MASTER'S THESIS

The characterization of hyperosomotic stress-induced signaling cascades and the downstream effectors in primary gill cell culture of Japanese eels, Anguilla japonica

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Date of Award:
2010

The Characterization of Hyperosmotic Stress-Induced Signaling Cascades and the Downstream Effectors in Primary Gill Cell Culture of Japanese Eels, *Anguilla japonica*

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A thesis submitted in partial fulfillment of the requirements

for the degree of

Master of Philosophy

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Hong Kong Baptist University

August 2010

Abstract

In mammalian tissues, most cells are bathed in extracellular fluid with well-controlled osmolarity. Notable exceptions include kidney medullary cells, intestinal cells and chondrocytes. However, under clinical disorders of plasma osmolarity, most cells are exposed to an-isotonic environment that cause cell shrinkage or swelling, consequently leading to a diverse group of disease states. Considerable number of studies has been carried out to decipher the osmosensing and the regulatory mechanisms in mammalian system, yet major gaps exist in our understanding of how animal cells detect volume perturbations and how the osmosignaling pathways activate cell volume regulatory mechanisms. Marine organisms are known to have well-developed osmoregulatory apparatus in fluid and ion transport. Euryhaline fish are naturally exposed to waters of changing salinity. Different from mammalian cells, the maintenance of constant cell volume in an-isotonic environment becomes a habitual and regular process in gill epithelial cells. Cell volume regulation is a salient and evolutionary conserved process. Gill of euryhaline fish is an excellent tissue in the understanding of the fundamental mechanisms of osmotic stress-induced cell volume regulation. Surprisingly there has been very limited study to decipher this mechanism in gill cells.

In the first part of study, it was aimed to investigate the early activations of osmotic stress-related protein kinase and to characterize the possible link of the kinases with the downstream effectors (i.e. transcriptional factors and osmolyte transporter). Freshwater primary gill culture was challenged by incubation with a hypertonic culture medium (500mOsmol l^{-1}) for 6hrs. The osmotic challenge evoked a pleiotropic stimulation of various signaling pathways (i.e. ERK, p38 MAPK, JNK, MLCK, MARCKS and CREB), H3 phosphorylation and the expressions of some downstream targets (i.e. Na^+/K^+-ATPase, Ostf and TauT). The importance of p38 MAPK and

MLCK pathways in the regulation of expressions of Na^+/K^+-ATPase, Ostf and/or TauT were addressed. Collectively this study deciphered possible functional links of the osmosensing-signaling cascades to the regulation of the downstream effectors.

In the second part of the study, the regulation of TauT in an-isotonic conditions was characterized, using both *in vivo* and *in vitro* approaches. The data indicated that TauT mRNA was detectable in both freshwater and seawater fish gills. The expression level of TauT mRNA increased in gills of seawater acclimating fishes. A high abundance of TauT protein was found to be localized in seawater gill chloride cells. Using primary gill cell culture, the expression of the gene was induced when the ambient osmolarity was raised from 320 to 500mOsmol l^{-1}. Hypertonic treatment of the culture caused an increase of F-actin distribution in cell periphery. Treatment of the cells with colchicine or cytochalasin D significantly reduced TauT transcript level following hypertonic exposure. The inhibition of MLCK by ML7 showed a significant additive effect to the hypertonic-induced TauT expression. We have demonstrated the involvement of ionic strength, cytoskeleton, and the MLCK in the regulation of TauT expression. Collectively the studies shed light on the understanding of osmosensing and hyperosmotic adaption in fish gills.

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