Eliot T Smith, J Glycobiol 2015, 3:3
http://dx.doi.org/10.4172/2168-958X.S1.002

OMICS International
conferenceseries.com

Glycobiology World Congress

August 10-12, 2015 Philadelphia, USA

Expression of recombinant human mast cell chymase with Asn-linked glycans in glycoengineered *Pichia pastoris*

Eliot T Smith
East Tennessee State University, USA

Human mast cell chymase is a potent serine protease with roles in inflammation and allergy response. Chymase has received attention for its ability to generate angiotensin II from angiotensin I or angiotensin (1-12). The primary natural source of human chymase is skin tissue and recombinant expression provides a safe and abundant alternative for chymase research. One drawback of many expression systems, however, is the inability to generate proteins with human glycosylation patterns. To generate recombinant human chymase (rhChymase) with a glycosylation pattern that more closely resembles the natural enzyme, rhChymase was expressed and secreted in active form with $(Man)_5$ $(GlcNAc)_2$ Asn-linked glycans using the SuperMan$_5$ strain of GlycoSwitch® *Pichia pastoris* (BioGrammatics). Five milligrams of active enzyme were recovered from one liter of fermentation medium by cation exchange and heparin affinity chromatography. Purified rhChymase glycoprotein appeared as a single band migrating at an apparent molecular weight of 30 kDa on SDS-PAGE and treatment to remove glycosylation reduced the apparent molecular weight to 25 kDa, consistent with properties of the native enzyme. Western blotting with antibodies against human chymase labeled rhChymase. Active site titration with the potent chymase inhibitor Eglin C followed by kinetic analyses with peptide synthetic substrates demonstrated that glycosylated rhChymase possesses enzymatic activity that closely resembles its native counterpart. This work provides a source of active rhChymase with glycosylation similar to the as yet unidentified human chymase glycan pattern(s) and offers a foundation for future production of chymase with true human glycosylation.

Biography

Eliot T Smith completed his PhD at the James H Quillen College of Medicine at East Tennessee State University in 2013. He currently works as a Postdoctoral Researcher at the University of Pennsylvania, where he studies NDR kinases in the laboratory of Francis Luca.

eliotsmi@vet.upenn.edu

Notes: