Development of a proteomic workflow for the identification of heparan sulfate proteoglycan-binding substrates of ADAM17

Matteo Calligaris[1], Donatella Spanò[1], Stephan Mueller[2], Simone Bonelli[1], Margot Lo Pinto[1], Giovanni Zito[3], Carl Blobel[4], S Lichtenthaler[2], Linda Troeberg[5], and Simone Scilabra[1]

[1]Ri.MED Foundation
[2]DZNE-Standort München
[3]ISMETT
[4]Hospital for Special Surgery Arthritis and Tissue Degeneration Program
[5]University of East Anglia Norwich Medical School

April 22, 2024

Abstract

Ectodomain shedding, which is the proteolytic release of transmembrane proteins from the cell surface, is crucial for cell-to-cell communication and other biological processes. The metalloproteinase ADAM17 mediates ectodomain shedding of over 50 transmembrane proteins ranging from cytokines and growth factors, such as TNF and EGFR ligands, to signaling receptors and adhesion molecules. Yet, the ADAM17 sheddome is only partly defined and biological functions of the protease have not been fully characterized. Some ADAM17 substrates (e.g. HB-EGF) are known to bind to heparan sulfate proteoglycans (HSPG), and we hypothesised that such substrates would be under-represented in traditional secretome analyses, due to their binding to cell surface or pericellular HSPGs. Thus, to identify novel HSPG-binding ADAM17 substrates, we developed a proteomic workflow that involves addition of heparin to solubilize HSPG-binding proteins from the cell layer, thereby allowing their mass spectrometry detection by heparin-secretome (HEP-SEC) analysis. Applying this methodology to murine embryonic fibroblasts stimulated with an ADAM17 activator enabled us to identify 47 transmembrane proteins that were shed in response to ADAM17 activation. This included known HSPG-binding ADAM17 substrates (i.e. HB-EGF, CX3CL1) and 17 novel HSPG-binding putative ADAM17 substrates. Two of these, MHC-I and IL1RL1, were validated as ADAM17 substrates by immunoblotting.

Hosted file

`PaperHEPSEC_vFinal.docx` available at https://authorea.com/users/773051/articles/860271-development-of-a-proteomic-workflow-for-the-identification-of-heparan-sulfate-proteoglycan-binding-substrates-of-adam17