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Molecular characterisation and inductive expression of a fish protein arginine methyltransferase 1 gene in response to virus infection☆

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Abstract

Protein arginine methyltransferase 1 (PRMT1) is currently thought as an effector to regulate interferon (IFN) signalling. Here *Paralichthys olivaceus PRMT1* (*PoPRMT1*) gene was identified as a virally induced gene from UV-inactivated *Scophthalmus maximus* Rhabdovirus (SMRV)-infected flounder embryonic cells (FEC). *PoPMRT1* encodes a 341-amino-acid protein that shares the conserved domains including post-I, motif I, II and III. Homology comparisons show that the putative *Po*PMRT1 protein is the closest to zebrafish PMRT1 and belongs to type I PRMT family (including PRMT1, PRMT2, PRMT3, PRMT4, PRMT6, PRMT8). Expression analyses revealed an extensive distribution of *Po*PMRT1 in all tested tissues of flounder. In vitro induction of *Po*PRMT1 was determined in UV-inactivated SMRV-infected FEC cells, and under the same conditions, flounder *Mx* was also transcriptionally up-regulated, indicating that an IFN response might be triggered. Additionally, live SMRV infection of flounders induced an increased expression of *Po*PRMT1 mRNA and protein significantly in spleen, and to a lesser extent in head kidney and intestine. Immunofluorescence analysis revealed a major cyptoplasmic distribution of PoPRMT1 in normal FEC but an obvious increase occurred in nucleus in response to UV-inactivated SMRV. This is the first report on in vitro and in vivo expression of fish PRMT1 by virus infection, suggesting that *Po*PRMT1 might be implicated in flounder antiviral immune response.

Keywords: Protein arginine methyltransferase 1; *Paralichthys olivaceus*; *Scophthalmus maximus* rhabdovirus virus (SMRV); Virus infection; Differential expression; Antiviral immune response

1. Introduction

Protein arginine methylation is a widespread post-translational modification that transfers methyl groups from *S*-adenosyl-L-methionine (AdoMet) to the guanidino nitrogen atoms of arginine residues, increasing the structural

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diversity of modified proteins in order to modulate their function in the living cells. This modification is catalysed by protein arginine methyltransferases (PRMT). Based on their reaction product specificity, these enzymes are divided into two major classes: type I PRMTs promote the formation of ω-monomethylarginine and asymmetric ω-N^G,N^G-dimethylarginine (aDMA), and type II PRMTs catalyse the formation of ω-monomethylarginine and symmetric ω-N^G,N^G-dimethylarginine (sDMA) [1]. In mammals, six type I PRMT enzymes, such as PRMT1 [2], PRMT2 [3], PRMT3 [4], CARM1 (PRMT4) [5], PRMT6 [6] and PRMT8, have been identified, and type II enzymes identified to date include the Janus kinase-binding protein JBP1/PRMT5 [7], and PRMT7 [8,9]. The recently identified FBXO11/PRMT9 should belong to type II PRMT family although it contains a unique N-terminal F-box domain that does not appear in the other known PRMT enzymes [10]. Structurally, PRMT proteins possess double E and THW loops, an *S*-adenosylmethionine binding site (motif I), a catalytically active region (motif III), and two function-unknown motifs termed ''post-I'' and ''motif II'' [11]. The structural basis for functional characterisation of the PRMT family is further revealed by the crystal structure analyses of rat PRMT3 catalytic core [12] and yeast Hmt1/Rmt1 [13]. Until now, eight orthologues of human *PRMT* genes have been identified in zebrafish (*Danio rerio*) and pufferfish (*Fugu rubripes*) by data mining of genomic sequences or assembled EST sequences [14,15].

Arginine methylation by PRMTs has been reported to regulate signal transduction [16–20], transcription [5], RNA transport [21,22], and possibly splicing [23]. PRMT1 is the predominant and essential arginine methyltransferases in human cell, responsible for at least 85% of all arginine methylation reactions [24]. This result is consistent with the findings that most of the identified substrates are catalysed by PRMT1 [25,26], and that PRMT1 is vital for the early development of mice in that *PRMT1*$^{-/-}$ mice die beyond embryonic days 6.5 [27]. PRMT1 is believed to preferentially catalyse proteins that interact with nucleic acids, such as histone H3 and H4, components of hnRNP, fabrillarin and nucleolin, to regulate protein-protein interactions [26]. Further analysis shows a predominantly cytoplasmic localisation of PRMT1 in normal cells but a significant nuclear accumulation when protein arginine methylation is inhibited [28]. Interestingly, PRMT1 was constitutively associated with the interferon (IFN)-α receptor [20] and methylated STAT1 on Arg-31 to suppress the interaction with the negative regulatory protein PIAS in HeLa cells [29]. Abrogation of PRMT1 expression by antisense technology decreased the antiviral and antiproliferative abilities of type I IFN [17], and HCV directly modulated NS3 helicase activity through inhibition of cellular PRMT1 to interfere with the cellular defense against viruses [30]. These studies show that in mammalian system, PRMT1 should be implicated in IFN signalling and limitation of virus replication.

It is well known that vertebrate IFN system is the first line of defence against virus infection. In fish, IFN genes and many IFN stimulated genes have been identified, indicating that fish also possess a complete IFN system with the antiviral function. The finding of PMRT1 involved in IFN-mediated antiviral immune response motivated us to investigate the role of fish PRMT1 in response to virus infection. In this study, using Japanese flounder (*Paralichthys olivaceus)* as a model organism to study the fish immune system, an orthologue of mammalian *PRMT1* gene, termed *PoPRMT1,* was cloned from cultured flounder embryonic cells (FEC). Further, the up-regulation of *Po*PRMT1 was confirmed at mRNA and protein levels in virally infected FEC cells and flounder.

2. Materials and methods

2.1. Cells and virus

Flounder (*Paralichthys olivaceus*) embryonic cells (FEC) [31] and carp leucocyte cells (CLC) [32] were maintained in DMEM and medium 199 at 24 °C, respectively. All media was supplemented with 10% fetal calf serum (FCS). Turbot (*Scophthalmus maximus* L.) rhabdovirus (SMRV) was propagated in CLC cells. The supernatant containing SMRV was harvested and centrifuged to eliminate cell debris. UV-inactivation of SMRV was performed as described previously [33,34].

2.2. Gene cloning

Rapid amplification of cDNA end (RACE)-PCR was used to clone the full-length cDNA of *PoPRMT1* gene. During screening of a subtracted cDNA library that was constructed with mRNA made from UV-inactivated SMRV-infected and mock-infected FEC cells [35], one EST homologous to mammalian *PRMT1* gene was retrieved. A pair of primers