FUNCTIONAL GENOMICS AND FISH HEALTH

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FUNKCIONALNE GENOMIKA I ZDRAVLJE RIBA

Abstrakt

Korišćenje metoda funkcionalne genomike daje uzbudljive mogućnosti za istraživa nja u akvakulturi. Profilisanje multiple genske ekspresije se koristi i za razumevanje molekularnih mehanizama i za razvoj novih dijagnostičkih markera i protokola. Ova metodologija je posebno korisna za proučavanje bolesti riba, s obzirom na dramatične promene u ekspresiji gena i složenosti odgovora na patogene. Ovaj rad predstavlja kratak pregled funkcionalnih genomičkih istraživanja koja su urađena na salmodnim ribama u institutu Nofima Marin.

Ključne reči: *mikroarej, losos, bolesti riba, patogeni*

INTRODUCTION

Diseases caused with viruses, bacteria and parasites are the main risk factor and source of losses in fish farming. Norwegian salmon aquaculture is spending substantial resources and effort for the disease control. The key activities are monitoring of fish health and detection of pathogens, improvement of resistance by breeding, vaccination and use of feed additives. One of the main problems in health management is a limited availability of diagnostic tools tuned for different tasks. Methods of multiple gene expression analyses can provide most efficient approach to this problem as can be shown by several examples from our work.

To date, microarray is the most commonly used method of gene expression profiling. Microarray (chip) is a glass slide, onto which genes are printed in a form of cDNA or oligonucleotides; each spot is used as a probe to compare gene expression in two samples, test and control. Hundreds or thousands of genes are analyzed simultaneously. We are using own salmonid fish microarray, SFA2 or immunochip, which includes 1800 genes (K o s k i n e n et al., 2004; K r a s n o v et al., 2005). Careful selection of genes made possible to cover the key functional classes, such as immune and stress responses, cell cycle and apoptosis, oxidative stress and protein folding and various pathways of metabolism and signal transduction. Each gene is printed in 6 spot replicates, which

ensures high accuracy and reproducibility of analyses. Most important findings are routinely verified with an independent method, real-time qPCR. This system has been used for a wide range of studies including, responses to pathogens, contaminants and stressors, nutrition, embryonic development and differentiation of cells. Our gene expression database includes more than 400 samples. Meta analyses or comparison of large data sets help to separate true responses from random fluctuations thus increasing value of each new study. Fish diseases is the research area, in which microarray technology is especially useful. Infections cause dramatic and highly reproducible changes in gene expression. Given extreme diversity and complexity of responses to pathogens, only high-throughput methods are able to provide a comprehensive picture. Uncritical use of immune assays is at best useless or can lead to erroneous conclusions.

Gene expression and virus diagnostics

At present diagnostics of infectious diseases is based exclusively on the finding of pathogens. Therefore new diseases, which arise continuously can be detected not earlier than infectious agents are identified and characterized. No wonder that fish mortality often remains unexplained. Furthermore, finding of a pathogen does not necessarily mean a disease state since virulent and protracted strains of viruses and bacteria often have minor molecular differences. Studies of host-pathogen interactions can help to find solution to this problem. The task is to select genes that react to viruses much greater than to any other stressors and ideally, discriminate between the pathogenic and non-pathogenic strains.

To date, we have analyzed Atlantic salmon responses to all important viruses: ISA – infectious salmon anemia (S c h i ø t z et al., 2008; J ø r g e n s e n et al., 2008), IPN (infectious pancreatic necrosis) and PD (pancreatic disease). Rainbow trout infected with IPNV –infectious hematopoietic necrosis virus was studied in collaboration with partners from Spain (M a c K e n z i e et al., 2008). We found a panel of genes with strong responses to viruses that have not reacted to other treatments. Most VRG (virus responsive genes) have unknown roles and they are equally stimulated with viruses in different tissues and cell cultures. Comparison of IPN with different virulence showed that VRG respond to the pathogenic strains. Importantly, induction of these genes was observed in fish with CMS – cardiac myopathy syndrome and HMSI – heart and muscle systemic inflammation. Etiology of these diseases is unknown though virus nature is suspected. Our results provide additional evidence in favor of this hypothesis.

To develop diagnostic assays, we have screened with qPCR about 100 candidate genes. This set included VRG presented on the microarray and genes from the same multi-gene families. Studies continue with eight genes: several galectins and galectin binding protein, srk protein kinase and interferon (IFN) inducible protein 44. Antibodies to the VRG encoded proteins will be aseessed for diagnostics of viral diseases.

Mechanisms of resistance to virus

Resistance of salmon to viruses is characterized with high individual variation, which remains unexplained. We compared gene expression in ISA infected salmon with different times of survival after challenge. Salmon with early mortality was characterized with high viral loads and dramatic induction of genes known for the roles in the innate anti-viral responses, many of these are IFN-dependent. The intermediate mortality group had high virus titers but lower expression levels of genes involved in inflammation and

cellular stress. Finally, the group that survived to the end of challenge test was characterized with induction of immunoglobulins in heart and dramatic reduction of virus. Results suggested that the ability to endure high levels of infection for sustained periods could be associated with lower innate immune responses while subsequent protection and viral clearance was most likely conferred by activation of adaptive immunity.

Obviously, assays of innate immune parameters are unable to evaluate resistance of salmon to ISA. Our results suggested a pivotal role of adaptive immunity but unfortunately, we were unable to identify the immunoglobulins associated with resistance. Limited ability to discriminate similar transcripts is a well known drawback of cDNA microarrays. We hope that this problem will be resolved with an aid of oligonucleotide microarray that provides greater specificity of analyses.

Vaccine protection against furunculosis

To date, vaccines have been developed against most part of the important diseases of Atlantic salmon. However in many cases vaccination reduces mortality but does not achieve complete eradication of infection. Vaccine against *Aeromonas salmonicida*, the causative agent of furunculosis is a well known example of limited protection. To search for the changes associated with protection, we compared hepatic gene expression in vaccinated salmon with high and low resistance (HR and LR). Most immune genes showed greater induction in LR with except for several components of the complement system. HR fish was characterized mainly with up-regulation of genes for proteins involved in the protection of extracellular matrix, lipid metabolism, and clearance of endogenous and exogenous toxic compounds. The gene expression analyses suggested that active anti bacterial reactions did not improve resistance, which depended largely on the ability to evade damages from pathogen and acute immune responses. Based on results, we are able to suggest several gene markers of vaccine protection against furunculosis.

Immune stimulators or modifiers?

The use of feed additives acting on the immune system is considered a promising approach for improving resistance to pathogens. At present, their development is complicated with the limited knowledge on the mechanisms of action and the target functions that need modification. We conduced a pilot study using lentinan, a β-glucan from shiitake mushroom as a model. Rainbow trout with lentinan-supplemented and control (C) diets were injected with bacterial lipopolysaccharide (LPS), a classical inducer of inflammation. The microarray analyses in spleen showed that lentinan had rather inhibitory than stimulatory effects on immunity. A group of genes implicated in acute inflammatory responses showed greater expression levels in control. These were for example IFN and tumor necrosis factor (TNF) dependent genes. A similar trend was observed in metabolism of iron and xenobiotics, markers of oxidative and cellular stress. Interestingly, differences between C and L were similar to those observed between salmon with low and high resistance to ISA. A large number of immune genes showed equal responses to LPS in both study groups. Thus, lentinan decreased acute reactions to the inflammatory agent while major parts of the immune response remained unchanged. Our results are in line with the view that feed additive should rather modify than stimulate immunity by enhancing beneficial and reducing detrimental responses. Treatment of fish with inducers of inflammation followed with gene expression analyses is a promising approach to selection of immune modifiers.

Why Atlantic salmon in prone to infection with ectoparasite salmon louse?

The salmon louse, an ectoparasitic caligid crustacean is one of the major problems during the sea phase of salmon production. Atlantic salmon is characterized with high susceptibility to lice as opposed to other species, e.g. coho salmon. We analyzed gene expression in skin and inner organs of infected fish at different stages of salmon louse development. Results suggested that poor ability to expel parasites can be due to weak inflammatory responses. Rapid sensing witnessed by induction of a panel of immune genes was followed with the gene expression patterns that were characteristic for hyporesponsive T-cells. Cellular stress was prevalent in damaged skin as seen by highly significant up-regulation of heat shock proteins, other chaperones and mitochondrial proteins. Induction of the major components of extracellular matrix and other genes involved in wound healing was observed only at the terminal stage of lice development. Overall, the gene expression changes suggest a combination of chronic stress, impaired healing and immunomodulation as the main reason for high sensitivity of Atlantic salmon to lice.

From cDNA microarrays to oligo chips

The cDNA microarray platforms have been exclusively useful however their age is coming to the end. At present they are being substituted with oligonucleotide chips that have a number of advantages. These are an unlimited choice of genes (only sequences are required) and high quality of hybridization with a minimum risk of error. To develop salmonid oligonucleotide microarrays, we designed a comprehensive database – STARS (Salmon and Trout Annotated Reference Genes). It includes all identified Atlantic salmon and rainbow trout genes with annotations by structure, functions and cellular roles and provides tools for designing microarrays and mining of gene expression data. STARS will be used as a standard by partners from Norway, UK, Switzerland and Spain. This will greatly facilitate exchange of results produced in different laboratories. START can be easily adapted for any fish species, the only requirement is availability of mRNA (cDNA) sequences. Our pilot tests with an oligonucleotide microarray produced promising results and studies will continue at large scale.

CONCLUSIONS

High-throughput analytical methods are essential for research in fish health and diseases. Utility of microarrays has been demonstrated by our and other groups. There is little doubt that new technologies such as proteomics and metabolomics will come to this area in near future. "Omics" approaches are equally useful for understanding of mechanisms and development of diagnostic tools. Importantly, results may change vision and promote gradual revision of the existing paradigms. It is customary to regard resistance to diseases solely or mainly as a function of the immune system. Insufficient immune responses can exacerbate disease as seen in example with salmon louse. However our studies have also revealed negative correlation between survival of infected fish and a large number of immune genes. On the contrary, resistance was associated with expression of genes that have never been regarded in disease context. Collaboration between research teas from different countries will be of great importance for successful development of this area.

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