Characterization of the zebrafish orthologue of Klotho

by

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June 2011

Contents

Abstract

Klotho is a multi-functional anti-ageing protein. Its deletion causes accelerated ageing in mice while its overexpression increases mouse longevity. Klotho is a single pass transmembrane protein and its extracellular domain is cleaved at the cell surface by proteolytic enzymes and subsequently shed into the blood and cerebrospinal fluid, thereby functioning as a humoral factor.

The transmembrane form of Klotho is a co-receptor for fibroblast growth factor 23 (Fgf23). Fgf23 acts on the kidney to induce phosphate excretion into urine. However, in the absence of Klotho, Fgf23 cannot bind to its receptor. Therefore, Klotho deficient mice show hyperphosphataemia. The extracellular domain of Klotho released by proteolytic cleavage inhibits the insulin/insulin-like signaling pathway. The inhibition of the insulin pathway enhances protection of cells from oxidative stress and this is an evolutionarily conserved mechanism for extending life span. In addition to this function, secreted Klotho is known to activate calcium ion channels and inhibit the Wnt pathway. Taken together, Klotho acts as an ageing suppressor, but the precise mechanism of how Klotho exerts these functions is not fully understood. In particular, the function of Klotho against oxidative stress needs to be closely investigated as oxidative stress is a major contributor to ageing as well as many diseases associated with ageing, such as Alzheimer's disease.

The strength of zebrafish as a model system for human diseases has been displayed over the past two decades. Zebrafish possess relative simplicity of organ structure while preserving morphological and genetical similarity to higher vertebrates.

In this study, I characterize the zebrafish orthologue of Klotho. Chapter 2 (thesis chapter in the form of a manuscript) describes the identification of the candidate gene and confirmation of its orthologous relationship with Klotho genes in other organisms by bioinformatics approaches. It determines the temporal expression of *klotho* during embryonic development and the tissue specificity of its expression in adult zebrafish by RT-PCR. It also investigates the relative levels of *klotho* mRNA transcript in zebrafish embryos and adult tissues by quantitative real time RT-PCR. Chapter 3 describes exploration of expression of zebrafish *Klotho* in response to oxidative stress using an oxidative stress inducer, hydrogen peroxide. It also examines proteolytic cleavage of zebrafish Klotho as seen in humans and mice by Western Blotting analysis. Chapter 4 describes an attempt to create site-directed mutations in the *klotho* coding sequence in the genome of zebrafish using zinc finger nucleases.

Declaration

I, Yuya Sugano, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Identification and expression analysis of the zebrafish orthologue of Klotho (Springer, New York City)

Signed

Date 28/06/11

List of Publications

Identification and expression analysis of the zebrafish orthologue of Klotho

Yuya Sugano and Michael Lardelli

Development, Genes and Evolution, Manuscript accepted 14th June, 2011

Acknowledgement

I would like to express my gratitude to:

My supervisor, Dr. Michael Lardelli, for his invaluable assistance, support and guidance during the completion of the work.

Dr. Morgan Newman for her advice, assistance and support. Members of Lardelli lab, Dr. Simon Wells, Lachlan Wilson, Seyyed Hani Moussavi Nik and Anne Lim for their advice and support in any respect.

The Discipline of Genetics for providing a wonderful environment to learn science.

Prof. Shugo Watabe for generously encouraging me to pursue my enthusiasm to study abroad.

Rotary Foundation for sponsoring my study. Rotarians of Adelaide Light Rotary Club for hospitality in Adelaide.

My family and friends and especially John and Deborah for their support in numerous ways.

Without these people, I would not have made it.