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Short communication

Dynamic evolution of CIKS (TRAF3IP2/Act1) in metazoans

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ABSTRACT

CIKS (TRAF3IP2/Act1) is important for inflammatory responses and autoimmunity control through its dual functions in CD40L/BAFF and IL17 signaling in mammalians. In this study, we performed comparative and evolutionary analyses of CIKSs from metazoans. Although nematode (*Caenorabditis elegans*) and sea urchin (*Strongylocentrotus purpuratus*) have IL17 and IL17 receptors, we found no CIKS in their genomes. The ancient CIKS-like (CIKSL) genes from the invertebrates lottia (*Lottia gigantea*) and amphioxus (*Branchiostoma floridae*) have an additional DEATH domain compared with other CIKSLs/CIKSs. Our data suggest that the ancient CIKSL evolved into early chordate CIKS possibly through gene tandem duplication and gene fission. Based on phylogenetic and synteny analyses, vertebrate CIKS genes are divided into two groups, one of which is orthologous to human CIKS and the other is paralogous. Expression analysis indicated that cephalochordata amphioxus IL17 together with CIKS might play an ancient and conserved role in host defense against bacterial infections. During the evolutionary process, the CIKS genes have obtained more and more functions through cooperation with other genes.

1. Introduction

CIKS, also called Act1 or TRAF3IP2, was first cloned as a signaling molecule involved in NF-κB signaling (Leonardi et al., 2000; Li et al., 2000). Human CIKS contains a helix-loop-helix motif at the N-terminus, a SEFIR domain and a coiled-coil motif at the C-terminus. Two TRAF binding sites were identified in the N-terminus of human CIKS (Li et al., 2000). Further study suggested that the activation of NF-κB by overexpression of CIKS is through the TAK1, NIK and IKK signaling cascade (Qian et al., 2002).

CD40/BAFFR-mediated non-canonical NF-κB activation pathway plays a key role in B cell survival (Coope et al., 2002; Kayagaki et al., 2002). Dysregulation of CD40/BAFFR pathways lead to development and pathogenesis of systemic autoimmunity (Mackay et al., 1999; Mehling et al., 2001). Co-immunoprecipitation analysis showed that endogenous CIKS is recruited to BAFFR/CD40 in B cells and epithelial cells after stimulation with their ligands BAFF and CD40L, respectively (Qian et al., 2004). Studies on B cell-specific CIKS-deficient mice demonstrated that CIKS acts as a negative regulator in B cell function and humoral immune responses through its impact on CD40- and BAFF-mediated signaling (Qian et al., 2004).

The IL17/IL17R family is a novel SEFIR domain-containing subclass of cytokines. A number of evidences supported that the IL17/IL17R system plays a critical role in host defense against bacterial/fungal infections and in the pathogenesis of human autoimmune diseases (Agarwal et al., 2008; Iwakura and Ishigame, 2006; Lubberts et al., 2005; Onishi and Gaffen, 2010; Zhang et al., 2006). TRAF6 was the first intermediate signaling component that is implicated in the IL17-mediated signaling pathway (Schwandner et al., 2000; Yao et al., 1997). CIKS is an essential adaptor molecule for IL17R-mediated signaling through binding TRAF6 and heterotypic SEFIR–SEFIR interaction in mammalians (Kanamori et al., 2002; Qian et al., 2007). Using the CIKS-deficient mice, it was subsequently found that the IL17-dependent expression of pro-inflammatory proteins absolutely requires CIKS (Qian et al., 2007).

To clarify the evolutionary dynamics of CIKS genes, in this study, we identified near-complete repertoires of CIKS genes from genomic sequences of three invertebrates (sea anemones *Nematostella vectensis*, molluscs *Lottia gigantea*, amphioxus *Branchiostoma floridae* and ciona *Ciona intestinalis*), lamprey *Petromyzon marinus*, shark *Callorhinchus milii*, three teleost fishes (zebrafish *Danio rerio*, fugu *Takifugu rubripes* and medaka *Oryzias latipes*), clawed frog *Xenopus tropicalis*, chicken *Gallus gallus* and two

Abbreviations: CIKS, connection to IKK and SAPK/JNK; Act1, nuclear factor-κB activator 1; TRAF3IP2, TRAF3-interacting protein 2; CD40, CD40 molecule; TNF, receptor superfamily member 5; CD40L, CD40 ligand; BAFF, B-cell-activating factor, tumor necrosis factor ligand superfamily member 13b; BAFFR, B-cell-activating factor receptor, tumor necrosis factor receptor superfamily, member 13c; IL17, interleukin-17; IL17R, interleukin-17 receptor; SEFIR domain, SEF/IL-17R domain.

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Table 1
Sequence information of CIKS genes of different species.

Species	Sources	Length	TRAF2/3	TRAF6	HLH	CC	EST/mRNA
HoCIKS	Genebank NP_001157753.1	564	+	+	+	+	NM_001164281.1
MuCIKS	Genebank NP_598761.2	555	+	+	+	+	NM_134000.3
GaCIKS	Genebank XP_419782.1	532	+	+	+	+	XM_419782.2
XeCIKS1.1	Genebank NP_001016565.1	333	–	+	+	–	NM_001016565.2
XeCIKS1.2	JGI 23742[P]	ND	ND	ND	ND	ND	DT420479.1
DaCIKS1.1	Genebank XP_002662532.1	436	–	+	–	–	EE716742.1
DaCIKS1.2	Genebank XP_002663886.1	398	+	+	–	–	EH561047.1
DaCIKS1.3	Genebank XP_002662539.1	327	+	+	–	–	EE716742.1
FuCIKS1.1	JGI 562194	446	–	–	+	–	CA589318.1 CA332550.1
FuCIKS1.2	JGI 574494[P]	ND	ND	ND	ND	ND	No
FuCIKS1.3	JGI 616174[P]	ND	ND	ND	ND	ND	No
OiCIKS1.1	Ensembl ENSORLP00000018955[P]	ND	ND	ND	ND	ND	No
OiCIKS1.2	Ensembl ENSORLP00000004604[P]	ND	ND	ND	ND	ND	BJ712971.1
OiCIKS1.3	Ensembl ENSORLP00000016882[P]	ND	ND	ND	ND	ND	BJ743033.1
ShCIKS1.1	IMCB AAVX01038374.1[P]	ND	ND	ND	ND	ND	No
ShCIKS1.2	IMCB AAVX01080745.1[P]	ND	ND	ND	ND	ND	No
ShCIKS1.3	IMCB AAVX01475951.1[P]	ND	ND	ND	ND	ND	No
ShCIKS1.4	IMCB AAVX01600592.1[P] AAVX01179545.1[P]	ND	ND	ND	ND	ND	No
LaCIKS1.1	WUGSC Contig18015.1[P]	ND	ND	ND	ND	ND	EE736944.1
LaCIKS1.2	WUGSC Contig5414.6(5)[P]	ND	ND	ND	ND	ND	No
CiCIKS	Genebank XP_002129288.1	509	+	+	+	–	FF781403.1
BfCIKS1.1	JGI 78867	235	–	+	–	–	No
BfCIKS1.2	JGI 82312	238	–	+	–	–	No
BfCIKS1.3	JGI 225945[P]	ND	–	–	–	–	No
BfCIKSL	JGI 78868	696	–	+	–*	–	No
LoCIKSL	JGI 233367	607	+	+	–*	+	FC588202.1

Ho: human; Mu: mouse; Ga: gallus; Xe: xenopus; Da: zebrafish; Fu: fugu; Oi: medaka; Sh: shark; La: lamprey; Ci: ciona; Bf: amphioxus; Lo: lottia; +: present; –/No: absent; ND: not determined; HLH: helix-loop-helix; CC: coiled-coil; [P]: partial; *: The N terminus of amphioxus and lottia CIKSL was DEATH domain.

mammalians (human *Homo sapiens*, mouse *Mus musculus*) and analyzed their phylogenetic relationships. In addition, to verify the ancient function of CIKS, one of amphioxus CIKS genes was cloned and its expression was examined in amphioxus with no and stimulation with *Escherichia coli*. A potential evolutionary scenario for the CIKS gene was inferred based on the results of these analyses.

2. Materials and methods

2.1. Data extraction

Amino acid sequences of SEFIR domains of human CIKS/-related molecules were used as baits in BLASTP searches to extract homologous metazoan sequences from databases as follows: JGI database (http://genome.jgi-psf.org/) for clawed frog *X. tropicalis*, fugu *T. rubripes*, ciona *C. intestinalis*, amphioxus *B. floridae*, molluscs *L. gigantea* and sea anemones *N. vectensis*; NCBI (http://www.ncbi.nlm.nih.gov/sites/entrez) and Ensembl (http://www.ensembl.org/Multi/blastview) databases for chicken *G. gallus*, fishes (zebrafish *D. rerio* and medaka *O. latipes*), fly *Drosophila melanogaster* and nematode *Caenorhabditis elegans*; SP base (http://www.spbase.org/SpBase/search/)for sea urchin *Strongylocentrotus purpuratus*; the lamprey *P. marinus* contigs were obtained from Washington University Genome Sequencing Center (WUGSC) (http://genomeold.wustl.edu/tools/blast/). The amino acid sequences of human CIKS/-related molecules were also used as queries for TBLASTN searches from IMCB (http://blast.fugu-sg.org/) for shark *C. milii*. All returned sequences were reciprocally searched against the other genomes to further verify their identity. Some predictions were verified by searching the EST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome).

2.2. Sequence analysis

Human CIKS contains two TRAF binding sites at N-terminus and middle of the sequence respectively, which can interact with different TRAFs, including TRAF2, TRAF3 and TRAF6. The binding sites were detected with Minimotif Miner (MnM) (http://mnm.engr.uconn.edu/MNM/SMSSearchServlet). The coiled-coil motif was detected with COILS (Lupas, 1996). The helix-loop-helix motif was detected as described by Li et al. (2000).

Phylogenetic analyses were performed for SEFIR domains. Multiple protein sequence alignments were generated using ClustalX (version 1.81) (Thompson et al., 1997). Phylogenetic analyses using Neighbor-Joining (NJ) and Maximal Parsimony (MP) algorithms were performed with MEGA (4.0) package (Tamura et al., 2007). Data were analyzed using P-distance, and gaps were removed by pairwise deletion. The topological stability of the NJ and MP trees was evaluated by 1000 bootstrap replications. To gain an insight whether the genes adjacent to the identified vertebrate CIKS genes were evolutionarily conserved, synteny analysis was carried out with Ensembl (http://www.ensembl.org) and NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/mapview/) using human (NCBI Build 36.2), mouse (NCBI Build 37.1), chicken (WASHUC2), amphibian (JGI v4.1), zebrafish (Zv8), pufferfish (JGI v4.0), and *O. latipes* (NIG MEDAKA1) databases.

2.3. Expression of amphioxus CIKS gene detected by semi-quantitative RT-PCR

To determine the function of ancient CIKS gene, we carried out related expression experiments in Chinese amphioxus (Branchiostoma belcheri tsingtauense). For the tissue expression of CIKS gene, total RNAs of the intestine, hepatic diverticulum, gill, skin, testis and muscle from Chinese amphioxus were isolated. Bacterial challenge was performed in amphioxus as described by Pang et al. (2006). Bacteria *E. coli* (Gram-negative) were cultured in LB medium at 37 °C overnight. Bacteria were washed twice using sterile phosphate buffer solution (PBS), and then diluted to appropriate concentration in sterile PBS. The amphioxus was challenged in a rectangular tank by immersion exposure with freshly prepared culture of bacteria. At the time of challenge, the bacterial culture was added to the tank to a concentration