

Targeted MS Quantitation of Klotho, a Protein Biomarker of Aging

Jennifer N. Sutton¹, Mary F. Lopez¹, David Sarracino¹, Bryan Krastins¹, Amol Prakash¹, Orson Moe², Makoto Kuro-o², Michael Athanas³, Kevin Rosenblatt²

¹Thermo Fisher Scientific, Cambridge MA U.S.A., ² Department of Pathology, University of Texas Southwestern Medical Center at Dallas, Dallas TX U.S.A. ³Vast Scientific, Wayland, MA U.S.A.

Overview

Purpose: Previously developed ELISA and Western blot assays for the *Klotho* protein in plasma are unsatisfactory due to the lack of specific antibodies and imprecise mass measurements for verification of analyte identity. The development of an accurate and robust assay for *Klotho* would facilitate accurate detection and quantification in human samples for further study.

Methods and Results: We report here the development of a quantitative, selective reaction monitoring (SRM), SID-MS-based assay using triple quadrupole mass spectrometry and Aqua™ heavy isotope labeled peptides to detect and quantify *Klotho* protein in plasma from mice and human samples. A novel software algorithm was used to select target peptides and provide optimal analytical conditions for assay development. Alignment, probability scoring and ion ratio analysis for confirmation and quantitation of *Klotho* were carried out with automated data processing.

Introduction

The *Klotho* gene was originally discovered in mouse mutants that displayed accelerated loss of multiple functions resulting in a phenotype that resembled premature aging¹. More recently, the extension of lifespan in mice overexpressing *Klotho* suggests that *Klotho* acts as an aging suppressor in mammals². The extracellular domain of the *Klotho* protein is shed from the cell surface and circulates in the blood in mice and humans. In mice, it has been demonstrated to bind to a putative cell-surface receptor resulting in the inhibition of insulin and IGF1 signaling. Therefore, *Klotho* appears to act as a hormone that modulates insulin metabolism and aging.

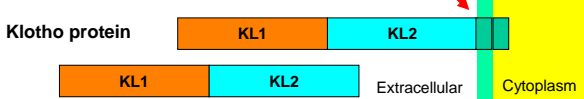
FIGURE 1. The *klotho* gene

Was originally identified as a gene mutated in the *klotho* mouse, which exhibited multiple aging-like phenotypes. Subsequent work by Kuro-o and Rosenblatt at UTSW has demonstrated that *Klotho* acts as a protein hormone

A goddess in Greek myth who spins the thread of life



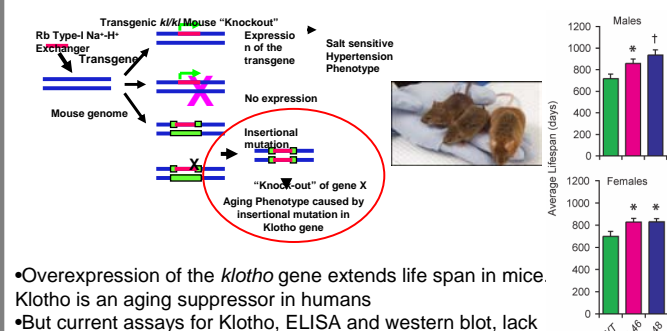
The gene encodes a single-pass transmembrane protein of ca 130kD
 • Short intracellular domain
 • Extracellular domain contains two homologous regions
 • Weak homology with B-glucosidase



• Highly expressed in kidney and brain
 • Extracellular domain shed into blood, urine and csf

Methods and Results

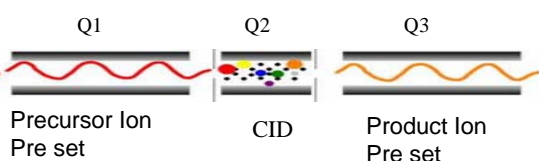
FIGURE 2. A defect in the *klotho* gene expression leads to a syndrome resembling aging in mice and is the first documented mammalian model for human aging that manifests multiple aging-like phenotypes in a single individual.



• Overexpression of the *klotho* gene extends life span in mice. *Klotho* is an aging suppressor in humans
 • But current assays for *Klotho*, ELISA and western blot, lack sufficient sensitivity/selectivity.
 • Better assays for *Klotho* protein are needed.

FIGURE 3. Schema for protein/peptide quantitation using SRM assays on a triple quadrupole mass spectrometer.

1. A "proteotypic" target peptide is selected by hypothesis-driven (chosen from publications or databases) or empirical (discovered through experimentation) data
2. Q1 is set to transmit only the parent m/z of the selected peptide.
3. Fragmentation is induced in Q2.
4. Q3 is set to transmit only a selected fragment ion.
5. SRM analyses for many targeted proteins can be carried out during an LC/MS/MS run.



TSQ Quantum Ultra™

- Sensitive, Robust, Peptide Assays
- Enhanced Selectivity
- QED MS/MS for peptide structure confirmation
- AQUA heavy peptide standards can be used for accurate quantification
- Up to 300 SRM transitions can be monitored /sec.

FIGURE 4. Development of a selective reaction monitoring SRM assay.

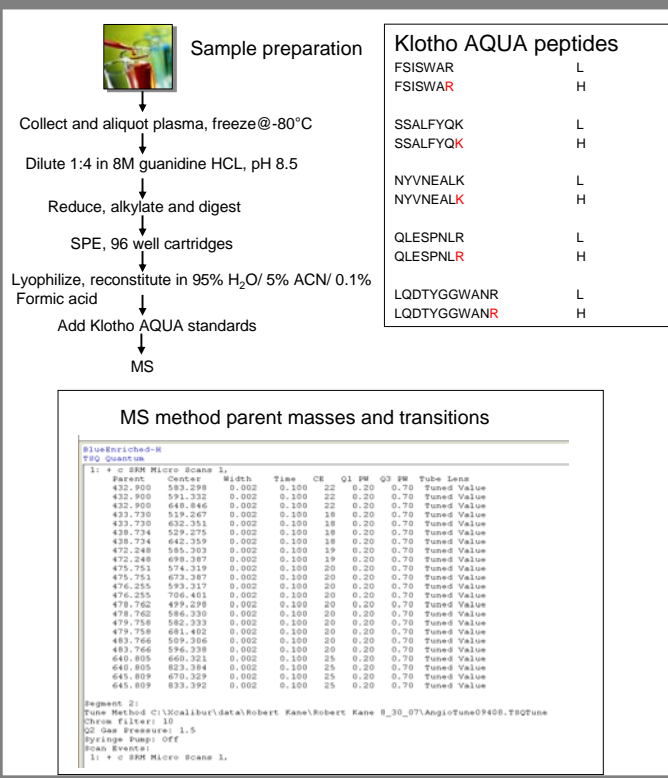
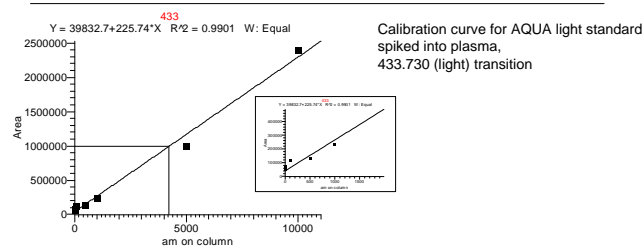
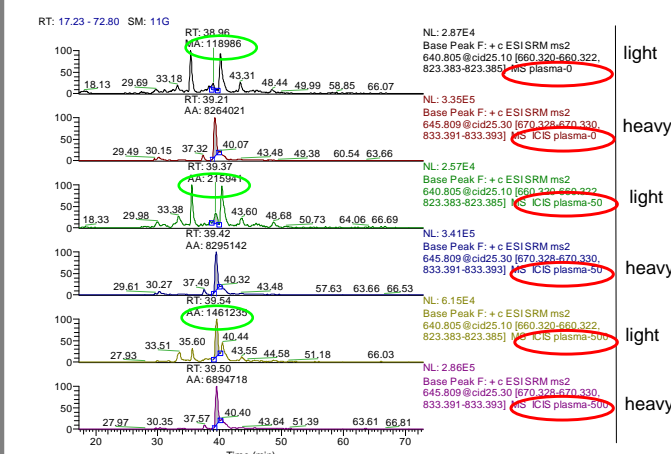
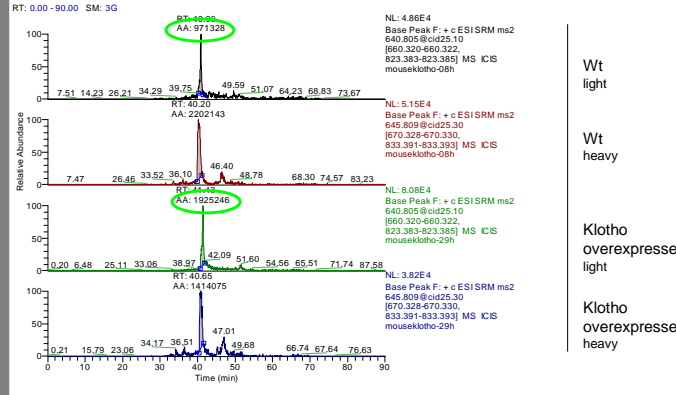


FIGURE 5. *Klotho* detection in plasma, mouse and human. Human plasma was spiked with AQUA standards at 0, 50 and 500 femto moles on column and detected using SRM-transitions at 640.805m/z and 645.809 m/z.



Mouse *klotho* mutants, plasma, 640.805, 645.809 SRM transitions



Conclusions

- A SRM – based assay for the detection of *Klotho* was developed and applied to the quantification of *Klotho* protein in human and mouse plasma.
- Limits of sensitivity in a background of raw plasma were determined to be on the order of 200-500 attomoles on column.
- Limits of sensitivity for neat peptides were established and are on the order of 10-20 attomoles on column.
- Further work will focus on optimization of enrichment strategies and automation for sample preparation

References

1. Mutation of the mouse *klotho* gene leads to a syndrome resembling ageing. M. Kuro-o *et al*, Nature 390,45 (1997).
2. Suppression of aging in mice by the hormone *Klotho*. H. Kurosu *et al*, Science 309,1829 (2005).
3. *Klotho*, an aging suppressor gene. K. Rosenblatt and M Kuro-o, Hormone Research (suppl 1) :191-203 92007).

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