Growth Hormone Secretagogue Peptide A233 Up-Regulates Mx Expression in Teleost Fish In Vitro and In Vivo

Rebeca Martinez Rodriguez (✉ rebeca.martinez@cigb.edu.cu)

Center for Genetic Engineering and Biotechnology: Centro de Ingenieria Genetica y Biotecnologia
<https://orcid.org/0000-0003-4021-6941>

Maria Alejandra Fernandez-Trujillo

University of Malaga: Universidad de Malaga

Liz Hernandez

Center for Genetic Engineering and Biotechnology: Centro de Ingenieria Genetica y Biotecnologia

Adrián Page

University of Malaga: Universidad de Malaga

Julia Béjar

University of Malaga: Universidad de Malaga

Mario Pablo Estrada

Center for Genetic Engineering and Biotechnology: Centro de Ingenieria Genetica y Biotecnologia

Research Article

Keywords: Growth hormone, secretagogue, peptide, teleost

Posted Date: November 23rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1079555/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Aquaculture constitutes an alternative source for food production and contributes to the reduction of indiscriminate catch of aquatic organisms from their natural environment (Diaz & Neira, 2005; Beveridge et al., 2013). However, high mortality during larval state remains a challenge in this sector, mainly because of factors like diets and diseases induced by pathogens (Helvik et al., 2009; Stentiford et al., 2017). Therefore, growth and health management is a key strategy for sustainable aquaculture (Martinez et al., 2016a).

Introduction

Aquaculture constitutes an alternative source for food production and contributes to the reduction of indiscriminate catch of aquatic organisms from their natural environment (Diaz & Neira, 2005; Beveridge et al., 2013). However, high mortality during larval state remains a challenge in this sector, mainly because of factors like diets and diseases induced by pathogens (Helvik et al., 2009; Stentiford et al., 2017). Therefore, growth and health management is a key strategy for sustainable aquaculture (Martinez et al., 2016a).

Synthetic growth hormone (GH) secretagogues (GHSs) consist of a family of ligands, initially termed GH-releasing peptides (GHRPs) (Momany et al., 1981). These synthetic compounds were developed to release GH *in vitro* (Bower et. al., 1977). Ghrelin is the endogenous ligand to the previous orphan GHS-receptor (GHS-R), and thus a potent stimulator of pituitary GH release. Since its discovery, it has become increasingly clear that ghrelin plays an integral role not only in the neuroendocrine control of GH release but also in the regulation of feeding, energy metabolism, cardiovascular performance, and immune responses in a variety of vertebrates, including fish (Kaiya et al., 2008; Kaiya et al., 2013).

Synthetic GHS can modulate the functions described for ghrelin as well (Hattori, 2009; Kaiya et al., 2011). On the other hand, GHS-R is expressed predominantly in the brain and pituitary gland, but it is also expressed in other peripheral tissues such as gut, gills, spleen, and head kidney (Kaiya et al., 2013). This receptor and the growth hormone receptor (GHR) are expressed in cells of the immune system of mammals and fish, which suggests a relation between ligands for this receptor and the immune system (Hattori, 2009).

Fish immune system is compound by physical and chemical barriers, humoral and cellular factors, which prevent the proliferation of the pathogen. The interferon (IFN) system is essential as part of the first line of defense against animal viruses (Verhelst et al., 2013). IFN I induces the transcription of hundreds of genes, some of which encode direct antiviral effectors, such as the Mx proteins (Fernández-Trujillo et al., 2015). Mx proteins are dynamin-like GTPases which are involved in basic cellular processes involving membrane remodeling (Das et al., 2019). They can interfere with the viral replication at different life stages of the virus, by forming oligomers, in which the GTPase activity is essential for viral inhibition

(Kochs et al., 2005; Das et al., 2019). Previously, it has been reported the clear antiviral specificity of these proteins in several fish species (Lin et al., 2006; Chen et al., 2008)

The A233 decapeptide is a GHS. So far, studies have demonstrated an impact of peptide treatment on growth, immune system function, and antioxidant defense in tilapia fish (Martinez et al., 2012). Also, A233 peptide treatment increases INF- γ levels in mouse splenocytes and decreased the viral load in dengue virus-challenged mice (Martínez et al., 2016). However, to our knowledge there is no previous report describing any GHSs with antiviral activity in fish.

The aim of the present study was to evaluate the ability of A233 to stimulate the antiviral activity in RTG-2 cell line *in vitro*, as well as in sea bream (*Sparus aurata*) and zebrafish (*Danio rerio*) *in vivo*. Our results demonstrate by the first time A233 potential antiviral effect on teleost fish.

The effect on the antiviral activity of A233 was firstly investigated using RTG-2 cell line (a fibroblast cell line derived from gonad of Rainbow trout (*Oncorhynchus mykiss*)). Cells were incubated with A233 (1 nM) and/or polyI:C during 0, 24, 48, and 72 hours. The antagonist peptide [D-Lys3]-GHRP6 (100nM) (Sigma) for GHS-R was included as negative control. A significant upregulation of the gene that encode for Mx3 was upregulated 24 hours after the treatment with A233 mixed with polyI:C (Fig. 1A). However, each independent stimuli did not upregulate this gene, suggesting the mix was essential. As it was expected no response was detected after the stimulation with the antagonist peptide (Control) (Fig. 1A).

Based on these first results, a additional tests were performed, where cells were incubated for 24 hours with different amounts of A233 (1, 10 and 100 nM) and/or polyI:C. As previously, the antagonist peptide was used as control. Once again, Mx3 was upregulated in cells incubated with the mix of A233 (1 nM) and polyI:C in comparison with the response detected with the Control + polyI:C. The stimulation of cells with the antagonist peptide alone showed no modulation of the Mx3 expression. (Fig. 1B).

Considering the upregulation of Mx3 by A233, next experiments were focused on study the antiviral effect of this peptide in fish through an *in vivo* experiment in gilthead sea bream (*S. aurata*). Fish were handled according to the European Union guidelines for the handling of laboratory animals (Directive 2010/63/UE). The lowest stress-generating conditions of temperature (18 °C), light (8-h light and 16-h darkness), oxygen (6 ± 0.5 ppm), 37% of salinity and feeding were applied. Fingerlings (n=10) of 20 g were intraperitoneally injected once with 1 μ g/g of body weight with A233 in a total volume of 100 μ L. The control group received the same volume of PBS. Samples from head kidney for total RNA extraction were taken at 24, 48 and 72 hours after the injection. The gene expression of Mx2 (González-Mariscal et al., 2014) and IgM were followed by qPCR. Our results showed that Mx2 was up-regulated at 24 hours by the action of A233, while IgM was up-regulated at 72 hours in the same group (Fig. 2).

Based on the results obtained in sea bream, we tested the ability of A233 to modulate the gene expression in the intestine of zebrafish (*D. rerio*). Zebrafish wild type AB line were maintained in standard conditions in the aquarium of the University of Málaga as previously described by (Durán et al., 2011) and experiments were performed at 28.5°C. Juvenile zebrafish (n=6) of 0.3 g were intraperitoneally

injected during 3 days with 1 µg/g of body weight with A233 in a total volume of 10 µL. The control group received the same volume of PBS. Samples from intestine for total RNA extraction were taken at 24 hours after the last injection. The gene expression of MxAB, MxC, IFN γ and IL-1 β were followed by qPCR. After 24 hours, MxAB was upregulated. However, MxC, IFN- γ and IL-1- β were not significantly up-regulated (Fig. 3).

In the present work, the combined treatment with the GHS A233 and polyI:C upregulated the expression of Mx3 gene in RTG-2 cell line over control and polyI:C groups at 24 hours. RTG-2 cells are a validated experimental system for the functional characterization of virus–host interactions (Tafalla et. al., 2007; González-Mariscal et al., 2016). This suggests that a higher protection against a viral infection could be achieved in the presence of this secretagogue. Differently, the treatment with A233 alone did not up-regulate the transcription of this antiviral gene. Similar results were reported by Picchietti et. al., (2013) where the treatment of SAF-1 cells with *Aloe* extract alone did not stimulate the transcription of Mx, while did in the presence of polyI:C and *Aloe*. Also, Kim et al., (2009) and Falco et al., (2014) reported that after the administration of β -glucans in the diet of grass carp, another stimulus such as virus or polyI:C respectively, were necessary to enhance the transcription of Mx. Thus, further studies should be conducted to explore the molecular mechanisms involved in the potentiating effect of A233 on polyI:C activated cells. The inclusion of the antagonist of the GHS-R showed that Mx3 expression was down-regulated when incubated alone and with Poly I:C. This result agrees with Martinez et. al., (2016b) where the addition of this molecule in a mice splenocytes cell culture, decreased the secretion of IFN-g compared to GHRP-6, another GHS. Previously, Martinez et. al., (2017) reported the action of another GHS as a candidate molecular adjuvant in mice and fish in the presence of different antigens. Considering this, future studies should be conducted to evaluate the possible actions of this secretagogue as a molecular adjuvant to enhance the effects of antigens in vaccines. The search for novel adjuvants that are safe and elicited the desired immune response in fish, remains an active research area in this field (Dalmo et. al., 2016; Wangkahart et. al., 2019).

In addition, in the presence of the antagonist for the GHS-R there was no up-regulation of Mx3 gene, which suggests that the effect of this GHS on the antiviral response is specific and mediated by the GHS-R. The expression of Mx2 in fingerlings of sea bream at 24 hours was up-regulated by the treatment with A233. Previously it has been demonstrated that this gene might play a major role in both innate and adaptive immune response in this species, being expressed in the head kidney, a lymph myeloid tissue (Fernández-Trujillo et al., 2011). In addition, it showed activity against DNA and RNA viruses (Fernández-Trujillo et al., 2013). Others authors have reported the effects of other immunostimulants (i.e: β -glucans or LPS) on the survival of virus-infected fish, where Mx expression have resulted also up-regulated (Wang et al., 2016; Bravo et al., 2013). The treatment with A233 also up-regulated the expression of IgM after 72 h. This molecule is an indicator of specific immune responses and provides systemic protection in fish (Munang'andu & Evensen, 2019). Other authors have reported the effect of other molecules involved in the neuro-endocrine-immune regulation, on the antibody levels. In tilapia, Lugo et al., (2010) reported that the treatment, with pituitary adenylate cyclase-activating polypeptide (PACAP), stimulated the secretion of IgM in serum. In addition, IgM levels have been related to a better diseases outcome against viral

infections. Caruffo et al., (2016) demonstrated that an oral vaccine against infectious salmon anemia based on recombinant antigens, modulated Mx expression levels in head kidney and stimulated IgM levels in serum, which was related to a higher survival in a challenge trial. Similarly, in grass carp vaccinated against grass carp reovirus, Mx and IgM were up-regulated in head kidney, while the survival rate was about 80% after the challenge (Zeng et al., 2016). Further studies should be conducted to evaluate the effect of A233 treatment in challenge trials. The results obtained in the present work with IgM stimulation are in agreement with previous studies conducted in tilapia, where the growth hormone secretagogue peptide-6 stimulated IgM levels in serum when administrated with different antigens (Martínez et al., 2017).

The intestine is a tissue continuously exposed to a complex community of microbiota and environmental pathogens. Thus, gut-associated lymphoid tissue has essential roles in maintaining mucosal homeostasis in digestive tract (Salinas, 2015; Rombout et al., 2011). In the present work, the treatment with A233 up-regulated MxAB levels in intestine of zebrafish. In this sense, different authors have reported the importance of this gene in the intestinal immune response against viral infections. For example, Wang et al., (2019) reported that in rainbow trout, distinct Mx isoforms were upregulated after a challenge with PolyI:C. Meanwhile, Dong et al., (2019) informed in the same species, the importance of this gene in the digestive tract, for the antiviral state developed after a bath infection with infectious hematopoietic necrosis virus (IHNV). Other studies have shown the up-regulation of Mx after the treatment with immunostimulants in teleost. For example, Falco et al., (2014) reported an increase in Mx expression levels in gut after feeding with β -glucans. Therefore, the results obtained in this work are of interest because different viruses can infect fish through the intestine, causing necrosis of epithelial cells, hemorrhagic or enteritis (Crane & Hyatt, 2011; Martin et al., 2016; Kopper et al., 2014).

Previous studies with A233 have shown that *in vitro* in a murine macrophages cell line, there was a stimulation of the production of MAVS proteins (Martínez et al., 2016). Meanwhile *in vitro* it stimulated the secretion of IFN- α in mice splenocytes cell culture; and *in vivo* decreased the viral load in DENV challenged mice. These *in vitro* and *in vivo* observed effects were specifically mediated through the GHS-R (Martinez et. al., 2016). Other authors have reported the importance of MAVS proteins as adaptors in the IFN antiviral mediated pathway, which leads to the transcription of different ISG, as Mx (Chen et al., 2015; Zou et al., 2014; Wu et al., 2020). In fish, it has been reported that IFN- α can stimulate the transcription of Mx proteins (Velázquez et al., 2017; Peng et al., 2016; González-Mariscal et. al., 2014). The results obtained in the present study shows that in the presence of A233, Mx expression was stimulated in RTG2-cell line, as well as in head kidney of sea bream and intestine of zebrafish, which suggests the stimulation of an antiviral state. Further studies should be conducted to evaluate the A233 control capacity on viral status under a viral challenge as well as its potential use as adjuvants in vaccines.

Declarations

Acknowledgment

This research was supported by the University of Malaga, Spain.

References

1. Beveridge MCM, Thilsted SH, Phillips MJ, Metian M, Troell M, Hall SJ (2013) Meeting the food and nutrition needs of the poor: The role of fish and the opportunities and challenges emerging from the rise of aquaculture. *J Fish Biol* 83(4):1067–1084
2. Bowers CY, Chang J, Momany F (1977) y F. K. Effect of the enkephalins and enkephalin analogs on release of pituitary hormones *in vitro*. *Molecular Endocrinology*, 287–292
3. Bravo J, Real F, Padilla D, Oliveira JG, Grasso V, Román L, Acosta F (2013) Effect of lipopolysaccharides from *Vibrio alginolyticus* on the Mx gene expression and virus recovery from gilthead sea bream (*Sparus aurata* L.) experimentally infected with Nodavirus. *Fish and Shellfish Immunology* 34(1):383–386
4. Caruffo M, Maturana C, Kambalapally S, Larenas J, Tobar JA (2016) Fish & Shellfish Immunology Protective oral vaccination against infectious salmon anaemia virus in *Salmo salar*. *Fish and Shellfish Immunology* 54:54–59
5. Chen WQ, Hu YW, Zou PF, Ren SS, Nie P, Chang MX (2015) MAVS splicing variants contribute to the induction of interferon and interferon-stimulated genes mediated by RIG-I-like receptors. *Dev Comp Immunol* 49(1):19–30
6. Chen YM, Su YL, Shie PS, Huang SL, Yang HL, Chen TY (2008) Grouper Mx confers resistance to nodavirus and interacts with coat protein. *Dev Comp Immunol* 32:825–836
7. Crane M, Hyatt A (2011) Viruses of fish: An overview of significant pathogens. *Viruses* 3(11):2025–2046
8. Das BK, Roy P, Rout AK, Sahoo DR, Panda SP, Pattanaik S, Dehury B, Behera BK (2019) Molecular cloning, GTP recognition mechanism and tissue-specific expression profiling of myxovirus resistance (Mx) protein in *Labeo rohita* (Hamilton) after Poly I: C induction. *Scientific Reports, December 2018*, 1–19. <https://doi.org/10.1038/s41598-019-40323-0>
9. Diaz NF, Neira R (2005) Biotecnología aplicada a la acuicultura. *Cien Inc Agr* 32(1):45–49
10. Dong S, Ding LG, Cao JF, Liu X, Xu HY, Meng KF, Yu YY, Wang Q, Xu Z (2019) Viral-Infected Change of the Digestive Tract Microbiota Associated With Mucosal Immunity in Teleost Fish. *Front Immunol* 10(December):1–13
11. Durán I, Marí-Beffa M, Santamaría JA, Becerra J, Santos-Ruiz L (2011) Actinotrichia collagens and their role in fin formation. *Dev Biol* 354(1):160–172
12. Falco A, Miest JJ, Pionnier N, Pietretti D, Forlenza M, Wiegertjes GF, Hoole D (2014) β -Glucan-supplemented diets increase poly(I: C)-induced gene expression of Mx, possibly via Tlr3-mediated recognition mechanism in common carp (*Cyprinus carpio*). *Fish and Shellfish Immunology* 36(2):494–502

13. Fernández-Trujillo MA, García-Rosado E, Alonso MC, Álvarez MC, Béjar J (2015) Synergistic effects in the antiviral activity of the three Mx proteins from gilthead sea bream (*Sparus aurata*). Vet Immunol Immunopathol 168(1–2):83–90
14. Fernández-Trujillo MA, García-Rosado E, Alonso MC, Castro D, Álvarez MC, Béjar J (2013) Mx1, Mx2 and Mx3 proteins from the gilthead sea bream (*Sparus aurata*) show in vitro antiviral activity against RNA and DNA viruses. Mol Immunol 56(4):630–636
15. Fernández-Trujillo MA, Novel P, Manchado M, Sepulcre MP, Mulero V, Borrego JJ, Álvarez MC, Béjar J (2011) Three Mx genes with differential response to VNNV infection have been identified in Gilthead sea bream (*Sparus aurata*). Mol Immunol 48(9–10):1216–1223
16. González-Mariscal JA, Fernández-Trujillo MA, Alonso MC, García-Rosado E, Álvarez MC, Béjar J (2016) Gilthead sea bream (*Sparus aurata*) Mx gene promoters respond differentially to IPNV and VHSV infections in RTG-2 cells. Vet Immunol Immunopathol 171:73–80
17. González-Mariscal JA, Gallardo-Gálvez JB, Méndez T, Álvarez MC, Béjar J (2014) Cloning and characterization of the Mx1, Mx2 and Mx3 promoters from gilthead sea bream (*Sparus aurata*). Fish Shellfish Immunol 38(2):311–317
18. Hattori N (2009) Expression, regulation and biological actions of growth hormone (GH) and ghrelin in the immune system.. In Growth Hormone and IGF Research, vol 19. Elsevier Ltd, pp 187–197
19. Helvik J, Hamre K, Hordvik I, van der Meeren T, Ressem H, Scharl M, Tveiten H, Øie G (2009) The fish larva: a transitional life form, the foundation for aquaculture and fisheries. The Research Council of Norway 5:13
20. Kaiya H, Kangawa K, Miyazato M (2013) Ghrelin receptors in non-mammalian vertebrates. Front Endocrinol 4(JUL):1–16
21. Kaiya H, Miyazato M, Kangawa K (2011) Recent advances in the phylogenetic study of ghrelin. Peptides 32(11):2155–2174
22. Kaiya H, Miyazato M, Kangawa K, Peter RE, Unniappan S (2008) Ghrelin: A multifunctional hormone in non-mammalian vertebrates. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology 149(2):109–128
23. Kim Y, sin, Ke F, Zhang QY (2009) Effect of β -glucan on activity of antioxidant enzymes and Mx gene expression in virus infected grass carp. Fish and Shellfish Immunology 27(2):336–340
24. Kochs G, Reichelt M, Danino D, Hinshaw JE, Haller O (2005) Assay and functional analysis of dynamin-like Mx proteins. Methods Enzymol 404:632–643
25. Kopper G, Mirecki S, Kljujev IS, Raicevic VB, Lalevic BT, Petrovic-Jovicic J, Stojanovski S, Blazekovic-Dimovska D (2014) Hygiene in Primary Production. In *Food Safety Management: A Practical Guide for the Food Industry*. <https://doi.org/10.1016/B978-0-12-381504-0.00023-8>
26. Lin CH, John C, Lin JA, Chang CY (2006) Inhibition of nervous necrosis virus propagation by fish Mx proteins. Biochem Biophys Res Commun 351(2):534–539
27. Lugo JM, Oliva A, Morales A, Reyes O, Garay HE, Herrera F, Cabrales A, Pérez E, Estrada MP (2010) The biological role of pituitary adenylate cyclase-activating polypeptide (PACAP) in growth and

- feeding behavior in juvenile fish. *J Pept Sci* 16(11):633–643
28. Martin SAM, Dehler CE, Król E (2016) Transcriptomic responses in the fish intestine. *Dev Comp Immunol* 64:103–117
29. Martinez R, Carpio Y, Morales A, Lugo JM, Herrera F, Zaldívar C, Carrillo O, Arenal A, Pimentel E, Estrada MP (2016a) Oral administration of the growth hormone secretagogue-6 (GHRP-6) enhances growth and non-specific immune responses in tilapia (*Oreochromis* sp.). *Aquaculture* 452:304–310
30. Martínez R, de Villavicencio-Díaz TN, Sánchez A, Ramos Y, Ferro JN, González LG, Méndez M, Rodríguez E, Marcos E, Sánchez B, Masforrol Y, Garay H, Albericio F, Hermida L, González LJ, Vonasek E, Estrada MP, Besada V (2016b) Comparative proteomic analysis of growth hormone secretagogue A233 treatment of murine macrophage cells J774A.2 indicates it has a role in antiviral innate response. *Biochemistry and Biophysics Reports* 5:379–387
31. Martínez R, Hernández L, Gil L, Carpio Y, Morales A, Herrera F, Rodríguez-Mallón A, Leal Y, Blanco A, Estrada MP (2017) Growth hormone releasing peptide-6 enhanced antibody titers against subunit antigens in mice (BALB/c), tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). *Vaccine* 35(42):5722–5728
32. Martinez R, Ubieta K, Herrera F, Forellat A, Morales R, De la Nuez A, Rodriguez R, Reyes O, Oliva A, Estrada MP (2012) A novel GH secretagogue, A233, exhibits enhanced growth activity and innate immune system stimulation in teleosts fish. *J Endocrinol* 214(3):409–419
33. Momany FA, Bowers CY, Reynolds GA, Chang D, Hong A, Newlander K (1981) Design, Synthesis, and Biological Activity of Peptides which Release Growth Hormone in Vitro. *Endocrinology* 108(1):31–39
34. Munang'andu HM, Evensen Ø (2019) Correlates of protective immunity for fish vaccines. *Fish and Shellfish Immunology* 85:132–140
35. Peng W, Lu D-Q, Li G-F, Zhang X, Yao M, Zhang Y, Lin H-R (2016) Two distinct interferon- γ genes in *Tetraodon nigroviridis*: Functional analysis during *Vibrio parahaemolyticus* infection. *Mol Immunol* 70:34–46
36. Picchietti S, Bernini C, Belardinelli MC, Ovidi E, Taddei AR, Guerra L, Abelli L, Fausto AM (2013) Immune modulatory effects of *Aloe arborescens* extract on the piscine SAF-1 cell line. *Fish and Shellfish Immunology* 34(5):1–10
37. Rombout JHWM, Abelli L, Picchietti S, Scapigliati G, Kiron V (2011) Teleost intestinal immunology. *Fish and Shellfish Immunology* 31(5):616–626
38. Salinas I (2015) The mucosal immune system of teleost fish. *Biology* 4(3):525–539
39. Stentiford GD, Sritunyalucksana K, Flegel TW, Williams BAP, Withyachumnarnkul B, Itsathitphaisarn O, Bass D (2017) New Paradigms to Help Solve the Global Aquaculture Disease Crisis. *Pathogens* 13(2):1–6
40. Tafalla C, Chico V, Perez L, Coll JM, Estepa A (2007) In vitro and in vivo differential expression of rainbow trout (*Oncorhynchus mykiss*) Mx isoforms in response to viral haemorrhagic septicaemia virus (VHSV) G gene, poly I: C and VHSV. *Fish Shellfish Immunol* 23(1):210–221

41. Velázquez J, Acosta J, Herrera N, Morales A, González O, Herrera F, Estrada MP, Carpio Y (2017) Novel IFN γ homologue identified in Nile tilapia (*Oreochromis niloticus*) links with immune response in gills under different stimuli. *Fish and Shellfish Immunology* 71(October):275–285
42. Verhelst J, Hulpiau P, Saelens X (2013) Mx Proteins: Antiviral Gatekeepers That Restrain the Uninvited. *Microbiol Mol Biol Rev* 77(4):551–566
43. Wang T, Liu F, Tian G, Secombes CJ, Wang T (2019) Lineage/species-specific expansion of the Mx gene family in teleosts: Differential expression and modulation of nine Mx genes in rainbow trout *Oncorhynchus mykiss*. *Fish and Shellfish Immunology* 90(February):413–430
44. Wang W, Sun J, Liu C, Xue Z (2016) Application of immunostimulants in aquaculture: current knowledge and future perspectives. *Aquac Res* 48(1):1–23
45. Wu XM, Zhang J, Li PW, Hu YW, Cao L, Ouyang S, Bi YH, Nie P, Chang MX (2020) NOD1 Promotes Antiviral Signaling by Binding Viral RNA and Regulating the Interaction of MDA5 and MAVS. *J Immunol* 204(8):2216–2231
46. Zeng W, Wang Q, Wang Y, Zhao C, Li Y, Shi C, Wu S, Song X, Huang Q, Li S (2016) Immunogenicity of a cell culture-derived inactivated vaccine against a common virulent isolate of grass carp reovirus. *Fish and Shellfish Immunology* 54:473–480
47. Zou PF, Chang MX, Xue NN, Liu XQ, Li JH, Fu JP, Chen SN, Nie P (2014) Melanoma differentiation-associated gene 5 in zebrafish provoking higher interferon-promoter activity through signalling enhancing of its shorter splicing variant. *Immunology* 141(2):192–202

Figures

Figure 1

qPCR of Mx3 expression levels in RTG2 cell line. A) Temporal expression pattern with A233 at 1nM. B) Assay with different concentrations of A233, including an antagonist peptide (ant) at 24h. Data represent media $\pm$ SD. Different letters indicate statistical differences among groups ($p < 0.05$) as ANOVA post-test Newman-Keuls.

Figure 2

Expression levels of Mx2 and IgM in Head kidney of A233-stimulated gilthead seabream (*Sparus aurata*). Data represent media $\pm$ SD of three individual fish. Asterisks represent significant differences with control at the same time ($p < 0.05$) as T-Student test.

Figure 3

Expression of different immune-related genes in intestine of zebrafish (*Danio rerio*) after the treatment with A233. Data represent media $\pm$ SD of three individual fish. Asterisk represents significant differences with control ($p < 0.05$) as T-Student test.