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Cloning and Characterization of BTG-1 Gene from Pacific Oyster (*Crassostrea gigas*)

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BTG 1 (B-cell translocation gene 1) gene was first identified as a translocation gene in a case of B-cell chronic lympocytic leukemia. BTG1 is a member of the BTG/TOB family with sharing a conserved N-terminal region, which shows anti-proliferation properties and is able to stimulate cell differentiation. In this study, we identified and characterized the pacific oyster *Crassostrea gigas* BTG1 (cg-BTG1) gene from the gill cDNA library by an Expressed Sequence Tag (EST) analysis and its nucleotide sequence was determined. The cg-BTG1 gene encodes a predicted protein of 182 amino acids with 57% 56% identities to its zebrafish and human counterparts, and is an intron-less gene, which was confirmed by PCR analysis of genomic DNA. Maximal homologies were shown in conserved Box A and B. The deduced amino acid sequence shares high identity with other BTG1 genes of human, rat, mouse and zebrafish. The phylogenic analysis and sequence comparison of cg-BTG1 with other BTG1 were found to be closely related to the BTG1 gene structure. In addition, the predicted promoter region and the different transcription-factor binding site like an activator protein-1 (AP-1) response element involved in negative regulation and serum response element (SRE) were able to be identified by the genomic DNA walking experiment. The quantitative real-time PCR analysis showed that the mRNA of cg-BTG1 gene was expressed in gill, heart, digestive gland, intestine, stomach and mantle. The cg-BTG1 gene was expressed mainly in heart and mantle.

Key words : Abundant in neuroepithelium area, B-cell translocation gene, *Crassostrea gigas*, PMRT1, protein arginine methyltransferase 1

Introduction

The cell cycle is a very important biological process controlled a set of proto-oncogenes, which promote cell division and genes that negatively regulate cell proliferation, which are 12 different sets of tumor suppressor genes. It is well known that proliferation-related genes such as retinoblastoma protein (Rb) and p53 are key regulators of the cell cycle progression or cell apoptosis [14]. However, some physiologically important anti-proliferative genes also have a role in cell cycle regulation, which controls cell growth, differentiation and apoptosis [3, 5, 9, 13, 14, 18, 21, 26, 30, 31]. In vertebrate, BTG family of anti-proliferative gene products consists of at least nine distinct members: *BTG1, BTG2/TIS21/PC3* (orthologs of human, mouse and rat respectively), *BTG3/ANA, TOB, TOB2, B9.10, PC3K, PC3B and B9.15.* This family has two short conserved domains (Box A and Box B).

The BTG1 was identified near the breakpoint of chromosomal translocation found in a B-cell chronic lymphocytic leukemia [21] and share high homology with the previously identified Tis21 [7] and Pc3 [2] genes. It is expressed by several types of non-endothelial cells and is highly regulated during cell growth and proliferation [15, 21, 28]. Expression of BTG1 was maximal in the G_0/G_1 phases of the cell cycle and down-regulated when cells progressed throughout G_1 [16, 28], and also increased in response to DNA damage [6, 28].

The deduced amino acid sequence of Btg1 displays a high degree of conservation in two regions of Box A and B [8], which determine the localization of Btg1 in cytoplasm and nucleus. Cytoplasmic retention sequence located in the first 43 amino acids residues of Btg1 and in a region encompassing the A Box [19]. The well-conserved Box B, a Nuclear Localization Sequence (NLS), whose activity is enhanced by

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the C-terminal domain of Btg1 showing a nuclear retention activity, mediate the interaction of BTG1 with the nuclear protein CAF1 [20]. In addition, the Btg1 amino-terminal domain bears an LXXLL motif acting as a weak Nuclear Localization Sequence [18].

The Box C (GXXXVLYXX) sequence is present only in Btg1 and Btg2 [10, 14], which interacts with the methyltransfease class I, Protein-arginine N-Methyltransferase 1 (PRMT 1). Both BTG1 and BTG2 may modulate signal transduction pathways leading to growth arrest and/or differentiation by activating PRMT1.

Recent data report that BTG1 may play an important role in the process of angiogenesis [11]. And act as a novel important co-activator involved in the regulation of myoblast differentiation [4]. It does not only stimulate the activity of myogenic factors, but also of nuclear receptors already known as positive myogenic regulators. Transcriptional cofactors Btg1 and Btg2 act as important regulators of B-cell differentiation, providing an important link to their role as tumor suppressors in B-cell malignancies [25, 29].

To date, more than 20 members of this family have been found from several species by other many laboratories. Though a biological common feature of these gene family was their ability to inhibit cell proliferation, their molecular role is not definite clearly. In pacific oyster, BTG1 could prompt the apoptosis in oyster hemocytes and vertebrate cancer cells, and possessed an analogous function of angiogenesis in adductor muscle of oyster [12].

The pacific oyster is one of the important aquaculture animals and is a physiologically well studied bivalve species. Because of the filter-feeder characteristics of pacific oyster, bacterial and non-bacterial pathogens become concentrated within them will threaten the human health [27]. The goal of our research is to define the set of genes which can determine the degree of pacific oyster's health quality from chemical contaminants, bacterial and viral pathogens. In this study, we have cloned and characterized cg-BTG1 gene from the pacific oyster and analyze its tissue specific expression. This is the report on cloning of Btg1 gene of invertebrates.

Materials and Methods

Animal and preparation of tissue

The Pacific oyster (*Crassostrea gigas*), weighed between 150 g and 200 g, was provided from the Department of Food Safety in National Institute of Fisheries Science, Republic of Korea (http://www.nifs.go.kr/). The following tissues were obtained by dissection: gill, heart, digestive gland, intestine, stomach and mantle. The tissues were kept on liquid nitrogen until preparation of total RNA.

cDNA library construction

Poly(A)$^+$ RNA was isolated from pacific oyster gill using the Micro-FastTrack^{TM} 2.0 (Invitrogen, USA) according to the manufacturer's instruction, and the cDNA library was constructed from this isolated mRNA using a ZAP-cDNA synthesis kit (Stratagene, USA) following the manufacturer's instructions. The size-fractionated cDNA fragments were ligated into the *EcoR* I and *Xho* I sites of the Uni-ZAP XR vector. Ligated vectors were packaged using Gigapack II Gold extract (Stratagene, USA).

Cloning of the pacific oyster BTG1 gene, nucleotide sequencing and phylogenetic analysis

Initially, full-length cDNA of pacific oyster BTG1 was obtained by the random sequencing of gill cDNA library and EST analysis. The cDNA library was sequenced on both strands with a capillary DNA sequencer (ABI PRISM® 3700 DNA Analyzer, Applied Biosystems, Foster, CA, USA) at Genotech (Deajeon, Republic of Korea). Nucleotide and pre-

Table 1. Oligonucleotide primers used for pacific oyster BTG1 amplification and expression studies

Primer name	Sequence	Information
poBTG1-GSP1	5′- AGG AAT GAT GGA AAA TAT GAC GGT CTC-3′	Primer walking for 5′-flanking region
poBTG1-GSP2	5′-CCT GTT GTT CAC TTA CAT GTT TAG AAG-3′	
poBTG1-IS-F	5′-GAA ACT CGA TCG TTC TAT GAA C-3′	Specific primer for intron searching
poBTG1-IS-R	5′-CAA ACA CGA CGG CTC TTC AA-3′	
poBTG-rF	5′-CTC TTG TGG CCT AAA CGA GAC CGT CAT-3′	Primers for real time quantitative PCR
poBTG-rR	5′-CTT CAA TCA GCT GTA CAC ATA GGC GGC -3′	
poβ-actin-F	5′-TGT CCC TGT AYG CTT CCG GTC GTA C-3′	qPCR control primer
poβ-actin-R	5′-GAT GGC TGG AAC ATG GCC TCT GG-3′	

(All oligonucleotide primers were designed using Primer3 [22] and synthesized from Bioneer, Korea)

dicted protein sequences were analyzed using BLAST programs in not-redundant databases of the National Center for Biotechnology Information (NCBI BLAST, http://www.ncbi.nlm.nih.gov/BLAST). For phylogenetic analysis, the deduced amino acids sequences between three conserved regions (Box A, Box B and Box C) of pacific oyster BTG1 gene and other BTG1 genes obtained from GenBankwere aligned using CLUSTAL omega. Multiple sequence alignment was utilized to generate the phylogenetic tree using neighbor-joining methods with 1,000 bootstrap replicates. A neighbor-joining tree was generated based on this genetic distance matrix by using MEGA4 to identify possible phylogenetic clade. The predicted transcription factor binding region was searched using the MatInspector at Genomatix http://www. genomatix.de).

Genomic DNA extraction from pacific oyster and primer walking for 5' flanking region

The genomic DNA of the pacific oyster gill was prepared by the method of Blin and Stafford [1].

To identify the existence of possible introns in BTG1 gene, two separate PCR reactions were performed by using pacific oyster genomic DNA and BTG1 cDNA clone as for PCR templates with same set of PCR primers (poBTG1-IS-F and poBTG1-IS-R). PCR was conducted under the following cycle parameters: initial denaturation for 5 min at 94℃, followed by 35 cycles of a 30 sec denaturation step at 94℃, a 30 sec annealing step at 55℃ and 1 min extension step at 72℃, with a final extension of 5 min at 72℃. The PCR product was analyzed on a 1.5% agarose gel stained with EtBr along with DNA 100 bp size marker (Bioneer, Republic of Korea).

Primer walking PCR primer sets was constructed using a sequence of pacific oyster BTG1 from degenerate PCR. For primer walking PCR, two specific primers of BTG1 were designed as follows; poBTG1-GSP1 and poBTG1-GSP2.

Primer walking was conducted using GenomeWalker™ Kit (Clontech, USA) following the manufacturer's protocol. Primer walking PCR reaction mixture was comprised of 1 ul of each adaptor ligated DNA, 1 pmol of AP1 and GSP1, each 10 mM dNTP mix, 10X Tth PCR reaction buffer, 50X Advantage Genomic Polymerase mix and deionized H_2O in a final volume of 50 ul.

PCR was conducted under the following conditions, two-step cycle parameters: initial denaturation for 5 min at 94°C, followed by 7 cycles of a 25 sec denaturation step at 94°C, a 3 min annealing and extension step at 72°C, and 32 cycles of 25 sec denaturation step at 94°C, a 3 min annealing and extension step at 67°C, with a final extension of 7 min at 67°C.

After amplification, the PCR product was cloned into pGEM®-T easy vector system (Promega, USA) and sequenced (Genotech, Republic of Korea).

RNA isolation and Expression profile by quantitative real-time PCR

Real-time quantitative PCR was used to evaluate the tissue distribution of pacific oyster BTG1 mRNA expression. The following tissues were obtained by dissection: gill, heart, digestive gland, intestine, stomach and mantle. The tissues were kept on liquid nitrogen until preparation of total RNA. Total RNA was prepared using the RNeasy RNA purification kit (Qiagen, USA). One microgram of RNA was used for cDNA synthesis using Superscript™ II RNase H- reverse transcriptase (Invitrogen, USA) in a reaction mixture containing Random Hexamer, pd(N)6 (Bioneer, Republic of Korea). Reactions were run at 42°C for 60 min and then inactivated at 75℃ for 10 min.

Each quantitative PCR reaction for pacific oyster BTG1 and β-actin gene was done in a 20 ul reaction mixture containing 80 ng of cDNA, a set of forward/reverse primers (1 pmol each), QuantiTect™ SYBR® Green PCR Kit (Qiagen, USA). The fluorescence was detected by DNA Engine Opticon 2 Real-Time PCR Detection System (Bio-Rad, USA). The set of primers used for pacific oyster BTG1 is poBTG-rF and poBTG-rR and for pacific oyster β-actin is poβ-actin-F and poβ-actin-R. The PCR protocol was as follows: 95℃ for 15 min, 45 cycles of 95℃ for 15 sec, 65℃ for 20 sec, 72℃ for 25 sec and 72°C for 5 min. Standard curves were obtained by amplifying known amounts of PCR fragments of pacific oyster BTG1 (35 ng ~ 0.0035 pg) and pacific oyster β-actin (7.04 ng ~ 0.074 pg) under the same PCR conditions. The real-time qPCR was analyzed on the Opticon Monitor 3 software (Bio-rad, USA) and PRISM4, statistical graph software (Graphpad, USA).

Results

Cloning, sequencing and structure of pacific oyster BTG1

The cg-BTG1 was identified from gill cDNA library during EST analysis. As shown in Fig. 1. A, the full-length

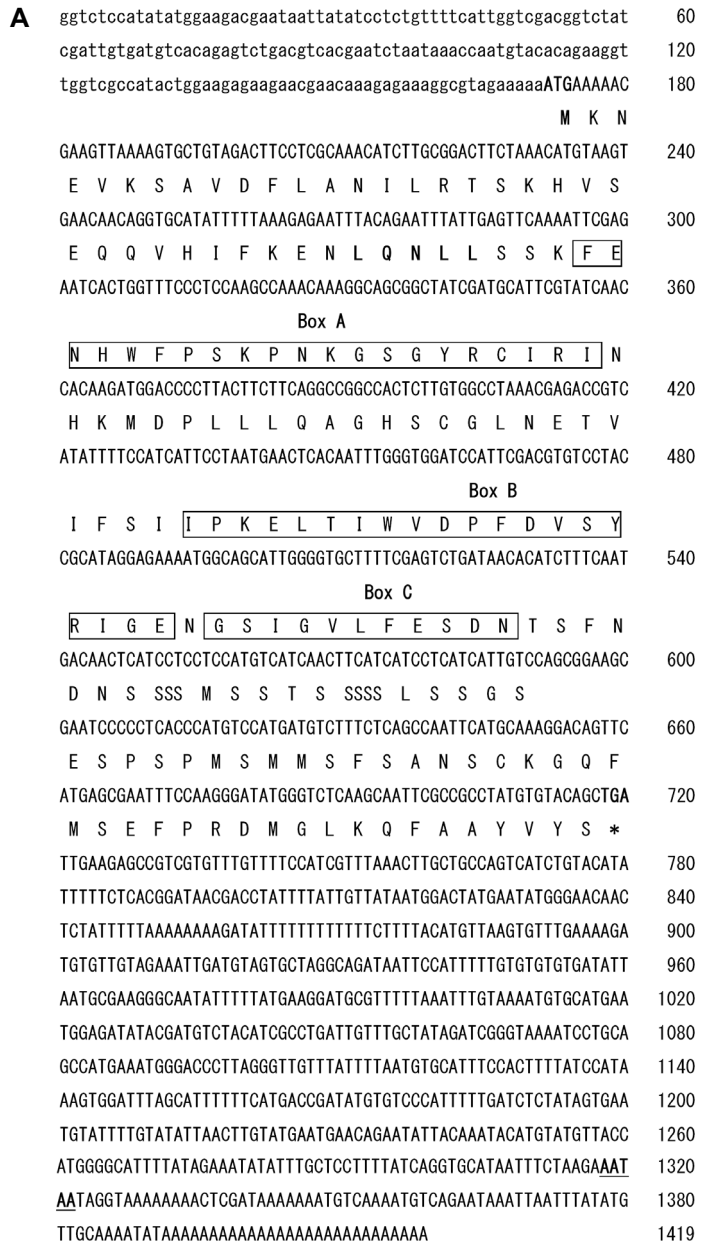

Fig. 1. A. cDNA and deduced amino acid sequence of pacific oyster (*Crassostrea gigas*) B-cell translocation gene 1 (BTG1). Start and stop codons are shown in bold. The Box A, B and C are boxed. Polyadenylation signal is underlined. B. The remarkable structural feature of the BTG1 protein residues in the existence of three highly conserved domains (Box A, B and C) in deduced amino acid sequence of pacific oyster.

cDNA of cg-BTG1 yielded a 1419-bp sequence that included an initiation codon ATG, and in-frame stop codon, and a poly (A) tail. The cDNA of cg-BTG1 comprised a 171-bp 5′-untranslated region (5′-UTR) and 549-bp coding region, followed by a 699-bp 3′-untranslated region (3′-UTR) containing on potential poly-adenylation signal, AATAA (nt 1318-1322).

The nucleotide of cg-BTG1 was predicted to encode 182 amino acids from the first methionine according to universal codon usage. A translational termination codon (nt 718-720, TGA) was observed in-frame following codon 182.

The deduced amino acid sequence of cg-BTG1 demonstrated that cg-BTG1 as like other BTG1 genes, contains a LXXLL-related sequence (LQNLL, aa 34-38), a Box A (aa 43-63), a Box B (aa 88-107) and Box C (aa 109-119), which were all previously defined as conserved elements in BTG1 family (Fig. 1). The multiple serine residues were found in C-terminus region (from aa 121 to aa 155) of cg-Btg1. It is known that the serine residue (aa 159) in C-terminus of human BTG1 acts as the phosphorylation site, which enhance to drive the BTG1 protein in the cytoplasm. The nucleotide sequence of cg-BTG1 ORF was submitted to NCBI Genbank with accession number of FJ009577.

To determine the existence of possible introns in the genome of cg-BTG1 gene, two separate PCR experiments were performed with pacific oyster genomic DNA and full length of cg-BTG1 cDNA clone by using primer set, poBTG1-IS-F and poBTG1-IS-R, which locate in the juxtaposition of BTG1 ORF region. PCR product obtained from pacific oyster genomic DNA showed exactly same nucleotide sequence with cg-BTG1 cDNA sequence. It is revealed that pacific oyster BTG1 is an intron-less gene.

Analysis of the predicted protein sequence of pacific oyster BTG1

The educed amino acid sequences of cg-BTG1was analyzed for multiple alignments (Fig. 2) and phylogenetic analysis (Fig. 3).

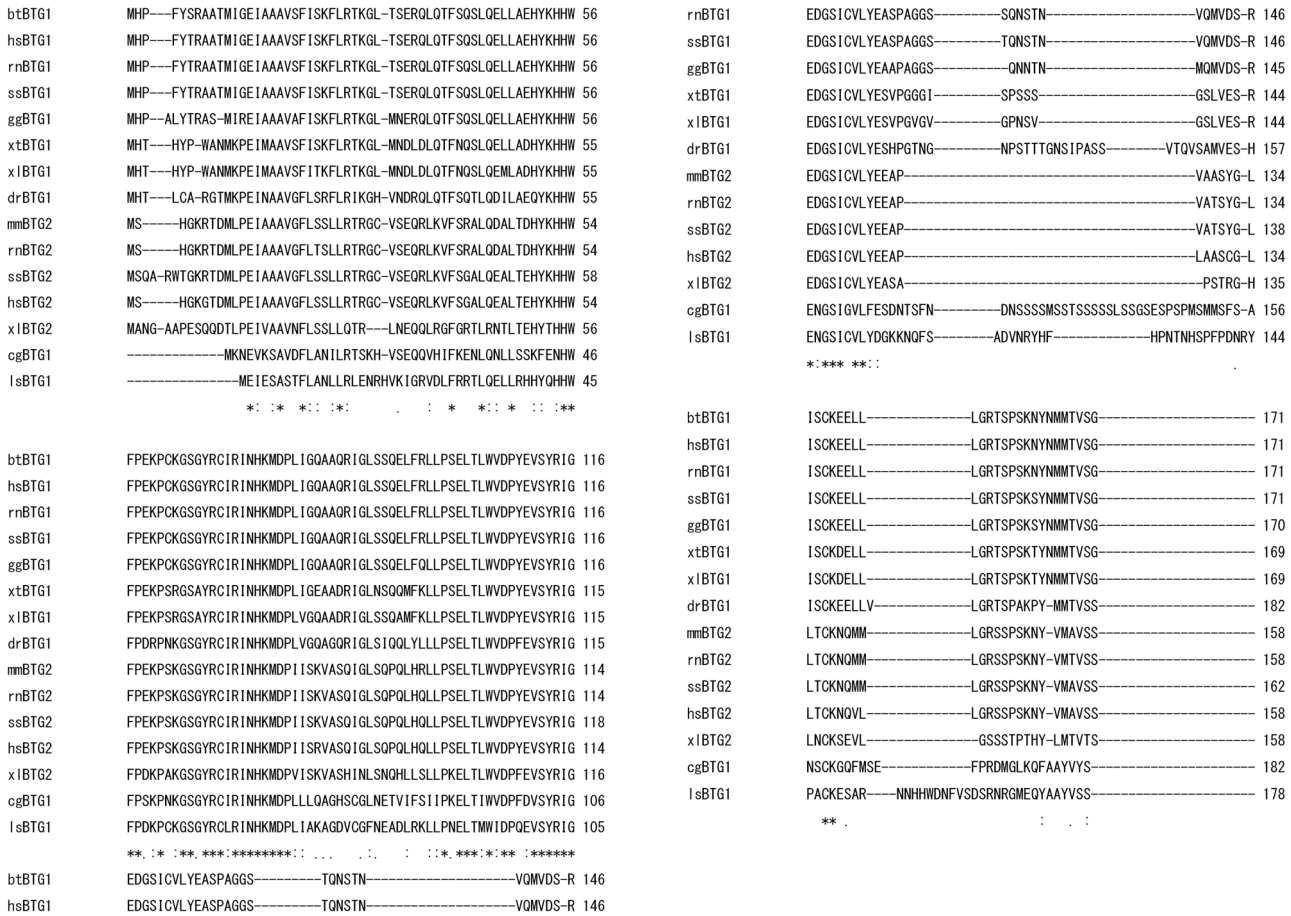

Fig. 2. The multiple alignment of the deduced amino acid sequence of the pacific oyster BTG1 with other BTG families (Table 2). An asterisk indicates the identical amino acids.

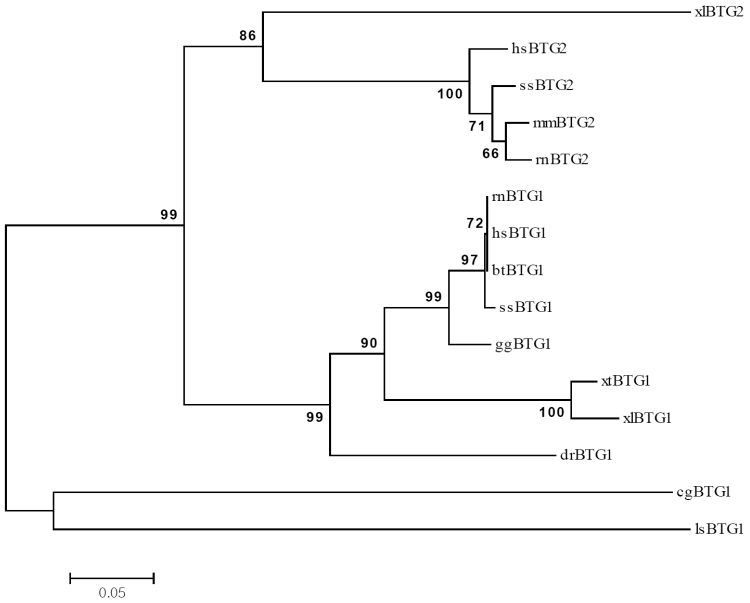

Fig. 3. Phylogenetic relationships of pacific oyster BTG1 (cgBTG1) with other BTG families (Table 2). In this neighbor-joining phylogram, all individuals are represented and the branches are based on the number of inferred substitutions as indicated by the bar.

The accession numbers of the amino acid sequences applied to the multiple alignments of cg-BTG1 (pacific oyster) were hsBTG1 (human), hsBTG2 (human), rnBTG1 (rat), rnBTG2 (rat), mmBTG2 (mouse), btBTG1 (cattle), ggBTG1 (chicken), ssBTG1 (pig), ssBTG2 (pig), lsBTG1 (spider), xlBTG1 (frog), xlBTG2 (frog), xtBTG1 (frog) and drBTG1 (zebrafish). The pacific oyster BTG1 gene showed 44%~53% amino acid sequence identity to BTG family of all available sequence information. As shown in Table 2, it shows about 53% of sequence identity and about 76% similarity to hsBTG2 (*Homo sapiens*), xlBTG2 (*Xenopus laevis*), and xtBTG1 (*Xenopus tropiclais*). This data appear to be some obscure to classified BTG1 and BTG2 gene. Although both BTG1 and BTG2 show very high sequence homologies and have three functional domains of same Box A, Box B and Box C region, it is different that BTG1 have longer amino acid residues than BTG2 in C-terminus region. Human Btg1 has 171 amino acids residues while human Btg2 has 158 amino acids, whereas cg-Btg1 has 182 amino acids residues, which is longer than human Btg1. From the phylogenetic relationship of BTG family as shown in Fig. 3, it reveals that cg-BTG1 is closely related to lsBTG1 (*Lycosas ingoriensis*) as a good example of invertebrate and shows more clear molecular classification of complex and similar BTG family. From these data, we designated our cDNA clone of cg-BTG1 gene as a member of BTG1 family not in BTG2.

Table 2. The amino acid sequence identity and similarity between BTG1 of pacific oyster (*Crassostrea gigas*) and other BTG families

Sequence for comparison	Species	Identity (%)	Similarity (%)	GenBank Accession No.
hsBTG1	*Homo sapiens*	47	65	NP_001722
rnBTG1	*Rattusnorvegicus*	47	65	NP_058954
btBTG1	*Bostaurus*	47	65	NP_776424
ggBTG1	*Gallus gallus*	46	66	NP_990681
ssBTG1	*Susscrofa*	47	65	NP_001093406
lsBTG1	*Lycosas ingoriensis*	44	64	ABX75488
xlBTG1	*Xenopus laevis*	48	66	NP_001079380
xtBTG1	*Xenopustropicalis*	52	75	NP_988926
drBTG1	*Danio rerio*	47	70	NP_956314
hsBTG2	*Homo sapiens*	53	75	NP_006754
mmBTG2	*Mus musculus*	44	66	NP_031596
rnBTG2	*Rattusnorvegicus*	44	66	NP_058955
ssBTG2	*Susscrofa*	44	66	ABW83760
xlBTG2	*Xenopus laevis*	53	76	NP_001088812

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atcgcaataatcatgttttgtttgtttaattcttcataatcagataataatctgataatt    60
ttgtccatatctttacatctttgatccttttttacgctgcacagtgatgataactaattca   120
cgcaaagaagttttcattactgccaacatttttttaaatgatcaaatagctcaattctaaa   180
ttatatagggtcatcgcaggatactcccaaattgtctgacacttgtcacgtcacctgcca   240
taccccttcggtggcctcattctgacgcgatgtttattgtgtgtgtatgtgttttgcgt   300
atcgtgtgtaaaatcacccgatgtcatgtcatgcagagccatttaatttaatttagtata   360
aattttgacaaatttcaacataaaaaagaagataccattggtcaagacgttcatcgcatt   420
tcataattctcatctgtacatttcacaaaaattacctaatatacgatctagcatattcca   480
gtgtttttatcgtacgtgaaaataggtcaaacagaggatatagcggtcacagtcgaataa   540
                          TBPF              SRFF
Taaacaaagcatgttgtgacgtctcattctataattagaggtctccatatatggaagacg   600
aataattatatcctctgttttcattggtcgacggtctatcgattgtgatgtcacagagtc   660
 CREB              CAAT
tgacgtcacgaatctaataaaccaatgtacacagaaggttggtcgccatactggaagaga   720
agaacgaacaaagagaaaggcgtagaaaaagttctggtagaaataaagggccgcagcgct   780
ccgagtcagcataagtaccaaaaaaattgaaaaggtttcatcaaacgataactgaagatc   840
aacttcatttatcctatttcccaacagatctgaagaaatcggaagaaatatttcgccatc   900
aaagaaactcgatcgttctatgaaca/ 926
                          /ATGAAAAACGAAGTTAAAAGTGCTGTAGACTTCC
                            M  K  N  E  V  K  S  A  V  D  F
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Fig. 4. Genome-walked 5′ flanking region sequence of pacific oyster BTG1. These contain the predicted binding site of transcription factor, Tata-binding protein factor (TBPF), cAMP response element binding protein (CREB), CCAAT binding factor (CAAT) and Serum response element binding factor (SRFF).

Analysis of the predicted transcription factor binding site

Primer walking was conducted using GenomeWalker™ Kit (Clontech, USA) following the manufacturer's protocol. Genome-walked 5′ flanking region sequence of cg-BTG1 is 750-bp upstream from the initiation codon. As shown in Fig. 4, the 5′-flaning region contains the predicted binding site of transcription factor, TATA-binding protein factor (TBPF, nt568-572), cAMP response element binding protein (CREB, nt 661-668), CCAAT binding factor (CAAT, nt 681-685) and Serum response element binding factor (SRBF, nt 589-599).

Expression profile study by quantitative real-time PCR

In order to determine the tissue specific mRNA expression of pacific oyster BTG1 genes, quantitative real-time PCR analysis was carried out using total RNA isolated from the gill, heart, digestive gland, intestine, stomach and mantle of pacific oyster. As shown in Fig. 5, mRNA transcripts of BTG1 were higher in heart, stomach, and mantle than gill, digestive gland, and intestine. This data suggests that cg-BTG1 mRNA is widely expressed in most tissues of the pacific oyster.

Discussion

In this study, the cDNA clone of cg-BTG1 was obtained from the gill cDNA library and the full-length sequence of the cg-BTG1 cDNA was determined, and the cg-BTG1 mRNA expression was analyzed by real-time quantitative PCR.

In vertebrate, BTG (or BTG/TOB) family consists of at least six distinct members and show high similarity to each other. Box A and Box B, the functional domains in BTG1, exist in BTG2, BTG3, BTG4, TOB and TOB 2 and involve in the cellular localization of the protein such as the cytoplasmic retention of Box A and the nuclear localization of Box B [17, 18, 20]. Box C, which binds with PRMT1 and is the third functional domain of BTG1 and BTG2, exists only in BTG1 and BTG2 [10, 14]. The predicted amino acids sequence of cg-BTG1 also shows strong similarity to other BTG1 and BTG2. The multiple sequence alignment of all available BTG1 and BTG2 proteins shows strong homology. Although it is not easy to classify the cg-BTG1 in this study

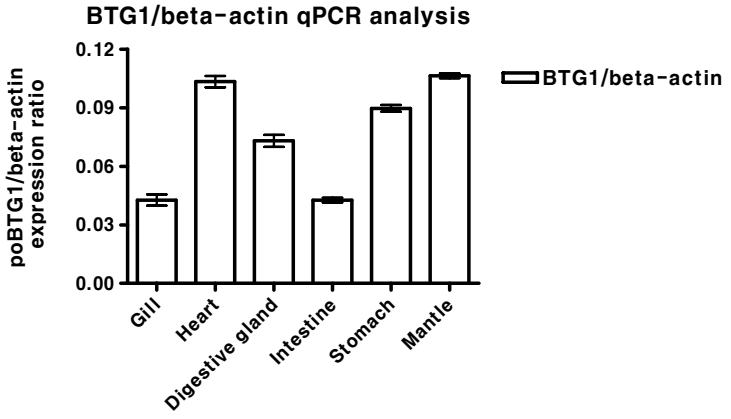

Fig. 5. Quantitative real-time PCR analysis of BTG1 in pacific oyster. BTG1 was measured in 6 different tissue, gill, heart, digestive gland, intestine, stomach and mantle. All data were taken from a representative assay and were normalized relative to β-actin, representing the mean (n=3) of three identical replicates ± standard deviation.

as BTG1 not as BTG family, the following reasons can support our designation of cg-BTG2. First, cg-BTG1 contains Box C (aa 109 -119), which is a typical domain in only BTG1 and BTG2. Second, the polypeptide of BTG1 is longer than BTG2 as shown in Fig. 2. Human, bovine, and pig BTG2 have 158 amino acids whereas BTG1 have 171 amino acids (for human, bovine, and pig), 170 amino acids (for chicken), 169 amino acids (for frog), 182 amino acids (for zebrafish), and 178 amino acids (for spider). Finally, the phylogenetic tree reveals that cg-BTG1 is closely related to spider BTG1 (Fig. 4.).

Interestingly, unlike the human and mouse, the genomic organization of the BTG1 in pacific oyster have not contained intron. Moreover, like the human and mouse sequences, the pacific oyster BTG1 cDNA contained a long AT-rich region in 3′ un-translated region which may be important in post-transcriptional regulation of the genes [21]. Some copies of ATTTA motif related RNA stability were found.

Also, transcripts for pacific oyster BTG1 in 6 different tissues of pacific oyster detected by real-time PCR, BTG1 was measured in all tissues, gill, heart, digestive gland, intestine, stomach and mantle. Most remarkably, expression of BTG1 genes mostly high detected in heart and mantle, this two tissues consists of many muscle, especially. Many other reports have already shown that BTG1 strongly inhibited myoblasts proliferation and stimulated myoblasts differentiation [17, 18] which suggested that BTG/TOB family may play a key role in muscle development. One or several members of BTG/TOB family may be related to muscle growth. BTG/TOB family in muscle development seems to be evolutionary conserved, as suggested by experiments in Xenopus [23], and by the expression pattern of the family in zebrafish [24]. In particular, the expression of BTG1 and BTG2 in the heart primordia suggests a novel role for BTG genes in cardiomyogenesis, and it is noteworthy that Btg1 expression precedes that of Btg2, which, in turn, is very restricted and transiently expressed.

Further studies will focus on the existence of BTG2 gene in oyster and the regulation of cg-BTG1 and cg-BTG2 by p53 activation which induced by hypoxia, genotoxic stress, and metabolic changes.

Acknowledgement

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초록 : 참굴(*Crassostrea gigas*)의 BTG1 유전자의 특성

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BTG1 (B-cell translocation gene 1)은 APRO family (anti-proliferative protein family)에 속하며, 이들은 공통의 생물학적 기능은 세포증식을 억제하는 것으로 알려져 있다. 본 연구에서, 굴의 gill cDNA library를 random sequencing을 통한 EST 분석과정에서 BTG1 clone을 확보하였으며, 분자생물학적 특성을 조사하였다. 굴의 BTG1은 182 개의 아미노산으로 구성되며, zebrafish와 57%, human과 56%의 상동성을 나타냈으며, 사람이나 설치류와 달리 ORF (open reading frame) 내에 intron이 존재하지 않았다. Genomic DNA walking을 통해 굴의 BTG1의 predicted promoter를 확인하였으며, 분석결과 AP-1 element와 SRE (serum response element) 부위가 존재하였으며, 5′flanking region에 cAMP response element (CRE) 부위가 확인되었다. 굴의 BTG1의 조직별 유전자발현 수준을 확인하기 위해 real-time PCR을 수행하였으며, 6 개 조직 모두에서 BTG1의 유전자발현이 나타났으며, 그 중에서 heart와 mantle에서 높은 수준의 mRNA 발현을 확인할 수 있었다.