatsuda *et al.*, 2006). However, no recombinant enzymes have been used in this study.

In a recent study, scientists have used the technique immunoscreening with the monoclonal antibody to synthesize a cDNA clone from L3 larvae of *Gnathostoma spinigerum*. From this clone, they have identified a gene of 732 bp encoding a metalloproteinase having 33 – 39 % similarity with MMP of *Caenorhabditis elegans* (Uparanukraw *et al.*, 2001).

From above discussion it has been assumed that parasitic nematodes have the ability to modify or degrade host's extracellular matrix by secreting metalloproteinases. This process of invasive nature assists to parasites that migrate through different host tissues and causing pathogenesis. Also, our knowledge related with characterization as well as pattern of expression of genes of these proteases in nematode parasites is not satisfactory.

In conclusion, it has been found that characterization of MMPs in parasitic nematodes have been evaluated but not with specific and sufficient information. Still we don't have enough knowledge about the different MMPs in many other of nematode parasites. Present study needs much attention towards more intense morphological and functional characterizations of gene or gene family of MMPs and all these studies related with parasitic helminthes secreting metalloproteinases. Study of gene family can help to investigate more intensively the pathogenicity as well as to develop anti-metalloprotease drug to combat against helminthic parasites.

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