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P-351.

High expression levels of the *MDM* gene are related to inflammatory response in the mussel *Mytilus galloprovincialis*

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Abstract

The vertebrate *MDM* paralog genes *MDM2* and *MDM4* encode for negative regulators of the p53 protein family and their over-expression is related to many different tumors. In invertebrate species, comparative genomics analyses showed the presence of a single *MDM* homolog gene, where its involvement in tumour pathogenesis was demonstrated only in the mussel *Mytilus trossulus*. Nevertheless, has been proposed MDM2 fulfill a variety of cellular functions, with other p53-independent activities. In particular, recently an additional pro-inflammatory role of MDM2 has been reported in mammals. In this work, we report the first isolation of the *mdm* cDNA in the mussel *Mytilus galloprovincialis* and its expression analysis in the digestive gland tissue of animals collected in four different coastal sites of the Campania region (Italy) during one year. The Real time RT-PCR results revealed an increased expression of the *mdm* gene in tissues displaying chronic inflammatory lesions, which were predominant during summer period. The observed results suggest a possible involvement of the *mdm* in inflammatory processes also in invertebrate species.

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P-193.

Cloning and functional characterization of three novel antimicrobial peptides from tilapia (*Oreochromis niloticus*)

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Abstract

Antimicrobial peptides constitute an important component of the innate immune system. Teleost fish represent a potentially fruitful resource for novel antimicrobial peptides discovery since these organisms rely significantly on their innate immune systems to combat the constant threat of infections in the aquatic environment. In the present study, we isolated three antimicrobial peptides-like transcripts from tilapia (*Oreochromis niloticus*) gills based on EST reported sequences. These peptides were named oreochromicins (Oreoch-1, Oreoch-2 and Oreoch-3). The cDNA sequences for these putative AMPs encode three pre-pro-peptides with the highest similarity with members of the piscidin family from teleost fish. The predicted three pre-pro-peptides consist of a signal peptide, a highly cationic mature peptide of 23, 25, and 32 amino acids, respectively and a carboxy terminal pro-domain. The synthetic peptides displayed a broad-spectrum of antimicrobial activity against Gram-negative, Gram-positive bacteria and Fungi. These peptides are constitutively expressed in brain, heart, head kidney, spleen and gut. Additionally, it was assayed their binding properties to lipopolysaccharide and cytotoxic activity in mammalian and fish cells.

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P-116.

Transfection of rainbow trout fin cells for expression of rhabdoviral antigens

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Abstract

The implementation of novel systems to study the role of non-leukocyte cell populations, particularly those of the skin, gill and gut mucosae, in fish immune responses is of great importance, since they constitute the first defence barriers against pathogens. In that context, we report the development of a method to transfect with great efficacy fin cell lines established from rainbow trout (*Oncorhynchus mykiss*). This transfected cells may be of application in in vitro assays to get further knowledge on the immune responses against fish viral diseases, using the Viral Haemorrhagic Septicaemia Virus (VHSV) as an experimental model.

Fin cell lines were obtained from subcultures of anal fin explants of rainbow trout. Cells from a fibroblastic-like fin cell line were transfected to express the glycoprotein G protein of VHSV (GVSHV) using pAE6-Gvhsv plasmid. Additionally, as positive controls, other fin cells batches were transfected with pMCV1.4-eGFP plasmid. Transfections were carried out using electroporation with Neon Transfection System (Invitrogen). The electroporated cells were plated in a 24-well plate with culture medium without antibiotics. Fluorescence cytometry was used to detect GVHSV expressing cells using a cocktail of anti-GVHSV monoclonal antibodies (C10, 3F1A2 and 116). GFP expression was detected by fluorescence microscopy.

Results confirmed that at 24 h post-transfection (p.t.) GFP expression was detectable in fin cells indicating a successful electroporation, which was estimated to be ≈39% at day 3 p.t. Also, successful transfection with GVSHV was achieved, the percentage of GVHSV-expressing cells being ≈17% as determined by flow cytometry at day 3 p.t.

These findings indicate that fin cell lines can be effectively transfected to express immune-relevant viral proteins, which are used to develop experimental viral vaccines. As genes involved in antiviral responses, such as Mx-1 protein and Ck-12 chemokine, are upregulated in GVSHV transfected fin cells, this model can be of application for in vitro studies of the role of the fin cells in VHSV infection, and also to carry out immune assays to speed out the development of such vaccines.

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P-265.

Characterization of Senegalese sole (*Solea senegalensis*) Mx promoter

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Abstract

Type I interferon (IFN) is a main component of the innate immune response against viral infections, promoting an antiviral state in cells. Mx proteins are the best-studied IFN stimulated genes (ISGs) in fish. The antiviral activity against different viruses has been demonstrated for

diverse fish Mx proteins, including Senegalese sole (*Solea senegalensis*) Mx protein (SsMx). To advance in the knowledge of the IFN pathway and the antiviral state in this species, is necessary to understand the regulatory mechanisms determining ISGs transcription. For this reason, the aim of the current study was the cloning and functional characterization of the SsMx promoter. To fulfill this objective, GenomeWalker™ Universal Kit was used to clone the SsMx promoter. A fragment of 1327 bp upstream of the transcriptional start site has been obtained. Sequence analysis showed a typical structure of an ISG promoter, including three ISREs (Interferon stimulated response element), a gamma activation sequence (GAS), a SP1 binding site, a STAT binding site and several GAAA/TTTC boxes. Then, the 1327-bp fragment obtained was cloned into a luciferase reporter vector, which was transfected into RTG-2 and CHSE-214 cells. The expression of luciferase was measured at different time points after stimulation of the IFN pathway with poly I:C. Interestingly, luciferase expression patterns differed depending of the cell line considered. In RTG-2 cells, the highest level of luciferase expression was observed at 24–48 h post-induction (p.i), decreasing afterwards, whereas in CHSE-214 cells a gradual increase of the luciferase expression up to 72 h p.i. was observed. Deletion and punctual mutation analyses have been performed to determine the contribution of each ISRE motif in the inducibility of the SsMx promoter. Results showed that ISRE1, sited closest to the transcriptional start site, is the main element contributing to the SsMx promoter response, while both, ISRE2 and ISRE3, have a minor additive effect on SsMx promoter induction.

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P-140.

Transcriptional activity of LGP2 promoter is enhanced by interferon regulatory factor 3 in Japanese flounder, *Paralichthys olivaceus*

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Abstract

LGP2 (laboratory of genetics and physiology 2), one of the pattern-recognition receptors, plays a very important role in innate immune response against viruses by inducing the production of type I interferon (IFN) through the recognition of cytosolic viral RNAs. Although the expression of LGP2 mRNA in mammals and teleosts are strongly induced after virus infection or poly I:C stimulation, the mechanism of transcriptional control of LGP2 gene is still unknown. We have cloned the LGP2 gene from Japanese flounder (*Paralichthys olivaceus*), which spanned 5,474 bp containing 12 exons and 11 introns. The expression of LGP2 mRNA in whole kidney was dramatically induced by VHSV (viral hemorrhagic septicemia virus) infection and poly I:C-stimulation *in vitro*. Japanese flounder LGP2 exhibited strong antiviral activities against VHSV, HIRRV (hirame rhabdovirus) or IPNV (infectious pancreatic necrosis virus) infected flounder natural embryo (HINAE) cells. However when the RD (regulatory domain) of the LGP2 was deleted, this function was lost. In addition, the expression of Mx and ISG15 in LGP2-overexpressed HINAE cells were strongly induced by poly I:C co-transfection but not by addition of poly I:C into the culture medium. To better understand why the Japanese flounder LGP2 gene expression was strongly induced, the transcriptional control region of Japanese flounder LGP2 gene was identified and its transcriptional activity analyzed by luciferase reporter assay. Numerous canonical motifs of IFN-regulatory factors (IRFs) were found in the 5′-upstream region (−1,337 bp) of LGP2 gene. Reporter assay showed that the poly I:C-responsive region regulating LGP2 transcription was located at −506 to −398. The transcriptional activity of poly I:C-responsive region was strongly enhanced by IRF3, which could bind to IRF3*3 motif located at −480, suggesting that LGP2 transcriptional control is probably involved in IRF3 function. Interestingly, transcriptional activity of LGP2 promoter was also enhanced in MDA5- or LGP2-overexpressed cells. These results suggest that the enhancement was caused by the induction of IRF3 by MDA5 or LGP2 overexpression. Furthermore, LGP2 promoter was enhanced by VHSV infection in HINAE cells using GFP expression construct regulated by the LGP2 promoter including the poly I:C-responsive region. These results suggest that Japanese flounder LGP2 acts as a cytosolic viral RNA sensor and the functions are conserved with those in mammals in the induction of antiviral response in the innate immune system.

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P-325.

Extract of a Chinese medicinal herb, *Astragalus membranaceus* enhances the non-specific immune response of barramundi (*Lates calcarifer*)

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Abstract

Barramundi or Asian sea bass (*Lates calcarifer*) is a promising new species for European aquaculture. This species is less sensitive to the rearing conditions; therefore it can be reared in intensive systems. However, fish reared in these conditions are more susceptible to infectious diseases due to the higher stocking density, transportation and handling stress or poor water quality. Antibiotics and vaccines are the most commonly used agents against infectious fish diseases of bacterial origin. However, their application has some detrimental effects. Antibiotics and their residues can accumulate in fish meat and in the aquatic environment, and their excessive use can cause the development of resistant bacteria. Vaccines are usually effective against only one type of pathogen. These negative effects can be eliminated by the immunostimulants used in the appropriate dose and time of feeding. Based on our previous experiments with herbal extracts applied as immunostimulants, a four-week feeding experiment was carried out using two different doses of a Chinese medicinal herb, *Astragalus membranaceus*. *Astragalus* extract was mixed to the fish feed in 0.5% and 1.0% concentration. Feed containing agar-agar only was used as a positive control, whereas feed without agar-agar or herbal extracts was the negative control. Fish were fed with these feeds for four weeks and blood samples were taken once a week. Phagocytic cells and plasma were isolated from blood by centrifugation, and non-specific immune parameters were determined. Superoxide anion production and phagocytic activity of leukocytes, and lysozyme activity, total protein and immunoglobulin levels of plasma were measured. Out of the measured immunological parameters, phagocytic activity of leukocytes, total protein and immunoglobulin levels of blood plasma were significantly higher in the treated groups than in the control. Based on these results, *Astragalus* extract can be used for enhancing non-specific immune response of barramundi.

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P-402.

Identification of fish Toll-like receptor ligands with a NF-kB luciferase reporter system

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Abstract

Fish farming is hampered by infectious diseases. Efficient and cheap vaccines against several aquatic viruses are important for a sustainable fish farming industry. Environmental and regulatory concerns hamper development of live, attenuated viral vaccines. Non-living vaccine antigens are