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Full length article

Transcriptome analysis reveals a complex response to the RGNNV/SJNNV reassortant Nervous Necrosis Virus strain in sea bream larvae

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Abstract

The gilthead sea bream (Sparus aurata) is a marine fish of great importance for Mediterranean aquaculture. This species has long been considered resistant to Nervous Necrosis Virus (NNV), an RNA virus that causes massive mortalities in several farmed fish animals. However, the recent appearance of RGNNV/SJNNV reassortant strains started to pose a serious threat to sea bream hatcheries, as it is able to infect larvae and juveniles of this species. While host

response to NNV has been extensively studied in adult fish, little attention has been devoted to early life history stages, which are generally the most sensitive ones. Here we report for the first time a time-course RNA-seq analysis on 21-day old fish gilthead sea bream larvae experimentally infected with a RGNNV/SJNNV strain. NNV-infected and mock-infected samples were collected at four time points (6 h, 12 h, 24 h, and 48 h post infection). Four biological replicates, each consisting of five pooled larvae, were analysed for each time point and group. A large set of genes were found to be significantly regulated, especially at early time points (6 h and 12 h), with several heat shock protein encoding transcripts being up-regulated (e.g. *hspa5, dnaj4, hspa9, hsc70*), while many immune genes were down-regulated (e.g. *myd88* and *irf5* at T06, *pik3r1*, *stat3*, *jak1*, il12b and *il6st* at T12). A gene set enrichment analysis (GSEA) identified several altered pathways/processes. For instance, the formation of peroxisomes, which are important anti-viral components as well as essential for nervous system homeostasis, and the autophagy pathway were down-regulated at 6 h and 24 h post infection (hpi). Finally, two custom "reactomes" (i.e. significant gene sets observed in other studies) were defined and used. The first reactome integrated the transcriptomic response to NNV in different fish species, while the second one included all genes found to be stimulated either by interferon (IFN) or by IFN and Chikungunya virus in zebrafish. Genes in both reactomes showed predominant up-regulation at 6hpi and 12hpi and a general down-regulation at 24hpi. Such evidence suggest a certain degree of similarity between the response of sea bream and that of other fish species to NNV, while the observed down-regulation of IFN- and viral-stimulated pathways argues for a possible interference of NNV against the host response.

Introduction

The gilthead sea bream (*Sparus aurata*) is one of the most important farmed species in Europe (http://feap.info/wp-content/uploads/2020/10/20200930_feap-production-report-2020.pdf), with an annual production that reached 83 thousand tonnes in 2016 (representing ~6% of the total EU aquaculture production) for an economic value that was worth €445 million (10% of total EU value) [1]. As for other farmed fish, sea bream aquaculture is threatened by the occurrence of several pathogens, mainly bacteria (*e.g. Photobacterium damselae piscicida*) and parasites (*e.g. Sparicotyle chrysophrii*) that can cause outbreaks in hatcheries and/or at sea and can result in massive mortality of animals and extensive economic damage to farmers [2]. Until recently, *S. aurata* was considered resistant to one of the major viral pathogens in aquaculture, the Nervous Necrosis Virus (NNV) [3], affecting a wide range of fish species and causing high mortality rates [4]. Clinical signs of Viral Nervous Necrosis (VNN) include abnormal swimming behaviour, abnormalities of swimming bladder control (e.g. swim bladder hyperinflation), anorexia and lethargy. In addition, nervous signs due to vacuolisation and necrosis of the central nervous system caused by viral replication in brain and retina cells, are observable [3,[5], [6], [7]].

NNV is caused by betanodavirus, a naked positive-sense single-stranded RNA virus of the family Nodaviridae [8]. At present, four different genotypes of the virus are internationally

recognised: striped jack NNV (SJNNV), tiger puffer NNV (TPNNV), barfin flounder NNV (BFNNV) and red spotted grouper NNV (RGNNV). In 2007 Toffolo et al. [9] reported for the first time the appearance of a reassortant NNV genotype (named SJNNV/RGNNV), followed by the discovery of the RGNNV/SJNNV genotype in 2009 by Olveira et al. [10]. Unfortunately, this latter genotype is able to cause disease outbreaks in sea bream [3,7], a species that was until recently considered resistant to the disease [3]. All RGNNV/SJNNV outbreaks in gilthead sea bream farms have occurred in hatcheries or in nurseries (with the exception of a few field outbreaks [7,11]) and this has led to the hypothesis (still debated) that juveniles/adults may be less susceptible to this reassortant virus (RGNNV/SJNNV) than larval stages [3].

So far, several studies have been conducted to elucidate the molecular and physiological changes that occur in the various fish species infected with NNV (e.g. Refs. [5,6,[12], [13], [14], [15], [16], [17]]). These studies have highlighted that diverse mechanisms are triggered in the host while trying to respond to the virus. Interestingly, Liu, et al. [12], as well as Kim et al. [5], reported the induction of Heat Shock Proteins (HSPs), interferon and immune genes in sea bass epithelial cells and in sevenband grouper brain tissue in response to NNV infection. In addition, Li, et al. [15,18] discovered that the autophagy pathway is up-regulated in the late stage of infection and results in higher replication rates of RGNNV, suggesting that the virus might be able to interfere with cellular processes of the host. While host response to NNV has been extensively studied in adult fish, little attention has been devoted to early life-history stages, which are generally the most sensitive ones. Moreover, no evidence is available on the transcriptomic response of gilthead sea bream to the novel reassortant RGNNV/SJNNV. Here we report a time-course RNA-seq analysis on 21-day old sea bream larvae, experimentally infected with a RGNNV/SJNNV strain. NNV-infected and mock-infected samples were collected at four time points (6 h, 12 h, 24 h, and 48 h post infection). Four biological replicates, each consisting of five pooled larvae, were analysed for each time point and group to identify differentially expressed genes and significantly regulated cellular pathways. In addition, the sea bream transcriptomic response to NNV was evaluated in a rigorous comparative context with that of other species. To this end, custom “reactomes” (*i.e.* significant gene sets observed in other studies) were defined and used. The first reactome integrates the transcriptomic response to NNV in different fish species, while the second one includes all genes found to be stimulated either by interferon (IFN) or by IFN and Chikungunya virus in zebrafish. These reactomes were used in a Gene Set Enrichment Analysis (GSEA).

Overall, the analysis revealed a complex pattern, with time-dependent profile and several involved cellular pathways, shedding light for the first time on the transcriptome response of gilthead seabream larvae to NNV.

Section snippets

Virus propagation

For the experimental challenge, the reassortant betanodavirus strain (RGNNV/SJNNV) named VNNV/S.aurata/Farm1/461-1/Nov2014 was used [3]. The strain was isolated from a natural disease outbreak occurred in 2014 in the Mediterranean. The virus was replicated on E–11 cells [19] according to standard procedures [20]. Viral titre was calculated according to the Reed and Muench formula [21] and expressed as $TCID_{50}$/mL....

Experimental infection

The experimental infection protocol was evaluated by the Istituto Zooprofilattico...

Viral replication

Betanodavirus replication in pooled sea bream larvae was assessed by real-time qPCR estimating the number of RNA1 and RNA2 copies (CN) per reaction, corresponding to 20 ng of total extracted RNA. The trend of viral replication over time was consistent for both target genes, though quantitatively higher for RNA1 (p-value < 0.05) (Fig. 1). Larvae were checked by RT-qPCR immediately after challenge (i.e. 10 min after infection), to obtain the starting viral load, showing RNA1 and RNA2 levels equal ...

Discussion

In the present study, both genome replication of the virus and gene expression changes in the host were monitored. Overall, RNA1 levels were higher than RNA2 at all the time points, consistently with previous reports [32,33]. In the first 6 h post infection the viral titre displayed a slight increase and a high number of DEGs was detected. Viral titre, however, slightly decreased at T12, remained stable at T24, and then increased again at T48, while the number of DEGs reached its peak at T12...

Conclusion

The reassortant RGNNV/SJNNV is a “relatively” new viral strain of NNV that poses serious threats to sea bream aquaculture and the molecular changes induced by this virus in sea bream are still uncharacterised. In this work we followed the dynamics of viral replication and analysed the host response at the transcriptome level. Early down-regulation of important immune pathways (TLR, ISG, fish immune/viral reactomes) suggests that the reaction of the sea bream immune system might be rapidly...

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CRediT authorship contribution statement

L. Peruzza: Software, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **F. Pascoli:** Investigation, Methodology, Resources. **G. Dalla Rovere:** Investigation. **R. Franch:** Investigation. **S. Ferraresso:** Investigation. **M. Babbucci:** Formal analysis. **L. Biasini:** Validation, Visualization, Investigation. **M. Abbadi:** Validation, Investigation. **V. Panzarin:** Writing – original draft, Conceptualization, Resources, Funding acquisition, Conceptualization. **A.**...

Declaration of competing interest

Authors declare no conflict of interest....

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