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REVIEW

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Role of Cathepsin B in *Schistosoma japonicum* Infection

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Abstract

Proteases, including cysteine-, serine-, threonine-, aspartate-, and metallo-proteases, are a group of enzymes that have the catalytic ability to hydrolyze or digest peptide bonds of proteins. Proteases play important roles in host invasion, hemoglobin degradation, transformation and immune invasion of schistosomes. The gut cathepsin B of all schistosomes including *S. japonicum*, *S. mansoni* and *S. haematobuim* is involved in hemoglobinolysis. Since *Schistosoma japonicum* cercariae show quicker migration through host skin as compared to *S. mansoni* and *S. haematobium*, it has been proposed that cathepsin B residing in the acetabular gland of *S. japonicum* might be one of the factors that facilitates its rapid skin penetration. This review focuses on the potential roles of cathepsin B for hemoglobin degradation and skin penetration of schistosomes. Defining the role of cathepsin B might be useful in identifying the drug target for schistosomiasis.

Keyword: *Schistosoma japonicum*, cathepsin B, skin penetration

Mechanisms of host penetration in schistosomes

Three major schistosomes, *Schistosoma mansoni, S. japonicum* and S. *haematobium*, are the blood parasites of humans and all have a complex life cycle, i.e., snail intermediate host is required in addition to its definitive host. For *S. japonicum*, humans and other definitive hosts are infected by cercariae which are shed from freshwater snail intermediate hosts. The cercariae in water infect the host by direct skin penetration and rapidly transform into schistosomules, a larva stage, in the blood circulation. It takes several days for the cercariae to pass through the skin, enter the blood vessels and migrate to the heart and the lungs. In the lungs, the schistosomules undergo developmental changes into young adults. Then, young adults move to the liver where they feed on red blood cells, develop into an adult worm and find a partner. After pairing-up, male and female worms migrate in pairs to the mesenteric vein of the small intestine for egg production. The eggs penetrate to lumen of intestines via small venules and are passed to the external environment in the feces [1].

For initiation of the host penetration process, the cercariae attach to the host skin using their ventral suckers. The schistosome cercariae have a particular head organ in the anterior part that can extend and pull back slightly. The head organ of cercariae has three types of gland cells where secretory vesicles are found: the acetabular gland; the head gland; and the sub-tegumental

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cell bodies [2]. These glands are important for host penetration and parasite transformation. The acetabular gland contains abundant cercarial protease enzymes that facilitate skin penetration [3]. The specialized movement of the head organ and the secreted protease enzymes from the head gland and acetabular gland assist in the access of the parasite through the host skin in the initiation process [3-6]. The migratory pattern of cercariae occurs after attachment, at which point the cercariae lose their tails and transform into schistosomules at the stratum corneum layer which is the outer layer of the epidermis. This layer is an aquatic environment and is surrounded by lipid lamellae; therefore, the cercariae can-easily pass between the cells of this layer without requiring any protease enzymes. When cercariae reach the stratum spinosum, the middle part of the epidermis, they must pass through condensed cell interactions. Thus, the secreted protease enzymes and cercarial elastase (serine protease) from the acetabular gland are required for the disruption of this epidermis layer [7-9]. However, the movement of cercarial penetration is retarded when they access the epidermal basement membrane, the interactive protein matrix barrier. Finally, the basement membrane protein matrix must be digested via the proteolytic activity from enzymes in the acetabular gland. Then, the cercariae migrate through the extracellular matrix of the dermis by using the activity of secreted protease enzymes that degrade collagen and elastin [6,10]. After entering the dermis, the cercariae must encounter small venules or lymphatic vessels to exit into the vascular space (Fig. 1). In addition, during penetration into a small venule or a lymphatic vessel, the enzymes from the acetabular gland are required for disruption. *In vivo* experiments showed that the cercariae reached the epidermal basement membrane within 30 minutes and the cercariae have no problem disrupting this layer. After that, the cercariae are retained in the basement membrane because of the laminin and collagen networks that cannot be digested by the secreted enzymes from the acetabular gland [11,12]. Therefore, the acetabular gland contents may not be involved in the process of basement membrane barrier digestion. Thus, head gland hydrolytic activity may play an important role in the mechanical disruption of the basement membrane and assisting the access of parasites to a blood vessel because it has been found in both cercariae and schistosomules. The mechanisms of skin penetration of the schistosome in various hosts seem to be different during these processes, including the duration of host penetration and the presence of enzyme.

Skin migratory patterns of three species of schistosomes

Skin migration is an initiation step for host infection of schistosomes. This step requires many factors such as host searching mechanism and type of head gland enzymes. Although three major schistosomes show different patterns of skin penetration, most of them successfully infect their hosts and can complete their life cycles. The cercariae of schistosomes can enter the host skin within minutes. After 2 hours of exposure on the host skin, more than 50% of schistosomules of *S. japonicum* are present in the dermis of both humans and mice, and almost 100% of the parasites are not observed in the mouse epidermis after 24 hours [12,13]. The migration behavior of *S. japonicum* through human skin shows a similar pattern to that of mouse skin, but the schistosomules are slightly delayed during migration through the human epidermis. On the other hand, *S. japonicum* migrates through host tissue and exits blood vessels and the lung faster than *S. mansoni* and *S. haematobium*, and develops into adult stage earlier. It was reported that *S. japonicum* lays eggs within 24-27 days after infection, whereas 30-35 and 60-63 days are required by *S. mansoni* and *S. haematobium*, respecvtively [14,15].

The skin penetration pattern of *S. mansoni* cercariae through host skin seems to be distinct from those of *S. japonicum*. After 8 hours of human skin exposure, 94% of *S. mansoni* schistosomules are present in the human epidermis, while nearly 59% are present in the skin epidermis of

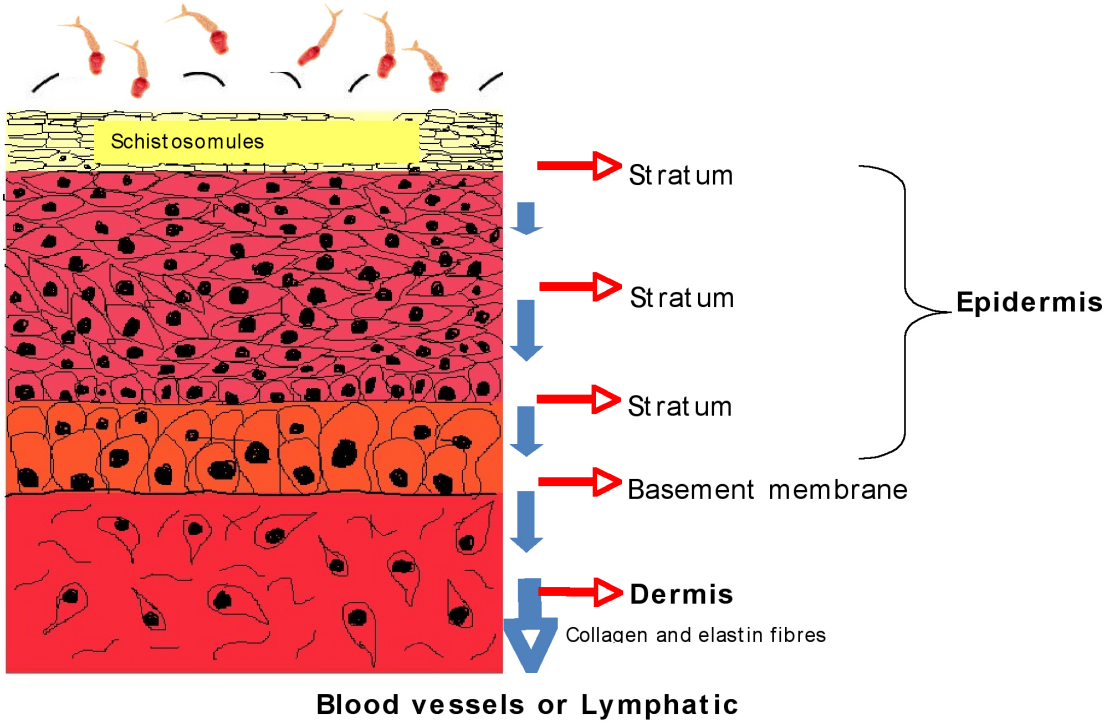

Fig 1 Skin penetration processes of schistosomes [Skin penetration occurs after the cercariae attach to the host skin and transform to schistosomules in the stratum corneum, the outer layer of epidermis. Then, the schistosomules can easily pass though the epidermis; however, their migration is retarded in thebasement membrane layer. Thus, protease enzymes are required for the disruption of matrix proteins. After that, schistosomules migrate to the dermis and exit to blood vessels or lymphatic vessels, respectively.]

mice. Examination at 24 hours after infection showed that approximately 89% of the *S. mansoni* schistosomules are still in the epidermis of human skin. The schistosomules reached the dermis within 48 hours after infection, with 68% and 60% of schistosomules being found in both mouse and human skin samples, respectively. At 72 hours after infection, 90% (mouse skin) and 71% (human skin) of schistosomules are observed in the dermis, mostly close to the blood vessels. It has been indicated that the migration of *S. mansoni* schistosomules through human and mouse epidermis is slower than that of *S. japonicum* [16]. On the other hand, skin penetration patterns of S. *haematobium* schistosomules in humans and hamster are similar to those of *S. mansoni*. Almost 93% of *S. haematobium* schistosomules could be found only in the epidermis 8 hours post cercariae exposure in human skin. Approximately 48-72 hours after infection, the majority of *S. haematobium* schistosomules enter the dermis and, within 72 hours, several of these schistosomules are close to the blood vessels [15,17]. Thus, schistosomules of *S. mansoni* and *S. haematobium* take nearly three days to accomplish skin penetration, whereas schistosomules of *S. japonicum* complete skin penetration in just one day.

During skin penetration, not all cercariae successfully penetrate the host skin and some even die during this process. Interestingly, the death of schistosomules during skin penetration is significantly lower in *S. japonicum* (7%) compared with that in *S. mansoni* (28-43%) or *S. haematobium*

(30%) indicating that *S. japonicum* has better fitness than other schistosomules species, which may enhance its ability to infect various types of host [18,19]. From the above evidence, it is apparent that *S. japonicum* is more successful in host skin penetration than other species because the duration of skin penetration is shorter and the death rate of schistosomules is lower as compared to other species. This may depend on the different types of secreted enzymes from the acetabular gland and mechanical processes during skin penetration. During *S. mansoni* skin penetration, serine protease has been characterized as the major protease from the acetabular gland secretion which is implicated in the invasion process. On the contrary, *S. japonicum* processes cathepsin B as a major protease of the acetabular gland. Thus, cathepsin B is most likely to be a major protease enzyme that assists with the skin penetration of this species. The important role of cathepsin B in skin penetration of *S. japonicum* has been proposed and needs to be further investigated together with other cysteine proteases.

Cysteine protease in schistosomes

Cysteine proteases are an important protease enzyme for schistosomes, the activities of which are involved in immune evasion, transformation, and tissue invasion [20]. Cysteine proteases have a cysteine residue in their active site that is essential for hydrolysis. Cysteine proteases obtained from parasites are divided into two major groups: papain-like or Clan CA and legumain-like or Clan CD. Papain-like, or Clan CA proteases, are further divided into family C1 (cathepsin B and cathepsin L-like) and family C2 (calpain-like) which are most important parasite proteases [21].

Papain-like protease**s** of Clan CA, such as cathepsins B, C, H, K, L and S, are important digestive enzymes in the gut of schistosomes and are the main peptidase for hemoglobin degradation. These biological functions of Clan CA in schistosomes are characterized by the use of inhibitors and substrate specificity identification. The results of investigations showed that the cysteine proteases Clan CA family C1 (cathepsins B and L) are the major gut localization enzymes and cathepsin B may associate with skin penetration of schistosomes. However, these findings need further investigation. Thus, this review will concentrate on the cathepsin B cysteine proteases which are the most abundant proteases in the gut and acetabular gland of human schistosomes [21,22].

Asparaginyl endopeptidases (AE) or legumain-like cysteine proteases are found in Clan CD. Recently, legumain-like proteases have been reported in plants and mammals including humans, pig, rat, and mouse. Legumains are shown to be localized in lysosome-like compartments and may help in *trans*-processing of other proteins [23,24]. Moreover, both hepatic fluke (*Fasciola hepatica*) and blood fluke (*S. mansoni*) have been reported to process these proteases for their nutrient acquisitions. In *S. mansoni*, the asparaginyl endopeptidase is detected in the gut gastrodermis and the parasite lumen. The biological functions of AE in schistosomes may not be directly associated with hemoglobinolysis but may be involved in the *trans*-activation of other gut digestive peptidases, including papain-like proteases.

Role of cathepsin B in schistosomes

Structure of cathepsin B protease in schistosomes

Cathepsin B is a lysosomal cysteine protease belonging to Clan CA and family C1, and is the major protease of many parasites. In schistosomes, cathepsin B is most abundant in the gut and is important for hemoglobin degradation in the process of nutrient acquisition [25]. Moreover, cathepsin B is the major enzyme in the acetabular gland of *S. japonicum,* and, therefore, may play an important role in skin penetration by degrading macromolecular barriers of the host tissue [6,26]. The structures of schistosome cathepsin B and human cathepsin B are very similar. The cathepsin B of schistosomes was first identified in *S. mansoni* (SmCB1) which was demonstrated by its migration at 31 kDa on SDS-PAGE [27,28]. Moreover, a unique motif (YWLIANSWxxDWGE)

in cathepsin B enzymes has been identified in blood-feeding helminthes, including in cathepsin B of schistosomes, which is proposed to function in hemoglobinolysis [25]. SmCB1 is formed from the folding of a single polypeptide chain and the molecule is divided into L and R domains. The active site contains the catalytic residues Cys-100, His-270, and Asn-290, which are spanned between both domains. In addition, the unique characteristic of cathepsin B is an occluding loop, which is an inserted peptide loop in the active site. The occluding loop (residues 175–194) contains two histidine residues (His-180 and His-181) that are important in substrate-enzyme interactions [29]. The property of the occluding loop has been attributed to dipeptidylcarboxy peptidase or exopeptidase but endopeptidase activity has also been found for this enzyme using the synthetic peptides; for example, benzoyl-glycinyl-histidinyl-leucine (Bz-Gly-His-Leu) as a substrate of dipeptidylcarboxy peptidase. Exopeptidases digest proteins from the N- or C-terminal amino acids, whereas endopeptidases digest internal peptide bonds [20,29]. For exopeptidase activity, two adjacent histidine residues in the occluding loop are required. The histidine residues bind to the C-terminal carboxyl group of the substrate and move the C terminal dipeptide into the active site for hydrolysis. The actions of two histidine residues in the occluding loop are pH-dependent, and occur mainly at acidic pH, with the function of the histidine residues being diminished at neutral pH [20,22,30]. The occluding loop of cathepsin B makes it less susceptible to the inhibitors as covers its active site. Thus, substrate specificity analysis for the active site of cathepsin B may contribute towards a better understanding of its structure, which is important for inhibitor development and drug targets.

Processing procathepsin B of schistosomes

Procathepsin B may require other enzymes, such as asparaginyl endopeptidase and cathepsin C, and optimal pH for its activation to the mature form. The preproenzyme of cathepsin B is composed of 340 amino acids, and is processed into a mature enzyme of 250 residues [31]. The molecular mass prediction from the amino acid sequence of cathepsin B is about 28 kDa. However, a product of 31 kDa is apparent on SDS-PAGE, which is larger than the predicted size. This may be due to the N-linked glycosylation of the mature cathepsin B. *In vitro,* procathepsin B cysteine protease could not be activated suggesting that this enzyme may not undergo autocatalytic activity but is processed by another protease. To date, the *S. mansoni* asparaginyl endopeptidase (SmAE) has been reported to have endopeptidolytic action and contain autocatalysis at acidic pH (23, 32, 33). Autocatalytic activation of asparaginyl endopeptidase orthologs was also observed in plant and mammalian species and the activation process is similar to that of the SCB1proenzyme. SmAE has been hypothesized to *trans*-activate other zymogens such as SmCB1 and SmCL1 to their mature catalytic forms. *In vitro,* the SmCB1 proenzyme was rapidly converted to its mature catalytic form by SmAE. Moreover, rat cathepsin L can process SmCB in the final step to complete mature SmCB *in vitro*. Therefore, this enzyme may also be important for cathepsin B processing *in vivo* [24,32]. Although catalytic activity of these digestive peptidase enzymes is important for hemoglobin degradation and nutrient acquisition in schistosomes, this action depends on optimal pH, substrate specificity and susceptibility to host protease inhibitors. Thus, their functions in hemoglobin degradation should be extensively investigated to develop drugs or vaccines for schistosomiasis.

Functions of cathepsin B as a digestive peptidase in schistosomes

Schistosome digestive peptidase enzymes are essential for host hemoglobin degradation, and include endopeptidase enzymes, such as cathepsins B, D and L, and exopeptidase enzymes such as cathepsin C (20). Schistosomes use digestive peptidase enzymes for digesting host erythrocytes to obtain hemoglobin and convert this into amino acids that are important for growth, development, and reproduction. All

digestive peptidase enzymes are synthesized as zymogens and are activated through autocatalytic activity or by acidic pH in the schistosome gut. All digestive peptidases have a low pH optima for their actions with either synthetic peptidyl substrates (generally at pH 5.0-6.0) or protein substrates, including hemoglobin (at pH 3.5-5.0) [25]. Most studies have focused on the cathepsin B cysteine protease from *S. mansoni*, but orthologs of this enzyme also have been identified in other schistosome species. The cathepsin B proteases of schistosome have proteolytic activity like that of mammalian cathepsin B, which has been reported to be associated with tumor cell invasion and inflammatory diseases. Following SCB1 identification in schistosomes, the second major cathepsin B protease (SmCB2) was identified with 49% identity to SmCB1 and proteases showed high immunogenicity in infected humans and mice [34]. The cathepsin B of schistosome or Sm31 is more abundantly expressed than other proteases in the gut and gastrointestinal contents (GIC). Therefore, SmCB1 is believed to be secreted into the gut of the parasite, while SmCB2 is not present in the gut but is found at the outer surface of tegument and body tissue. As mentioned above, cathepsin B in schistosomes has optimal pH values for its activity, ranging from acidic to neutral pH [24,25]. This evidence strongly supports the suggestion that cathepsin B protease could function effectively both in the gut of flukes, which has a slightly acidic pH, and in the skin, which has neutral pH. Although a major function of cathepsin B is associated with hemoglobin degradation in the gut of schistosomes, it has been reported that aspartic protease and other cysteine proteases such as cathepsin L are associated with hemoglobin degradation in schistosomes [25,35,36]. Moreover, cathepsin D, an aspartic protease, has been identified in *S. japonicum* and its function has been shown to be involved in host hemoglobin degradation [35]. Therefore, it is not surprising that other proteolytic enzymes have also been found for the degradation of hemoglobin and other blood component in schistosomes.

In addition to its role in hemoglobin degradation, the cathepsin B cysteine protease was reported to be the major protease in the acetabular gland of S. *japonicum* and is expressed at levels that are 40-folds greater than in *S. mansoni* [6]. Thus, *S. japonicum* may use cathepsin B during skin penetration, while *S. mansoni* uses serine protease (elastase) for the same purpose. However, the functions of cathepsin B in the acetabular gland secretions are still unclear. Although the cercariae of *S. japonicum* do not contain a serine protease, *S. japonicum* takes less time than *S. mansoni* for skin penetration. This may be due to a different mechanism of skin penetration and different enzymes in the acetabular gland that facilitate skin penetration in the hosts, such as cathepsin B.

Biological functional studies of cathepsin B in schistosomes using inhibitors and the RNAi pathway

Cathepsin B of schistosomes is a gut-associated peptidase that has been investigated for drug and vaccine development. Recently, inhibitors were used to study the biological functions of cathepsin B both *in vitro* and *in vivo*. The cathepsin B inhibitors included CA074, peptidylfluoromethyl ketones, peptidy1 (acyloxy) methyl ketones and vinyl sulfones [20,37]. An *in vitro* study showed that most inhibitors are highly effective for the reduction of hemoglobin degradation in cultured schistosomules. Moreover, the newly transformed schistosomules died 3 days after incubation with vinyl sulfones. However, these inhibitors have not been studied to determine their effects in animal models [29]. The inhibition of cathepsin B and cathepsin L activities using peptidy1 (acyloxy) methyl ketones and peptidy1 (acyloxy) methyl ketones was investigated in mice. These inhibitors reduced the schistosomules burden by approximately 50%, decreased egg production and hepatomegaly, and eliminated granuloma formation. On the other hand, both inhibitors were less effective for the reduction of the worm burden after incubation with adult worms compared with the effect observed with those of early transformed schistosomules [37]. However,

the consequences of this experiment were not conclusive regarding the functions of cathepsin B because the activity of cathepsin L was also inhibited. Although cathepsin B inhibitors have a high efficacy for killing schistosomules *in vitro*, their activity *in vivo* has not been determined and the result may be beneficial in schistosomiasis treatment.

The other technique that has been used to determine the biological functions of cathepsin B in schistosomes and other helminthes is RNA interference (RNAi) silencing. RNAi post-transcriptionally modulates genes and has been used to investigate the biological functions of gene products in many organisms, including parasitic helminthes. The functions of RNAi include the inhibition of gene expression, anti-viral infection and chromatin modulation. The RNAi pathway processes double-stranded RNA (dsRNAs) which designate the degradation of homologous mRNA. In schistosomes, several developmental stages have been studied with regards to the biological functions of genes using RNAi-induced gene suppression, including miracidium, sporocysts, schistosomules and adult worms [38-44]. Cathepsin B functions were mostly demonstrated in cultured schistosomules. The RNAi-induced gene suppression was used to demonstrate cathepsin B functions on hemoglobin degradation in schistosomes [38-40]. *In vitro* experiments showed that the RNAi mechanism successfully suppressed cathepsin B expression (>80%) in schistosomules culture. The dsRNAs revealed a 10-folds reduction of cathepsin B transcripts and resulted in growth restraint in cultured schistosomules and animal models compared with that of the control group [38]. However, treating schistosomules with the long dsRNA of cathepsin B did not reduce hemoglobin degradation, but 3 hour-transformed schistosomules may be less susceptible to dsRNA. In addition, the newly transformed schistosomules had low levels of cathepsin B expression when compared to schistosomules or adult worms and the cathepsin B expression was slightly decreased (30%) after treatment with long dsRNA [38]. For this reason, hemoglobin degradation may still occur in the gut of schistosome. Moreover, *in vitro* studies of cathepsin B transcript suppression showed that cathepsin B suppression via RNAi silencing seemed to be dependent on the age of schistosomules. Seven day-cultured schistosomules are more susceptible to the long dsRNA of cathepsin B after emergence from the snail than those newly transformed schistosomules [39,40]. In addition, adult worms (49 days) are also more susceptible to dsRNA than newly transformed schistosomules. To understand the process of long dsRNA after electroporation, the fluorescein-labeled dsRNA was used for examination. It was shown that long dsRNA initially enter the caecum of cultured schistosomules (7 days) through the mouth and entered newly transformed schistosomules via the pre- and post-acetabular glands [39]. Thus, the mouth may be essential for long dsRNA delivery by electroporation in schistosomules. Additionally, the delivery method of cathepsin B long dsRNA for newly transformed schistosomules should be further improved because DNA delivery by electroporation slightly decreased cathepsin B expression. The delivery methods of long dsRNA that have been commonly used include soaking, electroporation, liposome solution, feeding and directly injection into a parasite body. It has been reported that the electroporation showed a 100-1000 fold higher efficiency for long dsRNA delivery than that of simple soaking and that the liposome solution did not enhance long dsRNA delivery [40]. In spite of successful gene function determined by the use of long dsRNA in schistosomes, long dsRNA does not function well in many parasites. Besides using long dsRNA, there is strong evidence of siRNA in reducing cathepsin B transcript (>75%) in cultured schistomules [39]. Moreover, successful siRNA-mediated knockdown has been demonstrated in tyrosinase genes, which are important for egg shell protein sclerosis and the serotonin transporter in *S. japonicum* and *S. mansion,* respectively. Therefore, siRNA-mediated knockdown could be an alternative pathway to determine gene function in schistosomes and other parasites. Although, successful RNAi

silencing and inhibitors of cathepsin B have been used to determine the function in schistosomes, the complete suppression of cathepsin B transcript and its activity are still needed. The RNAi silencing and specific inhibitors of cathepsin B can be used to study the function of cathepsin B in skin penetration.

Degradation of extracellular matrix by cathepsin B in schistosomes

Cathepsins are known to be extracellular matrix (ECM) degradation components, and act as the other classes of protease activator (Table 1). Cathepsins are normally expressed with tissue and cell-type specificity. Cathepsins B, L, and H are mostly found in all cell types and tissues and have been involved in non-specific protein degradation in lysosomes. In contrast, cathepsins S, K, V, F, C, and W, are more specifically expressed and exhibited in certain cell type functions [45,46]. Many studies have indicated that cathepsins are involved in ECM degradation, for example, cathepsins B and L have been shown to degrade collagen IV, fibronectin, and laminin components of the basement membrane [45,47]. In addition, cathepsin B contributes to tumor cell proliferation, angiogenesis, invasion and metastasis [47,48]. In schistosomes, ECM will be degraded by secreted protease enzymes from acetabular gland. The serine protease from the acetabular gland of *S. mansoni* has been only described to cleave human skin components [8,9]. In contrast, the function of cercarial cysteine peptidases, cathepsins L1 and B1, in schistosomes are not well understood. However, these enzymes are proposed to be involved in the disruption of the outer keratinized layer of skin. In 2007, Kas et al. reported the activity of the cysteine proteases from cercarial extraction of *S. mansoni* and bird schistosome, *Trichobilharzia regenti* [49]. The peptidases of both parasites were capable of degrading native keratinocytes and collagens (types II, IV), and these peptidases were identified as cathepsin B-like enzymes by specific inhibitors and fluorometric assays for enzyme activity. Thus, the ability of these cysteine peptidases to degrade skin proteins supports their role in skin penetration process. Moreover, the skin penetration pattern of *T. regenti* is similar to that of *S. japonicum*, where cathepsin B was mainly identified from acetabular gland. Moreover, the cathepsins B1 and B2 of *T. regenti* are associated with hemoglobin degradation and host skin penetration [50]. This finding demonstrated that the degradation of keratin and collagen (type II and IV) by cercarial cathepsin B-like protease may support the skin invasion theory. However,

Table1 Types of cathepsin for extracellular matrix protein degradation.

ECM proteins	Cathepsins
Proteoglycan	Cathepsin B. L
Aggrecan	Cathepsin K, L, S
Fibers: collagen	Cathepsin S
Fibrillar (Types I, II, III, IV, XI)	Cathepsin B, L
Basement membrane (Type IV)	Cathepsin L
Other types (VI VII XIII)	
Elastin	Cathepsin S, L, V, K
Fibronectin	Cathepsin B, L, S
Laminin	Cathepsin B, L
Osteocalcin	Cathepsin B, S, L, D
Osteonectin	Cathepsin B, K

Adopted from Brömme and Wilson [45]

the activity of cercarial cathepsin B of *S. japonicum* for skin components degradation should be further investigated in order to support its role in skin penetration.

Conclusion and Future perspectives

Proteases of schistosomes are essential for many biological processes including nutrient acquisition and host penetration. Skin penetration of schistosomes is an initiation process for host infections and there are many factors involved in this process, including host and parasite factors. Serine and cysteine proteases of parasites play an important role in the skin penetration of schistosomules. *S. mansoni* uses serine proteases during skin penetration, while *S. japonicum* may secrete the cathepsin B cysteine protease in this process. As a consequence of the different types of enzyme in the acetabular gland and the process of host penetration, the migratory behavior of schistosomules seems to be different among *Schistosoma* spp. Skin penetration of *S. japonicum* takes less time than that of other species. Since the acetabular gland of this parasite species contains a higher amount of cathepsin B protease, cathepsin B protease is considered the most likely enzyme facilitating skin penetration of *S. japonicum*. However, the factors that help the schistosomules of *S. japonicum* to migrate through the skin more rapidly and become established in the host at an earlier stage are still unknown. Thus, understanding the mechanism of skin penetration will help identifying a potential target for drug development or vaccine development aimed at the initial step of infection (skin penetration). Recently, RNAi-mediated gene silencing has been used as a molecular tool to inhibit gene expression. The successful suppression of cathepsin B transcripts using RNAi in *S. mansoni* promisingly indicates that cathepsin B is essential for hemoglobin degradation, parasite growth, parasite burden and egg production. Thus, it can be employed to investigate cathepsin B protease activity for skin penetration in *S. japonicum* (Figure 1). Moreover, cathepsin B is a major protease that has been reported to associate with hemoglobin degradation in schistosomes. Therefore, this enzyme may be used as a target for anti-schistosomiasis. Although the inhibition of cathepsin B expression did not cause the total death of schistosomes *in vivo*, it might be a target candidate for reducing the morbidity and transmission of schistosomiasis.

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