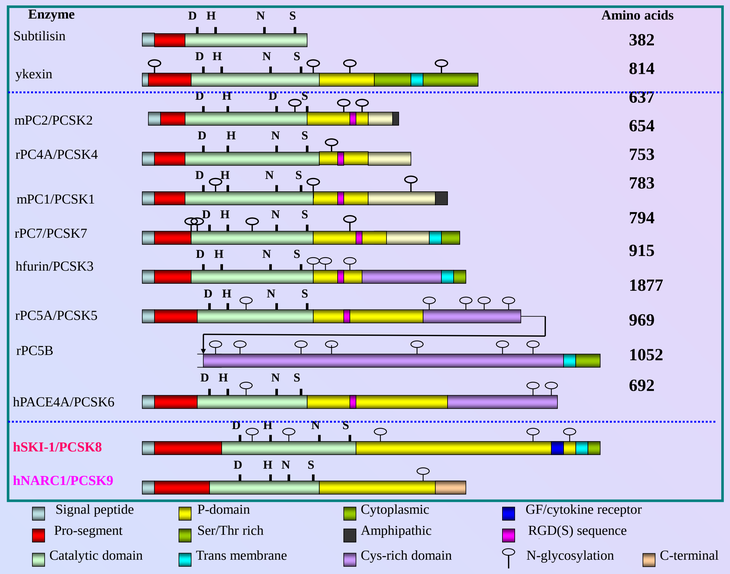
An efficient and selective in vitro solid phase assay for PCSK4 activity: Potential diagnostic tool in fetal and placental growth restriction

Caroline Muia, Chunyu Lu and Dr.Ajoy Basak
Health Science Department, University of Ottawa

Introduction

Nearly half of all infant mortality occur in the first 28 days after birth with Intrauterine Growth Restriction (IUGR) syndrome being one of the leading causes. Even today despite advancements in research and our better understanding of the mechanism of this condition, it still remains in the forefront of reproduction research. IUGR, also called Fetal Restricted Syndrome (FRS), leads to abnormal development and growth of placenta and fetus. It is linked to the proteolytic activity of PCSK4 (Proprotein Convertase Subtilisin/Kexin type 4) enzyme, also known as PC4. This enzyme is exclusively present in reproductive tissues/organs including ovary and placenta. It is present on the surface of plasma membrane overlying the acrosome of the sperm. It cleaves inactive larger pro-Insulin growth factor2 (proIGF2) to generate its mature shorter form IGF2, which plays a crucial role in fetal and placental growth/ development during pregnancy. Decreased PCSK4 activity leads to less production of mature IGF2 causing IUGR condition. *Thus monitoring PCSK4 activity using a selective and sensitive method will be useful for early detection of IUGR.*



Hypothesis

Decreased level or impaired PCSK4 protease activity is responsible for production of low mature IGF2 level leading to IUGR.

Objective

Develop an efficient, highly selective and rapid solid phase fluorescence based method for monitoring PCSK4 protease activity in vitro.

Aim 1: Design and Synthesis of a Highly Flourescent Peptide

Aim 2: Application of the Fluorescent Peptide for selective *In Vitro* Assay of PCSK4 Activity

Aim 3: Potential Application of the above method for monitoring PCSK4 activity in biological samples under IUGR vs Control conditions

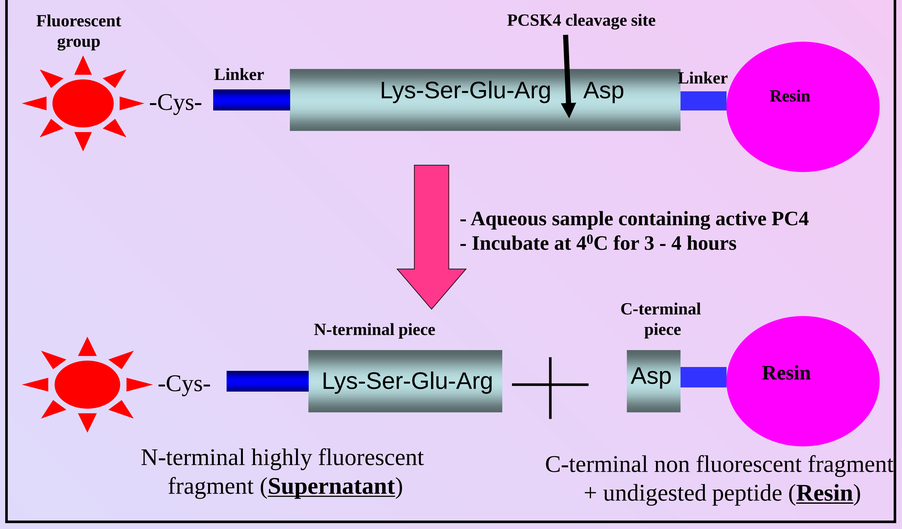
Methods

Design & Preparation of a fluorescent peptide: A 5-amino acid long peptide sequence mimicking the recognition motif of PCSK4 enzyme was selected from the cleavage site of proIGF2 (human) which is described as the most potent physiological substrate of PCSK4.
-The above peptide also contains a linker (Adoa) and Cys-attached Fluorescence moiety as shown below.
-The peptide thus designed was synthesized using Solid Phase Peptide Chemistry using Intavis MultiPep instrument and PEGA resin on which the it remain immobilized.

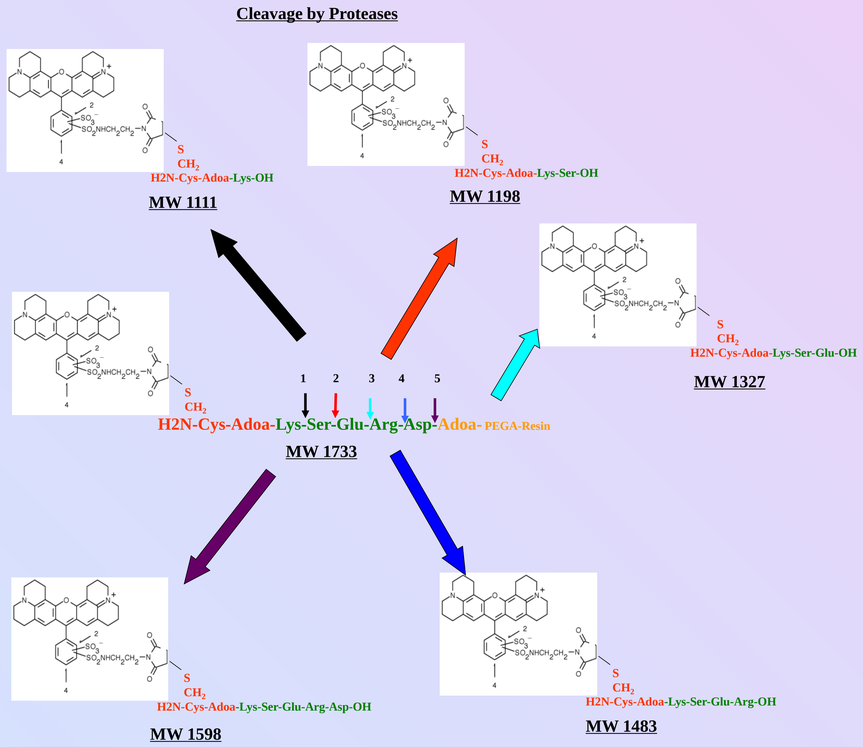
Enzyme assay: The above immobilized fluorescent peptide on resin (~100 μg) was incubated with various enzymes including recombinant PCSK4 available in the lab

Adoa=8-Amino 3,6-Dioxa-Octanoic acid), serves as an 8-atom linker/spacer chain

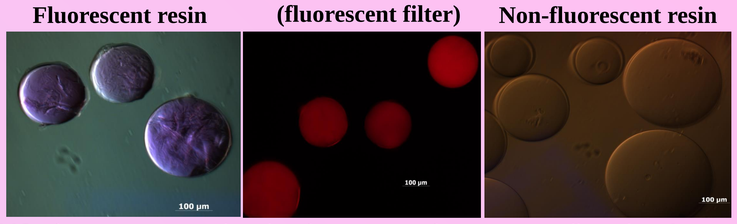
RESULTS



All possible cleavages and the expected Mol Wts of all fragments thus produced and released into the medium (supernatant).

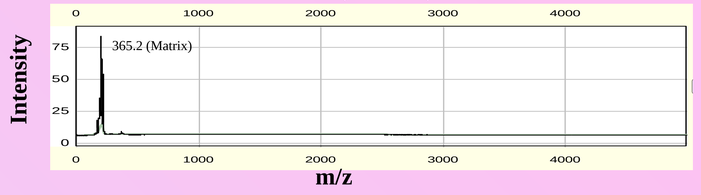


Fluorescent & non-fluorescent PEGA resins under microscope

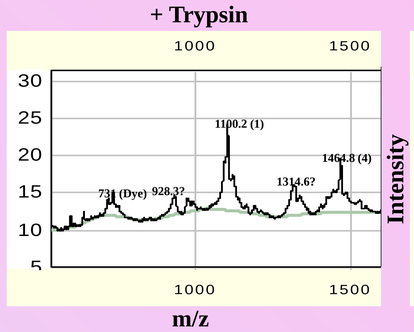


MS of supernatants showing fragments after enzyme digestion & control

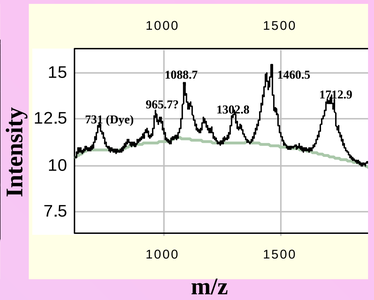
+ Water (Control)



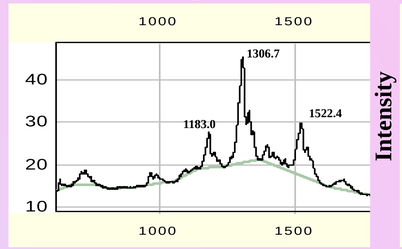
+ Trypsin



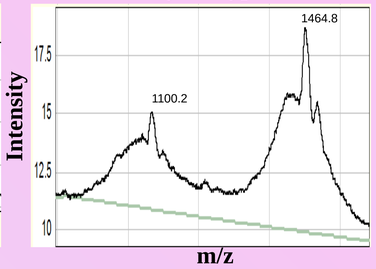
+PCSK4



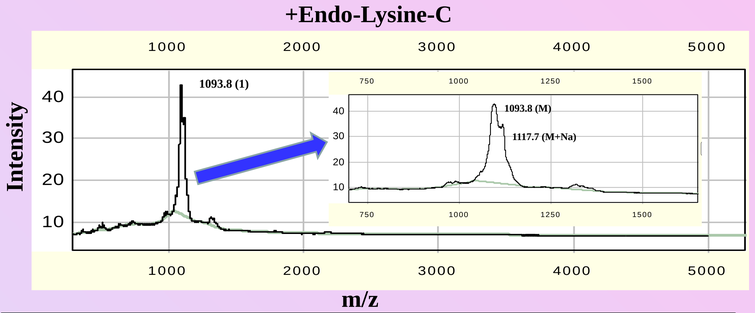
+ Pepsin



+ Chymotrypsin



+Endo-Lysine-C



Summary

- *A novel solid phase fluorescence based method has been developed for in vitro assay of PCSK4 activity*
- *The method is very simple and can be made selective and sensitive by using dextro amino acid incorporation and the use of protease inhibitors*
- *Application of this method for examination of PCSK4 activity in Placenta tissue is under progress.*

Acknowledgement

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References

Qiu et al PNAS, 102, 11047-52, 2005; DeChiara et al Cell 64, 849-59, 1991; Baker, J. et al Cell 75, 73-82, 1993; Constancia, M. et al Nature 417, 945-48, 2002; Fowden, A. L. Placenta 24, 803-12, 2003; Saving Lives at Birth: A Grand Challenge for Development, Grand Challenge Development, 2014; Basak et al Methods in Molecular Biology 768