ELSEVIER

Fish & Shellfish Immunology 20 (2006) 419–426

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

Short sequence report

Characterization of a NK-lysin antimicrobial peptide gene from channel catfish

Qun Wang [a,b], Baolong Bao [a,1], Yaping Wang [a,2], Eric Peatman [a], Zhanjiang Liu [a,*]

[a] *The Fish Molecular Genetics and Biotechnology Laboratory, Department of Fisheries and Allied Aquacultures and Program of Cell and Molecular Biosciences, Aquatic Genomics Unit, Auburn University, Auburn, AL 36849, USA*

[b] *East China Normal University, Shanghai, China*

Received 3 March 2005; accepted 11 May 2005
Available online 7 July 2005

Keywords: Catfish; Fish; NK-lysin; Granulysin; Antimicrobial peptide; Immunity; Disease

Natural killer (NK) cells and cytotoxic T lymphocytes (CTL) are important and powerful components of mammalian host defenses against a broad range of microbial assaults. These cell types, upon stimulation, are known to release cytolytic granules toward target cells (reviewed in [1,2]). These granules contain proteins, including perforin and the granzyme family, capable of causing membrane perturbation and eventual cell apoptosis. Given the potency and widespread efficacy of these molecules, immunologists have long been interested in capturing and investigating other protein components contained within cytolytic granules. Examination of genes expressed late (3–5 days) after T-cell activation [3] led to the identification of an mRNA initially designated 519. Further characterization of 519 localized its protein to cytolytic granules of CTLs and NK cells and found that it possessed lytic activity against tumor cells [4,5]. The renamed protein, granulysin (reviewed in [6]), was subsequently discovered to have broad lytic abilities against bacteria, fungi, protozoa, and parasites [7–9]. Most notable was the effective killing of intracellular *Mycobacterium tuberculosis* by granulysin in combination with perforin [7].

NK-lysin, the probable porcine homolog of human granulysin, was isolated from pig intestine and possesses similar structure and antimicrobial properties [10–13]. Both granulysin and NK-lysin are

* Corresponding author. 203 Swingle Hall, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849, USA. Tel.: +1 334 844 4054; fax: +1 334 844 9208.
E-mail address: zliu@acesag.auburn.edu (Z. Liu).
[1] Permanent address: College of Aquatic Life Sciences and Technology, Shanghai Fisheries University, Shanghai 200090, China.
[2] Permanent address: Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China.

1050-4648/$ - see front matter
doi:10.1016/j.fsi.2005.05.005

members of the saposin-like protein (SAPLIP) family. Saposins (reviewed in [14]) are involved in sphingolipid catabolism and contain conserved cysteine and hydrophobic residues. Amoebapores, lytic peptides contained within the cytolytic granules of the protozoan parasite, *Entamoeba histolytica*, also fall within the saposin family, demonstrating the conservation of the saposin-like NK-lysin proteins.

A single copy gene for granulysin in humans encodes two alternatively spliced mRNAs, 519 and NKG5 [15]. Because of the shorter peptide sequences of 519, it has been used in much of the follow-up work. The 519 mRNA encodes a 15 kDa protein non-lytic precursor molecule predominantly present at the low pH condition of the cytolytic granules. This precursor is post-translationally processed to a 9 kDa lytic molecule (74 amino acids) which only attains its full activity upon release into the higher pH of the extracellular environment. This mature lytic molecule of 74 amino acids corresponds closely to the conserved SAPLIP domain [4,16]. This processing, therefore, represents a self-protective mechanism for the cell. Similar processing steps result in the generation of the lytic 9 kDa (78 amino acids) NK-lysin molecule from the 129 amino acid precursor in porcine. The active peptide in NK-lysin also corresponds with the SAPLIP domain and shares 35% amino acid identity to granulysin.

Although the mechanism of granulysin/NK-lysin induced cell death is not completely understood, some steps are clear. Granulysin disrupts the cell membrane, perhaps with the aid of perforin [7], increases intracellular Ca^{2+}, and directly damages the mitochondria, leading to eventual cell apoptosis [17–19].

With the emergence of antibiotic-resistant bacteria, and the scarcity of new classes of useful antibiotics, there is an increasing need to identify novel antimicrobial peptides like NK-lysin from various species for the development of alternative therapeutics for both human and animal medicine. However, sequences have been reported from only a minimal number of fish species [20–26], the largest vertebrate group containing over 23,000 species [27].

Although a bovine homolog has recently been identified [13], the status of the NK-lysin/granulysin antimicrobial class in lower vertebrates is unknown. As part of a larger effort to characterize the catfish innate immune system, we report here identification, molecular cloning and characterization, and expression of a NK-lysin gene from channel catfish (*Ictalurus punctatus*).

Three clones of channel catfish NK-lysin cDNA were initially identified from analysis of expressed sequence tags (ESTs) [28]. Sequence alignment revealed that all three cDNAs were transcripts from a single gene. All three clones harbor complete cDNA and they were completely sequenced from both strands by using universal and reverse sequence primers to obtain the full cDNA sequences. High-density filters of a channel catfish BAC library were purchased from Children's Hospital of the Oakland Research Institute (CHORI, Oakland, CA). Each set of filters contained 10-genome coverage of the channel catfish BAC clones from BAC library CHORI 212 (http://bacpac.chori.org/library.php?id=103). As part of ongoing efforts to physically map important genes, an overgo probe was designed based on the shared cDNA sequence and hybridized to the catfish BAC library. Sequences of the overgo primers are shown in Table 1.

Table 1
Primers and their sequences used in this study

Primer name	Sequences (5′ to 3′)
Overgo primers	CCTGTGCAATGCACATGGAATACC
	GCAGAGTCAACTCTCAGGTATTCC
RT-PCR primers	CCTGTGCAATGCACATGGAATACC
	TCTTGCAGAGACCTCGAAGG
PCR Primer for TA-cloning	TGGAACCTCCTCGTTGCTTC
	ATCTGCTTGTGGTGTTGTGG
Beta-actin primers	AGAGAGAAATTGTCCGTGACATC
	CTCCGATCCAGACAGAGTATTTG
Additional sequencing primers	AACAGGCACCAGGGAGTAGC
	CCTGTGCAATGCACATGGAATACC