ENVIRONMENTAL BIOTECHNOLOGY 12 (2) 2016, 40-42; doi:10.14799/ebms287

Functional investigation of MiR92b-3p for diagnosis and miRNA-based cure in chemically induced liver injury in fish: a project description

January 2017 - January 2020
Decision No. DEC-2016/21/B/NZ9/03566, National Science Centre of Poland
Principal investigator: Prof. Dr. Eng. Paweł Brzuzan

Paweł Brzuzan, Maciej Woźny, Maciej Florczyk

Department of Environmental Biotechnology, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, Poland

Corresponding author: Paweł Brzuzan, Department of Environmental Biotechnology, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, Słoneczna 45G, 10-709 Olsztyn, Poland; Phone +48 89 523 41 87; Fax: +48 89 523 41 31; E-mail: brzuzan@uwm.edu.pl

Key words: hepatotoxicity mechanism, liver regeneration, Locked Nucleic Acids, microcystin, RNA interference, toxicogenomics, whitefish

ABSTRACT

The continued lack of knowledge concerning the molecular background of adverse effects caused by microcystin-LR (MC-LR) is surprising. This toxin requires additional attention, not only for its ability to cause acute poisoning, but also for its ability to initiate cancer in acute doses, and potentially, to promote cancer via chronic exposure to low concentrations in drinking water. Our recent studies on whitefish (*Coregonus lavaretus*) revealed that long-term exposure to MC-LR resulted in severe liver injury, followed by regeneration of the liver and its unexpected resilience to further toxin uptake. These effects were accompanied by perturbations of hepatic microRNAs (miRNAs) that have target genes involved in cytoskeletal remodeling, cell metabolism, cell cycle regulation, and apoptosis. Among the most pronounced individual alterations, the reduction of MiR92b-3p expression was the most remarkable, and we suggest roles for the miRNA in the aberrant processes of liver cells. This project addresses potential involvement of MiR92b-3p in the as yet unknown regulatory network of MC-induced hepatotoxicity in fish. After a suite of biochemical, physiological, anatomical, and transcriptomic analyses *in vitro* and *in vivo*, we will show how MiR92b-3p works in a damaged liver and which processes it targets. Finally, the research will confirm if and how MiR92b-3p can be targeted therapeutically. We expect it to be shown effective enough to pave a way for its use as a tool for treatment of liver damage in fish. What is more, the RNA-based silencing technique that will be used should yield exciting data for our understanding of the system-level biology of vertebrates.

ABBREVIATIONS

antimir	microRNA antisense oligonucleotide (inhibitor)
MC	microcystin
MC-LR	microcystin-LR
mimic	microRNA sense oligonucleotide (inducer)
MiR92	microRNA 92 precursor family
MiR92b-3p	microRNA92b-3p
miRNA	microRNA
SOB-15	rainbow trout liver epithelial cell line

© UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

FUNCTIONAL INVESTIGATION OF MiR92b-3p IN FISH

Naturally-occurring chemicals like the cyanotoxin microcystin (MC) can contaminate water and food, and thus induce potentially fatal hepatotoxic effects in animals and humans that consume the toxin. Despite recent advances, the molecular mechanisms underlying MC toxicity remain elusive, and there is still a knowledge gap concerning specific MC target/interacting proteins, signaling pathways triggering cell responses to MC, and downstream pathways of toxicity and cell injury.

Our recent studies on whitefish *(Coregonus lavaretus*) revealed that long-term exposure to MC-LR resulted in severe liver damage, followed by regeneration of the organ and its unexpected resilience to further toxin uptake (Woźny et al. 2016). Interestingly, these effects were accompanied by perturbations of hepatic microRNAs (miRNAs) that have target genes involved in cytoskeletal remodeling, cell metabolism, cell cycle regulation, and apoptosis (Brzuzan et al. 2016). We found that, after 14 or 28 days of the challenge, dozens of hepatic miRNAs were significantly up-regulated or suppressed, but the reduction of MiR92b-3p expression was the most pronounced of these alterations. In normal vertebrate liver, this miRNA is abundantly expressed (thousands of copies per cell). The MiR92 family plays important roles in cell proliferation, and several members confer an anti-apoptotic phenotype. Thus, targeting MiR92b-3p with antisense oligonucleotides (*antimir*) could provide a potential therapeutic approach to slow down or even prevent the adverse effects of MC-LR, with MiR92b-3p serving as both a predictive marker and an operative therapeutic target.

To elucidate the potential involvement of the still unknown regulatory network in MC-induced hepatotoxicity, in this project we will 1) characterize the functions of hepatic MiR92b-3p *in vitro* and *in vivo*, and use bioinformatic tools to identify its upstream regulatory elements and downstream targets, which may be useful markers of chemically-induced liver injury, and 2) evaluate the attractive possibility of targeting MiR92b-3p for therapeutic use in fish. To gain insight into the function of MiR92b-3p in fish liver, we will test the following hypotheses:

Hypothesis #1: Exposure of fish-liver cell-line SOB-15 (expressing normal steady-state levels of MiR92b-3p) to MC-LR will induce dose dependent changes in gene expression and in phenotype that are connected with pivotal biological processes (cell proliferation, apoptosis, cell cycle, and oxidative stress);

Hypothesis #2: Administering a *mimic* to the SOB-15 cell line will increase MiR92b-3p abundance, which will increase active transport/weaken cellular self-defense mechanisms, thus increasing the cytotoxicity of MC-LR;

Hypothesis #3: Down-regulation of MiR92b-3p (through delivery of MiR92b-3p antimir) before MC-LR treatment of the SOB-15 cell line will suppress adverse phenotype changes and gene expression, thus strengthening cellular resilience to MC-LR (through reduced uptake or increased biotransformation and elimination);

Hypothesis #4: miRNA intervention by delivery of MiR92b-3p antimir in healthy or MC-LR treated whitefish will prevent or cure pathological liver changes, respectively, providing proof of principle for the innovative therapy. Conversely, delivery of MiR92b-3p *mimic* to MC-LR–treated fish will aggravate the adverse effects in the liver;

Hypothesis #5: The effects of MiR92b-3p interventions will be associated with altered expression of a list of target genes for MiR92b-3p. These associations will provide information on biological functions of MiR92b-3p and evidence for the role of this miRNA in MC-LR–dependent liver injury in fish.

To meet the research objectives, complementary in vitro and *in vivo* studies will run simultaneously. In vitro SOB-15 cell based bioassays (cell cycle, proliferation, apoptosis, oxidative stress assays) will be performed in different exposure variants (MiR92b-3p *antimir* or *mimic*, with or without MC-LR) to understand the functional role of MiR92b-3p in the mechanism of hepatotoxicity. In order to comprehend how MiR92b-3p affects expression of its major mRNA targets, liver samples collected from whitefish treated with MiR92b-3p *antimir* or *mimic* alone will be subjected to high-throughput mRNA sequencing. Then, to understand how MiR92b-3p may act together with MC-LR and affect the global gene expression pattern, samples collected after co-exposure to MC-LR and *antimir* or *mimic* will also be mRNA profiled. Targeting MiR92b-3p with antisense oligonucleotides could provide a potential therapeutic approach to slow down or even prevent the adverse effects of MC-LR. In parallel, biochemical, physiological, and hematological markers, and levels of circulating MiR92b-3p will be determined to document MC-LR induced changes in fish. The genome-wide screen for regulated mRNAs should yield candidate mRNAs for further profiling (qPCR), followed by functional analyses to test if MiR92b-3p is involved in post-transcriptional regulation of a (target) gene, using a luciferase reporter assay. Finally, the role of microRNA 92b-3p in MC-LR induced hepatotoxicity in fish will be determined.

Discovering the role of MiR92b-3p in mediating actions in various cellular processes in fish liver will help to improve our overall understanding of MC-LR–dependent hepatotoxicity and drug-induced liver injury. This project will contribute original insights, not only into the aberrant physiology of the liver cell, but also into the evolution of the MiR92 family and how it operates on crucial signaling determinants of non-mammalian pathways, including mechanisms of oncogenesis. Moreover, if MiR92b-3p is linked to the hepatotoxic effects of MC-LR, this could provide new avenues for diagnostic purposes and pave the way for the possible translation of the MiR92b-3p antimir to aquaculture as a deliverable reagent for the treatment of liver diseases. What is more, the RNA-

based silencing technique that will be used should yield exciting data for our understanding of the system-level biology of vertebrates.

ACKNOWLEDGEMENTS

The project is funded by the National Science Centre of Poland (decision number: DEC-2016/21/B/NZ9/03566) between January 2017 and January 2020.

REFERENCES

Brzuzan, P., M. Florczyk, A. Łakomiak, M. Woźny. 2016. Illumina sequencing reveals aberrant expression of microRNAs and their variants in whitefish (*Coregonus lavaretus*) liver after exposure to microcystin-LR. PLOS ONE 11: e0158899.

Woźny, M., B. Lewczuk, N. Ziółkowska, P. Gomułka, S. Dobosz, A. Łakomiak, M. Florczyk, P. Brzuzan. 2016. Intraperitoneal exposure of whitefish to microcystin-LR induces rapid liver injury followed by regeneration and resilience to subsequent exposures. Toxicology and Applied Pharmacology 313: 68–87.