第 33卷 第 6期 水 生 生 物 学 报 Vol. 33, No. 6
2009年 11月 ACTA HYDROBIOLOGICA SINICA Nov., 2009

DOI号: 10.3724/SP.J.0000.2009.61031

病毒感染草鱼胸腺的 EST分析和免疫相关基因的鉴定

李洋洋　张义兵　桂建芳
(中国科学院水生生物研究所,淡水生态与生物技术国家重点实验室,武汉 430072)

摘要:以感染草鱼(*Ctenopharyngodon idellus*)出血病病毒(GCHV)的草鱼胸腺为材料,构建了草鱼胸腺的 SMART cDNA文库。筛选文库获得到 1933条有效 EST序列。BLASTX分析显示,583条序列在公共数据库中能找到同源基因(E-value≤1.00E 10-3, Identities≥30%),另外 1350条序列则找不到显著同源性。已知基因按具体功能可划分为 6类,大部分与细胞内的各种生理过程、细胞结构以及免疫防御相关。研究结果从分子水平上表明鱼类的胸腺在机体感染病毒的免疫反应中发挥重要作用,同时也表明胸腺组织在病毒感染后可能表达很多目前还不清楚功能的新基因。

关键词:草鱼;胸腺;cDNA文库;EST;免疫相关基因
中图分类号:Q785　**文献标识码**:A　**文章编号**:1000-3207(2009)06-1031-07

草鱼(*Ctenopharyngodon idellus*),草食习性,肉质鲜美,生长快,是我国淡水养殖的主要经济鱼类,随着养殖密度的增大和养殖环境的恶化,病害发生频繁。例如草鱼鱼苗和低龄草鱼在遭受草鱼出血病病毒(Grass carp hemorrhage virus, GCHV)感染后能引发出血病,导致大量死亡。因此,草鱼免疫相关的研究就尤为重要。探索免疫防治措施,提高鱼体的自身免疫能力,才能发展健康养殖。

近年来,对于鱼类免疫系统的研究受到广泛的重视,并取得了重要进展。至今已构建了多种鱼的 cDNA文库,获得了一些重要的免疫相关基因。例如本实验室从灭活的草鱼出血病病毒诱导的鲫鱼胚胎细胞差减 cDNA文库中分离鉴定了一批抗病毒相关和免疫相关基因的 EST,并成功克隆了数十个干扰素系统基因,其中 TLR3能特异地识别 dsRNA和诱导 I型干扰素的产生,STAT1和 JAK1是干扰素信号通路因子,另外还有 Mx1、Mx2、Viperin、PKR和 IFI56等抗病毒基因[1—4]。这些免疫相关基因的发现和获得加快了对鱼类免疫机制的了解。

胸腺是大多数鱼类最早发育的中枢淋巴器官[5],也是产生功能性 T淋巴细胞的主要免疫器官[6]。胸腺直接参与机体防御,参与体液免疫和细胞免疫[7,8],在鱼类免疫和免疫系统的发育过程中发挥关键作用。目前对于草鱼胸腺的认识仅是对组织结构的了解[9],还没有胸腺组织的基因表达的相关研究。为了更清晰认识草鱼胸腺在病毒免疫中的作用,本实验室在最近构建了感染草鱼出血病病毒后的草鱼胸腺 SMART cDNA文库,并通过 EST分析,对其表达的基因特别是免疫相关基因进行了较系统分析。

1 材料与方法

1.1 材料　实验用草鱼为 1龄,体重 40 g左右,取自本所实验渔场,在实验室条件下暂养一周。采用腹腔注射草鱼出血病病毒(GCHV)的方法进行人工感染,攻毒后约 7d,鱼体出现明显出血病症状,分离胸腺组织。

1.2 方法

1.2.1 胸腺组织总 RNA的提取　用 SV Total RNA Isolation Kit提取胸腺组织总 RNA,具体操作按说明书进行。总 RNA提取后,溶于无 RNA酶的水中,经琼脂糖凝胶电泳及紫外荧光光度计测定其 A_{260}和

收稿日期:2008-05-12;**修订日期**:2009-02-23
基金项目:973计划项目(2004CB117403);863计划项目(2007AA09Z423)资助
作者简介:李洋洋(1982—),女,汉族,吉林省九台市人;硕士研究生;主要从事鱼类遗传学和分子免疫学的研究。E-mail: yangyang5626@yahoo.com.cn
通讯作者:桂建芳, E-mail: jfgui@ihb.ac.cn

A_{280}鉴定质量后,保存于 -70℃冰箱备用。

1.2.2 cDNA第1链的合成 取3 μL总RNA约500 ng,加入10 μmol/L SMART IV™寡核苷酸和10 μmol/L CDS III/3′端PCR引物各1 μL。混匀后,于72℃变性2min,冰上冷却2min,依次加入5×第1链缓冲液2 μL,20 mmol/L的DTT 1 μL,10 mmol/L的dNTP混合物1 μL及PowerScript逆转录酶1 μL,共计10 μL反应体系。混匀后,置42℃反应1h,经逆转录合成cDNA第1链。

1.2.3 双链cDNA的LD PCR扩增 取cDNA第1链产物2 μL,分别加入去离子水80 μL、10×Advantage 2 PCR缓冲液10 μL,50×dNTP混合物2 μL,5′端PCR引物2 μL,CDSIII/3′端PCR引物2 μL及50×Advantage 2聚合酶混合物2 μL,使反应总体系达到100 μL,混匀后,置于PTC-100 PCR仪上,循环参数为:95℃预变性1min,95℃变性15s,68℃复性延伸6min,进行21个循环扩增双链cDNA。取5 μL反应产物进行琼脂糖凝胶电泳检测。

1.2.4 SMART cDNA质粒文库的构建、重组子的PCR鉴定和测序 取双链cDNA PCR产物与pMD 18-T克隆载体(TaKaRa)连接,连接产物转化大肠杆菌DH5α,涂平板后挑克隆于LB培养基中培养。以菌液为模板进行PCR,用引物AACGCAGAGTACGCGGG和CAGAGTACT$_{16}$筛选重组子,产物经琼脂糖凝胶电泳分析。选取插入片段在500 bp以上的克隆用载体上的引物进行测序。

1.2.5 序列分析 应用Cross-match软件去除原始序列中的低质量序列、载体序列和重复序列,将预处理后长度大于220bp的EST序列利用BlastX软件与NCBI(http://www.Ncbi.Nlm.Nih.gov/Blast)上的非冗余蛋白质序列数据库进行同源比对分析。E-value≤1.00E 10—3以及Identities≥30%的序列被认为具有生物学意义的相似性,并对这部分EST序列按生物学功能进行分类。

2 结 果

2.1 SMART cDNA文库构建

制备的总RNA(图1A)经1%琼脂糖凝胶电泳检测可见清晰的28S和18S两条RNA条带以及典型的mRNA弥散带,表明组织总RNA完整性良好,可用于文库构建。双链cDNA产物呈现较为均匀的拖带,主要集中在500 bp以上,说明草鱼胸腺组织中所转录出的mRNA成分多样(图1B)。双链cDNA产物与载体连接转化大肠杆菌后利用菌液PCR验证重组情况,电泳估计插入片段的大小,结果显示阳性克隆中的片段大小在250—2000 bp之间(图1C)。挑选插入片段在500 bp以上的阳性克隆测序。

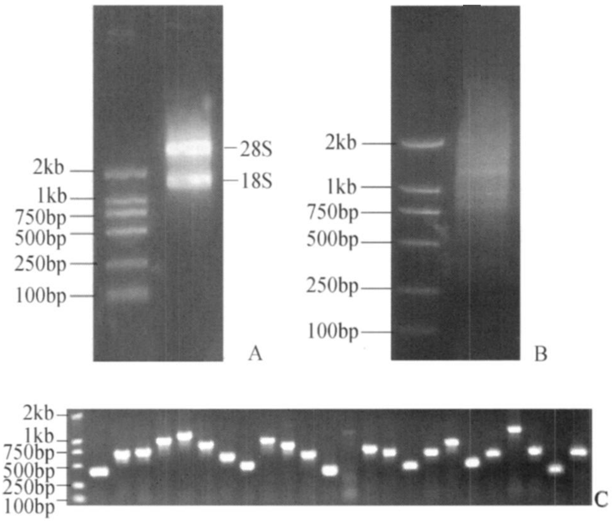

图1 A为胸腺中提取的总RNA,B为SMART法合成的双链cDNA,C为cDNA文库中插入片段的PCR鉴定

Fig. 1 Total RNA isolated from thymus (A); Double-stranded cDNA (B); PCR analysis of recombinant colonies from the cDNA library (C)

2.2 EST分析

去除测序结果中的低质量序列以及序列上的载体部分,共获得1933条大于220 bp的有效EST,对其进行BlastX分析,初步认为符合E-value≤1.00E 10—3, Identities≥30%的比对结果有意义。其中有583个EST能找到同源序列,340个完全找不到匹配结果,1010个与数据库中的蛋白匹配程度不高。找不到比对结果的序列中可能有首次在鱼类中鉴定的新基因。在有同源序列的583个EST中,有423个代表249个已知基因,它们参与细胞组成和细胞发生的各种生物学过程,按照功能可分为6类(表1):代谢活动相关、转录和翻译、细胞结构、细胞增殖和凋亡、信号传导以及免疫反应;另外的160个EST则与78个斑马鱼、河豚等基因组中预测的未知功能基因同源。

在423条已知功能的EST中,与RNA和蛋白质的合成相关的最多,有107条,代表57个基因,有核糖体蛋白、转录和翻译因子以及参与RNA转录后加工的基因。其中核糖体大亚基蛋白L11和L32分别出现了11次和9次。细胞内的代谢活动是复杂多样的,与代谢相关的EST有81条,就代表了基因57个,包括参与能量产生和转化的细胞色素c氧化酶、

NADH脱氢酶、ATP合成酶等,参与碳水化合物、核苷、蛋白和脂类代谢的各种酶类,还有参与胞内各种运输活动的成员。另外,代表着参与形成和维持细胞结构,调控细胞的增殖和凋亡的基因的 EST也占据了一定的比例,例如出现了 6次的前纤维蛋白(Profilin 2),是肌动蛋白结合蛋白,调节微丝骨架的结构和功能[10],出现了 10次的 granulin 1则在细胞的增殖中发挥调控作用[11]。

表 1　草鱼 SMATR cDNA文库的 ESTs的统计数据

Tab. 1　Summary statistics of ESTs from SMATR cDNA library of grass carp

ESTs分类 Category	EST数 Number of ESTs	基因数 Number of genes
代谢活动 Metabolism	81	57
转录和翻译 Transcription and translation	107	57
细胞结构 Cell structure	41	26
信号转导 Signal transduction	17	14
细胞增殖、分化和凋亡 Cell proliferation, differentiation and apoptosis	60	20
免疫相关 Immune related	100	59
其他已知蛋白 Other known protein	17	16
假定的蛋白 Hypothecial protein	160	78
完全无同源性的 ESTs No hits	340	219
同源相似程度低的 ESTs Low identity	1010	698
总计 Total	1933	1243

2.3　免疫相关基因的鉴定

从草鱼 SMATR cDNA文库中获得的免疫相关的 EST有 100个,代表 59个基因,约占所鉴定到的 249个已知基因的 23.7%。从文库中鉴定到的免疫相关 EST(表 2)。

这些免疫相关基因在免疫反应中发挥的作用不同,按照具体功能又可分为以下几类:有些参与抗原的加工和呈递,例如蛋白酶体(Proteasome)能够降解抗原产生被 MHC分子识别的肽类,使抗原被提呈至细胞表面而被细胞毒性淋巴细胞所识别[12];有些参与信号传导,如 Janus kinase 3(JAK3)通常表达于 T细胞和 NK细胞,其他免疫细胞中的 JAK3可以被诱导表达,JAK3主要参与 I型细胞因子受体家族(IL-2R, IL-4R等)介导的信号转导[13];有些在细胞识别和黏附中发挥作用,如 CD8-α与 MHC Ⅰ类分子相互作用,以保证抗原特异激活过程中的 T细胞受体与靶细胞紧密结合[14];有些是天然免疫的成员,如 lysozyme可以使具有吞噬功能的细胞更容易吞噬细菌;有些则具有多种免疫相关的功能,如 β thymosin(β胸腺素),最初从胸腺中分离得到,但后来发现是许多组织中都存在的一种小肽,是重要的肌动蛋白结合蛋白[15],能降低多核白细胞的侵润及炎症细胞因子和趋化因子的 mRNA表达水平[16],能够抑制中性粒细胞趋化和组织炎症反应[17],与血管生成和伤口的愈合相关[18];还鉴定到一些与趋化因子同源的序列。另外,在与数据库里的蛋白匹配程度不高的 EST中肯定也存在一些免疫相关的基因,只是仅凭相似性的分析还无法对其作出详细判断。大量免疫相关基因的表达充分说明胸腺作为免疫器官在鱼体的炎症反应中发挥着重要作用。

表 2　草鱼 cDNA文库中免疫相关 ESTs的具体分类

Tab. 2　Detail analysis of immune-related ESTs from the cDNA library

基因鉴定 Identification	功能 Function	同源物种 Species	E值 E-value	一致性 Identity (%)	出现频率 Frequency
MHC class II-associated invariant chain	Antigen processing and presentation	*Danio rerio*	2E-96	80	2
MHC class II antigen	Antigen processing and presentation	*Ctenomys haigi*	3E-33	89	1
MHC class II integral membrane protein alpha chain 2	Antigen processing and presentation	*Danio rerio*	8E-61	71.6	1
Beta-2 microglobulin	Antigen processing and presentation	*Danio rerio*	8e-39	70	2
Proteasome activator subunit 2	Antigen processing and presentation	*Danio rerio*	6E-71	97	1
Proteasome (prosome, macropain) subunit, beta type, 9a	Antigen processing and presentation	*Danio rerio*	4E-63	92	1
Proteasome (prosome, macropain) subunit, alpha type 3	Antigen processing and presentation	*Mus musculus*	2E-25	87	1
Proteasome subunit N3	Antigen processing and presentation	*Oncorhynchus mykiss*	7E-103	91	1
Proteasome 26S subunit, Non-ATPase, 13	Antigen processing and presentation	*Danio rerio*	5E-43	69	1
CC chemokine SCYA103	Chemotaxis	*Ictalurus punctatus*	1e-26	63	1

续表

基因鉴定 Identification	功能 Function	同源物种 Species	E值 E-value	一致性 Identity (%)	出现频率 Frequency
Interleukin-8*	Chemotaxis	*Danio rerio*	2e-10	59	3
Chemokine CXC protein*	Chemotaxis	*Danio rerio*	5e-36	75	2
Peripheral-type benzodiazepine receptor	Chemotaxis	*Oncorhynchus mykiss*	5E-81	80	1
Complement component 1, q subcomponent binding protein	Complement	*Bos taurus*	6E-45	72	1
Interferon-inducible protein Gig1	Innate immunity	*Carassius auratus*	5e-112	100	1
Interferon-inducible protein Gig2	Innate immunity	*Carassius auratus*	4e-9	41	1
interferon-inducible protein Gig2*	Innate immunity	*Danio rerio*	3E-96	81.5	1
C-type lectin*	Innate immunity	*Danio rerio*	6e-73	79	1
Novel C-type lectin domain containing protein	Innate immunity	*Danio rerio*	3e-123	89	1
lysozyme g	Innate immunity	*Cyprinus carpio*	2e-80	87	2
Ferritin H-3	Innate immunity	*Ictalurus punctatus*	7E-10	40	1
Ferritin middle subunit	Innate immunity	*Salmo salar*	6E-82	82	1
Pentraxin	Innate immunity	*Cyprinus carpio*	2E-71	72	1
Perforin	Innate immunity	*Ctenopharyngodon idella*	1e-68	95	1
Grancalcin	Innate immunity	*Mus musculus*	2E-72	75.3	1
Nonspecific cytotoxic cell cationic antimicrobial protein-1	Innate immunity	*Ictalurus punctatus*	5E-10	91.7	1
Janus kinase 3	Signal transduction	*Cyprinus carpio*	1E-102	92.4	1
Receptor of activated protein kinase C (RACK)	Signal transduction	*Danio rerio*	1E-137	99.6	1
Leukotriene B4 receptor*	Signal transduction	*Danio rerio*	7e-99	89	1
G protein-coupled receptor 26	Signal transduction	*Rattus norvegicus*	3E-49	57	1
CD81 antigen	cell adhesion and signal transduction	*Danio rerio*	1e-85	87	1
CD9 antigen	cell adhesion and signal transduction	*Danio rerio*	9e-39	85	1
Integrin alpha V subunit	cell adhesion and signal transduction	*Danio rerio*	9E-07	81.2	1
Vinculin	cell adhesion and signal transduction	*Mus musculus*	1E-57	83	2
T cell receptor beta 2	T cell recognition, adhesion and activation	*Carassius auratus langsdorfii*	9e-9	69	1
TCR-alpha J segment III-33	T cell recognition, adhesion and activation	*Danio rerio*	0.001	95	2
CD8 alpha2-2	T cell recognition, adhesion and activation	*Carassius auratus langsdorfii*	4e-54	75	1
CD4-like protein 2	T cell recognition, adhesion and activation	*Ictalurus punctatus*	2E-25	37.6	1
CD3epsilon	T cell recognition, adhesion and activation	*Salmo salar*	2e-17	55	2
CD79a	B cell recognition, adhesion and activation	*Ictalurus punctatus*	4E-38	70.3	1
Interferon-inducible and double-stranded-dependent eIF-2kinase	Various immune functions	*Carassius auratus*	8E-134	95	1
Apolipoprotein E precursor protein	Various immune functions	*Danio rerio*	1E-114	85.6	15
Cystatin B	Various immune functions	*Oncorhynchus mykiss*	1E-26	62	1
Cathepsin S	Various immune functions	*Cyprinus carpio*	1E-115	81.7	1
Cathepsin L, a	Various immune functions	*Danio rerio*	2E-53	96	2
Calreticulin	Various immune functions	*Danio rerio*	9E-45	89	1
B7-H4 protein*	Various immune functions	*Danio rerio*	1E-12	46.1	1
Interferon-inducible guanylate-binding protein	Various immune functions	*Oncorhynchus mykiss*	3E-52	44.6	1

续表

基因鉴定 Identification	功能 Function	同源物种 Species	E值 E-value	一致性 Identity (%)	出现频率 Frequency
Beta thymosin	Various immune functions	*Oncorhynchus mykiss*	2e-06	90	6
Thymosin beta a	Various immune functions	*Cyprinus carpio*	2e-7	89.2	4
Prothymosin, alpha	Various immune functions	*Danio rerio*	6e-08	96	1
Ubquitin-like protein 2	Various immune functions	*Carassius auratus*	4E-30	91	1
Tumor-associated calcium signal transducer	Various immune functions	*Danio rerio*	7E-43	61.7	2
HSP 90-beta	Various immune functions	*Danio rerio*	6E-77	87.5	3
Translationally controlled tumor protein	Various immune functions	*Labeo rohita*	4E-20	90	3
S100 calcium binding protein	Various immune functions	*Salmo salar*	8E-22	60	4
Ubiquitin-conjugating enzyme E2 I UBE2 I	Various immune functions	*Homo sapiens*	7E-24	91	1
Small ubiquitin-related modifier 2 precursor (SUMO-2)	Various immune functions	*Danio rerio*	7E-18	100	1
Neurotoxin/C59/Ly-6-like protein	Various immune functions	*Ctenopharyngodon idella*	4E-17	92.8	2

注: *为计算机自动预测的基因

Note: * Indicate molecules predicted by automated computational analysis

3 讨　论

胸腺作为免疫器官,直接参与机体的防御[7,8]。草鱼出血病是由草鱼出血病病毒引起的对草鱼鱼种阶段危害最严重的一种鱼病。受感染后的草鱼胸腺组织出现明显病变,直接影响鱼体免疫功能[19]。本研究的结果从分子水平上表明胸腺组织在受到病毒感染后有很多免疫相关基因的表达。

EST技术现已成为大规模获取功能基因的一种最为快捷有效的研究手段。许多鱼类新基因的发现以及克隆就是利用构建全长 cDNA文库并大量获得 EST这种方法实现的[20,21]。通过 BlastX同源比对分析,本研究获得的大部分 EST不能在数据库中找到同源序列,或是不能根据同源蛋白序列相似性比对鉴定功能。这部分序列中既有可能有大部分是已知基因,但因为鱼类基因与哺乳类的同源性太低而无法对应其同源关系,又极有可能是一些尚未报道的新基因序列,因此有必要通过一级结构的分析而初步预测其功能,并进行深入的研究。

跟哺乳动物一样,胸腺是最早出现 T细胞的淋巴器官[22],T细胞在胸腺中发育成熟并被运送到头肾、脾等外周免疫器官[5,23]。鱼类的胸腺也同样经历脂肪性退化及伴随着性成熟的生理性萎缩[24,25]。繁殖周期、季节变化及环境胁迫也会导致胸腺体积及细胞组成上的变化[26,27]。到目前为止胸腺退化的生理功能和意义还不太清楚。大量的未知 EST出现在我们的文库中,它们是否与草鱼胸腺以及免疫系统的发育相关,或与胸腺的退化相关,值得深入探索。

参考文献:

[1] Zhang Y B, Shi Y H, Gui J F. Construction of antiviral subtractive cDNA library of cultured fish cells [J]. *Acta Hydrobiol Sin*, 2003, **27**: 113—118 [张义兵, 石耀华, 桂建芳. 鱼类培养细胞抗病毒基因差减 cDNA 文库的构建. 水生生物学报, 2003, **27**: 113—118]

[2] Zhang Y B, Zhang Q Y, Xu D Q, *et al*. Identification of antiviral genes in the cultured fish cell induced by the inactivated virus [J]. *Chinese Science Bulletin*, 2003, **48**: 581—588 [张义兵, 张奇亚, 徐德全, 等. 从灭活病毒诱导的培养细胞中鉴定鱼类抗病毒相关基因. 科学通报, 2003, **48**: 581—588]

[3] Zhang Y B, Gui J F. Cloning, identification and characterization of interferon system genes tin crucian carp (*Carassius auratus* L.) [J]. *Journal of the Graduate School of the Chinese Academy of Sciences*, 2004, **21**: 418—426

[4] Zhang Y B, Jiang J, Chen Y D, *et al*. The innate immune response to grass carp hemorrhagic virus (GCHV) in cultured *Carassius auratus* blastulae (CAB) cells [J]. *Dev Comp Immunol*, 2007, **31**: 232—43

[5] Jósefsson S, Tatner M F. Histogenesis of the lympholid organs in sea bream (*Sparus aurata* L.) [J]. *Fish Shellfish Immunol*, 1993, **3**: 35—49

[6] Matsunaga T, Rahman A. In search of the origin of the thymus: the thymus and GALT may be evolutionarily related [J]. *Scand J Immunol*, 2001, **53**: 1—6

[7] Ortiz-Muniz G, Sigel M. Antibody synthesis in lymphoid organs of two marine teleosts [J]. *J Reti-endothel Soc*, 1971, **9**: 42—52

[8] Romano N, Taverne-Thiele J J, van Maanen J C, *et al*. Distribution of leukocyte subpopulations in developing carp (*Cyprinus carpio* L.): immunocytochemical studies [J]. *Fish and Shellfish Immunology*, 1997, **7**: 439—453

[9] Lu Q Z. Studies on the histology of thymus of the grass carp [J]. *Acta Hydrobiol Sin*, 1991, **15**: 327—334 [卢全章. 草鱼胸腺

组织学的研究. 水生生物学报, 1991, **15**: 327—334]

[10] Witke W, Podtelejnikov A, Di Nardo A, *et al*. In mouse brain profilin I and profilin II associate with regulators of the endocytic pathway and actin assembly [J]. *The EMBO Journal*, 1998, **17**: 967—976

[11] Bhandari V, Palfree R G, Bateman A. Isolation and sequence of the granulin precursor cDNA from human bone marrow reveals tandem cysteine-rich granulin domains [J]. *Proc Natl Acad Sci USA*, 1992, **89**: 1715—1719

[12] Stoltze L, Nussbaum A K, Sijts A, *et al*. The function of the proteasome system in MHC I class antigen processing [J]. *Immunol Today*, 2000, **21**: 317—319

[13] Johnston J A, Kawamura M, Kirken R A, *et al* Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2 [J]. *Nature*, 1994, **370**: 151—153

[14] Devine L, Sun J, Barr M, *et al*. Orientation of the Ig domains of CD8 alpha beta relative to MHC class I [J]. *J Immunol*, 1999, **162**: 846—851

[15] Safer D, Elzinga M, Nachmias VT. Thymosin B4 and Fx, an actin-sequestering peptide, are indistinguishable [J]. *J Biol Chem*, 1991, **266**: 4029—4032

[16] Sosne G, Szliter E A, Barrett R, *et al*. Thymosin beta4 promotes corneal wound healing and decreases inflammation in vivo following alkali injury [J]. *Exp Eye Res*, 2002, **74**: 293—299

[17] Young J D, Lawrence A J, MacLean A G, *et al*. Thymosin beta 4 sulfoxide is an anti-inflammatory agent generated by monocytes in the presence of glucoeorticoids [J]. *Nat Med*, 1999, **5**: 1424—1427

[18] Philp D, Goldstein A L, Kleinman H K. Thymosin beta4 promotes angiogenesis, wound healing, and hair follicle development [J]. *Mech Ageing Dev*, 2004, **125**: 113—115

[19] 卢全章, 郭琼林. 草鱼出血病胸腺组织病理的研究 [J]. 鱼病简讯, 1989, (2—3): 74—75

[20] Aoki T, Nam B H, Hirono II, *et al*. Sequences of 596 cDNA Clones (565, 977bp) of Japanese Flounder (*Paralichthys olivaceus*) Leukocytes Infected with Hirame Rhabdovirus [J]. *Mar Biotechnol*, 1999, **1**: 477—488

[21] Nam B H, Yamamoto E, Hirono I, *et al*. A survey of expressed genes in the leukocytes of Japanese flounder, *Paralichthys olivaceus*, infected with Hirame rhabdovirus [J]. *Dev Comp Immunol*, 2000, **24**: 13—24

[22] Miracle A L, Anderson M K, Litman R T, *et al*. Complex expression patterns of lymphocyte specific genes during the development of cartilaginous fish implicate unique lymphoid tissues in generating an immune repertoire [J]. *Int Immunol*, 2001, **13**: 567—580

[23] Tatner M F. The migration of labelled thymocytes to the peripheral lymphoid organs in the rainbow trout, Salmo gairdneri richardson [J]. *Dev Comp Immunol*, 1985, **9**: 85—91

[24] Chilmonczyk S. The thymus in fish: development and possible function in the immune response [J]. *Ann Rev Fish Dis*, 1992, **2**: 181—200

[25] Luer C A, Walsh C J, Bodine A B, *et al*. The elasmobranch thymus: anatomical, histological and preliminary functional characterization [J]. *J Exp Zool*, 1995, **273**: 342—352

[26] Tamura E, Honma Y, Kitamura Y. Seasonal changes in the thymus of the viviparous surfperch, Ditrema temmincki, with special reference to its maturity and gestation [J]. *Jap J Ichthyol*, 1981, **28**: 295—303

[27] Zapata A G, Varas A, Torroba M. Seasonal variations in the immune system of lower vertebrates [J]. *Immunol Today*, 1992, **13**: 142—147

EST ANALYSIS AND IMMUNE-RELATED GENE IDENTIFICATION OF GRASS CARP THYMUS IN RESPONSE TO VIRUS INFECTION

LI Yang-Yang, ZHANG Yi-Bing and GUI Jian-Fang

(State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, *China)*

Abstract: The thymus is the primary tissues for production of functional T lymphocytes in vertebrate and is directly involved in defense mechanism. The grass carp (*Ctenopharyngodon idellus*) is an integral part of fish culture and forms an important source of protein for human consumption in China. Histological studies showed that grass carp display thymus tissue, which is important for cellular immunity and humoral immunity and similar to mammals, becoming degenerated with fish ages. While the research from molecular level on grass carp thymus is rare. The underlying molecular mechanisms involved in the immune responses of fish thymus are poorly understood and its value as an indicator of immune competence is unknown. In order to obtain more molecular evidence for the physiological function of grass carp thymus, a cDNA library was constructed by using the SMART cDNA synthesis method with mRNAs derived from thymus of grass carp infected with grass carp hemorrhage virus (GCHV), and then EST (expressed sequence tags) analysis was performed. SMART-prepared cDNA was full-length-enriched and well suitable for generation of cDNA libraries for various applications. Expressed sequence tags (EST) were an efficient approach to characterize the transcripts of genes expressed in tissues exposed to a given set of environmental conditions. In this study, a total of 1933 ESTs longer than 220bp were yielded and sequenced. BLASTX analysis showed that 583 ESTs represent grass carp genes which were homologous to known genes in mammals (E-value≤1.00E10-3, Identities≥30%), while the other 1350 ESTs shared low or no similarity by search of public protein databases in NCBI. Further analyses revealed that these genes, based on the functions of the homologous mammalian genes, could be classified into 6 categories, including immune-related, transcription and translation, metabolism, cell structure, signal transduction, cell proliferation, differentiation and apoptosis. Most of them encode proteins involved in biological processes, cell structure maintaining and immune response. Relatively few teleost genes involved in immune functions have been sequenced, compared to those from higher vertebrates. This study obtained a large number of immune-related genes that had not been previously reported for *C. idellus*, and which might represent key molecules for the understanding of the immune response to infection in fish thymus. 100 ESTs representing 59 different immune-related genes had been identified from the cDNA library. These genes encoding proteins involved in antigen processing and presentation, cell recognition, adhesion and activation, innate immune and so on. Many of these immune related genes had various immune functions. This study revealed from molecular level that grass carp thymus was the important immune tissue and might play an important role in the immune response to virus infection. In addition, this result also showed that grass carp thymus expressed many novel genes that were functionally unknown so far. Further investigations are needed to determine the function of these genes, as well as their role in the immune response. The generation of a collection of ESTs clones from grass carp thymus will provide the basis for functional genomics studies in this important organ.

Key words: Grass carp; Thymus; cDNA library; EST; Immune-related gene