Antiviral activity of European sea bass ISG15 against betanodavirus infections

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Type I interferon system (IFN-I) is the first line of defense against viral infections, activating a group of genes known as interferon-stimulated genes (ISG). Interferon-stimulated gene 15 (ISG15) is an ubiquitin-like protein with proven antiviral activity, acting intracellularly by binding to target proteins (ISGylation) or extracellularly acting as cytokine. One of the main viral diseases affecting European sea bass (*Dicentrarchus labrax*) is the viral nervous necrosis, which is caused by the nervous necrosis virus (NNV, *Betanodavirus* family), a non-enveloped icosahedral virus with two single-stranded RNA segments coding the RNA-dependent RNA polymerase (RNA1) and the capsid protein (RNA2). Betanodaviruses have been clustered into four genotypes according to nucleotide differences within the T4 region (RGNNV, SJNNV, TPNNV, BFNNV), although only the RGNNV genotype has been reported to cause high mortality in *D. labrax*. To study the role of the sea bass ISG15 protein against betanodavirus infections, an *in vivo* transcription analysis has been performed after RGNNV infection and poly I:C inoculation, and the sea bass ISG15 activity has also been evaluated using an *in vitro* approach. The *in vivo* analyses revealed similar kynetics and levels of transcription in fish brain and head kidney after poly I:C injection; however, the induction caused by RGNNV started earlier in brain, where the upregulation of *isg15* gene transcription was higher. In both organs, the maximum transcription in response to RGNNV was at 72 h post-infection (p.i.). To evaluate the activity of the ISG15 protein *in vitro*, an E-11 cell line permanently expressing the sea bass ISG15 protein, called Dl-ISG15-E11, has been developed. The intracellular anti-NNV activity of ISG15 was evaluated by comparing viral replication and cellular survival after RGNNV and SJNNV inoculation on Dl-ISG15-E11 and E-11 control cells. The results showed absence of anti-RGNNV activity at all sampling times analyzed; however, a significant reduction in SJNNV RNA2 copy number was observed at 48 and 72 h p.i. in ISG15-expressing cells compared with viral replication in control E-11 cells. Moreover, the survival percentage of SJNNV-inoculated Dl-ISG15-E11 cells was statistically higher than the survival of SJNNV-inoculated E-11 cells at the same times p.i. Regarding the extracellular activity of the sea bass ISG15 protein, a preliminary analysis revealed that E-11 cells in contact with Dl-ISG15-E11 cell supernatants are partially protected against both NNV genotypes. These results contribute to increase the knowledge on ISG15 and IFN type I system against betanodavirus infections in sea bass and its role in the host-pathogen relationship. (Support: this study has been funded by the project AGL2014-54532-C2-1-R from Ministerio de Economía y Competitividad (Spanish Government).P. Moreno was supported by a grant from Ministerio de Educacion, Cultura y Deporte (FPU12/00265, Spanish Government).

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