Fish and Shellfish Immunology 84 (2019) 158–168

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

Characterization of four C1q/TNF-related proteins (CTRPs) from red-lip mullet (*Liza haematocheila*) and their transcriptional modulation in response to bacterial and pathogen-associated molecular pattern stimuli

W.K.M. Omeka[a], D.S. Liyanage[a], Thanthrige Thiunuwan Priyathilaka[a], Hyukjae Kwon[a], Seongdo Lee[a], Jehee Lee[a,b,*]

[a] Department of Marine Life Sciences & Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province, 63243, Republic of Korea
[b] Marine Science Institute, Jeju National University, Jeju Self-Governing Province, 63333, Republic of Korea

ARTICLE INFO

Keywords:
CTRP
Immune challenge
Red-lip mullet
Tissue-specific expression
Transcriptional modulation

ABSTRACT

The structural and evolutionary linkage between tumor necrosis factor (TNF) and the globular C1q (gC1q) domain defines the C1q and TNF-related proteins (CTRPs), which are involved in diverse functions such as immune defense, inflammation, apoptosis, autoimmunity, and cell differentiation. In this study, red-lip mullet (*Liza haematocheila*) CTRP4-like (MuCTRP4-like), CTRP5 (MuCTRP5), CTRP6 (MuCTRP6), and CTRP7 (MuCTRP7) were identified from the red-lip mullet transcriptome database and molecularly characterized. According to *in silico* analysis, coding sequences of MuCTRP4-like, MuCTRP5, MuCTRP6, and MuCTRP7 consisted of 1128, 753, 729, and 888 bp open reading frames (ORF), respectively and encoded 375, 250, 242, and 295 amino acids, respectively. All CTRPs possessed a putative C1q domain. Additionally, MuCTRP5, MuCTRP6, and MuCTRP7 consisted of a collagen region. Phylogenetic analysis exemplified that MuCTRPs were distinctly clustered with the respective CTRP orthologs. Tissue-specific expression analysis demonstrated that *MuCTRP4-like* was mostly expressed in the blood and intestine. Moreover, *MuCTRP6* was highly expressed in the blood, whereas *MuCTRP5* and *MuCTRP7* were predominantly expressed in the muscle and stomach, respectively. According to the temporal expression in blood, all *MuCTRPs* exhibited significant modulations in response to polyinosinic:polycytidylic acid (poly I:C) and *Lactococcus garvieae* (*L. garvieae*). *MuCTRP4-like*, *MuCTRP5*, and *MuCTRP6* showed significant upregulation in response to lipopolysaccharides (LPS). The results of this study suggest the potential involvement of Mullet CTRPs in post-immune responses.

1. Introduction

C1q is a pattern recognition protein that is mainly involved in the complement pathway. It is the key crosslinker between innate and adaptive immunity [1]. The gC1q domain is composed of C-terminal regions of its A, B and C chains, which contribute to its heterotrimeric organization [2]. This feature resembles a bouquet of tulips under the electron microscope [2,3]. The three-dimensional structure of the gC1q domain is nearly identical to the C-terminal of the tumor necrosis factor homology domain (THD) [3]. Therefore, the structural and evolutionary relationship between the THD and gC1q domains define the C1q and TNF-related protein (CTRP) superfamily, which is comprised of 16 non-complement CTRPs [2,4]. Typical CTRPs contain four different segments: an N-terminal signal peptide, a short variable region, an N-terminal collagenous domain with different G-Y-X repeats, and a C-terminal gC1q domain [5]. However, each CTRP member is composed of at least one C1q domain and distinct combinations of other segments [6].

Though C1q complement proteins and TNF proteins have been highly investigated for their functions in innate and adaptive immunity [7–10], the biological function of CTRPs have not been elucidated sufficiently [11]. Previous studies on CTRPs suggested that they have versatile and distinct functions in glucose and fatty acid metabolism [12]. However, recent studies propose that CTRPs might act as dual-functional proteins that link immunity to metabolism [13]. Recent findings have shown that mammalian CTRPs can activate a number of cellular signaling pathways, such as the NF-κB, STAT3, MAPK, AMPK, and Akt pathways [13]. For example, mouse CTRP1 and CTRP9 can

* Corresponding author. Marine Molecular Genetics Lab, Department of Marine Life Sciences, Jeju National University, Jeju Self-Governing Province, 63243, Republic of Korea.
E-mail address: jehee@jejunu.ac.kr (J. Lee).

https://doi.org/10.1016/j.fsi.2018.09.078
Received 9 June 2018; Received in revised form 31 August 2018; Accepted 29 September 2018
Available online 02 October 2018
1050-4648/

activate Akt and p44/42-MAPK signaling pathways [14,15]. Further, human CTRP4 has been reported to promote cancer cell survival and stimulate STAT3 and NF-κB signaling pathways [16].

When considering previous studies in fish, few studies have been conducted to elucidate the immune relevancy of CTRPs [17,18]. For example, C1q3-like from *Paralichthys olivaceus* have been reported to have antimicrobial properties [19]. Moreover, CTRP1 and CTRP9 from *Eudontomyzon morii* showed immune responses to LPS and mediated wound healing in the heart [4]. Studies on CTRPs will provide novel insight into fish immunity because the immune functions of CTRPs in humans are still under investigation.

Red-lip mullets are distributed around the west coast of Korea. Mullet capture fisheries has been reduced in recent years due to over-hunting and reclamation [20]. Therefore, mullet cultivation has been increasing rapidly to meet the demand of consumption. Furthermore, mullet farming contributes 8% of the total amount of cultivated fish in Korea [20]. Although red-lip mullets are considered as a valuable fish species in Korean aquaculture, their high mortality rate has a negative impact on cultivation. Disease outbreaks such as *L. garvieae, Myxobolus* sp., and *Amyloodinium* sp. infections are major reasons for the high mortalities [20,21]. Therefore, investigation of MuCTRP genes in red-lip mullet in relation to immune stress may provide new insight into their immune defense system.

In this study, MuCTRP4-like, MuCTRP5, MuCTRP6, and MuCTRP7 were explored for molecular and transcriptional characteristics to gain a better understanding on their expression patterns under normal conditions. Bacterial and pathogen-associated molecular patterns (PAMP) were characterized, which might be helpful for predicting the functional behaviors of these proteins.

2. Material and methods

2.1. Identification and verification of MuCTRP sequences

The cDNA sequences with the highest homology to *CTRP4-like*, *CTRP5*, *CTRP6,* and *CTRP7* were obtained from the red-lip mullet transcriptome database established using the Blast2Go sequence annotation software and PacBio platform sequencing technique (Insilicogen, Korea). The obtained sequences were compared and verified with ortholog sequences using NCBI Blast algorithms and RefSeq non-redundant protein databases [22].

All MuCTRP sequences were cloned into the T-vector PMD20 (TaKaRa) by using gene specific primers (Table 1). Then cloned MuCTRP sequences were validated and confirmed by capillary sequencing (Macrogen, Korea).

Table 2
Molecular properties of MuCTRPs.

Name	Molecular weight	Theoretical pI	Number of amino acids
MuCTRP4-like	41.86 kDa	9.33	375
MuCTRP5	26.73 kDa	6.65	250
MuCTRP6	27.17 kDa	8.55	242
MuCTRP7	31.46 kDa	6.94	295

2.2. In silico analysis of MuCTRP sequences

Open reading frames and amino acid sequences of MuCTRP4-like, MuCTRP5, MuCTRP6, and MuCTRP7 were obtained using Unipro UGENE bioinformatics software v1.26.1. Next, the amino acid sequences were verified using the NCBI blastp program [23]. Conserved predominant domains and important amino acid motifs were identified by the NCBI conserved domain database (NCBI CDD) [24] and Motif Scan online tool [25], respectively. The molecular properties and N-linked glycosylation sites of MuCTRPs were analyzed by ExPASy Protparam [26] and NetNGlyc 1.0 online servers [27], respectively. Furthermore, signal peptides and its cleavage sites were analyzed using SignalP 4.1 online tool [28]. Multiple sequence and pairwise sequence alignments were performed by the Clustal Omega [29] and EMBOSS needle [30] tools, respectively. The phylogenetic tree was reconstructed by the neighbor-joining method using MEGA7 software with 5000 bootstraps [31].

2.3. Collecting samples for tissue distribution and immune challenge

Red-lip mullets (average body weight of 100 g) were purchased from Sangdeok fishery, in Hadong, Korea and adapted in 300 L laboratory aquarium tanks at 20 °C for one week. Then five un-challenged mullets were selected for tissue distribution analysis. Fish were carefully dissected after using conventional anesthetics (Tricaine mesylate-MS-222; 40 mg/L). Blood was drawn using sterile syringes coated with heparin sodium salt (USB, USA), and then peripheral blood cells were isolated by centrifugation at 3000 × *g* for 10 min at 4 °C. In addition, the head kidney, spleen, kidney, brain, muscle, liver, gill, intestine, skin, heart, and stomach were collected and immediately frozen in liquid nitrogen and stored at −80 °C until future use.

Mullet fish were selected and grouped for immune challenge experiment. LPS (Sigma, USA; 1.25 μg/g), poly I:C (Sigma, USA; 1.5 μg/g), and *L. garvieae* (1×10^3 CFU/μL) were prepared with phosphate buffered saline (PBS), and a total volume of 100 μL were injected to each individual intraperitoneally. Additionally, 100 μL of PBS was

Table 1
Primers used for quantitative real-time PCR.

Primer Name	Sequence 5′–3′	Description (melting temperature-T_m and amplicon size)
CTRP4-like qPCR forward	CCCGGAGCTTACTTCTTTGCCTTC	T_m 60 °C, 138 bp
CTRP4-like qPCR reverse	GCTCTGCATCTCCCGTCTCTTTG	T_m 60 °C, 138 bp
CTRP4like TA cloning forward	GCGCTTTCTACAACTGATGGTGCTTT	Tm 59.9 °C, 1217 bp
CTRP4like TA cloning reverse	GCCTCTGATCCATCCACATGCAAATAAAC	Tm 59.8 °C, 1217 bp
CTRP5 qPCR forward	GACACGCGGTGGCATCTTACTTTC	T_m 60 °C, 133 bp
CTRP5 qPCR reverse	CCATTGTACTCCGAGAGAGCCATCTG	T_m 60 °C, 133 bp
CTRP5 TA cloning forward	CGACGCACACAAACATCAGCAG	Tm 59.2 °C, 959bp
CTRP5 TA cloning reverse	GCCCATTGACTCGCTTTATCCAGAATG	Tm 59.7 °C, 959 bp
CTRP6 qPCR forward	TGCACCTGATGCACAACGACAAG	T_m 60 °C, 150 bp
CTRP6 qPCR reverse	CGGCATTCTCCCTCTCCCGTTTATAG	T_m 60 °C, 150 bp
CTRP6 TA cloning forward	AGTGGTTACTCCTGACACCAGCG	Tm 60.8 °C, 854 bp
CTRP6 TA cloning reverse	GGCAGCATCCGTGGCAAAAA	Tm 59.4 °C, 854 bp
CTRP7 qPCR forward	CCCACAGGAGAGAATGGAGATGTGG	T_m 60 °C, 147 bp
CTRP7 qPCR reverse	TCCCACGGAGAAGGCAGATTTAGG	T_m 60 °C, 147 bp
CTRP7 TA cloning forward	GATAGGAGGACAGGCACAGCAAG	Tm 59.4 °C, 973 bp
CTRP7 TA cloning reverse	AAGAACGGTGACTAGCAGTTTTACTTTTGTG	Tm 59.1 °C, 973 bp
EF1α qPCR forward	CCCTGGTCAGATCAGTGCTGGTTAT	T_m 60 °C, 187 bp
EF1α qPCR reverse	AGCGTCGCCAGACTTTAGGGATTT	T_m 60 °C, 187 bp

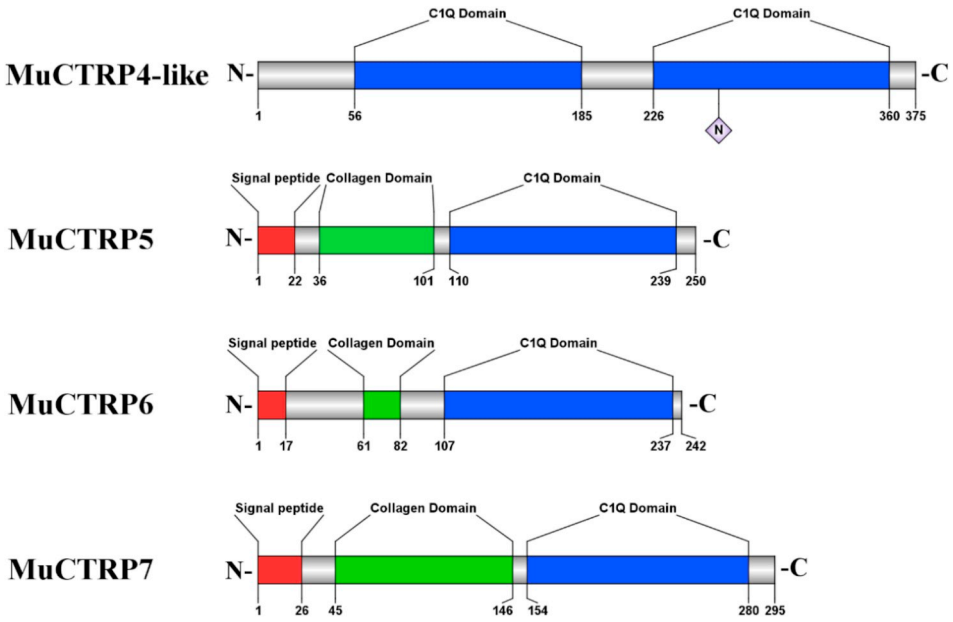


Fig. 1. Domain organization of MuCTRPs.

injected into each individual in the control group. Thereafter, peripheral blood cells were collected from five individuals from each group at 0, 6, 24, 48, 72 h post-injection (p.i.) following the same procedure in tissue distribution. All experiments in this study including mullet rearing and disease challenges were reviewed and approved by the Animal Care and Use Committee of Jeju National University.

2.4. RNA isolation and cDNA synthesis

Total RNA was collected from a pool of tissue samples (n = 5 for tissue distribution; n = 5 for the immune challenge) by RNAiso plus (TaKaRa, Japan) reagent followed by clean-up with RNeasy spin column (Qiagen, USA). RNA quality and concentration were examined by μDrop Plate (Thermo Scientific, USA) and subsequently ran 1.5% agarose gel to visualize RNA. First-strand cDNA was synthesized in a 20 μL reaction mixture containing 2.5 μg of total RNA using PrimeScript™ II 1st strand cDNA Synthesis Kit (TaKaRa, Japan). The synthesized cDNA was diluted 40-fold in nuclease-free water and stored at −80 °C until future use.

2.5. Quantitative real-time PCR (qPCR) analysis

The mRNA expression profiles of the *MuCTRP* genes were determined by qPCR using TaKaRa Thermal Cycler Dice Real Time System III (TaKaRa, Japan). All qPCR primers were designed according to minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines using IDT's online tool Primer Quest (https://sg.idtdna.com/Primerquest/Home/Index) [32]. The gene encoding mullet elongation factor 1α (*EF1α*) was used as an internal reference gene (GenBank accession no MH017208). The reaction was carried out in triplicate for each sample in a final volume of 10 μL containing 3 μL of cDNA template, 5 μL of Ex Taq™ SYBR premix (TaKaRa, Japan), 1.2 μL of PCR grade water, and 0.4 μL of primers (10 pmol/μL) as shown in Table 1. The qPCR thermal cycler profile are as follows: initial denaturation at 95 °C for 10 min, followed by 45 cycles of 95 °C for 5 s, 58 °C for 20 s, and 72 °C for 20 s. After the 45 cycles, a final cycle of 95 °C for 15 s, 60 °C for 30 s and 95 °C for 15 s was set to estimate the specificity of target amplification.

Relative expression levels of *MuCTRP* were determined according to the Livak ($2^{-\Delta\Delta CT}$) method [33]. For tissue-specific expression analysis, Ct values of the lowest *MuCTRP* expressed tissue used for normalization. For the immune challenge data analysis, the expression levels of *MuCTRP* at different time intervals were represented in terms of fold changes relative to the PBS control at each time point. All the data were represented as mean ± standard deviation (SD), and tissue-specific expression data was statistically compared among tissues by one-way ANOVA followed by Tukey's range test using the IBM SPSS 24 statistical software (UK.). All other experimental results were statistically compared with un-injected controls by the *t*-test using the Origin Pro 2016 (USA) software. Statistical significance was defined as $P < 0.05$.

3. Results

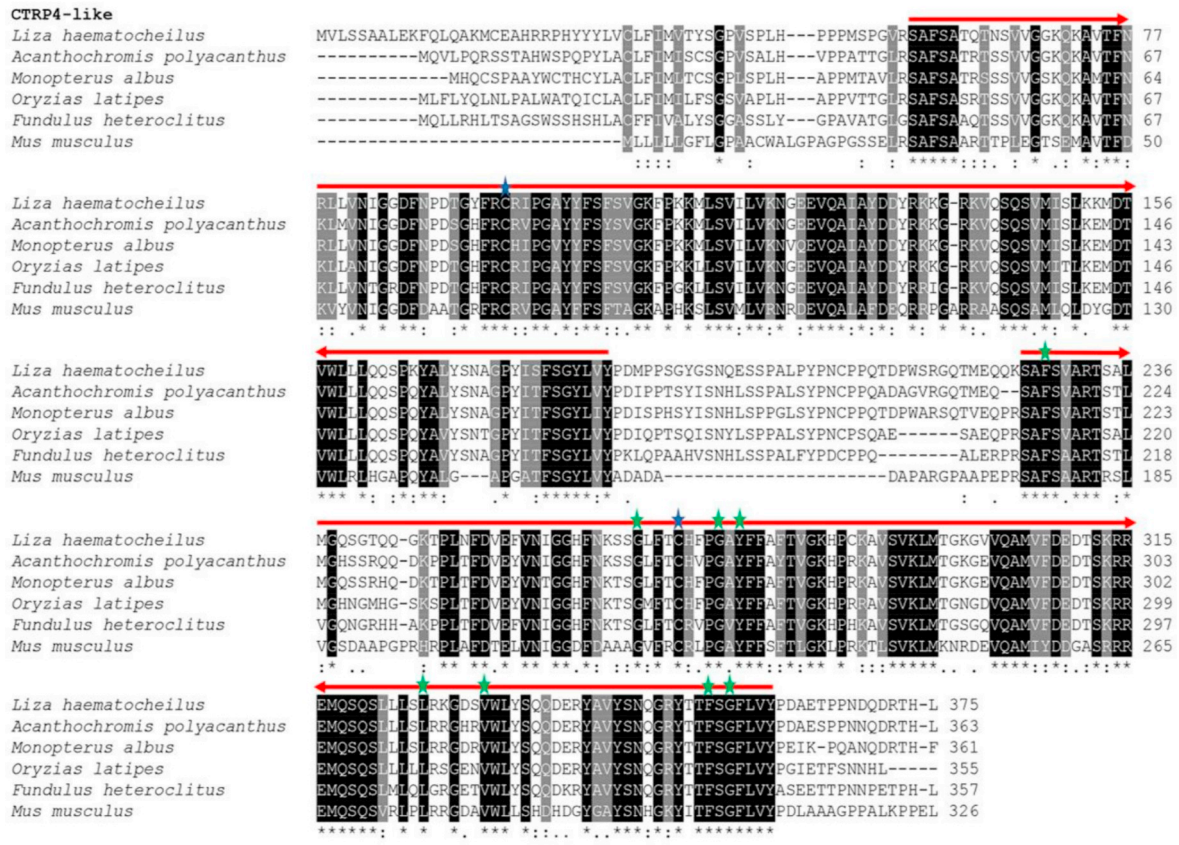
3.1. Sequence analysis of MuCTRPs

The lengths of the coding sequences of MuCTRP4-like (MH370116), MuCTRP5 (MH370117), MuCTRP6 (MH370118), and MuCTRP7 (MH370119) were 1128, 753, 729, and 888 bp, respectively. The theoretical isoelectric point (pI), molecular mass, and length of the encoded protein sequences are shown in Table 2. *In silico* analysis revealed that MuCTRP4-like contained two C1q domains between residues 56–185 and 226–360, respectively (Fig. 1). However, MuCTRP4-like did not contain a signal peptide, whereas a N-linked glycosylation site was found at the peptide sequence NKSS at residues 263–266. MuCTRP5 consisted of one collagen domain between residues 36–101 and one C1q domain at residues 110–239. (Fig. 1). Furthermore, a signal peptide cleavage site was found at residue 22, but no N-linked glycosylation sites were found. MuCTRP6 had one C1q domain between residues 107–237, one collagen domain between residues 61–82 (Fig. 1), and a signal peptide cleavage site at residue 17, but no N-linked glycosylation site. Finally, MuCTRP7 was comprised of a collagen domain at residues 45–146, a C1q domain at residues 154–280, a signal peptide at residues 1–26, but no N-linked glycosylation sites (Fig. 1).

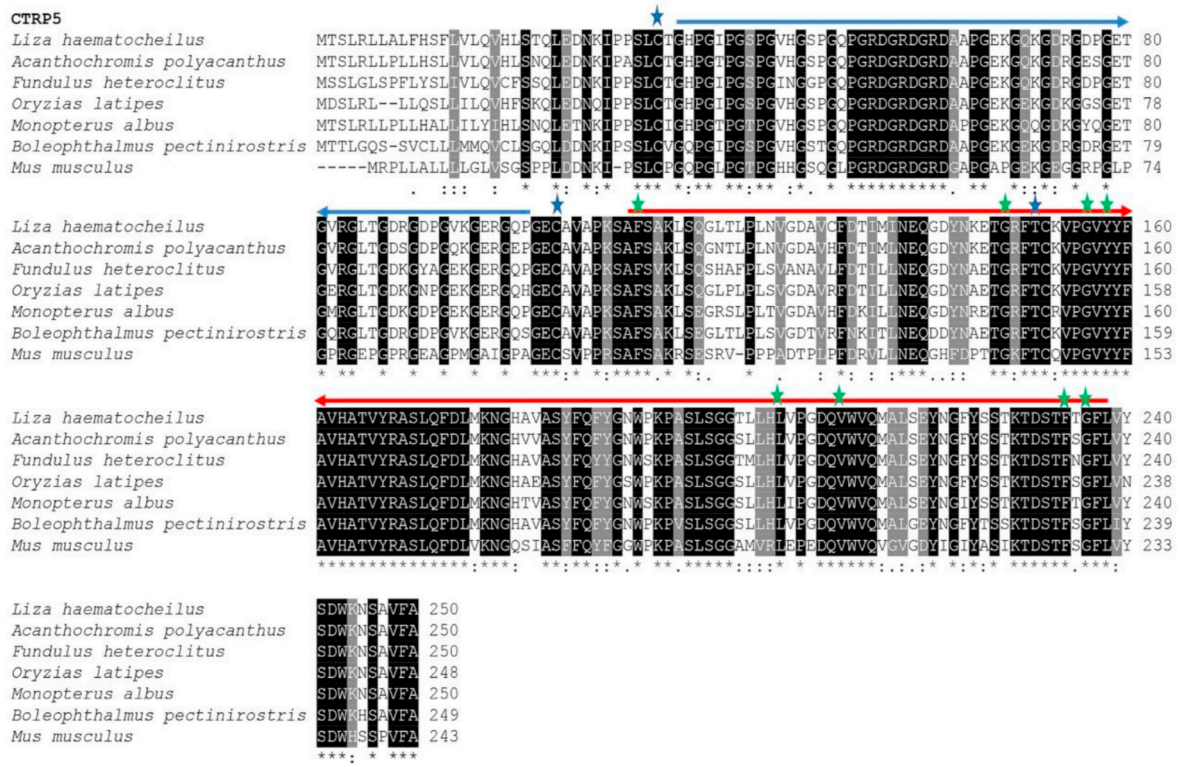
3.2. Multiple sequence alignment and phylogenetic analysis of MuCTRP sequences

According to the multiple sequence alignment, CTRP4-like consisted of two tandem C1q domains, which were combined with a non-conserved amino acid linker region. As depicted in Fig. 2A, two highly

A



B



(caption on next page)

Fig. 2. Multiple-sequence alignment of MuCTRP4-like, MuCTRP5, MuCTRP6, MuCTRP7, and its orthologs from selected organisms. Sequence alignments were performed using the Clustal Omega tool. Conserved residues are shaded in black. The conserved collagen domains are marked with a blue arrow, and conserved C1q domains are indicated on the sequences by the red arrow. Blue stars denote the conserved cysteine residues in the selected species, and green asterisks represent the conserved amino acids in the C1q family. Consensus symbols beneath the alignment; the asterisk (*) indicates the positions that have fully conserved residues, colon (:) indicates conservation between amino acids of strongly similar properties, and period (.) indicates amino acids with weak similar properties. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

conserved cysteine residues were observed in each C1q domain of CTRP4-like. CTRP5 sequences have 23 conserved GYX repeats that represent the collagen region (Fig. 2B). Furthermore, it was composed of one C1q domain and three conserved cysteine residues (Fig. 2B). CTRP6 was highly conserved among the selected species (Fig. 2C), with 14 conserved GYX repeats collagen region, one conserved C1q domain, and four conserved cysteine residues (Fig. 2C). The collagen domain of CTRP7 was represented by 34 conserved GXY repeats (Fig. 2D) and contained one C1q domain and four conserved cysteine residues (Fig. 2D). Additionally, eight conserved amino acid residues (Tyr, Gly, Phe, Val, and Leu) were observed in the C1q domain of all CTRP sequences (Fig. 2D).

Among the selected species, MuCTRP4-like, MuCTRP5, and MuCTRP7 shared the highest identity and similarity with *Acanthochromis polyacanthus* (Table 3). MuCTRP6 shared the highest identity and similarity with *Labrus bergylta* compared to the other

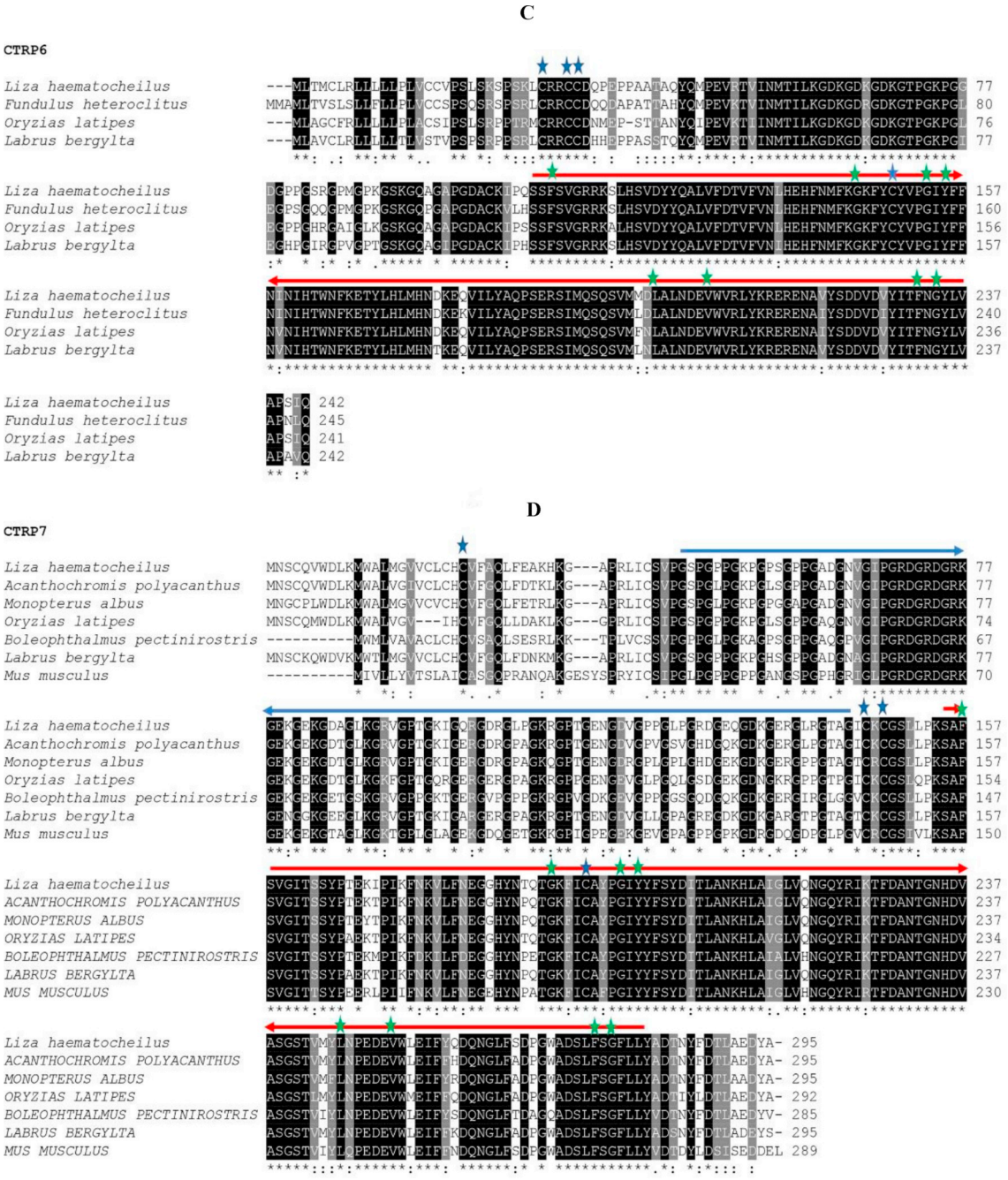


Fig. 2. (*continued*)

Table 3
Identity and similarity percentages of CTRP orthologs from different species compared to MuCTRPs.

Accession	Scientific name	Identity %	Similarity %
CTRP4-like			
XP_022056278.1	*Acanthochromis polyacanthus*	80.3	86.4
XP_020474212.1	*Monopterus albus*	76.4	83.2
XP_004070130.1	*Oryzias latipes*	72.4	81.6
XP_012731482.1	*Fundulus heteroclitus*	70.1	79.2
AAY21929.1	*Mus musculus*	42.9	56.1
CTRP5			
XP_022056645.1	*Acanthochromis polyacanthus*	93.5	95.2
XP_012723495.1	*Fundulus heteroclitus*	89.0	91.2
XP_020563951.1	*Oryzias latipes*	88.0	92.8
XP_020455834.1	*Monopterus albus*	86.0	92.4
XP_020773689.1	*Boleophthalmus pectinirostris*	82.0	88.2
AAH25174.1	*Mus musculus*	58.5	71.9
CTRP6			
XP_020499617.1	*Labrus bergylta*	88.0	93.4
XP_011476708.1	*Oryzias latipes*	86.8	93.0
XP_012704990.1	*Fundulus heteroclitus*	86.5	92.7
AAH20551.1	*Homo sapiens*	47.8	63.6
CTRP7			
XP_022055180.1	*Acanthochromis polyacanthus*	91.9	94.9
XP_020459139.1	*Monopterus albus*	88.8	92.2
XP_020486523.1	*Labrus bergylta*	86.4	91.9
XP_004068372.	*Oryzias latipes*	84.4	90.5
XP_020774550.1	*Boleophthalmus pectinirostris*	77.3	84.1
AAY21932.1	*Mus musculus*	65.9	76.5

selected species. According to the phylogenetic tree illustrated in Fig. 3, all of the MuCTRP paralogs clustered with the respective orthologs distinctly. All of the MuCTRPs were cladded into their respective fish CTRP groups. CTRPs from mammalians and fish appeared to have evolved from a common ancestor and then diverged into separate groups. Furthermore, CTRP7, CTRP2, CTRP9, and CTRP5 might have evolved from a common ancestor and clustered into different clades. Finally, the remaining CTRPs in the phylogenetic tree appeared to have evolved from a distantly related common ancestor and then diverged into separate clades.

3.3. Tissue-specific mRNA expression of MuCTRPs

The qPCR analysis revealed that all of the *MuCTRP* homologs were ubiquitously expressed in all of the tissues tested (Fig. 4). According to the tissue-specific mRNA distribution, *MuCTRP4-like* was highly expressed in the blood and intestine followed by muscle, while the lowest expression was observed in the liver (Fig. 4A). Prominent mRNA expression of *MuCTRP5* was observed in muscles followed by the intestine and stomach (Fig. 4B). Interestingly, the kidney, blood, head kidney, and liver had a low level of *MuCTRP5* mRNA expression. *MuCTRP*6 was highly transcribed in peripheral blood cells, whereas the muscles, skin, intestine, brain, and spleen exhibited moderate expression levels (Fig. 4C). A low level of *MuCTRP6* expression was observed in the stomach, gill, heart, head kidney, kidney, and liver. In the expression profile of *MuCTRP7*, the highest mRNA expression was detected in the stomach followed by muscles, while the liver, blood, head kidney, and kidney showed the lowest expression among the tissues tested (Fig. 4D).

3.4. Temporal mRNA expression of MuCTRPs upon immune stimulation

Temporal mRNA expression profile of *MuCTRP4-like* in the blood showed significant modulations towards all of the selected PAMPs and bacterial stimuli during the post-injection time period. After stimulation with poly I:C, *MuCTRP4-like* mRNA levels were significantly upregulated at 24 and 48 h p.i., whereas downregulated expression was observed at 6 h p.i. compared to the un-injected control (Fig. 5A). For LPS, *MuCTRP4-like* mRNA levels were significantly upregulated at 24 h p.i. Moreover, *MuCTRP4-like* expression was significantly upregulated at 6, 24, and 72 h p.i towards *L. garvieae* (Fig. 5A). Furthermore, *MuCTRP5* showed significant upregulation at 24–72 h p.i towards poly I:C (Fig. 5B). Upon LPS challenge, *MuCTRP5* expression was significantly upregulated and reached a peak (1.17-fold) at 24 h p.i, and then diminished up to 72 h p.i. After the *L. garvieae* challenge, significant upregulation was only observed at 24 h p.i (Fig. 5B). The temporal mRNA expression profile of *MuCTRP6* in blood cells upon immune stimulation is depicted in Fig. 5C. *MuCTRP6* expression was significantly upregulated at 24–48 h p.i of poly I:C. For LPS, the *MuCTRP6* showed statistically significant modulation at 24 h p.i. For *L. garvieae*, the highest upregulation of *MuCTRP6* was observed at 6 h p.i., and transcription was reduced to normal levels at 24 h and then was gradually downregulated during the observed time period. Significant upregulation of *MuCTRP7* mRNA can be observed at 24 and 48 h p.i. upon poly I:C. Moreover, *MuCTRP7* showed significant upregulation of *L. garvieae* at 24 h p.i.

4. Discussion

The C1q family is comprised of complement proteins and a diverse range of non-complement proteins [2]. This study might be helpful for the elucidation of functional aspects of CTRP4-like, CTRP5, CTRP6, and CTRP7 in fish. Typically, CTRPs are recognized as paralogs of adiponectin, because adiponectin is structurally and functionally similar to most CTRPs [12]. For example, adiponectin is a trimer of β sandwich chains, each with a 10-strand jelly-roll folding topology [34]. This feature is conserved in the C1q/TNF family [1,35]. Furthermore, this feature is involved in many functional aspects such as inflammation, adaptive immunity, apoptosis, and energy regulation [34]. Therefore, most studies have predicted the function and structure of CTRPs based on adiponectin [35]. MuCTRP4-like, MuCTRP5, MuCTRP6, and MuCTRP7 share the characteristic domain architecture of adiponectin and C1q/TNF family. It has been demonstrated that CTRPs have a homo or heterotrimeric organization that is stabilized through a cluster of hydrophobic interactions [34]. For example, Human CTRP4 and CTRP5 are stabilized as homotrimers [13]. Furthermore, human CTRP6 and CTRP7 can form a heterotrimer with CTRP1 and CTRP2, respectively [13]. Four residues (Tyr, Gly, Phe, and Leu) are conserved through the C1q and TNF family, which were observed in MuCTRPs (Fig. 2) [2]. It has been reported that these conserved residues are important for packing the hydrophobic core of the promoters [3]. The gC1q domain of the CTRPs is located at the C-terminus of the collagen stalk [36], and the collagen stalk of CTRPs are represented by GXY repeats. MuCTRP5, MuCTRP6, and MuCTRP7 contained collagen regions with different GXY repeats that appeared to be conserved with those in mammals (Fig. 2) [12]. The collagen region implies a structural stability of the trimeric organization [34]. It has been suggested that this trimeric complex can form multimeric complexes that are assembled through conserved N-terminus cysteine residues [14]. Different CTRP multimeric complexes may have distinct signaling properties [14]. However, C1qTNF6 from Zebrafish (ZfCTRP6) showed slight variations in structure relative to MuCTRP6. ZfCTRP6 does not contain a signal peptide or collagen domain. It contains only a gC1q domain [6]. Therefore, the domain organization of MuCTRP6 is analogous to mammalian CTRPs rather than to ZfCTRP6. Even though MuCTRP7 and ZfCTRP7 have the same domain organization, the GXY repeats in the collagen domains of the two sequences are different. ZfCTRP7 contains 20 GXY repeats, and MuCTRP6 has 34 GXY repeats, which is similar to the collagen domain of mammalian CTRP6 [6,12]. Unlike other CTRPs, CTRP4 does not possess a collagen-like domain [16]. Normally, CTRP4 consists of a signal peptide and two tandem gC1q domains [37]. This unique feature is not shared by other CTRPs [16,37]. However, MuCTRP4-like does not contain a signal peptide and ZfC1qTNF4 also shares the same structural attributes [6]. Since signal peptides are required for protein secretion, MuCTRP4-like may not have extracellular functions [38].

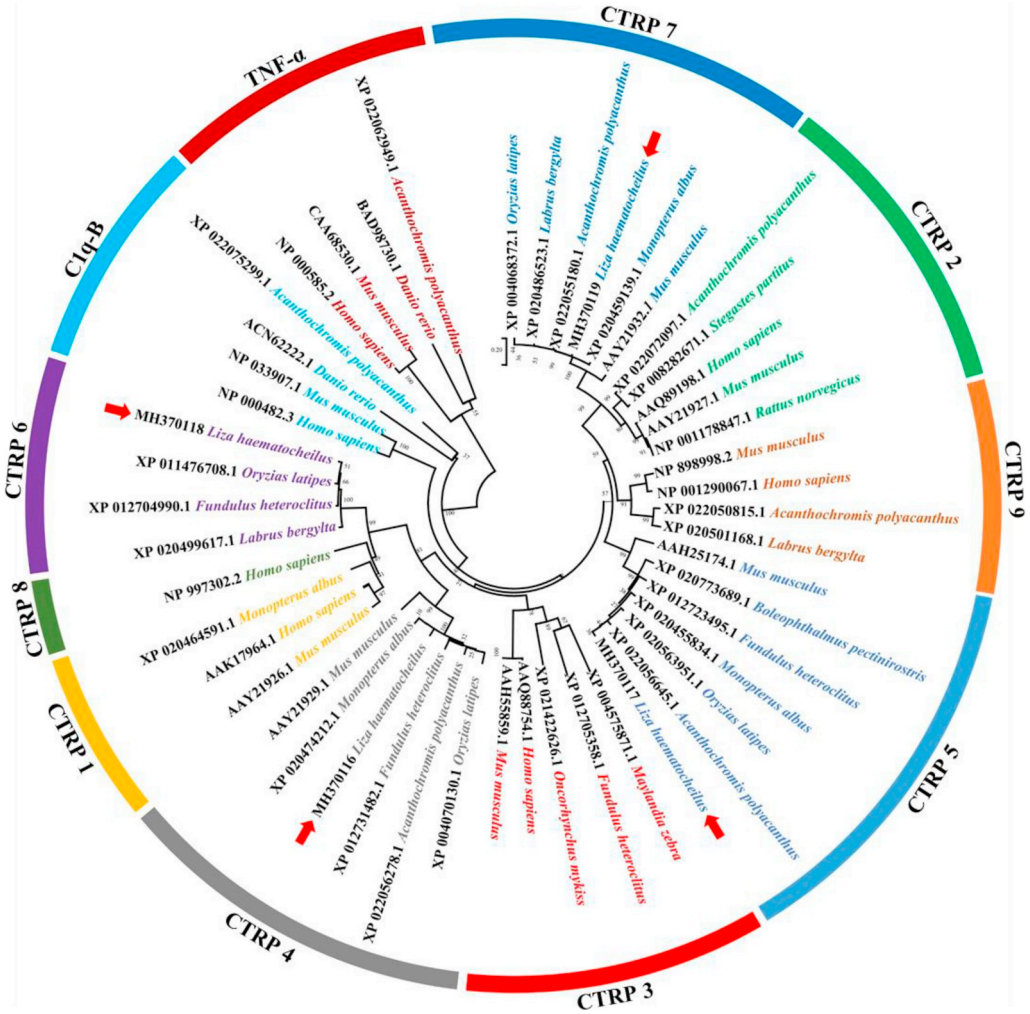


Fig. 3. Phylogenetic reconstruction of MuCTRP4-like, MuCTRP5, MuCTRP6, and MuCTRP7. The evolutionary development of MuCTRPs was analyzed with its different homologs and categorized under different taxonomic groups based on the multiple alignment profile of the protein sequences generated by the neighbor-joining method using MEGA 7.0 software. Bootstrap support values corresponding to each branch are indicated.

Phylogenetic results demonstrated that the TNF-α and C1q subcomponents evolved from a distantly related common ancestor, and that CTRPs evolved from C1q complement components. Many studies have demonstrated this based on crystal structure [3]. When comparing the CTRP orthologs from fish to those from mammals, they are significantly branched but clustered with respective CTRP orthologs, suggesting a divergence of the molecules during evolution. Therefore, we suggest that fish CTRPs have a distinct evolutionary pattern compared to mammals. Furthermore, it has been reported that the gC1 domain architecture is highly varied between Actinopterygii and mammals [39].

When considering the expression and function of CTRPs, many CTRPs are recognized as cytokines [13]. According to previous studies, mammalian CTRPs are mainly involved in a diverse range of metabolic, hormonal and immune functions [2,13,40]. It has been reported that *CTRP4* was highly expressed in the brain of humans, mice, and zebrafish [37]. In contrast, *MuCTRP4-like* was highly expressed in peripheral blood cells and the intestine. Previous study emphasized that normal tissue expression of CTRP4 was mainly involved in energy and other metabolic functions [37]. Compared to these results, a higher tissue expression of MuCTRP4-like in blood cells and the intestine can be related to glucose and lipid metabolism. Human and mouse *CTRP5* are mainly expressed in the retina of the eye and circulates in plasma [41]. Its physiological role in peripheral tissues are not known but there have been studies suggesting that CTRP5 leads phosphorylation of AMP-activated protein kinase (AMPK), thus stimulating glucose uptake and fatty acid oxidation in myocytes [42]. Activation of AMPK in skeletal muscles boosts fatty acid oxidation by phosphorylation of acetyl-CoA carboxylase [42]. Therefore, the highest expression of *MuCTR5* in muscles might be related to glucose and fatty acid metabolism in mullets. CTRP6 in mammals is mainly secreted from the placenta and adipose tissue, but *MuCTRP6* was predominantly expressed in peripheral blood cells [14,43]. However, it has been reported that CTRP6 can induce fatty acid oxidation by AMPK activation and is involved in glucose and lipid metabolism [44]. *MuCTRP7* is highly expressed in the stomach, whereas in human, CTRP7 is prominently expressed in lungs and adipose tissues [36]. CTRP7 has been identified as a regulator of whole body insulin sensitivity and glucose metabolism [45]. Furthermore, it has been suggested that the local expression and action of CTRP7 may differ between tissues depending on the metabolic context under normal physiological conditions [37]. Therefore, the high expression of MuCTRP7 in the stomach may be due to glucose metabolism or metabolic alterations.

MuCTRP4, MuCTRP5, MuCTRP6, and *MuCTRP7* were significantly upregulated in response to poly I:C, LPS, and *L. garvieae*. Therefore, MuCTRPs may have an immunological function in response to live bacteria and PAMPs stimuli. All of the *MuCTRPs* reached peak expression levels within 24 h p.i. Typically, the innate immunity responds within 0–96 h p.i [46]; therefore, we hypothesize that MuCTRPs may contribute to innate immune responses. It has been reported that the

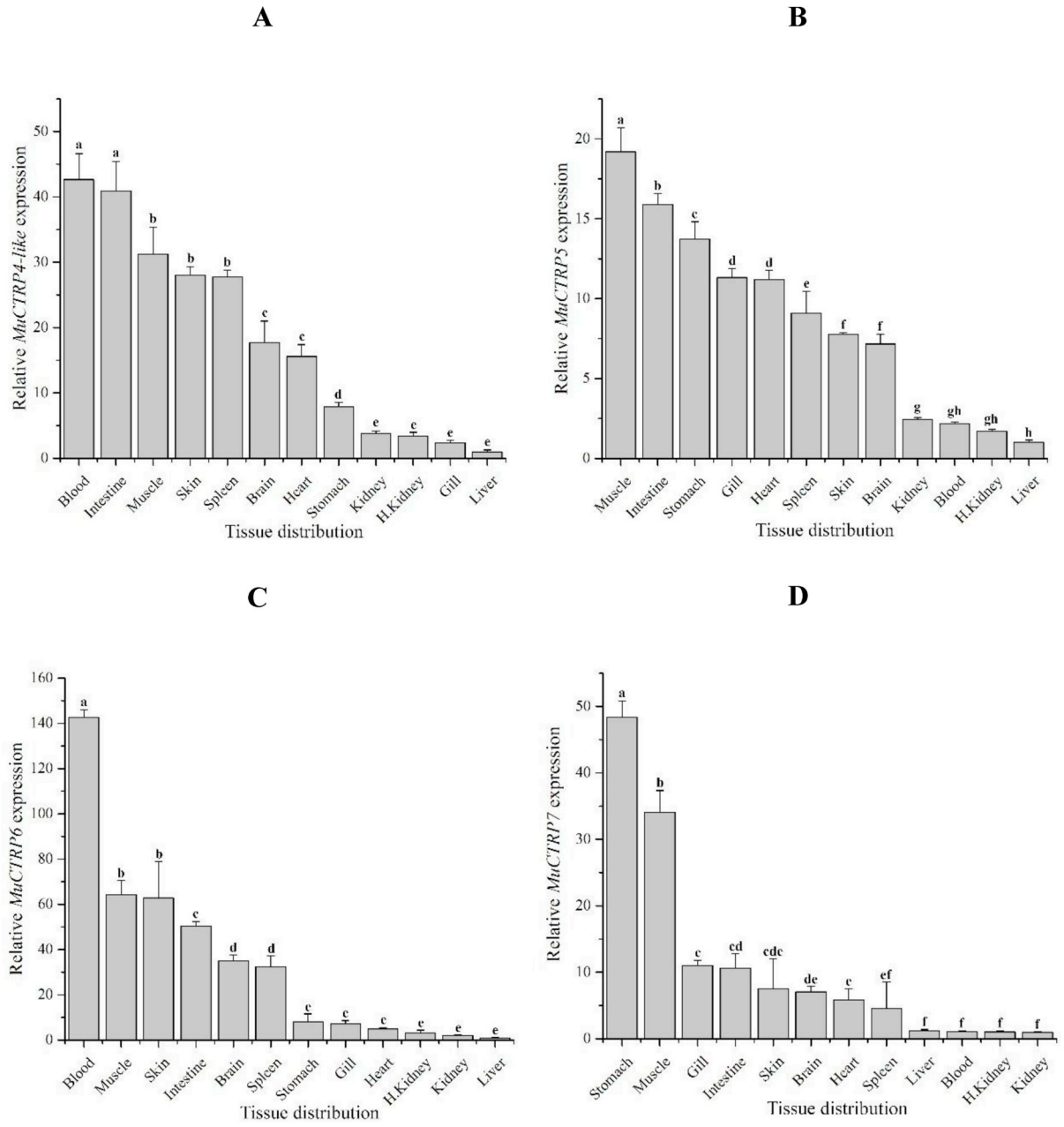


Fig. 4. Tissue-specific expression of (A) *MuCTRP4*-like, (B) *MuCTRP5*, (C) *MuCTRP6*, and (D) *MuCTRP7* under normal physiological conditions. The Livak method was used to calculate the relative mRNA expression of each tissue, and the Mullet *EF1α* gene was used as an internal control gene in the qPCR experiment. The data are represented as mean values (n = 3) ± standard deviation.

overexpression of CTRP4 in human endothelial and HEK293 cells induce the nuclear factor κB (NF-κB) activity [16]. NF-κB signaling can control the transcription of cytokines, antimicrobial effectors, and other genes that regulate cellular differentiation, survival, and proliferation [47], thereby contribute to various aspects of innate and adaptive immune responses. In addition, overexpression of CTRP4 can elevate the levels of TNF, IL-6, and STAT3 in HepG2 cells [13]. All of these transcription factors are involved in innate and adaptive immune responses in both healthy and carcinoma cells [16,48–51]. Therefore, modulation of *MuCTRP4-like* in response to PAMP and bacterial stimuli could induce the activation of cell survival factors such as NF-κB and STAT3.

According to the previous studies, CTRP5 expression was elevated in mitochondrial DNA-depleted myocytes, which can activate AMPK [52]. Activation of AMPK leads to a reduction in the accumulation of reactive oxygen species while attenuating the activation of the caspase 3 pathway, which prevents cell apoptosis under pathological conditions [52]. There is currently no prevailing data for CTRP5 expression in blood cells, but similar AMPK activation was observed in the lungs under chronic inflammatory conditions [53]. Therefore, modulation of *MuCTRP5* mRNA levels in response to PAMP and bacterial stimuli might indicate the activation of AMPK which prevent cell apoptosis.

CTRP6 induces IL-10 in Raw 264.7 cells [43]. IL-10 is an anti-inflammatory cytokine that constrains the function of Th1 cells, NK cells, and macrophages during infections [54], all of which are required for pathogen clearance but also involve in tissue damage during excessive activation [55–57]. Therefore, IL-10 was recognized as a key immunoregulator to prevent excessive immune activation during viral, bacterial, fungal, and parasitic infections [54]. Accordingly, MuCTRP6 might act as an important transcriptional factor that stimulates IL-10 expression during infections and thus regulate the immune system [43].

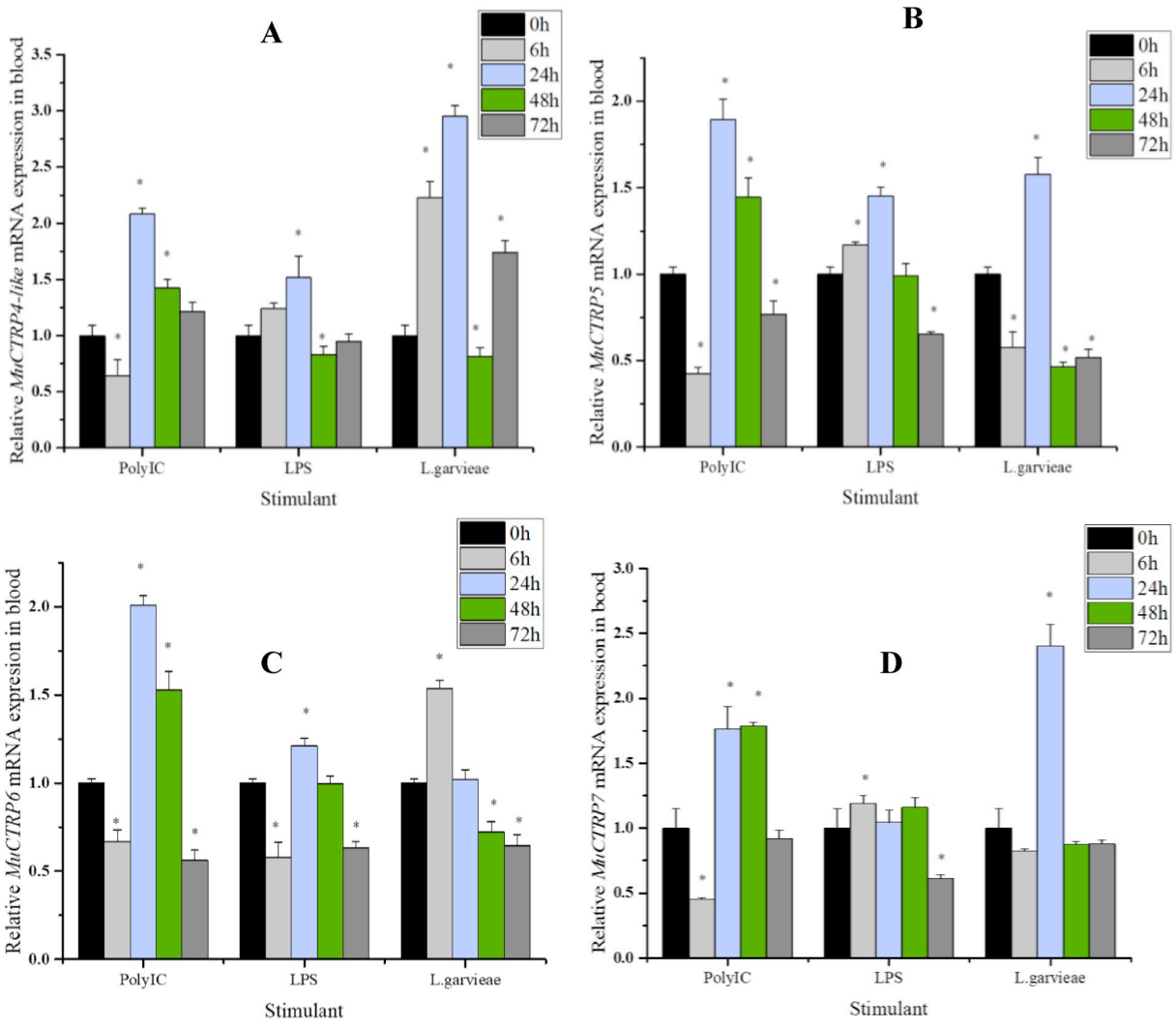


Fig. 5. Temporal expression profiles of (A) *MuCTRP4*-like, (B) *MuCTRP5*, (C) *MuCTRP6*, and (D) *MuCTRP7* in blood tissue after pathogen-associated molecular patterns (LPS and Poly I:C) and bacterial *(L. garvieae)* challenges. The Livak method was used to determine the relative mRNA expression levels of MuCTRPs using the Mullet *EF1α* gene as an internal control gene in the qPCR experiment. The fold changes in expression at different time points were compared with those at 0 h. The vertical bars represent the mean values (n = 3) ± standard deviation. Significant differences compared to the blank (0 h) are indicated with an asterisk for $P < 0.05$.

Many studies have suggested that CTRPs play a major role in metabolism. Cellular metabolism can be a regulator of the immune system [58]. Immune cells itself have to adapt to different microenvironments in tissues in order to act effectively. During a bacterial infection, competition for nutrients can occur in the host cells [59]. Since glucose is a key fuel for bacteria, the availability of glucose for immune cells can be depleted [60]. Viral infections can result in the depletion of glucose, and various cells at the inflammation site can compete for nutrients in the affected tissue. Therefore, metabolic alterations in infected tissues as well as in immune cells are required for pathogen clearance and recovery of the tissues [58]. It has been suggested that CTRPs are dual functional cytokines and hormones, which play roles in immunity and metabolism [13,36]. Therefore, upregulations of *MuCTRP4-like, MuCTRP5, MuCTRP6,* and *MuCTRP7* against pathogens suggest a link between metabolism and immunity.

In conclusion, we characterized four members of the CTRPs from *Liza haematocheila.* According to the tissue-specific expression profile, *MuCTRPs* were ubiquitously expressed in all of the tissues examined. Furthermore, transcriptional modulation of *MuCTRPs* in response to LPS, poly I:C, and *L. garvieae* reflect their possible involvement during immune responses.

Acknowledgment

This research was a part of the project titled 'Fish Vaccine Research Center,' funded by the Ministry of Oceans and Fisheries, Korea, and was supported by a grant from the Marine Biotechnology Program (20180430, Genome Analysis of Marine and Fisheries Organisms and Development of Functional Applications) Funded by the Ministry of Oceans and Fisheries, Korea.

References

[1] N.M. Thielens, F. Tedesco, S.S. Bohlson, C. Gaboriaud, A.J. Tenner, C1q: a fresh look upon an old molecule, Mol. Immunol. 89 (2017) 73–83, https://doi.org/10.1016/j.molimm.2017.05.025.
[2] U. Kishore, C. Gaboriaud, P. Waters, A.K. Shrive, T.J. Greenhough, K.B.M. Reid, R.B. Sim, G.J. Arlaud, C1q and tumor necrosis factor superfamily: modularity and versatility, Trends Immunol. 25 (2004) 551–561, https://doi.org/10.1016/j.it.2004.08.006.
[3] L. Shapiro, P.E. Scherer, The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor, Curr. Biol. 8 (1998) 335–340, https://doi.org/10.1016/S0960-9822(98)70133-2.
[4] L. Chen, F. Wu, S. Yuan, B. Feng, Identification and characteristic of three members of the C1q/TNF-related proteins (CTRPs) superfamily in Eudontomyzon morii, Fish Shellfish Immunol. 59 (2016) 233–240, https://doi.org/10.1016/j.fsi.2016.10.034.
[5] A. Kopp, M. Bala, J. Weigert, C. Büchler, M. Neumeier, C. Aslanidis, J. Schölmerich,

A. Schäffler, Effects of the new adiponectin paralogous protein CTRP-3 and of LPS on cytokine release from monocytes of patients with type 2 diabetes mellitus, Cytokine 49 (2010) 51–57, https://doi.org/10.1016/j.cyto.2009.10.001.
[6] J. Mei, J. Gui, Bioinformatic identification of genes encoding C1q-domain- containing proteins in zebrafish, J. Genet. Genomics 35 (2008) 17–24 doi:16/S1673-8527(08)60003-X.
[7] X. Zhang, P. Zhang, C. Li, Y. Li, C. Jin, W. Zhang, Characterization of two regulators of the TNF-α signaling pathway in Apostichopus japonicus: LPS-induced TNF-α factor and baculoviral inhibitor of apoptosis repeat-containing 2, Dev. Comp. Immunol. 48 (2015) 138–142, https://doi.org/10.1016/j.dci.2014.10.001.
[8] S. Li, X. Li, X. Gen, Y. Chen, J. Wei, J. Sun, Identification and characterization of lipopolysaccharide-induced TNF-alpha factor gene from Japanese flounder Paralichthys olivaceus, Vet. Immunol. Immunopathol. 157 (2014) 182–189, https://doi.org/10.1016/j.vetimm.2013.11.006.
[9] S. Li, Z. Jia, X. Li, X. Geng, J. Sun, Identification and expression analysis of lipopolysaccharide-induced TNF-alpha factor gene in Chinese mitten crab Eriocheir sinensis, Fish Shellfish Immunol. 38 (2014) 190–195, https://doi.org/10.1016/j.fsi.2014.03.019.
[10] L.L. Zhao, M. Jin, X.C. Li, Q. Ren, J.F. Lan, Four C1q domain-containing proteins involved in the innate immune response in Hyriopsis cumingii, Fish Shellfish Immunol. 55 (2016) 323–331, https://doi.org/10.1016/j.fsi.2016.06.003.
[11] O. Nakamura, Y. Wada, F. Namai, E. Saito, K. Araki, A. Yamamoto, S. Tsutsui, A novel C1q family member with fucose-binding activity from surfperch, Neoditrema ransonnetii (Perciformes, Embiotocidae), Fish Shellfish Immunol. 27 (2009) 714–720, https://doi.org/10.1016/j.fsi.2009.09.010.
[12] G.W. Wong, J. Wang, C. Hug, T.-S. Tsao, H.F. Lodish, A family of Acrp30/adiponectin structural and functional paralogs, Proc. Natl. Acad. Sci. Unit. States Am. 101 (2004) 10302–10307, https://doi.org/10.1073/pnas.0403760101.
[13] A. Schäffler, C. Buechler, CTRP family: linking immunity to metabolism, Trends Endocrinol. Metabol. 23 (2012) 194–203, https://doi.org/10.1016/j.tem.2011.12.003.
[14] G.W. Wong, S.A. Krawczyk, C. Kitidis-Mitrokostas, T. Revett, R. Gimeno, H.F. Lodish, Molecular, biochemical and functional characterizations of C1q/TNF family members: adipose-tissue-selective expression patterns, regulation by PPAR-γ agonist, cysteine-mediated oligomerizations, combinatorial associations and metabolic functions, Biochem. J. 416 (2008) 161–177, https://doi.org/10.1042/BJ20081240.
[15] G.W. Wong, S.A. Krawczyk, C. Kitidis-Mitrokostas, G. Ge, E. Spooner, C. Hug, R. Gimeno, H.F. Lodish, Identification and characterization of CTRP9, a novel secreted glycoprotein, from adipose tissue that reduces serum glucose in mice and forms heterotrimers with adiponectin, Faseb. J. 23 (2009) 241–258, https://doi.org/10.1096/fj.08-114991.
[16] Q. Li, L. Wang, W. Tan, Z. Peng, Y. Luo, Y. Zhang, G. Zhang, D. Na, P. Jin, T. Shi, D. Ma, L. Wang, Identification of C1qTNF-related protein 4 as a potential cytokine that stimulates the STAT3 and NF-κB pathways and promotes cell survival in human cancer cells, Cancer Lett. 308 (2011) 203–214, https://doi.org/10.1016/j.canlet.2011.05.005.
[17] C. Gestal, A. Pallavicini, P. Venier, B. Novoa, A. Figueras, MgC1q, a novel C1q-domain-containing protein involved in the immune response of Mytilus galloprovincialis, Dev. Comp. Immunol. 34 (2010) 926–934, https://doi.org/10.1016/j.dci.2010.02.012.
[18] H.H. Liu, L.X. Xiang, J.Z. Shao, A novel C1q-domain-containing (C1qDC) protein from Mytilus coruscus with the transcriptional analysis against marine pathogens and heavy metals, Dev. Comp. Immunol. 44 (2014) 70–75, https://doi.org/10.1016/j.dci.2013.11.009.
[19] L. Wang, C. Fan, W. Xu, Y. Zhang, Z. Dong, J. Xiang, S. Chen, Characterization and functional analysis of a novel C1q-domain-containing protein in Japanese flounder (Paralichthys olivaceus), Dev. Comp. Immunol. 67 (2017) 322–332, https://doi.org/10.1016/j.dci.2016.09.001.
[20] H.J. Han, N.S. Lee, M.S. Kim, S.H. Jung, An outbreak of lactococcus garvieae infection in cage-cultured red lip mullet chelon haematocheilus with green liver syndrome, Fish. Aquat. Sci. 18 (2015) 333–339, https://doi.org/10.5657/FAS.2015.0333.
[21] W.S. Kim, J.H. Kim, M.S. Jang, S.J. Jung, M.J. Oh, Infection of wild mullet (Mugil cephalus) with Myxobolus episquamalis in Korea, Parasitol. Res. 112 (2013) 447–451, https://doi.org/10.1007/s00436-012-3075-7.
[22] R. Agarwala, T. Barrett, J. Beck, D.A. Benson, C. Bollin, E. Bolton, D. Bourexis, J.R. Brister, S.H. Bryant, K. Canese, C. Charowhas, K. Clark, M. Dicuccio, I. Dondoshansky, S. Federhen, M. Feolo, K. Funk, L.Y. Geer, V. Gorelenkov, M. Hoeppner, B. Holmes, M. Johnson, V. Khotomlianski, A. Kimchi, M. Kimelman, P. Kitts, W. Klimke, S. Krasnov, A. Kuznetsov, M.J. Landrum, D. Landsman, J.M. Lee, D.J. Lipman, Z. Lu, T.L. Madden, T. Madej, A. Marchler-Bauer, I. Karsch-Mizrachi, T. Murphy, R. Orris, J. Ostell, C. O'sullivan, A. Panchenko, L. Phan, D. Preuss, K.D. Pruitt, K. Rodarmer, W. Rubinstein, E. Sayers, V. Schneider, G.D. Schuler, S.T. Sherry, K. Sirotkin, K. Siyan, D. Slotta, A. Soboleva, V. Soussov, G. Starchenko, T.A. Tatusova, K. Todorov, B.W. Trawick, D. Vakatov, Y. Wang, M. Ward, W.J. Wilbur, E. Yaschenko, K. Zbicz, Database resources of the national center for Biotechnology information, Nucleic Acids Res. 44 (2016) D7–D19, https://doi.org/10.1093/nar/gkv1290.
[23] R. Agarwala, T. Barrett, J. Beck, D.A. Benson, C. Bollin, E. Bolton, D. Bourexis, J.R. Brister, S.H. Bryant, K. Canese, C. Charowhas, K. Clark, M. DiCuccio, I. Dondoshansky, M. Feolo, K. Funk, L.Y. Geer, V. Gorelenkov, W. Hlavina, M. Hoeppner, B. Holmes, M. Johnson, V. Khotomlianski, A. Kimchi, M. Kimelman, P. Kitts, W. Klimke, S. Krasnov, A. Kuznetsov, M.J. Landrum, D. Landsman, J.M. Lee, D.J. Lipman, Z. Lu, T.L. Madden, T. Madej, A. Marchler-Bauer, I. Karsch-Mizrachi, T. Murphy, R. Orris, J. Ostell, C. O'Sullivan, V. Palanigobu, A.R. Panchenko, L. Phan, K.D. Pruitt, K. Rodarmer, W. Rubinstein, E.W. Sayers, V. Schneider, C.L. Schoch, G.D. Schuler, S.T. Sherry, K. Sirotkin, K. Siyan, D. Slotta, A. Soboleva, V. Soussov, G. Starchenko, T.A. Tatusova, K. Todorov, B.W. Trawick, D. Vakatov, Y. Wang, M. Ward, W.J. Wilbur, E. Yaschenko, K. Zbicz, Database resources of the national center for Biotechnology information, Nucleic Acids Res. 45 (2017) D12–D17, https://doi.org/10.1093/nar/gkw1071.
[24] A. Marchler-Bauer, M.K. Derbyshire, N.R. Gonzales, S. Lu, F. Chitsaz, L.Y. Geer, R.C. Geer, J. He, M. Gwadz, D.I. Hurwitz, C.J. Lanczycki, F. Lu, G.H. Marchler, J.S. Song, N. Thanki, Z. Wang, R.A. Yamashita, D. Zhang, C. Zheng, S.H. Bryant, CDD: NCBI's conserved domain database, Nucleic Acids Res. 43 (2015) D222–D226, https://doi.org/10.1093/nar/gku1221.
[25] M. Pagni, V. Ioannidis, L. Cerutti, M. Zahn-Zabal, C.V. Jongeneel, J. Hau, O. Martin, D. Kuznetsov, L. Falquet, MyHits: improvements to an interactive resource for analyzing protein sequences, Nucleic Acids Res. 35 (2007) 433–437, https://doi.org/10.1093/nar/gkm352.
[26] E. Gasteiger, C. Hoogland, A. Gattiker, S. Duvaud, M.R. Wilkins, R.D. Appel, A. Bairoch, The proteomics protocols handbook, Proteomics Protoc. Handb. (2005) 571–607, https://doi.org/10.1385/1592598900.
[27] R. Gupta, S. Brunak, Prediction of glycosylation across the human proteome and the correlation to protein function, Biocomputing 2002 (322) (2001) 310–322, https://doi.org/10.1142/9789812799623_0029.
[28] T.N. Petersen, S. Brunak, G. Von Heijne, H. Nielsen, SignalP 4.0: discriminating signal peptides from transmembrane regions, Nat. Methods 8 (2011) 785–786, https://doi.org/10.1038/nmeth.1701.
[29] F. Sievers, A. Wilm, D. Dineen, T.J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert, J. Söding, J.D. Thompson, D.G. Higgins, Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega, Mol. Syst. Biol. 7 (2011), https://doi.org/10.1038/msb.2011.75.
[30] W. Li, A. Cowley, M. Uludag, T. Gur, H. McWilliam, S. Squizzato, Y.M. Park, N. Buso, R. Lopez, The EMBL-EBI bioinformatics web and programmatic tools framework, Nucleic Acids Res. 43 (2015) W580–W584, https://doi.org/10.1093/nar/gkv279.
[31] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (2016) 1870–1874, https://doi.org/10.1093/molbev/msw054.
[32] S.A. Bustin, V. Benes, J.A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M.W. Pfaffl, G.L. Shipley, J. Vandesompele, C.T. Wittwer, The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments, Clin. Chem. 55 (2009) 611–622, https://doi.org/10.1373/clinchem.2008.112797.
[33] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[34] U. Kishore, K.B.M. Reid, C1q: structure, function, and receptors, Immunopharmacology 49 (2000) 159–170, https://doi.org/10.1016/S0162-3109(00)80301-X.
[35] J.L. Bodmer, P. Schneider, J. Tschopp, The molecular architecture of the TNF superfamily, Trends Biochem. Sci. 27 (2002) 19–26, https://doi.org/10.1016/S0968-0004(01)01995-8.
[36] M.M. Seldin, S.Y. Tan, G.W. Wong, Metabolic function of the CTRP family of hormones, Rev. Endocr. Metab. Disord. 15 (2014) 111–123, https://doi.org/10.1007/s11154-013-9255-7.
[37] M.S. Byerly, P.S. Petersen, S. Ramamurthy, M.M. Seldin, X. Lei, E. Provost, Z. Wei, G.V. Ronnett, G.W. Wong, C1q/TNF-related protein 4 (CTRP4) is a unique secreted protein with two tandem C1q domains that functions in the hypothalamus to modulate food intake and body weight, J. Biol. Chem. 289 (2014) 4055–4069, https://doi.org/10.1074/jbc.M113.506956.
[38] J. Coleman, M. Inukai, M. Inouye, Dual functions of the signal peptide in protein transfer across the membrane, Cell 43 (1985) 351–360, https://doi.org/10.1016/0092-8674(85)90040-6.
[39] R. Ghai, P. Waters, L.T. Roumenina, M. Gadjeva, M.S. Kojouharova, K.B.M. Reid, R.B. Sim, U. Kishore, C1q and its growing family, Immunobiology 212 (2007) 253–266, https://doi.org/10.1016/j.imbio.2006.11.001.
[40] A.P. Ortiz, E.C. Díaz-Toro, W.A. Calo, V. Correa-Fernández, A. Cases, M.C. Santos-Ortiz, C. Mazas, L. Mejía, D. Wetter, Characteristics of smokers accessing the Puerto Rico Quitline, Puert. Rico Health Sci. J. 27 (2008) 213–219, https://doi.org/10.1242/jcs.03292.Multiple.
[41] M.N.A. Mandal, V. Vasireddy, G.B. Reddy, X. Wang, S.E. Moroi, B.R. Pattnaik, B.A. Hughes, J.R. Heckenlively, P.F. Hitchcock, M.M. Jablonski, R. Ayyagari, CTRP5 is a membrane-associated and secretory protein in the RPE and ciliary body and the S163R mutation of CTRP5 impairs its secretion, Investig. Ophthalmol. Vis. Sci. 47 (2006) 5505–5513, https://doi.org/10.1167/iovs.06-0312.
[42] W.M. Yang, W. Lee, CTRP5 ameliorates palmitate-induced apoptosis and insulin resistance through activation of AMPK and fatty acid oxidation, Biochem. Biophys. Res. Commun. 452 (2014) 715–721, https://doi.org/10.1016/j.bbrc.2014.08.145.
[43] M.J. Kim, W. Lee, E.J. Park, S.Y. Park, C1qTNF-related protein-6 increases the expression of interleukin-10 in macrophages, Mol. Cell. 30 (2010) 59–64, https://doi.org/10.1007/s10059-010-0088-x.
[44] W. Lee, M.J. Kim, E.J. Park, Y.J. Choi, S.Y. Park, C1qTNF-related protein-6 mediates fatty acid oxidation via the activation of the AMP-activated protein kinase, FEBS Lett. 584 (2010) 968–972, https://doi.org/10.1016/j.febslet.2010.01.040.
[45] P.S. Petersen, X. Lei, R.M. Wolf, S. Rodriguez, S.Y. Tan, H.C. Little, M.A. Schweitzer, T.H. Magnuson, K.E. Steele, G.W. Wong, CTRP7 deletion attenuates obesity-linked glucose intolerance, adipose tissue inflammation, and hepatic stress, Am. J. Physiol. Endocrinol. Metab. 312 (2017) E309–E325, https://doi.org/10.1152/ajpendo.00344.2016.

[46] M. Kenneth, P. Travers, M. Walport, Innate Immunity : the first lines of defense, Janeway's Immunobiol. (2017) 37–73 doi:9780815342434.
[47] M.S. Hayden, A.P. West, S. Ghosh, NF-κB and the immune response, Oncogene 25 (2006) 6758–6780, https://doi.org/10.1038/sj.onc.1209943.
[48] N.L. Vujanovic, Role of TNF superfamily ligands in innate immunity, Immunol. Res. 50 (2011) 159–174, https://doi.org/10.1007/s12026-011-8228-8.
[49] B. Siegmund, TNF and its impact on adaptive immune mechanisms and chronic inflammation, Front. Gastrointest. Res. 34 (2015) 56–61, https://doi.org/10.1159/000381410.
[50] T. Tanaka, M. Narazaki, T. Kishimoto, Il-6 in inflammation, Immunity, and disease, Cold Spring Harb. Perspect. Biol. 6 (2014) 1–16, https://doi.org/10.1101/cshperspect.a016295.
[51] E.J. Hillmer, H. Zhang, H.S. Li, S.S. Watowich, STAT3 signaling in immunity, Cytokine Growth Factor Rev. 31 (2016) 1–15, https://doi.org/10.1016/j.cytogfr.2016.05.001.
[52] S.Y. Park, J.H. Choi, H.S. Ryu, Y.K. Pak, K.S. Park, H.K. Lee, W. Lee, C1q tumor necrosis factor ??-related protein isoform 5 is increased in mitochondrial DNA-depleted myocytes and activates AMP-activated protein kinase, J. Biol. Chem. 284 (2009) 27780–27789, https://doi.org/10.1074/jbc.M109.005611.
[53] D. Li, Y. Wu, P. Tian, X. Zhang, H. Wang, T. Wang, B. Ying, L. Wang, Y. Shen, F. Wen, Adipokine CTRP-5 as a potential novel inflammatory biomarker in chronic obstructive pulmonary disease, Medicine (United States) 94 (2015) 1–5, https://doi.org/10.1097/MD.0000000000001503.
[54] K.N. Couper, D.G. Blount, E.M. Riley, IL-10: the master regulator of immunity to infection, J. Immunol. 180 (2008) 5771–5777, https://doi.org/10.4049/jimmunol.180.9.5771.
[55] V. Dardalhon, T. Korn, V.K. Kuchroo, A.C. Anderson, Role of Th1 and Th17 cells in organ-specific autoimmunity, J. Autoimmun. 31 (2008) 252–256, https://doi.org/10.1016/j.jaut.2008.04.017.
[56] A. Poggi, M.R. Zocchi, NK cell autoreactivity and autoimmune diseases, Front. Immunol. 5 (2014) 1–15, https://doi.org/10.3389/fimmu.2014.00027.
[57] K.C. Navegantes, R. de Souza Gomes, P.A.T. Pereira, P.G. Czaikoski, C.H.M. Azevedo, M.C. Monteiro, Immune modulation of some autoimmune diseases: the critical role of macrophages and neutrophils in the innate and adaptive immunity, J. Transl. Med. 15 (2017) 36, https://doi.org/10.1186/s12967-017-1141-8.
[58] R.M. Loftus, D.K. Finlay, Immunometabolism: cellular metabolism turns immune regulator, J. Biol. Chem. 291 (2016) 1–10, https://doi.org/10.1074/jbc.R115.693903.
[59] V.H. Smith, T.P. Jones, M.S. Smith, Host nutrition and infectious disease: an ecological view, Front. Ecol. Environ. 3 (2005) 268–274, https://doi.org/10.1890/1540-9295(2005)003[0268:HNAIDA]2.0.CO;2.
[60] N.P. Vitko, N.A. Spahich, A.R. Richardson, Glycolytic dependency of high-level nitric oxide resistance and virulence in staphylococcus aureus, MBio 6 (2015) 1–10, https://doi.org/10.1128/mBio.00045-15.