MASTER'S THESIS

Effects of osmotic stress on molecular responses of gill cells from Japanese eels, Anguilla Japonica

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Abstract

Japanese eels (Anguilla japonicas) are snakelike fishes living in waters in the Asian region. In contrast to most fishes which are stenohalines that can only live in waters with a narrow range of salinity, Japanese eels are classified as euryhalines that can habitat in a broad range of salinity. As the lifecycle of Japanese eels consists of stages across fresh and seawater districts, a well-developed osmoregulation mechanism is needed to balance the intra- and extra-cellular osmolarity of the fishes throughout the seawater acclimation process. While fish gills are one of the organs that separating the ambient water and the inner body fluid of the fish, the fish gills of the Japanese eels have been studied as one of the most crucial organs for osmoregulation purposes. Yet, the osmoregulation and survival strategies of Japanese eels under hyperosmotic stress has not been fully elucidated.

In chapter 2, this study has performed a transcriptome study on the *ex vivo* gill filament model of the Japanese eel to profile the molecular responses after a hypertonic treatment of 4 hours or 8 hours. The experiment is aimed to mimic the gill cells exposed to seawater in the seawater acclimation process of Japanese eels. A profile of differential expressed genes (DEGs) has been revealed that 577 DEGs were commonly upregulated and 711 DEGs
were commonly downregulated in both 4- and 8-hours hypertonic treatment. Functional analysis and annotation have been processed with these DEGs, including Ingenuity Canonical Pathways analysis and gene ontology. These analyses have revealed that the cellular homeostasis of the gill cells has been disrupted and cell death responses has been induced by osmotic stress. The

results have raises a concern that the maintenance of cellular viability and a cell death regulation mechanism are needed for the fishes to survive in the early stage of seawater acclimation.

In chapter 3, this chapter demonstrated that gill cells in Japanese eels are susceptible to apoptosis when they are exposed to hyperosmotic treatments in both in vitro gill cell and the ex vivo gill filament model. To maintain the viability of the gills cells, two inhibitors of apoptosis, XIAP, and survivin, were seen to be expressed in gills cells. The expression of XIAP and survivin were upregulated by dexamethasone, which is an agonist mimicking the effect of cortisol on fishes in seawater acclimation. Meanwhile, the expression levels of the apoptosis executor, caspase 3, were downregulated. These data suggested that with the regulation of cortisol express in the fishes, XIAP and survivin are effective apoptosis regulators in the gill cells of Japanese eels.

The study has demonstrated the molecular responses of the gills of Japanese eels exposed to hyperosmotic stress at the transcriptional level and post-translational level by using transcriptome studies and protein study respectively. The study has paved cell death regulation to be another the key field to study in understanding the ability of salinity tolerance in euryhalines.

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