DOCTORAL THESIS

Characterization of in-situ Ca^{2+} -sensing mechanisms and stanniocalcin-1 target cells in gills of Japanese eels

Gu, Jie

Date of Award:
2014

Abstract

Calcium ion has diverse beneficial roles in living organisms. Failure in Ca^{2+} homeostasis affects a variety of molecular and cellular processes, ultimately leading to many pathological consequences. In mammals, body Ca^{2+} homeostasis is maintained by the coordinated calcium (re)absorption that occurs in the small intestines, kidneys and bones, and is under tight hormonal control. In fish, two special organs, Corpuscles of Stannius (CS) glands and gills form a regulatory circuit to detect and regulate blood Ca^{2+} homeostasis. However, the underlying molecular mechanism in the regulation of gill Ca^{2+} uptake has not been fully examined. Moreover, some putative biological active substances in CS glands have not been identified. To address these research questions, a euryhaline fish, Japanese eel (*Anguilla japonica*) was used as an animal model for the study.

Fish gill is equipped with epithelial calcium channel (ECaCl) as gatekeeper of Ca^{2+} entry, and membrane Ca^{2+}-ATPase (PMCA) for Ca^{2+} efflux. To test if branchial ECaCl and PMCA responded to change in water Ca^{2+} level, we investigated the changes in fish adapted in artificial freshwater (AFW), Ca^{2+}-deficient AFW (D-AFW) or high Ca^{2+}-AFW (H-AFW). Our data illustrated both short-term and long-term effects on modulations of the transporters. The changes correlated with expression levels of stanniocalcin-1 (STC-1) in CS glands. This part of study supports the regulatory circuit between gills and the glands. In primary cell culture of Japanese eel gill cells, Ca^{2+} sensing was shown to be mediated by Ca^{2+} sensing receptor (CaSR) coupled to phospholipase C (PLC)-extracellular signal-regulated kinase (ERK) and PLC-inositol triphosphate (IP_3)-Ca^{2+}/calmodulin-dependent protein kinase-II (CaMK-II) pathways. And CaSR-STC-1/cyclo-oxygenase-2 (COX-2) mediated protective pathway in gill cells that exerts a possible protective mechanism against an increase in intracellular Ca^{2+} levels associated with transepithelial Ca^{2+} transport. Apparently, the protective effects against Ca^{2+}-mediated cytotoxicity of gill cell were mediated by STC-1 binding on gill cells that led to elevations of cytosolic cAMP. In a follow-up experiment of using Ca^{2+}-imaging system in a model of thapsigargin (TG)-induced elevation of cytosolic Ca^{2+}, a hypocalcemic action of STC-1 was demonstrated and was found to be mediated by cAMP and COX-2 pathway. To further determine the gene expressed in CS gland responsive to changes in water salinity, the first transcriptome database of CS glands from fish adapted in freshwater or seawater condition. A *de novo* assembly of RNA sequencing data generated 11747 unigenes and revealed 475 genes that were differentially expressed. Three functional clusters: (1) Ca^{2+}-metabolism, (2) blood pressure and (3) ion-osmoregulation were revealed. Gene targets, in addition to STC-1 in related to the regulation of calcium metabolism and blood pressure, like calcitonin, atrial natriuretic peptide-converting enzyme and endothelin-converting enzyme 1 were identified. Taken together this thesis described a comprehensive study on the functional circuit between gills and CS glands to decipher the regulation and functions of transporters and hormones in calcium metabolism in fish.

Acknowledgments

I would like to express my sincere gratitude to my principal supervisor, Prof. Chris KC Wong, for his guidance, support, and many helpful comments throughout the study. I am also grateful to Prof. NK MAK, my co-supervisor, for his advice and support. Also, special thanks to Prof. Ricky NS Wong, Prof. J Zhang for their precious advice and generous support.

Besides, I would also like to thank Dr. Bonnie Yeung, Ms. Eman. Lee, Ms. Louise Ng, Ms. Olivia Chau, Mr. KW Chan, Mr. WS Chung, Mr. KK Ma, Mr. WK Ip, and other technicians in the Biology Department (Hong Kong Baptist University), for giving their help and their precious suggestion. And I also appreciate Dr. GG Zhao, Dr. X Wei, Dr. Jamie Wan, Dr. Alice Law, Dr. William Tse, Mr. Oscar Chow and other lab colleagues, for their technical assistances and advices.

Last but not least, I would like to thank my family, colleagues and friends for their encouragement and understanding.

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