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First description of programmed cell death10 (PDCD10) in rock bream (*Oplegnathus fasciatus*): Potential relations to the regulation of apoptosis by several pathogens

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ABSTRACT

In this study, we isolated and characterized programmed cell death10 (PDCD10), which is known to be related to apoptosis, from rock bream (*Oplegnathus fasciatus*). The full-length rock bream PDCD10 (RbPDCD10) cDNA (1459 bp) contains an open reading frame of 633 bp that encodes 210 amino acids. Furthermore, multiple alignments revealed that the six of the α-helix bundles were well conserved among the other PDCD10 sequences tested. RbPDCD10 was significantly expressed in the liver, RBC (red blood cell), gill, intestine, trunk kidney and spleen. RbPDCD10 gene expression was also examined in several tissues, including the kidney, spleen, liver, and gill, under bacterial and viral challenges. Generally, all of the examined tissues from the fish that were infected with *Edwardsiella tarda* and the red sea bream iridovirus (RSIV) exhibited significant up-regulations of RbPDCD10 expression compared to the controls. However, RbPDCD10 expression exhibited dramatic down-regulations in all of the examined tissues following injections of *Streptococcus iniae*, which is major bacterial pathogen that is responsible for mass mortality in rock bream. Our results revealed that rock bream PDCD10 may be involved in the apoptotic regulation of rock bream immune responses.

1. Introduction

Rock bream (*Oplegnathus fasciatus*) belong to the Perciformes order and the Oplegnathidae family and are widely distributed in the Pacific and Indian Oceans, primarily in the southern seas of Korea. Rock bream are regarded as an economically important species in the Korean aquaculture industry (Kim et al., 2012). However, unlike other commercially important fishes in Korea, the total production of rock bream is considered unsatisfactory (Kim et al., 2010) due to the occurrences of streptococcosis, red sea bream iridovirus (RSIV) disease, and white spot disease, which are major causes of mass mortality in rock bream (Ishitani et al., 1996; Jung and Oh, 2000; Sohn et al., 2000; Kim et al., 2002). Furthermore, due to stress from various limitations in farming environments, the frequencies of these diseases have increased and caused significant economic damage to the rock bream farming industry (Ko et al., 2004).

The term apoptosis was first coined in 1972 and is generally applied to programmed cell death in which cells die due to an active process that is characterised by nuclear fragmentation and cell shrinkage with the maintenance of cell membrane integrity (Dockrell, 2001). Apoptosis is a physiologic process that removes cells that have completed their primary function. Micro-organisms modulate apoptosis; the induction of apoptosis prevents the host cells from performing key functions, and its inhibition permits intracellular microbial persistence (Dockrell, 2001). Apoptotic cells provide a source of antigens for the adaptive immune response, but the relative importance of the antigens derived from apoptotic as opposed to necrotic cells requires clarification.

Cerebral cavernous malformations (CCM) induce haemorrhagic stroke and are indicative vascular malformations of the central

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nervous system that leak blood from the blood vessels. Three expressions of three genes, i.e., Krit1 (CCM1), OSM (CCM2), and PDCD10 (CCM3), are known to be correlated with development of CCM (Dibble et al., 2010). Among these genes, CCM3 codes for the smallest of the CCM proteins, which has a size of 25 kDa and consists of 212 amino acids (Dibble et al., 2010). CCM3 was initially classified as TF-1 apoptosis-related gene-15 (TFAR15), but it was observed to be up-regulated upon the induction of apoptosis by serum withdrawal in TF-1 human premyeloid cells (Bergametti et al., 2005; Guclu et al., 2005). This result is thought to have no relation with the apoptosis reaction; thus, the name was changed again (Bergametti et al., 2005; Guclu et al., 2005).

PDCD10 has been found to have a role in apoptosis because it induces apoptosis through the caspase 3 pathway (Chen et al., 2007). Moreover, PDCD10 can be regulated through phosphorylation and dephosphorylation because PDCD10 can be phosphorylated by serine/threonine kinase 25 (STK25) and dephosphorylate when it combines with the phosphatase domain of Fas-associated phophatase-1 (Voss et al., 2009). Similarly, PDCD10 is also known to regulate the activation of the extracellular signal-regulated kinase (ERK) pathway (Whitehead et al., 2004). Together, the results of these varied studies (Whitehead et al., 2004) suggest that PDCD10 plays significant roles in protein synthesis and mobilization, apoptosis, and cell proliferation and increase and also likely performs an important function in cancer (Dibble et al., 2010).

Although many PDCD10 sequences from various types of fish are registered in GenBank, information about the molecular features of the PDCD10 gene and analyses of its expression are extremely insufficient. Moreover, in terms of rock bream, which is a highly economically significant fish species for the Korean fish aquaculture industry, there are no reports on the existence of the PDCD10 gene or its molecular features.

In the present study, we firstly describe the molecular identification and expression of the PDCD10 gene in a teleost and then related the expression of this gene with apoptosis regulation. This research was conducted to provide improved information about the PDCD10 gene of a fish for which the molecular features and functions are not widely known. To achieve these goals, we used quantitative real-time PCR to observe changes in the expression of the PDCD10 gene according to tissue and experimental infections with pathogens. Furthermore, this study intended to facilitate the understanding of research on the immune systems of fish and provide useful basic data via analysis of the functions of immune genes in rock bream.

2. Materials and methods

2.1. Molecular characterisation and bioinformatics

The full-length cDNA of the rock bream programmed cell death10 (RbPDCD10) gene was obtained through expressed sequence tag (EST) analysis of a lipopolysaccharide (LPS)-stimulated rock bream liver cDNA library (Kim et al., 2010).

The molecular characterisation of the RbPDCD10 cDNA was performed using several programs and an online server according to the methods reported in our previous studies (Kim et al., 2012; Hwang et al., 2014). Briefly, multiple sequence alignments were created using GENETYX ver. 8.0 (SDC Software Development, Tokyo, Japan) with RbPDCD10 and several PDCD protein sequences from various species. The Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/) and the PROSITE profile database (http://www.expasy.org/tools/scanprosite/) were used to investigate the characteristic domains and specific motifs. Phylogenetic trees were constructed using the Mega 4 software with the neighbour-joining method (Tamura et al., 2007). Support for each node was derived from 2000 resamplings.

2.2. Gene expression analysis of RbPDCD10 in healthy rock bream

Tissue-specific gene expression was analysed to evaluate the expression of RbPDCD10 in peripheral blood leukocytes (PBLs), red blood cells (RBCs), head kidney, trunk kidney, spleen, liver, intestine, gill and muscle. All of these tissues were isolated from three healthy rock bream weighing ~200 g according to the methods reported in our previous study (Kim et al., 2012). The PBLs and RBCs were separated by density-gradient centrifugation using Percoll (Sigma–Aldrich) as described previously (Park et al., 2003). The total RNA from each sample was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA), and the first-strand cDNA synthesis was performed using a first-strand cDNA synthesis kit (Takara, Kyoto, Japan) according to the manufacturer's instructions.

The RbPDCD10 mRNA expression levels were analysed by quantitative real-time PCR (qRT-PCR) with gene-specific primers (forward: 5′-GAATAGACGGGTGCTGGAAA-3′, reverse: 5′-TCATGTTGGTTTGGTGGATG-3′) on a Thermal Cycler DICE Real-Time System (Takara Bio, Inc.) using SYBR™ Green Master Mix (Takara, Kyoto, Japan). RbPDCD10 expression was analysed using the comparative Ct ($2^{-\Delta\Delta CT}$) method with elongation factor (EF)-1α as a control. EF-1α was amplified as a control using EF-1α forward (5′-CCCCTGCAGGACGTCTACAA-3′) and EF-1α reverse (5′-AACACGACCGAC GGGTACA-3′) primers according to the methods reported in our previous study (Hwang et al., 2014). The data from each group were tested with a one-way analysis of variance (ANOVA) test using the SPSS version 17 software (SPSS, Chicago, IL, USA). All samples were analysed in triplicate, and the results are reported as the means ± the standard deviations (SDs).

2.3. Expression analyses following pathogen infection

The gene expressions of RbPDCD10 in the whole kidneys, spleens, livers, and gills of the infected rock bream were measured by qRT-PCR according to the methods reported in our previous study with minor modifications (Hwang et al., 2014). Briefly, the fish in the experimental group were intraperitoneally injected with *Edwardsiella tarda* (*E. tarda*, 1.5×10^5 cells/fish), *Streptococcus iniae* (*S. iniae*, 1.5×10^5 cells/fish), or red sea bream iridovirus (RSIV, 1.1×10^4 copies/fish). The control fish were injected with the same volume of phosphate-buffered saline (PBS). Five fish were sampled from each group at 1, 6, 24 and 48 h post-infection (hpi), and the whole kidneys, spleens, livers, and gills were harvested. Additionally, the total RNA extraction, cDNA synthesis, and qRT-PCR were conducted as described above (section 2.2).

3. Results and discussion

3.1. Characterisation of the RbPDCD10 cDNA

PDCD10 is one of the important mediating factors that induce apoptosis, and it is an immune gene that plays significant roles, such as inducing cell death following external invasion and inducing apoptosis. Moreover, PDCD10 is a factor that determines the survival of cells and may cause cancer when regulation fails (Bergametti et al., 2005).

This study analysed the molecular features of PDCD10 in terms of its involvement with the immune functions of rock bream, which is an economically important fish for the Korean aquaculture industry.

The full-length cDNA of the rock bream PDCD10 gene was 1480 bp, which included a 5′UTR of 134 bp and a 3′UTR that included a poly A(+) tail, was 532 bp, and consisted of an ORF of