JMLS 한국해양생명과학회지[Journal of Marine Life Science]
October 2016; 1(2): 88-94
http://jmls.or.kr

Identification and Expression of Retroviral Envelope Polyprotein in the Dogfish *Squalus mitsukurii*

Soo Cheol Kim[1], Kanij Rukshana Sumi[2], Myeong Rak Choe[1], Kang Hee Kho[2*]

[1]Department of Biomedical and Electronic Engineering, Chonnam National University, Yeosu 59626, Korea
[2]Department of Fisheries Science, Chonnam National University, Yeosu 59626, Korea

Corresponding Author
Kang Hee Kho
Department of Fisheries Science,
Chonnam National University, Yeosu
59626, Korea
E-mail : kkh@chonnam.ac.kr

Received : October 20, 2016
Revised : October 21, 2016
Accepted : October 31, 2016

Determining the infection history of living organisms is essential for understanding the evolution of infection agents with their host, particularly for key aspects such as immunity. Viruses, which can spread between individuals and often cause disease, have been widely examined. The increasing availability of fish genome sequences has provided specific insights into the diversity and host distribution of retroviruses in fish. The shortspine spurdog (*Squalus mitsukurii*) is an important elasmobranch species; this medium-sized dogfish typically lives at depths of 100~500 m. However, the retroviral envelope polyprotein in dogfish has not been examined. Thus, the aim of the present study was to identify and analyze the retroviral envelope polyprotein in various tissues of dogfish. The 1334-base pair full-length novel cDNA of dogfish envelope polyprotein (dEnv) was obtained by 3' and 5'-rapid amplification of cDNA end analysis from *S. mitsukurii*. The open reading frame showed a complete coding sequence of 815 base pairs with a deduced peptide sequence of 183 amino acids that exhibited 34~50% identity with other fish and bird species. It was also expressed according to reverse transcription and real-time polymerase chain reaction in the kidney, liver, intestine, and lung, but not in the gill. This distribution can be assessed by identifying and analyzing endogenous retroviruses in fish, which consists of three main genes: *gag*, *pol* and *env*. Dogfish envelope polyprotein sequence is likely important in evolution and induces rearrangements, altering the regulatory and coding sequences. This is the first report of the identification and molecular characterization of retroviral envelope polyprotein in various tissues of *S. mitsukurii*.

Keywords: Envelope polyprotein, Dogfish, *Squalus mitsukurii*, Kidney

Introduction

Viruses form the most numerous and diverse group of genetic entities. They are generally constituted by single- or double-stranded RNA or DNA genomes embedded within a protein coat. Among them, retroviruses consist in single-stranded positive-sense RNA viruses with a DNA intermediate. After infection, a retrovirus reaches the cytoplasm of the host cell and produces DNA by reverse-transcribing its RNA genome into complementary DNA (cDNA). This step is catalyzed by reverse transcriptase (RT) and RNA-dependent DNA polymerase, which is generally encoded by the retrovirus itself. Subsequently, the cDNA molecule is integrated into the host nuclear genome via the action of the retroviral integrase, forming a provirus. Provirus genes are transcribed and translated along with classical cellular genes by the host machinery.

Retroviruses are delimited by long terminal repeats (LTRs), which carry a promoter sequence and interact with the integrase for insertion. Retrovirus genomes classically contain three open reading frames: *gag* (5', group-specific antigen), encoding core and structural proteins, *pol* (polymerase), producing a polyprotein with RT, protease, and integrase domains, and *env* (3', envelope), which codes for coat proteins (Fig. 1). Additional accessory proteins are encoded by more complex retroviruses. After integration, ectopic homologous recombination can occur between both LTRs. This eliminates one LTR copy and the intervening sequence, generating solo LTRs that are frequently found in genomes. Evolutionary switching can occur between retroviruses and non-infectious LTR retrotransposons through the gain or loss of envelope genes (Malik et al., 2000).

Envelope (Env, gene) is a viral protein that forms the viral envel-

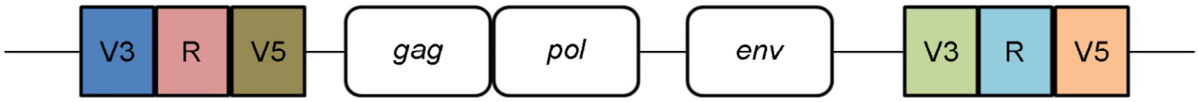

Fig. 1. Three major proteins encoded within the retroviral genome: Gag, Pol, and Env. Gag is a polyprotein and is an acronym for Group Antigens. Pol is the reverse transcriptase. Env is the envelope protein, which resides in the lipid layer and determines viral tropism.

ope. Some viruses have viral envelopes covering their protective protein capsids. The envelopes are typically derived from portions of the host cell membranes (phospholipids and proteins), but include some viral glycoproteins. They may help viruses avoid the host immune system. During infection, Env mediates binding of the virus to its receptor in the cell plasma membrane and subsequent fusion of the viral and cellular membranes. Env undergoes large-scale conformational changes during these events (Eckert and Kim, 2001; Colman and Lawrence, 2003).

Env is the major viral protein present on the surface of retroviral particles. Env is translated as a polyprotein and is subsequently extensively post-translationally modified during trafficking through the biosynthetic pathway. Briefly, Env is cleaved by a furin-like protease in the Golgi into its two subunits: the surface unit (SU) protein and transmembrane (TM) protein. These two proteins remain non-covalently associated in most retroviruses and further assemble into a homotrimeric complex to construct the active form of Env (Coffin et al., 1997).

Retroviruses have a number of features that make them unique as gene delivery vehicles. Their life cycle includes an integrated state in the DNA of the host chromosome. Depending on the local chromatin contexts, which is often influenced by cell type-specific promoter events, the retroviral promoter can direct high-level, efficient expression of genes encoded within the confines of its genome. As revealed by the discovery of oncogenes within certain strains of *leukemia* and *sarcoma*-inducing murine retroviruses, the retroviral genome can accommodate changes to its configuration (Jonathan et al., 2014).

Thus, retroviruses can be used for gene transfer and gene therapy to deliver genes to target cells at high efficiency in a manner that allows for long-term, stable expression of introduced genetic elements.

Moreover, similarly to other vertebrates, fish are infected by exogenous retroviruses. Retroviruses have been found to be associated with tumors in fish intensively cultured for food, wild fish populations showing signs of sickness, and fish reared in the laboratory (Lepa and Siwicki, 2011; Coffee et al., 2013).

The family *Squalidae* (Chondrichthyes: Squaliformes) includes two currently recognized genera, *Cirrhigaleus* with three species and *Squalus* with 24 species subdivided among three well-defined species subgroups as detailed in Ward et al. (2007). These subgroups include the *Squalus acanthia*, *Squalus mitsukurii*, and *Squalus megalops* groups. The *Squalus mitsukurii* group (Bigelow and Schroeder, 1948) was formerly termed as the *blainville-fernandinus*, *S. fernandinus*, or *S. blainville* group, but the name was changed because of identification problems with *S. blainville* (Risso, 1827) and the synonymous labeling of *S. fernandinus* (Molina, 1782) with *S. acanthias*. This group comprises at least 15 species, including the nominal *S. blainville sensu* (Chen et al., 1979) from the western North Pacific. Among this group, the shortspine spurdog is an elasmobranch species. It inhabits continental and insular shelves and upper slopes, as well as seamounts and ridges, typically at depths of 100~500 m. This fish is a high-trophic-level predator, feeding primarily on cephalopods, teleosts, and caridean shrimp (Ebert et al., 1992; Cortes, 1999). However, similar to many deep-water fishes, little molecular biological information is available for this species, although a few studies on the age, growth, and reproduction have been reported in the literature (Litvinov, 1990; Wilson and Seki, 1994; Fischer et al., 2006).

In this study, the shortspine spurdog *S. mitsukurii* was examined to characterize the Env protein in various tissues to provide a foundation for the identification and molecular characterization of the Env protein.

Materials and Methods

1. Experimental fish

Dogfish (*S. mitsukurii*) were caught in Yeosu Bay, Korea by a local fisherman, placed in a holding jar with water, and transported immediately to the Department of Fisheries and Oceanography, Chonnam National University.

All animal experiments were approved by the University of Chonnam National Animal Care and Use Committee and per-

formed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2. Experimental fish

Squalus mitsukurii was injected by cardiac puncture with 250 IU of heparinized saline (NaCl 280 mM; KCl 6 mM; $CaCl_2$ 5 mM; $MgCl_2$ 3 mM; $NaSO_4$ 0.5 mM; $NaHPO_4$ 1 mM; $NaHCO_3$ 8 mM; urea 350 mM; glucose 5 mM; trimethylamineoxide 70 mM; Foster et al., 1972) 40 min prior to surgery. Each fish was anesthetized using ethyl 3-aminobenzoate methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO, USA) to collect tissue samples. The cardiac cavity was opened, and the ventral aorta (VA) was cannulated using a 'Y' connector. The body was immediately perfused with 50 ml of heparinized saline to flush out erythrocytes from the various tissues. The gills, heart, liver, intestine, kidney, and muscle tissues were then detached from the rest of the body and immediately placed in liquid nitrogen and stored at -80℃ for subsequent use.

3. RNA extraction

Total RNA was extracted from various tissues (kidney, liver, intestine, and lung) of dogfish using an RNeasy mini kit (Qiagen, Hilden, Germany). RNA was treated with RNase-free DNase (Promega, Madison, WI, USA) to eliminate any genomic DNA contamination. cDNA synthesis was conducted using the Superscript® III First-Strand synthesis kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

4. Molecular cloning of dogfish envelope polyprotein

For molecular cloning, reverse transcription (RT) primers (forward: 5'-CAGTTCCAYTTCCAYTGGG-3' and reverse: 5'-GCIARNCCRTCISWYTTRTC-3') were selected based on the CA 12 sequence retrieved from previous experiments. RT-polymerase chain reaction (PCR) were performed using the EmeraldAmp® GT Master Mix (2X Premix) (Takara, Shiga, Japan). PCR was carried out using 1 μl cDNA template obtained from the kidney tissue in reaction mixtures containing 1 μl (20 pmole) of each forward and reverse primer, 8 μl Master Mix, and sterile distilled water (dH_2O) in a total volume of 20 μl. The PCR conditions were as follows: initial denaturation at 95℃ for 2 min, followed by 35 cycles of denaturation at 95℃ for 45 s, annealing at 60℃ for 45 s, and extension at 72℃ for 1 min, with a final extension for 5 min at 72℃. The PCR products were purified using a gel extraction kit (Promega), ligated into the pTOP Blunt V2 vector (Enzynomics, Daejeon, Korea), and transformed into competent *Escherichia coli* DH5α cells (Enzynomics). Plasmid DNA was extracted using a plasmid mini kit (Qiagen) and sequenced by the Macrogen Online Sequencing System (Macrogen, Seoul, Korea).

5. Rapid amplification of cDNA ends analysis

Based on the sequences of the cDNA fragments cloned, primers were designed to extend the initial fragment lengths by 3' and 5' rapid amplification of cDNA ends (RACE). To extend the 3'-end of the cDNA sequence, 3'-RACE was performed using the 3'-Full Race Core Set (Takara); PCR was carried out according to the instructions provided by the manufacturer. PCR was performed using two primers (5'-TGCACTGCCCTATCTCTGATG-3' and 5'-TATATGGTGGTATTGCGAAGG-3'), respectively, using Takara EX Taq at the annealing temperature of 60℃. After PCR, the products were ligated into the pTOP Blunt V2 vector (Enzynomics) and sequenced as described previously. The sequenced 3'-RACE products were then combined by overlapping with the initial cloned cDNA fragment.

To obtain the full-length sequence, 5'-RACE PCR was performed based on the cloned sequence. The cDNA for 5'-RACE PCR was synthesized by RT from kidney total RNA using the SMARTer RACE 5'/3' kit (Clontech, Mountain View, CA, USA); PCR was carried out according to the manufacturer instructions using a gene-specific primer (5'-CAGCGGAGTCCAGGTAAATTCTGTCATC-3'). Touchdown PCR was performed in 3-steps, followed by 5 cycles at 94℃ for 30 s, 72℃ for 3 min, 5 cycles at 94℃ for 30 s, 70℃ for 30 s, 72℃ for 3 min, and 20 cycles at 94℃ for 30 s, 68℃ for 30 s, and 72℃ for 3 min. The 5'-RACE products were purified using a Nucleospin Gel and PCR Clean-Up kit (Takara) and then transformed into Stella™ competent cells (Clontech) and sequenced as described previously. The sequenced 5'-RACE products were then combined by overlapping with the results of initial cloned cDNA fragment and final output of 3'-RACE.

6. Sequence analysis

Protein homology was evaluated using the Basic Local Alignment Search Tool (BLASTP) in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/). Gene structure was analyzed using ProtParam (http://expasy.org/tools/protparam.html).

7. RT-PCR expression analysis

Semi-quantitative RT-PCR was performed using Prime Taq Premix (2x) (Genetbio, Daejeon, Korea). Gene-specific primers (forward: 5'-CTGCCCTATCTCTGATGTTC-3' and reverse: 5'-TGCACGTGGTATTTTTAGTG-3') were used for RT-PCR and β-actin primers (forward: 5'-ATGCCAACACTGTCCTGTC-3' and reverse: 5'-GACAGGGAAGCCAGGAT-3') from *S. acanthias* (Gilmour et al., 2007) were used as a control. PCR mixtures were prepared from the various tissues as described above. The samples were subjected to the following PCR conditions: pre-denaturing at 95℃ for 2 min followed by 35 cycles of denaturation at 94℃ for 45 s, annealing at 58℃ for 45 s, and extension at 72℃ for 1 min, with a final extension for 5 min at 72℃.

8. Quantitative PCR expression analysis

Quantitative real-time PCR (q-PCR) was performed using 1 μl

```
1    ATGTTCGGGGCAAAAATGAAAAATAGCAGGACTAAATGCGGCTTCCGTATTTCAATAGAC
      M  F  G  A  K  M  K  N  S  R  T  K  C  G  F  R  I  S  I  D
61   CAGTGGGCAACTCCCACTAATGGAACTGGGAACGACAAGATGGAGGAAGGACAGGGAGTT
      Q  W  A  T  P  T  N  G  T  G  N  D  K  M  E  E  G  Q  G  V
121  AAAATAATTGAAGTAAAAGATTTAGAGAAAGCCTTTGAGATAGAAACCGGCTATGAGGGA
      K  I  I  E  V  K  D  L  E  K  A  F  E  I  E  T  G  Y  E  G
141  CAGAATGCGTGGATTGAGTGGGTGAGGTATTCGGCCCAGACGCTGAAGAAAGACAATTGT
      Q  N  A  W  I  E  W  V  R  Y  S  A  Q  T  L  K  K  D  N  C
201  TATGCTTGCGCATCAGGTAGACCACAGGCCCATCTGGAGCCCTTTCCAATGGGGTGGAAA
      Y  A  C  A  S  G  R  P  Q  A  H  L  E  P  F  P  M  G  W  K
261  GAAAATAGAGGGAGCATGGAATGCATGATGGCCTTGTACCAAGGTGAGAACGCCTGGGGC
      E  N  R  G  S  M  E  C  M  M  A  L  Y  Q  G  E  N  A  W  G
321  AACAGGACCTGCACTGCCCTATCTCTGATGTTCCTTCCTGTTAAGCAAAAACAATCAGCA
      N  R  T  C  T  A  L  S  L  M  F  L  P  V  K  Q  K  Q  S  A
381  AACGCTCCCTCCTTCTTGGTTACCGTGACGCATCATAAATCGTGTATAAGTCGTTCTGAT
      N  A  P  S  F  L  V  T  V  T  H  H  K  S  C  I  S  R  S  D
441  ACGGGACTAAGTAAAGATATGGGAGAACTCAAGACTTGTCTTGCGACTAAGAACGTGACC
      T  G  L  S  K  D  M  G  E  L  K  T  C  L  A  T  K  N  V  T
501  ACCCAGGGCAATTACTCATCACTAAAAATACCACGTGCAGATATATGGTGGTATTGCGGA
      T  Q  G  N  Y  S  S  L  K  I  P  R  A  D  I  W  W  Y  C  G
561  GGAAAGATCCCAAGACCCACTCTACCTCCCCAATGGAAAGGGACATGCGCATTAGTTCAG
      G  K  I  P  R  P  T  L  P  P  Q  W  K  G  T  C  A  L  V  Q
621  CTAACCATACTGTTCAGTATAGCATATGAAAGACAAGAGGAGAAGGGGAGTGTAAAAGGG
      L  T  I  L  F  S  I  A  Y  E  R  Q  E  E  K  G  S  V  K  G
681  AAACGATCTAAACGAAGCATACCCCCCTCATTTGATGACAGAATTTACCTGGACTCCGCT
      K  R  S  K  R  S  I  P  P  S  F  D  D  R  I  Y  L  D  S  A
741  GGCATCCCCAGAGGAGTACCAGACGAGTTCAAAGCCCGAAATCAGATCGCAACTGGGTTA
      G  I  P  R  G  V  P  D  E  F  K  A  R  N  Q  I  A  T  G  L
801  GAGTCCCTCTTCTAG
      E  S  L  F  *
```

Fig. 2. Nucleotide and deduced amino acid sequences of the dogfish envelope protein (dEnv). The deduced amino acid sequence is reported in one-letter code. The start and stop codons (asterisks) are in bold font. The primer pair used in RT-PCR and real-time PCR is marked in the sequence by an underline.

cDNA in a 20-μl reaction employing 2X qPCRBIO SyGreen Mix Lo-Rox (PCR Biosystems Ltd., London, UK). The gene-specific and β-actin RT-primers provided above were used as q-PCR primers. β-actin was used as a control to ensure that all cDNA synthesis were carried out with similar efficiencies. The q-PCR conditions were pre-incubation for 5 min at 95℃ followed by 3-step amplification at 95℃ for 30 s, 60℃ for 30 s, and 72℃ for 30 s for 40 cycles. The melting temperature was 95℃ for 10 s, 65℃ for 60 s, and 97℃ for 1 s as a default setting. For quantification, the fluorescence reading was recorded at the last step of each cycle. Three replicates (N = 3) were performed per sample. The LightCycler® 96 System (Roche, Mannheim, Germany) was used for amplification detection and data analysis. β-actin, an internal reference, was used in all analyses, and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Results and Discussion

1. Cloning and sequencing of dogfish envelope polyprotein

In the present study, a retroviral envelope polyprotein was successfully cloned from various tissues of dogfish. Moreover, the dogfish retroviral envelope polyprotein (dEnv) was demonstrated to be present within the dogfish, especially in kidney.

To obtain the full sequence of dogfish envelope polyprotein, the cDNA fragment obtained from cloning was combined with 3'- and 5'-RACE PCR fragments. This resulted in the acquisition of a 1,334-base pair (bp) cDNA from dogfish kidney tissue, with an open reading frame consisting of a complete coding sequence of 815 bp and deduced amino acid sequence of 284 residues (Fig. 2). Searching the NCBI database using BLASTP showed that this 284 amino acid protein resembles retroviral envelope polyprotein, sharing 28~48% identity with endogenous retrovirus group Env sequences for other fish species (48% identity: Killi fish, *Austrofundulus limnaeus*, GenBank accession no. XP_013886217.1; 36% identity: Burton's mouthbrooder, *Haplochromis burtoni*, GenBank accession no. XP_005949952.1; 34% identity: Zebra mbuna, *Maylandia zebra*, GenBank accession no. XP_012771319.1; 28% identity: Amazon molly, *Poecilia formosa*, GenBank accession no. XP_016517389.1), and sharing 46~50% identity for bird species (50% identity: fowl, *Gallus gallus*, GenBank accession no. XP_015155917.1; 48% identity: mallard, *Anas platyrhynchos*, GenBank accession no. XP_012965528.1; 46% identity: wild turkey, *Meleagris gallopavo*, GenBank accession no. XP_010711279.1). Additionally, the NCBI database showed that the protein examined in this study resembles the envelope polyprotein, sharing 43% identity with the human envelope polyprotein (GenBank accession no. ABB52637.1), 43~45% identity with primates (43% identity: chimpanzee, *pan troglodytes*, GenBank accession no. ABB52638.1; monkey, *macaca nemestrina*, GenBank accession no. ABB52641.1 and *chlorocebus anthiops*, GenBank accession no. ABB52640.1, 44% and 45%, respectively), 50% and 30% identity for other fish species (yellow croaker, *Larimichthys crocea*, GenBank accession no. KKF15995.1 and zebrafish, *Daniio rerio*, GenBank accession no. AAL78047.1, respectively). This new information, in conjunction with the results of our previous work in which Carbonic anhydrase (CA) was demonstrated to localize and in dogfish (Kim et al., 2016), offers convincing support for a model in which shortspine spurdog is ideal for molecular biological research of deep-water fishes.

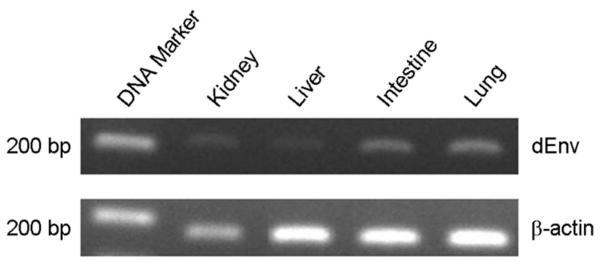

Fig. 3. mRNA expression of dogfish envelope polyprotein in several tissues. Analysis of mRNA expression of dogfish envelope polyprotein in several tissues using reverse transcription polymerase chain reaction. β-actin was used as an internal control. The tissues shown include the kidney, liver, intestine, and lung.

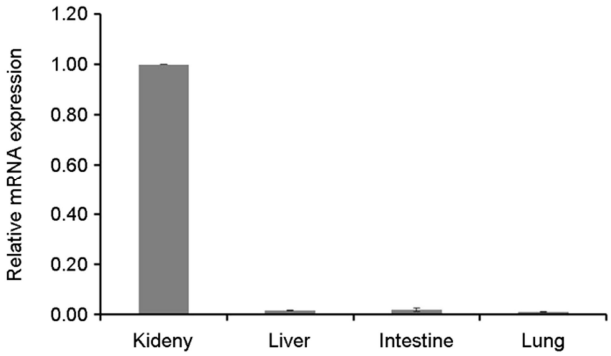

Fig. 4. Relative mRNA expression (means ± SD, N = 3) for the envelope polyprotein in dogfish tissues as determined by q-PCR. The data were compared with those from the kidney, which was given a relative value of 1.

2. mRNA expression of dogfish envelope polyprotein in various tissues

The tissue-specific expression and relative mRNA expression of dogfish envelope polyprotein were examined using gene-specific primers for dogfish envelope polyprotein and β-actin primers from spiny dogfish (*S. acanthias*) by semi-quantitative RT-PCR and q-PCR, respectively. The results of semi-quantitative RT-PCR illustrated that the dogfish envelope polyprotein gene was expressed in the kidney, liver, intestine, and lung, but not in the gill (data not shown) (Fig. 3). These results were quantified and confirmed by q-PCR. The relative mRNA abundance of dogfish envelope polyprotein was highest in the kidney and lowest in the lung (Fig. 4). Interestingly, the dogfish envelope polyprotein was highly expressed in the intestine and lung followed by the kidney and liver according to semi-quantitative RT-PCR; the highest result of relative mRNA abundance was observed in the kidney.

Retroviruses have highly variable nucleotide sequences (Doolittle et al., 1989; Xiong and Eickbush, 1990), and therefore, to ensure the results of cloning and sequencing of dEnv, several type of PCR were used in this study. RT-PCR is the technique to analyze mRNA expression derived from various sources. Real-time PCR is highly sensitive and allows quantification of rare transcripts and small changes in gene expression. Furthermore, it is easy to perform, provides the necessary accuracy and produces reliable as well as rapid quantification results (Michael, 2001). Therefore, we used these techniques and our results provide a rare example of retroviral envelope polyprotein in deep-water fishes, especially in dogfish.

According to earlier study by other researchers, they showed that expression of the Jaagsiekte sheep retrovirus (JSRV) envelope protein, causes a contagious lung cancer in sheep and goats with significant animal health and economic consequences, in lungs of mice by using a replication-incompetent adeno-associated virus vector, results in tumors with a bronchiolo-alveolar localization like those seen in sheep (Sarah et al., 2005). Moreover, there have been previous reports of endogenous retroviral gene expression in mammals (Larrson et al., 1993; Boyd et al., 1993; Venables et al., 1995), but only a few examples have clearly shown this expression to be important in host biology (Limjoco et al., 1993; Best et al., 1996). In case of the past research on retrovirus of fish, for example, the zebrafish, *Danio rerio*, a popular model system for vertebrate developmental studies, the application of retroviral vector technology to the zebrafish system was not feasible because of the limited host range of the standard vectors. However, a relatively recent report has demonstrated that a pseudotyped retroviral vector, which can be concentrated to very high titer, can infect cultured fish cells, including those derived from zebrafish embryos (Burns et al., 1993). Like the case of the above-mentioned studies, we cautiously suppose that the results of our present study will be a fundamental data of the progress of molecular biology using deep-water fishes.

Determination of the dEnv sequence will improve understanding of retrovirus diversity and evolution in aquatic animals. Additional sequence information together with functional characterization of fish retroviral envelope polyprotein is required to clarify the evolutionary relationships.

Acknowledgments

This research was a part of the project titled "Honam Sea Grant Program", funded by the Ministry of Ocean and Fisheries, Korea.

References

Best S, Le Tissier P, Towers G, Stoye JP. 1996. Positional cloning of the mouse retrovirus restriction gene Fv1. Nature 382: 826-829.

Bigelow HB, Schroeder WC. 1948. Fishes of the western North Atlantic. Part 1 (Sharks), Fishes of the Western North Atlantic. Memoirs Sears Foundation for Marine Research 1: 56-576.

Boyd MT, Bax CM, Bax BE, Bloxam DL, Weiss RA. 1993. The human endogenous retrovirus ERV-3 is upregulated in differentiating placental trophoblast cells. Virology 196: 905-909.

Burns JC, Friedmann T, Driever W, Burrascano M, Yee JK. 1993. Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. Proceedings of the National Academy of Sciences of the United States of America 90: 8033-8037.

Chen C, Taniuchi T, Nose Y. 1979. Blainville's dogfish, *Squalus blainville*, from Japan, with notes on *S. mitsukurii* and *S. japonicus*. Japanese Journal of Ichthyology 26: 26-42.

Coffee LL, Casey JW, Bowser PR. 2013. Pathology of tumors in fish associated with retroviruses: a review. Veterinary Pathology 50: 390-403.

Coffin JM, Hughes SH, Vamus HE. 1997. Retroviruses. Cold Spring Harbor Laboratory Press, New York.

Colman PM, Lawrence MC. 2003. The structural biology of type I viral membrane fusion. Nature Reviews Molecular Cell Biology

4: 309-319.

Cortés E. 1999. Standardized diet compositions and trophic levels of shark. ICES Journal of Marine Science 56: 707-717.

Doolittle RF, Feng DF, Johnson MS, McClure MA. 1989. Origins and evolutionary relationships of retroviruses. The Quarterly Review of Biology 64: 1-30.

Ebert DA, Compagno LJV, Cowley PD. 1992. A preliminary investigation of the feeding ecology of squaloid sharks off the west coast of southern Africa. African Journal of Marine Science 12: 601-609.

Eckert DM, Kim PS. 2001. Mechanisms of viral membrane fusion and its inhibition. Annual Review of Biochemistry 70: 777-810.

Fischer AF, Veras DP, Hazin FHV, Broadhurst MK, Burgess GH, Oliveira PGV. 2006. Maturation of *Squalus mitsukurii* and *Cirrhigaleus asper* (Squalidae, Squaliformes) in the southwestern equatorial Atlantic Ocean. Journal of Applied Ichthyology 22: 495-501.

Gilmour KM, Bayaa M, Kenney L, McNeill B, Perry SF. 2007. Type IV carbonic anhydrase is present in the gills of spiny dogfish (*Squalus acanthias*). American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 29: R556-R567.

Jonathan DS, Anne-Sophie K, Ronald CM. 2014. Structural and functional comparisons of retroviral envelope protein C-terminal domains: still much to learn. Virus 6: 284-300.

Kim SC, Sumi KR, Kim JW, Choi MR, Min BH, Kho KH. 2016. Carbonic anhydrase I in a cartilaginous fish, the shortspine spurdog (*Squalus mitsukurii*). Ocean Science Journal 51: 373-379.

Larsson E, Anderson AC, Holmberg L, Ohlsson R, Kato N, Callicio J, Cohen M. 1993. Expression of an endogenous retrovirus, HERV-R, in human tissues. Journal of Cancer Research and Clinical Oncology 119 (suppl.1), 6.

Lepa A, Siwicki AK. 2011. Retroviruses of wild and cultured fish. Polish Journal of Veterinary Sciences 14: 703-709.

Limjoco TI, Dickie P, Ikeda H, Silver J. 1993. Transgenic Fv-4 mice resistant to Friend virus. Journal of Virology 67: 4163-4168.

Litvinov FF. 1990. Ecological characteristics of the dogfish, *Squalus mitsukurii*, from the Sala-y-Gomez seamounts. Journal of Ichthyology 30: 104-115.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25: 402-408.

Malik HS, Henikoff S, Eickbush TH. 2000. Poised for contagion: evolutionary origins of the infectious abilities of invertebrate retroviruses. Genome Research 10: 1307-1318.

Michael WP. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research 29: e45.

Molina GI. 1782. Saggio sulla storia naturale del Chile, del Signor Abate Giovanni Ignazio Molina. Bologna, Saggio Chile, 306 pp.

Risso A. 1827. Histoire naturelle des principales productions de l'Europe méridionale, et particulièrement de celles des environs de Nice et des Alpes maritimes. F. G. Levrault, Paris & Strasbourg, 480 pp.

Sarah KW, Christine LH, Miller AD. 2005. Sheep retrovirus structural protein induces lung tumors. Nature 434: 904-907.

Venables PJ, Brookes SM, Griffiths D, Weiss RA, Boyd MT. 1995. Abundance of an endogenous retroviral envelope protein in placental trophoblasts suggests a biological function. Virology 211: 589-592.

Ward RD, Holmes BH, Zemlak TS, Smith PJ. 2007. DNA barcoding discriminates spurdogs of the genus Squalus. In: Last PR, White WT, Pogonoski JJ (eds.), Descriptions of new dogfishes of the genus Squalus (Squaloidea: Squalidae). CSIRO Marine and Atmospheric Research Paper 014, 130 pp.

Wilson CD, Seki MP. 1994. Biology and population characteristics of *Squalus mitsukuii* from a seamount in the central North Pacific Ocean. Fishery Bulletin 92: 851-864.

Xiong Y, Eickbush TH. 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO Journal 9: 3353-3362.