Abstract: A novel member of the cystatin family, nippocystatin (NbCys), was identified from excretory-secretory (ES)-products of a nematode Nippostrongylus brasiliensis, and the cDNA was cloned and sequenced. The mRNA of NbCys was confirmed to be expressed in both larvae and adults of the parasite. NbCys was translated as a proform with a single domain for secretion and was detected as a 14-kDa mature form in ES-products of the adult worm. Recombinant protein of NbCys profoundly inhibited the activity of cysteine proteases such as cathepsin L and B, but not that of cathepsin D, an aspartic protease. Furthermore, the ES-products had also been confirmed to inhibit cysteine proteases. Taken together, NbCys may play a role in evasion of N. brasiliensis from host defense systems, since cysteine proteases are known to participate in immune systems of infected hosts.

Keywords: Cystatin, Nippostrongylus brasiliensis, cloning

1. Parasites

Nippostrongylus brasiliensis (N. brasiliensis) is a nematode parasitic to humans, and is the most important nematode of the family Strongyloidea. It is transmitted to the human host through the ingestion of contaminated food or water. The life cycle consists of free-living, soil-inhabiting stages of the nematode in which the eggs are laid, and a vertebrate host (primarily humans or other mammals) in which the larvae develop into adults. The parasite is a common cause of giardiasis in humans, and is also responsible for the development of allergic reactions and chronic inflammatory bowel disease.

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2. cDNA cloning of NbCys

Pending work is conducted to clone the NbCys genes from N. brasilienisis and N. brasiliensis. The cDNA library is constructed from the mRNA isolated from the adult worms. The NbCys genes are amplified by PCR using degenerate primers based on the conserved cystatin domain. The amplified products are then cloned into a vector for further analysis. The cloning strategy is shown in Figure 1.

3. Evaluation of cystatin mRNA expression

To evaluate the expression of NbCys mRNA, RT-PCR is performed using total RNA isolated from the adult worms. The NbCys-specific primers are designed based on the cloned sequences. The PCR products are analyzed by gel electrophoresis. The expression level is quantified by densitometry and normalized to the expression of a housekeeping gene. The results indicate that NbCys is highly expressed in the adult worms.

4. Expression of rNbCys

The NbCys genes are expressed in E. coli. The recombinant protein is purified and characterized for its cystatin activity. The results show that the recombinant NbCys has similar enzymatic properties to the native protein.

5. Measurement of protease activities and their inhibition

The protease activities of N. brasilienisis and N. brasiliensis are examined using a substrate specific for cystatins. The protease activities are inhibited by the recombinant NbCys. The IC50 values are determined and the inhibition profile is analyzed. The results suggest that NbCys plays a role in regulating the protease activities in the nematodes.
Onchocerca volvulus  
Acanthocheilonema viteae  
DZTUBUJO  
and Brugia malayi  

C. elegans  
N. brasiliensis  

XBT DMPOFE CZ VTJOH

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The graph illustrates the activity (% of control) of Cat.B, Cat.L, and Cat.D in response to varying concentrations of rNbCys (10⁻⁴ M). The data show a significant decrease in activity with increasing concentration of rNbCys, indicating a dose-dependent effect.

The bar chart compares the activity (%) of ES and BSA at different dose levels (0, 2, 20 μg). The results indicate a higher activity of ES compared to BSA across all dose levels, suggesting a specific interaction with ES.

The data suggest that the activity of Cat.B, Cat.L, and Cat.D is reduced in the presence of rNbCys, while ES shows higher activity compared to BSA.
Cloning of a cystatin from nematode

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A cystatin has been cloned recently from N. brasiliensis and N. brasiliensis is a parasitic nematode that causes strongyloidiasis in humans and other animals. The cloning of a cystatin from this parasite is significant because cystatins play a role in regulating protease activity and therefore the immune response. The cloning of this cystatin provides insights into the immune evasion strategies employed by this parasite.

The cloning process involved the isolation of a cDNA library from N. brasiliensis and the identification of a gene that codes for a cystatin. The gene was then sequenced and its amino acid sequence was determined. This sequence was compared with other cystatin sequences to ensure its identity.

The cloning of a cystatin from N. brasiliensis and other nematodes such as C. elegans and Brugia malayi has opened up new avenues for research into the immune response of these parasites and the development of new treatments for parasitic diseases.
Nippostrongylus brasiliensis が、上記の実験で誘発された寄生虫の感染を示す。実験の過程では、宿主の免疫反応に対して寄生虫がどのように反応するかを観察することで、寄生虫の感染のメカニズムを理解することができる。